A new virus-like disease of barley : its etiology, epidemiology, and the ultrastructure of associated virus-like particles
by Nancy Lee Robertson

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University
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Abstract:
In 1982, a virus-like disease of barley was discovered in Montana; a yield loss of 25-30% was attributed to the disease. The disease was undiagnosable, although barley leaves had parallel light green to yellow dashes and streaks and unique intracellular virus-like particles (VLPs).

The VLPs were hypothesized to be the causal agent of the disease because of their consistent association with diseased tissue and not healthy tissue. The ultrastructure of the VLPs was studied by electron microscopy of leaf extracts and ultrathin sections from diseased plants. How the causal agent moved from plant to plant was determined by using field collected and/or laboratory reared aphids, leafhoppers, thrips, and mites; seed derived from diseased plants; soil tests; and by exposing healthy trap seedlings to fields of diseased plants.

Field surveys conducted from 1983 through 1986 found the disease to be confined to Pondera, Teton, Glacier, and Toole counties in North Central Montana. It increased in intensity and expanded its geographical range to the north and to the south, reaching epidemic levels in 1986. Barley was a preferred host, while wheat and three annual grasses had small incidences of infection. The disease had a greater affinity to drought-stressed sites. Recrop barley usually had the highest incidence of infection, while summer-fallow and rotations into wheat reduced the incidence.

Successful transmission tests occurred only with Petrobia latens Muller, the brown wheat mite. Healthy plants which had been exposed to field mites or the larvae hatched from diapausal eggs developed the diagnostic symptoms and contained the VLPs. Transovarial transmission was therefore assumed.

The VLPs resembled some plant viruses with an overall tubular shape with diameters ranging from 81 to 52 nm (ave. 64 nm) which were enclosed within a membrane. The VLPs were unlike plant viruses in that they attained apparent lengths over 4,0OO nm and had an indistinct internal ultrastructure. The morphological uniqueness of the assumed causal agent (VLPs) and the first implication that P. latens is a vector of a plant pathogen provided evidence for a newly described plant disease.

# A NEW VIRUS-LIKE DISEASE OF BARLEY: ITS ETIOLOGY, EPIDEMIOLOGY, AND THE ULTRASTRUCTURE OF ASSOCIATED VIRUS-LIKE PARTICLES

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Nancy Lee Robertson

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## ABSTRACT

In 1982, a virus-like disease of barley was discovered in Montana; a yield loss of 25-30% was attributed to the disease. The disease was undiagnosable, although barley leaves had parallel light green to yellow dashes and streaks and unique intracellular virus-like particles (VLPs).

The VLPs were hypothesized to be the causal agent of the disease because of their consistent association with diseased tissue and not healthy tissue. The ultrastructure of the VLPs was studied by electron microscopy of leaf extracts and ultrathin sections from diseased plants. How the causal agent moved from plant to plant was determined by using field collected and/or laboratory reared aphids, leafhoppers, thrips, and mites; seed derived from diseased plants; soil tests; and by exposing healthy trap seedlings to fields of diseased plants.

Field surveys conducted from 1983 through 1986 found the disease to be confined to Pondera, Teton, Glacier, and Toole counties in North Central Montana. It increased in intensity and expanded its geographical range to the north and to the south, reaching epidemic levels in 1986. Barley was a preferred host, while wheat and three annual grasses had small incidences of infection. The disease had a greater affinity to drought-stressed sites. Recrop barley usually had the highest incidence of infection, while summer-fallow and rotations into wheat reduced the incidence.

Successful transmission tests occurred only with Petrobia latens Müller, the brown wheat mite. Healthy plants which had been exposed to field mites or the larvae hatched from diapausal eggs developed the diagnostic symptoms and contained the VLPs. Transovarial transmission was therefore assumed.

The VLPs resembled some plant viruses with an overall tubular shape with diameters ranging from 81 to 52 nm (ave. 64 nm) which were enclosed within a membrane. The VLPs were unlike plant viruses in that they attained apparent lengths over 4,000 nm and had an indistinct internal ultrastructure. The morphological uniqueness of the assumed causal agent (VLPs) and the first implication that P. latens is a vector of a plant pathogen provided evidence for a newly described plant disease.

# INTRODUCTION

In 1982 a new virus-like disease of barley was discovered in Montana. The author surveyed a field with abnormally discolored barley plants near Conrad, Montana after the producer had reported the diseased barley.

The leaves of the affected barley plants had symptoms which were virus-like, yet unique from those of other virus diseases known to be present in the small grains in this region. A leaf mosaic pattern was produced by light green to yellow dashes, streaks, stripes, or bands distributed along the parallel axis of the leaf veins. Samples of these leaves were collected and processed for electron microscopy. Ultrathin sections of diseased tissue contained unique long, filamentous structures which resembled virus particles in size. More specifically, they resembled a group of plant rhabdoviruses, except that some of the structures had extraordinarily long lengths that are not attained by any present member of the plant viruses. Since the structures appeared to be only associated with diseased tissue and were morphologically similar to some plant viruses, they were hypothesized to be virus-like particles (VLPs) (74).

Further interest in and concern for the virus-like disease of barley was indicated when the producer reported a 25-30 percent yield loss from the affected barley. That the very nature of the apparent disease was undiagnosable with abnormal mosaic discolorations in leaves

containing unique VLPs led to an investigation of the disease. Specific objectives pertaining to the study of this virus-like disease are listed below:

1) Determine the natural host range, prevalence, and geographical distribution of the disease in North Central Montana.
2) Determine how the causal agent of the disease moves from plant to plant.
3) Study the unique virus-like particles found inside the affected plant tissue by ultrathin sections, sap extractions, and by isolation and partial purification techniques.
4) Determine if the virus-like particles are the causal agent of the disease.

# CHAPTER I

# THE ASSOCIATED VIRUS-LIKE PARTICLES: THEIR MORPHOLOGY AND STRUCTURE, DISTRIBUTION IN TISSUE, AND THEIR ISOLATION AND PARTIAL PURIFICATION FROM DISEASED PLANTS

## Materials and Methods

It was the intent to seek the identity of the unique virus-like structures associated with the disease, and determine their relationship with symptomatic plants. It was hypothesized that since these structures were only found in symptomatic leaves and never in healthy control leaves, and because they were morphologically similar to viruses, they were somehow involved in the etiology of the newly reported disease of barley. Moreover, it was believed that the structures were intact virus particles of a yet undescribed plant virus and were, in fact, the causal agent of the disease. Henceforth, these structures will be referred to as virus-like particles (VLPs) for the purpose of this report.

Inasmuch as the VLPs had sizes and shapes similar to those of other known virus particles, plant virological techniques were used in an attempt to characterize and identify the VLPs.

## Electron Microscopy of Ultrathin Sections and Extracted Sap from Diseased Plant Tissue

Fresh plant tissue was diced into 1 mm squares on a drop of cold 3% glutaraldehyde (analytical grade, Ted Pella, Inc.) in 0.1 M phosphate ($NaK_2PO_4$) buffer, pH 7.2. The tissue was then transferred to a small vial of 3% glutaraldehyde and aspirated in a vacuum chamber at $158.7 \times 10^3$ Pa for 15 min. before refrigerating overnight. Subsequently, the fixed tissue was rinsed three times with the same phosphate buffer, and placed in 2% osmium tetroxide in 0.1 M $NaK_2PO_4$, pH 7.2 for four hours at room temperature. The diced tissue was then rinsed three times in the same phosphate buffer, and serially dehydrated through 50% (2X), 70%, 95%, and 100% (3X) of ethanol, respectively. Next, the material was placed in propylene oxide, followed by the gradual infiltration of Spurr's epoxy resin (88); individual leaf sections were embedded in the same resin within plastic Beem capsules (Pelco, Inc.) and baked at 65 C. The ultrathin sections were cut with a Dupont diamond knife (Dupont Instruments) on a Reichert Om U2 ultramicrotome. After ribbons of sections were placed on a copper grid (mesh-300, Ted Pella, Inc.), the sections were double stained with uranyl acetate (previously saturated in 100% methanol) for 5 min. and then in Reynold's lead citrate, pH 12.0 for 3 min. (73); they were thoroughly rinsed after each stain. The ultrathin sections were viewed with either a Zeiss EM 9S-2 or an EM 10 CA electron microscope, each operating at an accelerating voltage of 60 KV. Both cross- and longitudinal-sections were cut with a special added effort to obtain

epidermal, mesophyll, phloem, and xylem cells all within the same section. The majority of the tissue was derived from leaves, while a few sections were from the root, awn, and sheath. Healthy control tissue was also processed in the manner described previously.

Field samples were immediately fixed in glutaraldehyde on the site, or the whole plants were removed from the soil and placed in plastic bags before storing in an ice chest up to two-three days. The material was collected throughout the growing season from mid-July 1982 through 1986.

Containerized seedlings (trap plants), which were sown under greenhouse conditions and then transported and exposed to diseased fields for a specific period of time before returning to the greenhouse, were also sampled. Leaves were detached from these trap plants and control plants while they were maturing in the greenhouse and immediately fixed in glutaraldehyde.

Leaves from plants exposed to the brown wheat mite were also processed for ultrathin sectioning (see Chapter III, page 59 for more details).

In vitro preparations of the VLPs were obtained by grinding diseased leaf tissue in 3 mls of cold 0.05 M Tris-HCl, pH 7.2 or 0.2 M sodium citrate, pH 7.6 with 10% sucrose in a chilled mortar with a pestle. Only fresh leaf material was used whereby a 1.5 cm diameter circle of tissue was cut with a no. 8 cork borer. Parlodian (0.5% parlodian in amyl acetate) -coated copper grids were placed on a drop of ground sap for 30 min. Each grid was rinsed in four drops of the Tris-HCl buffer, then rinsed on four drops of distilled water before it

was stained with 5% uranyl acetate in 50% ethanol and a final rinse of 95% ethanol. Some of the VLPs were treated with a negative stain, 2% phosphotungstic acid (PTA), pH 6.5, whereby a drop of PTA was applied to a grid with the VLPs preparation and allowed to dry, after which the excess stain was removed with filter paper.

Diseased field leaf samples were routinely checked for wheat streak mosaic virus (WSMV) by immunosorbent electron microscopy (8, 24) because WSM disease had been reported to occur in the same region as this new disease (10). The antiserum was kindly provided by J. Uyemoto and diluted to 1/1000.

Isolation and Partial Purification of VLPs from Diseased Leaf Tissue

The procedure used to isolate and partially purify the VLPs from barley leaves was based upon methods previously described by Jackson and Christie (44) and El Maataoui, Lockhart and Lesemann (26). Eighteen separate purification attempts were made using symptomatic barley plants; healthy barley control plants were similarly processed. Klages and Clark were the only barley cultivar plants sown and grown under field conditions and subsequently used in the purification scheme. The rest of the symptomatic leaf material was derived from trap plants that had developed the disease after being exposed to diseased plants in the fields for two to four weeks before returning them to the greenhouse. Five Ellis, four Dicktoo, four Alpine, two Kearney, and one Reno barley plants were all individually tested. Since these cultivars (except Clark and Klages) were all winter barleys, and had never been subjected

to a vernalization period, they continued to grow in the tillering habit with a proliferation of leaves.

The plants ranged from 1.5 to 4 months old when their leaves were removed for purification. The leaves were diced with scissors, and 15 g of leaf tissue was homogenized in 60 ml of 0.2 M sodium citrate, pH 7.6 containing 10% sucrose. The homogenate was filtered through two layers of cheesecloth, and placed in an ice bucket on a shaker for 15 minutes. The plant homogenate was then centrifuged at 5,000-*g* for 10 minutes in a fixed-angle Sorvall SS 34 rotor using a Sorvall RC2-B centrifuge. The supernatent was gently vacuumed through a 545 Celite pad. The pad was previously made by mixing 4 g of Celite with 60 ml of 0.2 M sodium acetate (pH 7.6) and pouring it onto two Whatman filters (qualitative 2) in a Buchner funnel. The filtrate was then overlayed on a continuous 10-40% (w/v) or step 30/60% (w/v) sucrose density gradient in Seton polyallomer tubes. The step gradient consisted of 8 ml of 60% and 5 ml of 30% sucrose in 0.2 sodium citrate, pH 7.5 (w/v) with an overlay of 5, 3, or 2 ml of filtrate. After centrifuging for 30 min. in a Beckman SW 25.1 rotor at 23,000 rpm, on a Beckman Spinco Model L ultracentrifuge, the zone between the 30/60 interface was removed and diluted 1:1 with the extraction buffer, and overlayed in a continuous 10-40% (w/v) sucrose gradient. The filtrates directly from the Celite pad or the zone removed from the step gradient were overlayed in aliquots between 5-8 ml onto the 10-40% (w/v) gradients. They were centrifuged for 30 min. in a SW 25.1 rotor at 22,000 rpm. The 10-40% (w/v) density gradients were made of 4 ml of 40%, 7 ml of 30%, 7 ml of 20%, and 5 ml of 10% sucrose in 0.2 M sodium citrate, pH 7.6.

If 25-55% (w/v) sucrose density gradients were later used, they consisted of 4 ml of 55%, 7 ml of 45%, 7 ml of 35%, and 4 ml of 25% sucrose in the same buffer (w/v). The 10-40% and 25-55% gradients were allowed to diffuse overnight in the refrigerator before overlays were applied. Some of the gradients contained respective sucrose solutions (10/40 mg/ml) of 0, 0.5, 1.0, and 2.0% glutaraldehyde (Ted Pella, analytical grade).

Successive layers of the gradients were removed and assayed for the presence of VLPs by EM. If VLPs were present in a specific sample, then that sample was used as part of another overlay on 24-55% (w/v) density gradient. They were then centrifuged for 1 hr. at 22,000 rpm, after which successive layers were assayed by EM. The tissue extracts were refrigerated or on ice at all times. Controls using healthy leaves were processed simultaneously.

In addition, after rate zonal centrifugation, some of the gradients were fractionated in an ISCO Model 640 fractionator (Instrumentation Specialities Company, Lincoln, Nebraska). The fractions were simultaneously monitored at an UV absorbance of 254 nm. Selected fractions were then assayed for the presence of VLPs by EM.

Each of the distinct steps employed to extract the VLPs and the selected fractions of the density gradients were assayed by placing a parlodian (0.5% parlodian in amyl acetate) -coated copper grid (Ted Pella, Inc.) on a drop of the extraction sample for 30 min. The grid was then rinsed in four drops of 0.2 M sodium citrate buffer, followed by four drops of distilled water; these grids were routinely stained with 5% uranyl acetate in 50% ethanol. Ultrathin sections were also

processed from the original leaves used in some of the purification attempts.

## Results and Discussion

Electron microscopy studies of affected leaf tissues may shed light on the identity of the VLPs. Specific organ, tissue, and cell type of a plant and the presence and/or absence of the VLPs in each may help explain disease symptoms and mode of transmission within the plant, and from plant to plant, respectively. For example, barley yellow dwarf virus is transmitted to a barley plant only by specific phloem-sucking aphids. The virions are confined to the phloem tissue and the virus apparently moves systemically through the plant in the phloem tissue (35). In addition, maturation and replication events of the VLPs may be vividly seen in ultrathin sections of plant tissue which would in turn help classify them to specific entities. The negative and positive staining of the VLPs in crude sap and/or partially purified preparations may give detailed ultrastructural details of the intact VLPs.

A total of 126 symptomatic plants was used for the tissue source in the EM studies of ultrathin sections. Plants which were sown under field conditions included 57 barley, 18 wheat, and 9 weedy grasses (three species). An additional 18 barley, 3 wheat, and 3 grasses of healthy appearing field plants were also tested.

Particle Structure

The identity and classification of a virus is partly based upon the particle morphology and size (39,49,56). Plant viruses may have rod-shaped, filamentous, isometric, or bacilliform particles with distinct dimensions for each specific virus member. Therefore, in order to best determine the overall morphological features of the VLPs in question, pictures were taken of them *in situ* and *in vitro* as they were viewed with the electron microscope. The resulting printed micrographs were then used with the help of Zidas (Carl Zeiss, Inc.) to measure the lengths and diameters of the VLPs which were simultaneously recorded on a printer.

*In situ (ultrathin sections)*. The morphology of the VLPs *in situ* was based upon ultrathin sections derived from 126 symptomatic plants of varying ages and environments; i.e., field versus greenhouse grown (Figure 1). The third leaf of six different diseased Dicktoo barley plants were used to determine the foregoing data on the particle length and diameter. Of the 104 particles measured, the particle diameter varied from 52 to 81 nm with an average of 64 nm, while the particle length varied much more dramatically with particles ranging from 126 nm to an extraordinary 4,095 nm. However, it was extremely difficult, if not impossible, to determine which of the structures constituted an intact particle. The plane of sectioning of the tissue and a limited one-dimensional view of a particle with long lengths often produces only a segment or section of a particle, i.e., the structure may be cut at an oblique angle giving the effect of a tapered or relatively short

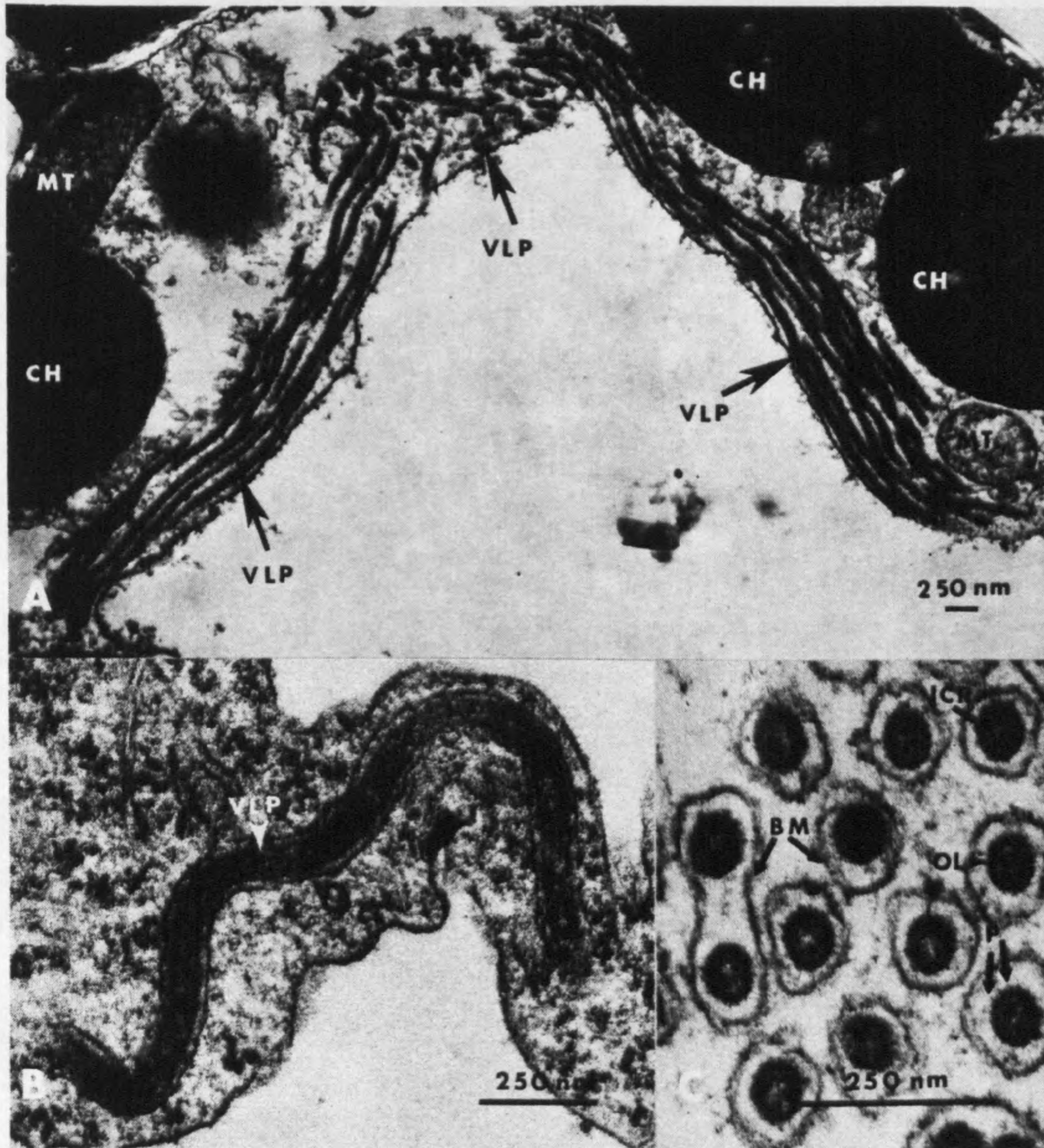


Figure 1. Virus-like particles (VLPs) in the cytoplasm of diseased barley leaf cells. (A) A cluster of VLPs depicting long particle lengths. Chloroplasts (CH) and mitochondria (MT) are also shown. (B) Ultrastructural features of a magnified VLP depicting the outer loose membrane and an inner core of zigzag material. (C) Cross-sections of the VLPs, revealing a loose, bilayer membrane (BM), projections (P), outer layer (OL), and an inner circular ring (ICR) of material. Each scale bar = 250 nm.

particle. An unusually long particle sometimes appeared to have 'fused' with neighboring like particles, while other long particles may have appeared to undulate in and out of the plane of sectioning, producing the appearance of a row of relatively small particles. The ends of the particles were at times rounded, tapered, or indistinct blurs. Extensive serial sectioning would reveal a three-dimensional view of the VLPs; however, the time and cost involved in doing such a project would be exorbitant, and, even then, only indirect evidence pertaining to the precise nature of the VLPs would be presented.

Occasionally small projections between 5 to 10 nm radiated out from the outer perimeter of the particles in cross-section. Each particle seemed to be covered by a tightly bound band 5 to 10 nm in diameter, which was in turn usually enclosed within a very loose double-layered membrane.

Internal structures were at times nondescript, whereas some longitudinal sections of the particles contained a broad central axis of a continuous electron opaque zigzag or crisscross structure. Cross-sections of other particles revealed an electron-dense inner ring composed of 5-10 nm isometric structures, while other particles appeared to be completely filled with electron dense material with a star-shaped electron opaque central core.

In vitro (leaf extracts). Crude leaf extractions of infected leaves were made to provide additional information on the morphological outline of a particle; and grinding the leaf with a mortar and pestle instead of with an homogenizer produces more intact, undamaged particles. Partially purified preparations of VLPs with the

appropriate stain may further elucidate fine structural features diagnostic of a particle.

The particles present in crude leaf extraction with a mortar and pestle were often difficult to find in relatively large numbers. Sometimes, if the grids were pre-treated with normal serum, particle detection improved. In contrast, Protein A and bovine serum albumin appeared to have little effect on enhancing the attachment of the VLPs to the grids. If the extraction buffer contained 1% glutaraldehyde, the particles and host material tended to clump and stick together, making it difficult to see single VLPs. The VLPs assumed a range of lengths up to 2,845 nm; the widths varied along the length of a particle between 41 and 74 nm, but usually were 50-60 nm in diameter.

After subjecting the particles to centrifugation in sucrose density gradients, relatively long particles over 2,000 nm still were prevalent. Moreover, the diameters of the particles often alternated in exaggerated 'pinched' and 'inflated' widths along the longitudinal axis.

Uranyl acetate (UA) was more dependable than phosphotungstic acid (PTA) for staining <u>in vitro</u> particles. PTA usually caused the VLPs to break apart, forming globular masses. However, if the VLPs were previously fixed with glutaraldehyde, an occasional stable particle was found. Generally PTA or UA did not penetrate into the surface of the VLPs to reveal any distinct fine structural features. However, when the UA stain did penetrate occasionally, an electron-dense zigzag structure located between both sides of the outer layer and along the entire length of the particle was visible (Fig. 1).

Based upon the described *in situ* and *in vitro* studies on the particle morphology of the VLPs, representative particles were found to have the following features in common: 1) the average particle diameters were 64 nm in ultrathin sections and 55 nm in leaf extractions, 2) the lengths varied from 126 nm to extremely long flexuous particles over 4,000 nm, 3) the structure was closely bounded by a 5-10 nm band, and 4) the internal structure of the particle consisted of a zigzag structure bounded by the outer particle layer. In addition, cross-sections of the VLPs *in situ* revealed an inner circle of nondescript electron-dense isometric structures, or an electron-opaque star-shaped central core was solidly surrounded by electron-dense material. Projections 5-10 nm long and about 5 nm in diameter were occasionally found radiating out of the particle's outer perimeter.

Given these ultrastructural parameters and details for the VLPs in question, an attempt to place such structures in one of the presently established 24 groups and two families of plant viruses failed (57). It had initially been assumed that the VLPs were related to virus members in the plant rhabdovirus family due to the similar tubular morphology and dimensions exhibited by both. A typical intact rhabdovirus particle is considered to be bacilliform in outline, and consists of an outer envelope enclosing a long, helically wound nucleocapsid which appears as cross-striations in negative stains (28,29,30,43,45, 58,68). The enveloping lipoprotein bilayer contains 6-10 nm spikes projecting outward. Members of viruses belonging to this group may

have particle dimensions differing from each other; diameters range from 45-100 nm and lengths vary from 190-430 nm.

Therefore, the tubular-like structure and diameter of the VLPs fit into the general scheme of the Rhabdoviridae; and the occasional appearance of structural projections on the VLPs indicate another similarity. However, it is not clear if the 5-10 nm outer layer of the VLPs is a lipid protein membrane, a protein coat, or constructed of yet another material. That the VLPs *in vitro* are stabilized with the addition of glutaraldehyde gives indirect evidence to the possibility of a lipid material. Furthermore, preliminary treatments of *in vitro* VLPs with the nonionic detergent, Triton-X 100, produced particles with frayed 'coats.' Evidence that the VLPs have a lipoprotein envelope may not be mandatory for rhabdovirus members, since orchid fleck virus appears to have a nucleocapsid similar to the rhabdoviruses but the particle is not enclosed in a membrane (25).

Perhaps the most convincing evidence that the VLPs are not a member of the Rhabdoviridae is the difference of fine structural detail. Instead of having inner helical cross-striations, the VLPs appear to have a zigzag structure. The nature of this structure is uncertain as it may be artifactual; nonetheless, it was found to occur on the particles *in situ* and *in vitro*.

However, the inner electron dense circular ring structure noticeable in cross-sections of the VLPs could correspond to the inner protein coat of the Rhabdoviridae. In contrast, the small isometric structures of the ring could be strands of nucleic acid if the genetic material constituted a segmented genome. This is true for the virus

members in the tomato spotted wilt group, in which an intact particle consists of an enveloped 85 nm structure with 3 to 4 strands of RNA (30).

These VLPs are very unique and distinct from any other known plant virus particle in that the lengths of the particles appear to be variable and exhibit the longest dimensions reported to date. Currently, citrus tristeza virus has the longest particles with diameters of 10-11 nm and lengths of 2,000 nm, resulting in flexuous, long virions (70).

Several animal viruses are also known to have extraordinarily long lengths in infected tissue. The Marburg and Ebola viruses have been documented to attain lengths up to 14,000 nm in ultrathin sections of tissue (67). Initially, they too were thought to belong to the Rhabdoviridae, but now are considered to be quite different, so a new family, the Filoviridae, has been proposed (51). Later, it was found that the true modal length and diameter of the Marburg and Ebola particles were only 790 x 80 nm and 970 x 80 nm, respectively (72).

Experts at the Center for Disease Control in Atlanta, Georgia (M. P. Kiley, personal communication) have compared micrographs of the ultrastructure of the VLPs from ultrathin sections of barley leaves with the structures of the Marburg and Ebola particles. Based upon the micrographs, they suggested that the barley VLPs and the two animal viruses resembled each other only in their long lengths, and perhaps the VLPs were more similar to the Rhabdoviridae.

Location and Distribution of the VLPs within the Plant

The plant organs in which the VLPs were located included the root, sheath, leaf, and awn of barley. Additional leaf tissue from three symptomatic weedy grass species and symptomatic wheat cultivars were also comparatively studied. Root sections from one grass species, Setaria viridis (L.) Beauv., were sectioned and found to contain the VLPs. However, the majority of the ultrathin sections were from the leaf tissue of barley, and, therefore, the following statements concerning localization and concentration of the VLPs within specific cell types are biased toward the leaf tissue of barley plants.

In the leaf tissue, the VLPs were only detected intracellularly within the cytoplasm. The most striking and unavoidable evidence for their appearance included large masses of aggregated long and short particles in the mesophyll cells. Interestingly, the VLPs were always found to be enclosed by a membrane structure, either as an individual particle or as a cluster of 'naked' particles bounded by a membrane. The large masses usually contained a combination of membrane-bounded individual particles and clusters of particles; often the masses of particles were delimited with another membrane.

The particles were also detected in nearly all of the other leaf cell types, including those of the epidermis, phloem, and xylem. However, large membrane-bound masses of the particles were rarely detected, and the numbers of VLPs, in general, were less than those seen in the mesophyll cells. Only occasionally were VLPs noticed in the epidermal cells, including the guard cells. The phloem sieve

elements, companion cells, and associated parenchyma cells also contained the VLPs, and were more often detected in the latter two cell types. The xylem vessels and xylem parenchyma cells had some VLPs, but in relatively low numbers. Differences of particle length and morphology were not noticeable among the cell types. The VLPs were never detected inside chloroplasts, mitochondria, or nuclei. Tissue that was derived from healthy-looking plants never contained the VLPs. Moreover, tissue that was removed from a green, nonsymptomatic leaf growing on a diseased plant never had the VLPs.

Unlike the root cells of a few barley roots, Setaria viridis contained large clusters of apparently 'naked' VLPs in some of the cortex cells of roots; membrane-bound particles were also detected. These cells displayed an unusually great number of intertangled, long particles with lengths over 4,000 nm. The VLPs were also seen in the other cell types of the root, but in very small numbers.

The distribution of virus particles within a plant organ or cell type varies with the susceptible host and infecting virus. Furthermore, the distributional pattern observed in a particular tissue is partly determined by what stage the infection process was undergoing at the time the sample was taken.

Rhabdoviruses infecting Gramineacous hosts are frequently found in all tissue and cell types (45). Therefore, the presence of the VLPs in nearly all cell types of the leaf lamina was not a unique phenomenon.

The ultrastructural features within an infected cell appeared to be normal except for the presence of the VLPs. However, extensive parallel studies between healthy control and diseased plants that

include all stages of the infection process may reveal subtle differences.

Apparent Particle Maturation

The VLPs appeared to have a close association with the endoplasmic reticulum complex (ER) of the cell. Apparent mature VLPs were contained individually or in small groups in cytoplasmic membranous cisternae, while other particles actually appeared to be forming within dilated cisternae. The nascent-forming VLPs sometimes seemed to be coming out of the cytoplasm, and never produced any evidence of 'budding' through a host membrane; often ribosomes were attached to the ER which surrounded the assumed developing VLPs. However, at no time were ribosomes found on the membrane that enveloped a mature VLP. The apparent developing particles were often found near densely stained membrane-bound areas and within regions of densely packed ribosomes and ER. Occasionally, masses of dark globular spherical structures between 20-30 nm in diameter were intimately associated with the particles. Vesicles of varying size also contained these spherical structures and/or the VLPs. Similar structures have been described as small cytoplasmic masses and spherical vesicles in barley cells infected with barley yellow striate mosaic virus (16).

Some particles appeared to be in the process of fusing together; this was noticed between individually membrane-bound particles or between VLPs sharing the same membrane. Binary fission was never detected between two forming particles.

Evidence for virus replication and maturation in ultrathin sections of tissue is often substantiated with the appearance of viroplasms and partially formed particles. Some of the plant rhabdoviruses of the Gramineae have been reported to contain viroplasms in the cytoplasm with associated particles budding from the perimeter (16, 92). No such structures were observed in the cytoplasm with the VLPs.

Plant rhabdoviruses may be further subdivided into two categories where assembly and accumulation of the particles occur in the nucleus or the cytoplasm (28). The VLPs did not appear to have an intimate physical association with the nucleus. The VLPs were not seen inside the nucleus and were never detected between the membranes of the perinuclear space; VLPs were never seen budding through the perinuclear membranes. In contrast, the membranes assumed to be from the ER were always found to envelope an apparent nascent-forming particle. Budding _per se_ was not observed, in that the particles did not appear to be protruding through a membranous structure. It is possible that the phenomenon of budding is occurring at a time when both the particle and membrane are simultaneously being formed together. This is thought to occur with barley yellow striate mosaic virus (BYSMV) or northern cereal mosaic virus (NCMV) infecting barley and wheat leaves, respectively (16,92). Interestingly, BYSMV and NCMV were serologically compared and were found to be two distinct, yet related viruses (60).

An ultrastructural study of BYSMV infecting its planthopper vector, _Laodelphax striatellus_ Fallen, revealed evidence for the occurrence of incomplete particles (17). One stage consisted of an electron-dense inner core separated from a loose envelope by an

electron-transparent space; they formed in long flexuous tubules of varying length and were 18 to 20 nm wide. The space between the core and membrane was later filled with more electron-dense material which was thought to be a second coat. Constriction of the long tubule then formed mature particles (17). The incomplete BYSMV particles with the described loose membrane closely resembled the VLPs I observed in leaf tissue. It is possible that the VLPs represent particles that have lost their ability to add the 'second' coat in the barley host plant. In fact, researchers in the USSR reported that when winter wheat mosaic virus (WWMV) infects millet plants, only subviral rhabdovirus particles are formed, and not mature virions (27). However, if WWMV infects wheat plants, normal mature virions are formed. Thus, it appears that the maturation of WWMV virions is blocked in millet plants.

Additional research on the existence of the VLPs in the vector tissue may show particles that are morphologically similar or dissimilar to the VLPs in barley tissue. The vector could also serve as host to the VLPs.

Tomato spotted wilt viruses also undergo particle maturation inside cytoplasmic cisternae (30). It appears that double enveloped particles occur only at the early stages of infection from budding through viroplasms or from regions of paired parallel membranes. The final maturation stage occurs when the double membranes surrounding the particles coalesce together, forming cisternae with the outer membrane and resulting in several mature enveloped particles inside (41). Similar events could produce the frequently observed clusters of VLPs found in the cytoplasm.

Partial Purification

Of 18 separate purification attempts to isolate the VLPs from symptomatic plants, ten attempts failed. No VLPs were seen on the assayed samples of leaf extracts which were removed in each major purification step, and no VLPs were seen on selected fractions of the sucrose density gradients. During one of the negative trials, the homogenate was initially frozen by grinding the leaf tissue in a frozen mortar and pestle. In another trial, 0.25% 2-mercaptoethanol was added to the extraction buffer and resulted in no distinguishable VLPs. However, the VLPs were readily detected in ultrathin sections of leaf samples and also in the homogenates that were not subjected to freezing or the addition of the reducing agent, 2-mercaptoethanol.

The remaining partial purification tests proved to be positive in that VLPs were found in distinct layers on the sucrose density gradients. Unfortunately, no distinct light scattering band was visible, and the VLPs were distributed over a relatively broad band in the estimated 25-30% density region of the 10-40% gradients. The lengths of the particles were usually longer in the denser regions. Host material was always present with the VLPs. Moreover, the greater the titer, the greater the amount of contaminating host material. For example, at the interface of the step 30/60% gradients, 24 to 12 VLPs per grid hole were visible with much contaminating host debris. If the zonal interface was removed and diluted with buffer, and then overlayed onto a 10-40% (w/v) sucrose gradient, a drop in titer to 6 VLPs per hole was encountered with much less visible host material. And if the

VLPs-containing bands were further centrifuged on a 25-55% (w/v) sucrose gradient, only a few to zero VLPs per grid and very little host material was apparent.

Therefore, the main problem in obtaining a partially purified preparation of VLPs was due to the failure to concentrate the VLPs in a relatively discrete band on the density gradients and the constant presence of contaminating host material. Clarification of the extracted plant material through a pad of high-purity analytical grade Celite (Johns-Manville Corp.) removed most of the visible host material along with most of the VLPs. And if extraction buffer was vacuumed through this pad, some of the VLPs were washed off, but so was host material. Activated charcoal mixed with the homogenate also did not help to separate the host material from the VLPs. Therefore, even though a considerable amount of plant material was filtered through the 545 Celite pad (medium grade), the maximum number of VLPs was also obtained.

Differences in the size and shape of the VLPs were found as they were subjected to greater gravitational forces and longer centrifugation times. Crude sap preparations usually consisted of apparent intact particles with near constant diameters throughout their length; some had a small bleb at one or both ends. In contrast, the VLPs in ultracentrifugation preparations often had alternating pinched and inflated diameters occurring throughout their entire length. The addition of glutaraldehyde (EM grade, Ted Pella, Inc.) to the density gradients appeared to help stabilize the VLPs and prevent them from

falling apart. However, the glutaraldehyde also appeared to cause aggregation and clumping of the VLPs and host material.

The UV absorption at 254 nm for the successive fractions of the 10-40% (w/v) sucrose density gradients containing the diseased or healthy leaf preparations was nearly the same. Usually the first 12 ml of media below the meniscus would have a high reading, while the sequential 5 ml samples would have declining absorption, reaching zero and then remaining at that level. However, based upon EM examination of each fraction (1.2 ml) which was derived from diseased leaves, the VLPs were visible only when the absorption began to drop. Few VLPs were detected in those first declining fractions, while the numbers gradually increased as the absorption approached or reached zero. The maximum number of VLPs was found about 18 ml below the meniscus, at which time the absorption was near zero and the density was estimated to be between 25-30%. The actual density should be further measured with a refractometer.

In summary, the VLPs were distributed over a relatively broad band of about 8 ml which did not have a significant UV absorption at 254 nm. The fact that the VLPS were only detected in this region may be interpreted in several ways: 1) the VLPs consisted of little, if any, nucleic acid, 2) the VLPs were distributed over a broad zone instead of a small concentrated light-scattering zone because of the variable size and shape of the VLPs, and 3) the original leaf titer was too low for detectable UV absorption.

The latter statement of initial low titers occurring in the original leaf material was a constant concern. Even though the

youngest symptomatic leaves were used, the plants used for the purification ranged from 1.5 to 4 months old. Matthews (56) states that upon infection, the virus titer rises rapidly in infected leaves, and then drops. Moreover, leaves infected at different times on the same plant will vary dramatically in virus concentration at a given time. I found that younger plants were more dependable sources for detection of VLPs in both ultrathin sections and crude sap. Occasionally an older plant would have a symptomatic leaf with a relatively large number of VLPs. Apparently healthy, green leaves were never found to have the VLPs in both young and older diseased plants. However, much more work needs to be conducted on the correlation between symptom severity and the titer of the VLPs, as I did not find an absolute relationship. Future studies may also involve another barley cultivar and/or plant species that may be more conducive to high VLPs concentrations.

Spectrometric difficulties are frequently encountered in UV absorption with rhabdoviruses because of the excessive light-scattering induced by the size and shape of the virions (45). Rhabdoviruses of the Gramineae have a bacilliform structure which is relatively large with *in vitro* diameters ranging from 45 to 100 nm and lengths between 120 to 355 nm with each specific virus within the group containing virions of equal dimensions. Therefore, it is possible that the VLPs may also produce inaccurate spectrometric measurements. It was suggested (M. Brakke, personal communication) that the VLPs may be concentrated using equilibrium density gradients. By changing from a rate zonal to a equilibrium density gradient, the density and not the size and shape of the VLPs will be considered. However, if the VLPs

are salt-labile, then the intact structures may lose their integrity in the commonly used gradient media, cesium chloride or cesium sulfate.

More importantly, if the VLPs do not, in fact, contain nucleic acid, they cannot be classified as a virus or a viroid. So some of the gradients were fractionated at 280 nm to test for the presence of proteins. Again, the results were the same for both diseased and healthy plants, and followed about the same absorption spectrum as those fractions being measured for nucleic acid content at 254 nm. It is possible that morphologically the VLPs appear to be a mimicry of viruses, but are composed of different substances. A recent report (48) on VLPs found in sick slugs, gave chemical evidence that those VLPs were composed solely of galactogen and glycogen. The study also provided evidence for the presence of VLPs in healthy slugs.

Moreover, it is possible, but not likely, that the VLPs are the result of an undetectable virus or viroid. The presence of this unknown causal agent could induce the plant host to form these unique VLPs, although such morphologically unique structures have not been reported to date.

Further studies using young diseased plants, using different extraction buffers and additives, and running equilibrium density gradients may be an effective way to increase the titer and concentrate the VLPs for partial purification. Once this is accomplished, direct tests involving the chemical nature of the VLPs may be ascertained. Extensive study of the physicochemical nature of isolated VLPs from diseased plant tissue is essential before the VLPs may be definitively

placed into the existing classification scheme used for viruses (39,56,57).

# CHAPTER II

# GEOGRAPHICAL DISTRIBUTION OF THE VIRUS-LIKE DISEASE AND OBSERVATIONS OF DISEASED PLANTS IN BARLEY AND WHEAT FIELDS

## Materials and Methods

Surveys for the virus-like disease were limited to North Central Montana, which included areas within Pondera, Teton, Toole, and Glacier counties. A 'roadstop' survey was conducted in 1983-86, whereby barley, *Hordeum vulgare* (L.), and wheat, *Triticum aestivum* (L.), fields along major road systems were observed for the presence of the disease. Additional fields reported to have the disease by producers were also monitored. Plants within a field were randomly checked with emphasis on crop edges and 'open-spaced' growing plants. The percentage of symptomatic plants was estimated and the distributional pattern was observed within a field or sections within a field. Pastures and roadside grasses adjacent to diseased fields were also surveyed for diseased plants. Leaf samples were removed from plants thought to have the disease and processed for electron microscopy. Positive diagnosis of the disease was based upon symptomatic leaves and/or the presence of the associated virus-like particles (VLPs) in the tissue.

Two specific sites of study, near Conrad and Valier, were monitored throughout the growing seasons for symptom development, pattern of disease spread, and incidence of infection. The Conrad site

was located about 10 km NE of Conrad and included the area in which the virus-like disease of barley was first reported. The general area consisted of about 160 hectares and included dryland spring barley and winter wheat, irrigated spring wheat and spring barley, and summer-fallow.

The Valier site was located about 24 km north of Valier and consisted of about 200 hectares. At least 150 hectares was in continuous, irrigated recrop barley, and the rest was sown to dryland spring wheat or barley which was grown on summer-fallowed land.

## Results and Discussion

1982-1983. The first report of the virus-like disease originated from a 30-hectare field of the barley cultivar Morex on the Conrad site in 1982. Plants within the field were examined by the author in mid-July, at which time most of the plants were at the headed stage. The disease appeared to be randomly distributed in the field, but more concentrated in the southwestern corner of the field. The field of diseased Morex barley had not been irrigated due to equipment failure. A yield loss of 27 hl/ha was estimated for the 1982 crop by the grower.

Four hectares of Norstar winter wheat were seeded by the producer next to the southern boundary of the Morex barley field in September, 1982. The remaining 30 hectares were planted with Fortuna spring wheat in 1983, and approximately 24 rows of Morex barley were sown into the Fortuna wheat adjacent to the Norstar wheat and near the western border of the Fortuna. In 1983, symptoms developed in an estimated 100% of the Morex barley, 25% of the Fortuna wheat, and 10% of the Norstar

wheat. Based upon ultrathin sections of field plants, it was later found that at least 50% of the Fortuna wheat affected was most likely attributed to a rhabdovirus infection. Diseased Norstar and Fortuna wheat plants were most commonly found in 'open-spaced' plants, along the Fortuna-Morex-Norstar border, and next to diseased volunteer Morex and Shabet barley plants. Patches of volunteer barley plants were distributed throughout the Fortuna wheat field and nearly 100% of the volunteer barley plants were affected. An annual grass, Setaria viridis (L.) Beauv., germinated among the Fortuna wheat and volunteer barley in July and had diagnostic leaf symptoms with the associated VLPs inside the cells.

Another field of Morex barley, located 10 km northeast of the Conrad site, was found to have a few scattered affected plants. No other fields were reported or found to have the virus-like disease in 1983.

1984. During the 1984 growing season, the disease again occurred on the Conrad site. The incidence was only about 1% in 20 hectares of Fortuna wheat. This year only the unique VLPs and no rhabdovirus particles were found in affected field wheat leaf samples. The volunteer Morex and Shabet barleys growing within the crop not only were less abundant this year, but also had dropped from 100% (1983) to an estimated 50-75% of infection. Another annual grass, Echinochloa crusgalli (L.) Beauv. displayed symptoms and had the associated particles. A field of Piroline barley (about 40 hectares) directly to

the west of the Fortuna wheat field had a few randomly scattered diseased plants.

Within 19 km north and northeast of the Conrad site, two Morex barley fields had a few scattered diseased plants throughout the crop. Late emerging Clark barley that was growing in a 0.4 hectare dry section within a Clark barley field had at least 75% incidence of infection. Approximately 45 km northwest of the Conrad site, more diseased Clark plants were observed in several fields on the Valier site. The affected plants were distributed randomly along irrigation ditches within the crop and on the crop edges; dry areas within barley fields contained over 50% infection.

The last infection site observed in 1984 was located near Brady (24 km south of the Conrad site) in fields of Morex. The majority of the disease was found in a late seeded strip of Morex barley or on margins of the crop; one symptomatic barley plant was found in a field of Robust barley.

An additional 'roadstop' survey was conducted in 1984 south of Brady, through Fairfield, Black Diamond, Ft. Benton, Lewistown, Moore, and Judith Gap. No evidence of this disease was found in these southern parts of the Triangle.

<u>1985</u>. The growing season of 1985 not only had widespread occurrence of this new virus-like disease throughout Pondera County, but fields of diseased barley were also found to the north in Toole County and to the south into Teton County. The number of barley fields infected and the incidence within each field was much higher than in

the previous three years. For example, on the Conrad site, two fields of Piroline barley had a few symptomatic plants in 1984 and 100% incidence of infection in 1985. Symptom severity was enhanced on the 14 hectares of dryland Piroline relative to that of the 15 hectares of irrigated Piroline. The 20 hectares of Lew spring wheat which replaced the 1984 crop of Fortuna wheat had no volunteer barley plants in 1985, and only an estimated 0.5% incidence of infection occurred in the Lew wheat field.

Similarly, barley fields on the Valier site and fields surrounding the site, which were observed to have a low incidence of infection in 1984, had incidences varying from 50 to 100% in several hundred hectares of Clark and Klages barley fields in 1985. Several kilometers from the Valier site, a 20 hectare field of Clark barley had symptomatic plants restricted along the easterly edge on 1 June, but by 15 June diseased plants were visible across the entire field, resulting in greater than 50% incidence of infection. Lew wheat growing adjacent to infected barley fields on the Valier site had only about 0.5% incidence of infection.

The newly reported northern region to have the virus-like disease was located about 64 km north of the Conrad site and to the east of Sunburst. The incidence of diseased barley plants varied from field to field; 18 hectares of Moravian III had 0.1%, two fields of Horsford had 60% in 43 hectares and 30% in 62 hectares, and 20 hectares of Clark had 30% incidence of infection.

The apparent southern boundary of the disease in North Central Montana was near Choteau or about 56 km southwest of the Conrad site.

One hundred fifty acres of Klages was reported to have about 5% of the plants affected; however, most of the disease was concentrated in two dry sections within the crop.

A summary of 17 fields of diseased barley crops that were observed at least once during the growing season of 1985 is given in Table 2 (Appendix). The information provided was collected through a mail survey and in person. From these data, it was noted that those fields seeded after 16 April had the greater incidence of infection. Irrigated versus non-irrigated fields did not appear to show differences in percentages of infection. The yield per hectare was far greater in irrigated fields than in non-irrigated fields. Comparable data with barley fields not affected by the disease was not available, and, therefore, it was not possible to determine how much influence the disease had on the actual yield reduction. Water availability appeared to be the dominant factor involved with greater yields.

1986. The virus-like disease was at epidemic levels in North Central Montana during the 1986 growing season. Every field of barley that was observed in Teton and Pondera counties had plants with the disease. At least 3,000 hectares of barley were affected, and over 200 producers in Pondera County reported the disease (J. Baringer (County Agent), personal communication). In contrast, no disease was observed in barley crops in the Sunburst area of Toole County, although several producers later reported a trace of the disease (Figure 2).

The Valier and Conrad sites were monitored every two weeks from 23 May to 1 August 1986. The Conrad site consisted of 30 hectares of

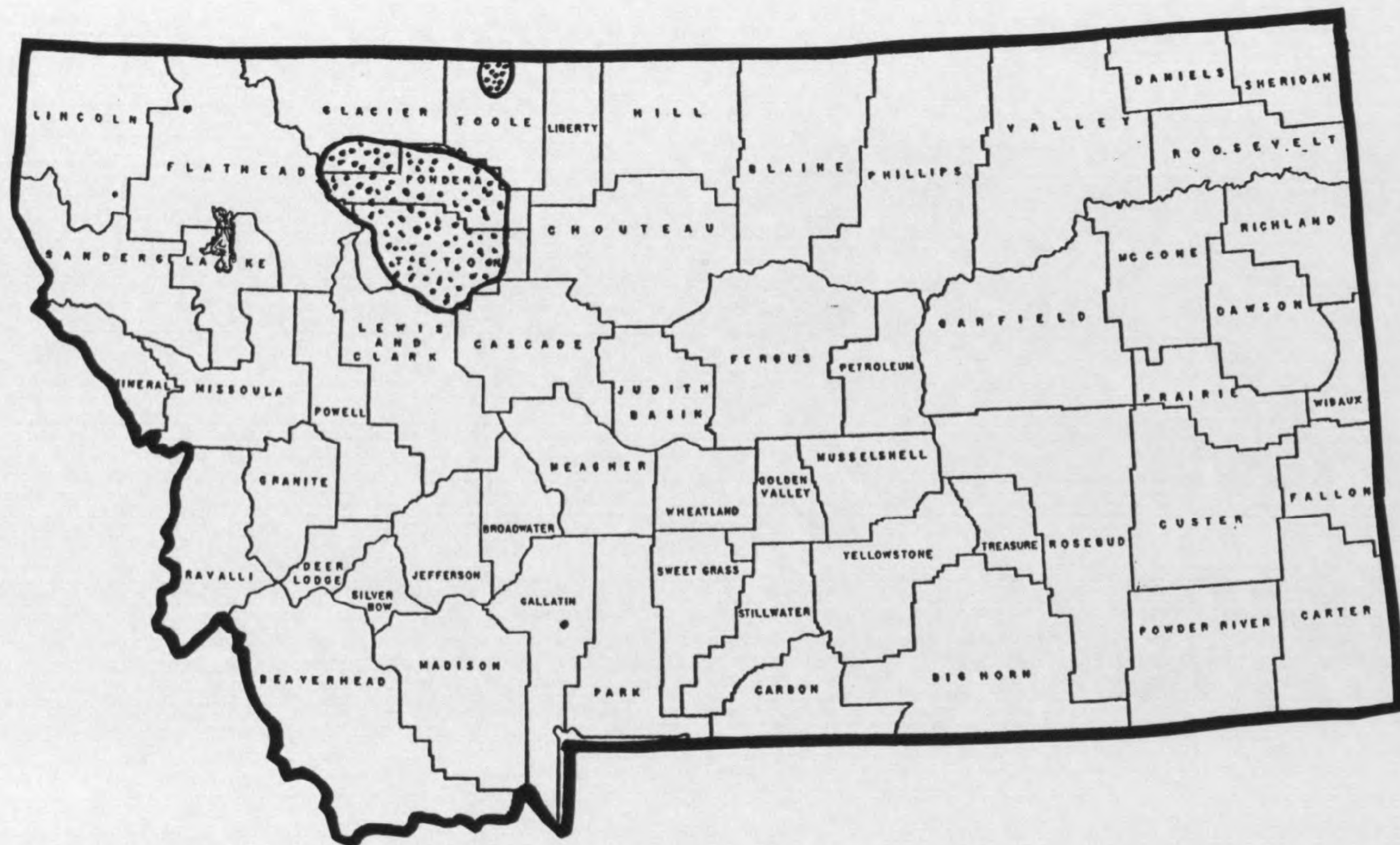


Figure 2. The 1986 distribution of the virus-like disease of barley in Montana.

recrop Lew spring wheat, four hectares of Norstar winter wheat, and about 30 hectares of Piroline barley with an estimated incidence of infection of 0.1%, 1%, and 25%, respectively, by 6 June; later, the Lew wheat and Piroline barley increased to an estimated 1% and 100% incidence of infection, respectively. On 23 May, no insect or mite infestations were noticeable on the crops. However, the brown wheat mite, Petrobia latens Müller, was found in great numbers on volunteer barley in an adjacent summer-fallow field. Subsequently, by 6 June, the Piroline barley was heavily invested with the mites, while the wheat had a relatively low number of mites. Symptoms in the barley were quite severe, resulting in dried, scorched leaves. However, both the size of the mite population and the severity of the symptoms were lessened with the advent of water applied by line-roll irrigation to the barley in the latter part of June. The affected Lew plants appeared to be moderately to severely stunted, with much chorotic banding.

The Valier site contained about 150 hectares of irrigated recrop barley and about 50 hectares of dryland barley that had been previously in summer-fallow. Nearly 100% incidence of infection occurred throughout the barley fields (Klages) by 7 June.

An eight hectare area, which had been in continuous recrop barley since 1980, was seeded with Klages in mid-April. The plants were at the 2- to 3-leaf stage with no symptoms on 23 May, but were tattered by extreme spring winds and soil erosion. By 6 June, all the plants had the virus-like symptoms and were infested with the brown wheat mite.

However, after the field was flood-irrigated on 29 and 30 June and the plants had matured, symptom severity and the mite population decreased.

Other areas on the Valier site included four center-pivots (14 hectares each) which were contiguously surrounded by dryland barley. All the plants were diseased, and the mite numbers and disease severity dramatically increased within the pivot boundaries, resulting in four distinct yellow circles. The sharp contrast depicting disease severity between the center-pivots of Klages barley and the surrounding dryland areas of Klages was, in part, attributed to soil moisture availability. The center-pivots of Klages had been in recrop barley, and, therefore, less water was initially available to the seedlings relative to the barley seedlings growing in the contiguous dryland areas which previously had been in a summer-fallow rotation.

The remaining fields of Klages barley on the Valier site were seeded in the first week of May, and water was applied with line-roll irrigation beginning in July. Again, nearly all of the plants were affected, but symptom severity was variable, with increased severity occurring in patchy areas. Areas within the field which could not be reached by irrigation had enhanced symptom severity and more mites on the plants. Mite numbers appeared to decrease with the application of water, and in those remaining dry sections of the field the plants continued to be infested with the mites throughout the growing season.

A third diseased annual grass, Lolium persicum Boiss. and Hohen, was found several kilometers north of the Valier site. The symptomatic grass species was first noticed on 18 June growing along the edge of a

spring wheat field in cultivated soil, but none of the wheat plants ever developed symptoms.

Other observations of interest in Pondera County included barley strips that had been treated with an insecticide and a field which had been partially burned. A producer had seeded four strips (five hectares each) with Harrington barley on 1 May, and each strip was between two summer-fallow strips. When three of these strips of barley were at the 2- and 3-leaf stage, and two weeks hence, the insecticide, carbofuran (Furadan 3 G), was sprayed on the plants for grasshopper control. The treated strips of barley had a low incidence of the disease, while the untreated strip was nearly 100% affected. In addition, more brown wheat mites were found on the untreated barley than on the Furadan-treated barley. Therefore, this provided indirect evidence of an association between the brown wheat mite and the severity and incidence of diseased plants.

Another producer had about 57 hectares that were partially burned before the entire field was sown with Klages barley on 1 May. The disease incidence was lower in the burned portion of the field, containing about 1% incidence of disease infection, while those plants growing in the non-burned portion had greater than 90% incidence of infection with patches of severely diseased plants. However, the difference in disease incidence probably was not due to the burning, but instead attributed to soil moisture. The burned area consisted of a low, depressed area with regions of 'standing' water and wet soil. That the spring burning of cropland appeared to have negligible effect on the disease was further substantiated by the numerous fields of

diseased barley observed in the Fairfield bench region which had been previously burned.

Symptomology. Affected plants growing in the field contained symptoms that were similar to symptoms induced by plant viruses infecting the monocotyledons (56), and especially by rhabdoviruses of the Gramineae (45). A developmental sequence of different types or intensity of symptoms progressed as the diseased plant matured.

Since field observations were conducted every two to three weeks, it was impossible to see and take note of the subtle changes of plant symptoms. However, no symptoms were ever observed in field plants at the 1,2-leaf stage, and usually were not visible until the 5-leaf stage. At the later leaf stage, the leaves had light green to yellow dashes, streaks, and stripes parallel to the mid-rib, producing an overall mosiac pattern (Figure 3). The yellow streaks and stripes were distinct from the surrounding green tissue. Although the discolored areas included the leaf veins, more often the dashes and streaks were interveinal. Usually during and after the boot stage, some of the younger leaves developed a prominent banding pattern parallel to the leaf veins. The banded portion progressed in discoloration from light green to yellow to occasional necrosis, and often consisted of a light green background with yellow dashes and streaks. The position occupied by the band of discolored tissue on the leaf appeared to be indiscriminate, in that at times it included the mid-rib and several millimeters of tissue on both or one side of the leaf, while other leaves had banding that did not include the mid-rib. The entire leaf area to one

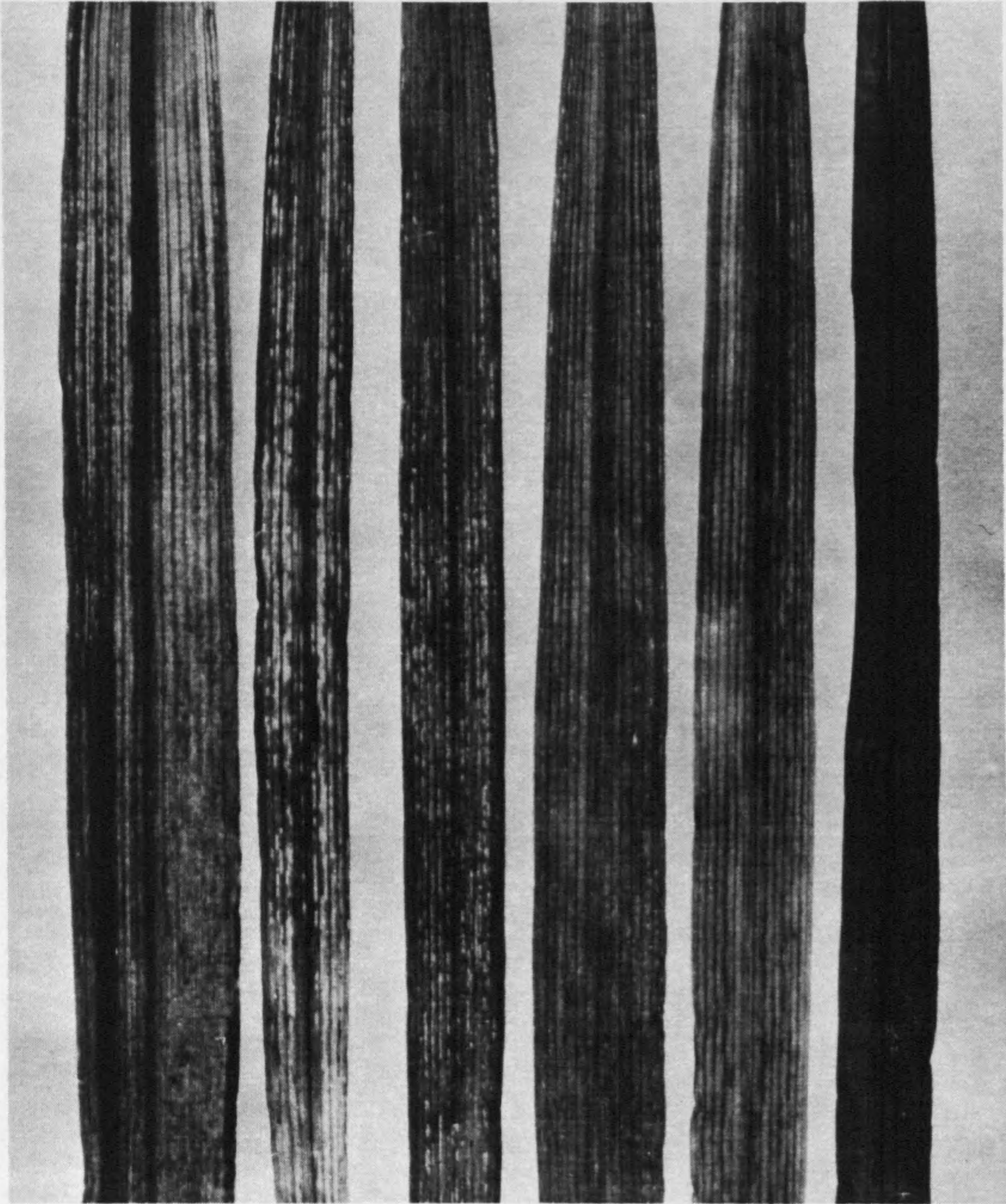

Figure 3. Barley leaves with symptoms of the virus-like disease; healthy leaf is to the right.

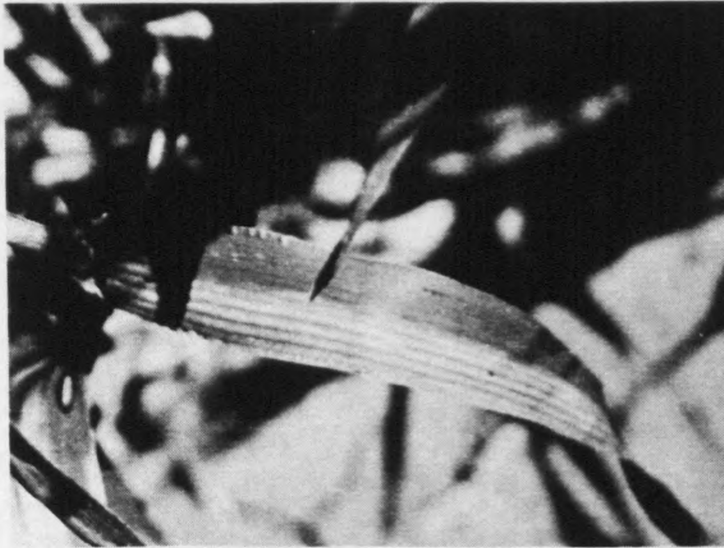

Figure 4. Symptoms of a diseased field plant depicting parallel streaks and stripes on one side of the leaf mid-rib.

side of the mid-rib may have also been discolored or streaked, whereas the other side appeared green and healthy, giving the whole leaf a two-toned appearance (Figure 4).

On the Conrad site in 1984 and 1985, diseased plants often had necrotic leaf tips and an overall 'scorched' appearance. I had assumed at that time that this was a sign of chemical burn. The producer had sprayed an herbicide, glyphosate (Roundup), next to the diseased barley plants, and it was possible that some of the spray drifted onto the diseased plants. However, in 1986, the majority of the barley fields throughout Pondera and Teton counties had barley plants with 'scorched,' dried leaves. Moreover, banding and necrotic dashes and tips on leaves of barley seedlings at the 3,5-leaf stage were evident.

Dwarfing or a noticeable reduction in plant size was not apparent in most field barley plants. Frequently, patches of diseased plants within an irrigated field were stunted, or barley plants under dryland field conditions contained stunted plants. Based upon producers' testimonials and field observations, the patches and fields of severely stunted plants were induced by lack of moisture. In contrast, the diseased Lew spring wheat on the Conrad site was moderately to severely stunted and had fewer tillers than the immediate surrounding healthy wheat plants. In addition, the affected wheat plants contained the parallel dashes, streaks, and bands but rarely had scorched leaves.

Comparisons of the total number of seeds per plant produced between diseased and healthy plants grown under the same conditions were not possible. The seed viability appeared not to be significantly affected, in that when a total of 22,458 seeds were harvested from field diseased Morex barley plants and then sown in the greenhouse, 85% (19,021) of the seeds germinated and produced apparently healthy plants (see Chapter III, page 63).

## Summary

### Geographical Distribution

Based upon the surveys conducted and observations made from 1982 through 1986, it was believed that the virus-like disease was confined primarily to North Central Montana. Curiously enough, however, in 1986 the disease was detected in two small experimental plots of barley at the A. H. Post Farm near Bozeman, Montana. Researchers from Canada

also reported that the disease was found in barley fields in southern Alberta, but based their diagnosis on symptomology alone (D. Gaudet, personal communication). However, I was later able to transmit the assumed causal agent using mites derived from the diapausal eggs found in Canadian soil. The occurrence and existence of this disease has not been reported elsewhere.

The disease appeared to have expanded its geographical boundaries since it was first diagnosed from a single Morex barley field in 1982. In 1985 and 1986, not only did previously diseased sites of barley result in a higher incidence and severity of infection, but additional barley fields were observed and reported to have the disease for the first time. Therefore, it appeared that a definite disease 'buildup' within barley fields and disease spread to apparent healthy barley fields had taken place. Nevertheless, it is also possible that affected plants were not reported or detected in previous years. The producer may have had such a low incidence of infection that the disease was not noticed, or, in fact, the affected plants were misdiagnosed for another disease or nutritional problem.

## Host Range and Cultural Practices

The natural host range of the disease, to date, includes barley, wheat, and three annual grasses. Morex, Piroline, Clark, Klages, Shabet, Premier, and Moravian III were all susceptible malting barleys, while Horsford was a susceptible hay barley. All the barley cultivars appeared to be equal in susceptibility, and none showed resistance. One winter wheat cultivar, Norstar, and three spring wheat cultivars,

Fortuna, Lew, and Leader, were found to be susceptible, but with low levels of incidence of infection. Diseased wheat plants were restricted to wheat fields that were near diseased volunteer barley plants or next to a field of diseased barley plants.

Echinochloa crusgalli, S. viridis, and L. persicum were the only weedy grasses found to have the disease. They all had diagnostic symptoms and were found in cultivated soil growing among diseased barley plants or near a diseased barley field. The diseased grasses have an annual habit, and germinate in the later part of June. Therefore, they probably do not play an important role in the overall epidemiology of the disease, in that they cannot act as an overwintering host or even a primary host of the causal agent. Overwintering reservoirs for plant viruses and for their vectors often include perennial roadside and pasture grasses (69); such symptomatic grasses were never observed relative to this disease. However, since symptomless grasses are common natural hosts of many plant viruses, it cannot be ruled out that asymptomatic perennial grasses of this disease do exist, and may contribute significantly in perpetuating a continuous disease cycle.

Recrop fields of barley nearly always had a reoccurrence of the disease, and usually at a much higher incidence and severity. Drought stressed areas within a barley field also appeared to produce a relatively high incidence and severity of the disease. Moreover, when water was applied to diseased crops, symptoms were not nearly as severe. However, by the time the producers first applied water to

their diseased crops, the disease had already taken effect on most of the barley plants within the field.

Rotation from barley into wheat resulted in a substantial drop in incidence of infection within wheat fields. Also, most barley fields sown in the early part of April or earlier had fewer plants affected by the disease.

# CHAPTER III

# TRANSMISSION STUDIES

How the causal agent moves from plant to plant, and how the disease is subsequently spread from field to field and from region to region, must be determined to fully understand the etiology and epidemiology of this new barley disease. Earlier, it was hypothesized that the causal agent was the unique VLP which was consistently only found in diseased tissue. Logically, if these VLPs could be transmitted to a healthy barley plant, resulting in disease development and the presence and replication of the VLPs in the plant tissue, the etiology of the disease might be partly elucidated.

This chapter describes various attempts to transmit the assumed causal agent of the virus-like disease of barley to healthy indicator test seedlings. Mechanical inoculation of diseased tissue, seed from diseased plants, soil from field sites of diseased plants, and insects and mites collected from diseased plants growing in fields were all tested in transmission experiments involving the new barley disease.

## Materials and Methods

The Conrad and Valier sites (previously described in Chapter II, pages 28-29) were extensively used for on-site studies for collections of soil, plant material, and arthropods.

## Mechanical Transmission Tests

Symptomatic barley leaves were detached from diseased field plants or from potted diseased plants growing under greenhouse conditions which had previously been exposed to diseased plants in fields. The leaves were ground in a cold mortar and pestle with various buffers and additives. The resulting leaf suspension was applied with a cotton Q-tip to dusted carborundum (Grit 600, Silicon Carbide) 2,3-leaf stage seedlings.

Control seedlings were mechanically inoculated with the appropriate leaf-buffer suspension from healthy plants in each of the experiments. Lighting was natural and supplemented with fluorescent (10,000 lux illumination) lights for 16 hr./day; room temperature varied from 15-30°C. Weekly observations were made until the spring barley and other grasses had matured to at least the boot stage and the winter barley had tillered for two months.

## Seed Transmission Tests

Seed from diseased plants was collected from the Conrad site in 1982 and 1983, and then sown in the greenhouse from 1983 through 1985 to determine if the presumed agent was transmitted by seed. The grower gave us a seed sample of his 1982 harvest of Morex barley on the Conrad site. Additional seeds from diseased plants on the same site were manually collected in 1983. These seeds were derived from symptomatic plants which were tagged for identification on 2 July 1983. Included were Morex barley, Fortuna spring wheat, and Norstar winter wheat (100

plants each); most were in the boot stage or had headed. At this time leaf samples were removed from ten plants of each cultivar for ultra-thin sectioning and immunosorbent electron microscopy against wheat streak mosaic virus antiserum; the same leaf was used in both assays.

After harvesting the seed in late August, these seeds were sown in steam sterilized greenhouse soil in the greenhouse in metal flats (30 cm x 20 cm x 9 cm or 31 cm x 45 cm x 9 cm). The lighting was natural and supplemented with cool white fluorescent (10,000 lux illumination) or metal-halide incandescent (40,000 lux illumination) lights for a 16 hr./day photoperiod. Room temperature varied from 15-30° C. Close observations were made until the plants were at the 5-leaf stage or had matured. Leaf samples were prepared for ultrathin sectioning. Certified seed controls were sown simultaneously in separate flats; leaf samples were prepared for ultrathin sectioning.

Soil Transmission Tests

In April, 1983, about 90 kg of soil was removed from the Conrad site, sieved to remove stones and stubble, and placed inside plastic bags. One-half of the soil was autoclaved at 120°C for one hour. Morex and Klages barley seeds were germinated in the autoclaved and non-autoclaved soil. As a soil control, greenhouse soil (steam sterilized) was used to germinate certified Morex seeds. All were grown under greenhouse conditions as described for seed transmission tests.

In 1984, field soil was removed from the roots of symptomatic Clark barley plants which had previously grown in a diseased field on the Valier site. The soil was placed in a 30 cm x 20 cm x 9 cm metal

flat with 60 certified Morex seeds. An additional 60 Morex seeds were sown in greenhouse soil as a control. All seedlings were grown to maturity under greenhouse conditions.

Plots of soil were fumigated on the Conrad site on 23 May 1984. Four 3 m x 6 m plots were raked clean of plant stubble and bordered with 30 cm deep trenches. Each plot was fumigated under plastic tarps with 1360 g of 98% methyl bromide, 2% chloropicrin (Dowfume, Dow Chemical, Midland, Michigan). Six days later, the plastic was removed from the plots; each plot was divided in half and seeded with Fortuna wheat and Morex barley, respectively. One month after the initial seeding, 24 greenhouse sown Morex and Fortuna seedlings were transplanted into three of the four plots; and a row of about 100 Morex seeds were sown between two of the plots. In July, the number of healthy and diseased plants were estimated and leaf samples were prepared for electron microscopy diagnosis.

Interaction Between Healthy and Diseased Plants under Greenhouse Conditions

Transplanted Field Sown Diseased Plants, 1984. To determine if the assumed causal agent would move from transplanted diseased plants to healthy indicator plants under greenhouse conditions, whole plants were removed from fields (Conrad and Valier sites) in July 1984, and replanted in containers of various sizes using greenhouse soil. Certified seed or healthy greenhouse sown seedlings were then introduced into some of the containers with diseased plants; or the diseased and healthy plants were grown in separate containers but within close

proximity of each other. The plants were grown in a Conviron growth chamber (Controlled Environments, Winnipeg, Canada) under a 16 hour photoperiod with cool fluorescent lights at 20°C or in an isolation room under metal-halide lamps or cool fluorescent lights supplemented with natural daylight for a 16 hour photoperiod at 20-30°C.

The healthy indicator test plants and the field transplants were exposed to each other for nearly three months (30 July - 30 October, 1984). Leaf samples from indicator test plants having abnormalities were processed for electron microscopy examination.

Diseased Trap Plants and Healthy Indicator Test Seedlings, 1986. Similar tests involving the exposure of diseased plants to healthy indicator test plants in the greenhouse were conducted in 1986. However, in this experiment, 174 healthy trap seedlings were sown in 20 plastic pots (16.5 cm diam.) and exposed to a diseased barley field for two weeks from 24 May to 7 June. The containerized plants were then removed from the field and grown in an isolation room under identical conditions as the previous tests conducted in 1984, except that the plants were sub-irrigated from a aluminum cake pan instead of being watered from the top; healthy indicator test seedlings at the 2-leaf stage were randomly distributed in separate pots among the trap plants from 9-25 June, and removed 3 August.

## Trap Seedlings

During the 1985 and 1986 growing seasons, trap seedlings were cycled onto and off of the Valier and Conrad sites to test for disease

spread. Barley and wheat cultivars were germinated in separate 16.5 cm plastic pots in the greenhouse at Bozeman, Montana. Trap wheat plants were included to compare their relative disease susceptibility to that of barley. Most of the seedlings were at the 1,2-leaf stage when they were introduced onto the sites. Initially in 1985, 2 to 65 seedlings were sown in each pot to test the possible effect of plant density on "disease capture." However, the number of seedlings was later kept to no more than 16 seedlings per pot. Clusters of the trap seedlings were strategically placed in different environmental surroundings within each site. In addition to using diseased barley fields within which to distribute the trap seedlings, a field of Lew spring wheat was also tested for movement of disease. This was the site where the disease was first recorded in Morex barley in 1982; since that time it had been planted only with spring wheat. Each pot of seedlings was placed in a freshly dug hole in which the rim of the pot was level with the soil surface of the field. The field crops varied as to cultivar, plant developmental stage, and soil moisture. If a specific location within a site was used to replace trap plants, the new seedlings were introduced in the exact position as the previous pot of seedlings. The trap seedlings were left on the site between two to four weeks before returning them to the greenhouse for further observation and fumigation with Vapona (dichlorvas) insecticide. One exception to fumigation included a set of trap seedlings exposed to the Valier site from 24 May - 7 June. These plants were instead placed in an isolation room with healthy indicator test seedlings to test for disease spread (discussed

in "Interaction between Healthy and Diseased Plants under Greenhouse Conditions," page 48).

In 1985, on the Conrad site, two sets of trap plants were cycled into and out of a Lew spring wheat field from 19 May to 1 June and 1 June to 18 June, respectively. Some of the plants had been treated with aldicarb (Temik) pesticide at the 2-leaf stage before field exposure.

The Valier site had trap plants exposed to diseased Clark and Klages fields from 17 June to 19 July 1985. Aldicarb had been applied to 15 pots of seedlings, while 5 other pots of seedlings were enclosed in a nylon monofilament screen (297 micron mesh opening).

The following year (1986), on the Conrad site, a Lew spring wheat field, a Piroline barley field, and a summer-fallow field were all used for trap plant exposures. The trap plants were arranged in five clusters of four pots (20 pots total) per field location. Each of the pots within a cluster was about 60 cm from one another. Three distinct sets of trap plants were cycled in and out of each field between 23 May and 31 July.

The Valier site had five cycles of trap seedlings placed about every two weeks in a diseased Klages barley field from 24 May to 1 August in 1986. The seedlings were distributed on the field in clusters of eight pots that were arranged in two parallel rows with each pot about 60 cm from each neighboring pot.

Transmission Tests Using Laboratory Reared Aphids

New York biotypes of three aphid species, *Rhopalosiphum padi*, *R. maidis*, and *Macrosiphum avenae*, originated from W. F. Rochow, Cornell University, Ithaca, New York, and were used to test for the transmission of the virus-like particles from diseased Morex leaves to healthy indicator test seedlings based upon Rochow's detached leaf method (75,76).

Transmission Tests Using Field Collected Leafhoppers

Leafhopper species were collected by sweeping the canopy of diseased barley fields and adjacent roadside grasses and pasture with insect nets made of solid cloth muslin or medium mesh fabric. Leafhoppers were quickly sucked into an aspirator apparatus from the net and then gently blown or tapped into caged healthy indicator test seedlings (2-leaf stage); the species were not separated from one another during the transmission tests. The cages were constructed of a cylinder of clear acrylic resin (25 cm long x 8 cm diameter) with a Nitex nylon screen top. Each cage enclosed 2-20 seedlings in an 8 cm diameter clay pot.

Leafhopper species were collected on a Conrad site or in a nearby pasture and roadside grasses during the growing season of 1983, 1984, and 1985. However, nearly all of the leafhoppers were collected from the pasture in 1984 and 1985 due to the paucity of leafhoppers in diseased barley and wheat crops in those two years.

All caged leafhoppers collected in 1983-84 were exposed to a 16 hr./day photoperiod under cool, white, fluorescent or metal-halide incandescent lights at 20-27°C. Inoculation access time ranged from five to 21 days, before Vapona fumigation.

In 1985, the field collected leafhoppers were exposed to healthy seedlings in rectangular dacron screened cages (37.5 cm x 18.75 cm x 17.5 cm) with inoculation access periods from 7 to 10 days. Some of the leafhoppers were cycled onto a fresh set of healthy seedlings, instead of being fumigated. An additional collection of leafhoppers were exposed to field transplanted diseased Piroline and Clark barley plants for a 5 day acquisition period, before being cycled onto healthy seedlings for one to two week exposures for a total of four weeks.

Subsamples of the collected leafhoppers in 1983 and 1984 were identified in 1983 by the late Dwight DeLong, Ohio State University, Columbus, Ohio, and by Derrick Blocker, Kansas State University, Manhattan, Kansas, in 1984.

Transmission Tests Using Greenhouse Reared Leafhoppers of *Endria inimica*

*Endria inimica*, the painted leafhopper, was specifically reared under greenhouse conditions and subsequently used in transmission trials for the virus-like disease of barley. The nonviruliferous leafhopper population originated from R. G. Timian, Fargo, North Dakota.

Leafhoppers were all allowed to feed on caged field transplanted diseased barley plants and then transferred to healthy indicator test

seedlings.

In addition, a subsample of the leafhopper population was allowed to feed on detached wheat striate mosaic virus infected Durum wheat leaves (provided by R. G. Timian, Fargo, North Dakota) for 24 hours after which time the leafhoppers were exposed to two sequential sets of caged healthy barley or wheat seedlings for 7 days each (84).

## Transmission Tests Using Greenhouse Reared Leafhoppers of *Macrosteles fascifrons*

The parent *Macrosteles fascifrons* leafhoppers were provided by L. Nault, Ohio Agricultural Research and Development Station, Wooster, Ohio. A nonviruliferous population of *M. fascifrons* was increased and maintained on Klages barley.

*M. fascifrons* leafhoppers were exposed to field transplants of diseased Morex barley for 4 days, at which time healthy indicator wheat and barley seedlings were added to the screened cage for an additional 3 weeks. Malathion (cythion) was then sprayed on all of the plants to terminate the leafhoppers. Greenhouse conditions were as previously described for the field collected leafhoppers.

## Transmission Tests Using Field Collected Thrips

Thrips species were collected with a solid muslin insect net in diseased barley and wheat fields in July 1985. They were immediately aspirated into caged healthy indicator 2-leaf Clark or Klages barley seedlings. The cages consisted of plastic cylinders with a nylon top, each enclosing 7-20 seedlings in a 20.5 plastic pot, or dacron screened

rectangular cages (37.5 cm x 18.75 cm x 37.5 cm) with pots of healthy barley seedlings inside. Thrips exposure time varied from 7 to 58 days under a 16 hr. photoperiod of natural light and metal-halide lights at 10°C to 27°C. All of the plants were fumigated with Vapona for two hours to terminate thrips exposure.

Subsamples of the thrips population were sent to Ramona Beshear, University of Georgia, Experiment, Georgia, for species identification.

## Transmission Tests Using Petrobia latens Müller, the Brown Wheat Mite

Mites Collected from Fields. During the 1986 growing period, vector transmission studies were concentrated on the brown wheat mite, *Petrobia latens* Müller. Mites were collected from diseased barley fields between 7 June and 1 August in 1986, using a version of the 'Jarring' method (21) whereby quick hand tappings against plants caused the mites to drop onto white paper. From the paper, the mites were quickly tapped into a plastic vial and covered with parafilm and a sponge stopper; field diseased leaves and stubble were added to the vials. The vials of mites were transported from the field site of collection to the laboratory in Bozeman. In the laboratory, the mites were identified and separated from other arthropods by briefly anesthetizing the vial contents with carbon monoxide gas with a pressurized 6.36 kg gas cylinder (Perlick Co., Inc., Milwaukee, Wis.). Active mites were then quickly placed upon healthy indicator test seedlings (1,2-leaf stage) by using a camel hair paint brush or human hair loops before enclosing the mites and plants within a cage. Caged seedlings were also exposed to mites by releasing previously examined mites

directly from a vial onto the potted plants' soil surface. All of the transmission experiments were subjected to a 16 hr./day photoperiod and natural lighting was supplemented with metal-halide incandescent lights (40,000 lux illumination) at temperatures between 20-40°C, unless stated differently.

The first mite transmission test involved greenhouse sown barley plants and mites 'en masse' coexisting together inside screened cages for 6 to 8 weeks. Mites of all stages were collected 7 June and 1 August 1986 from diseased barley (Klages) fields on the Valier site and were then exposed to healthy barley seedlings at the 1-leaf stage inside four separate cages: cage 'A' was 90 cm high x 60 cm wide and 120 cm long and made of Nitex nylon; the nylon screened cages of 'B', 'C', and 'D' were 37 cm long x 18.75 cm wide x 17.5 cm high. All plants were exposed to mites between 8-12 June to 2 August before fumigation with Vapona (dichlorvas).

The second type of test involved the exposure of adult and pre-adult mites to single test seedlings of healthy plants. The mites were collected on the Valier site on 3 and 18 July 1986 from diseased barley plants. Each seedling was grown in a plastic cone shaped container and enclosed with a 2.54 cm diameter x 25.5 cm long clear butyrate tube, with the top sealed with a sponge rubber stopper. Ten active mites per barley seedling were allowed to feed on the plant for a specific time period which varied between 12 and 168 hours; a total of 178 plants were included. After mite exposure, these plants were transplanted to 15.5 cm diameter pots.

In the 'mass transfer' and single plant tests, the plants were fumigated with dichlorvas and/or sprayed with malathion to terminate the mite exposure. The plants were grown in the greenhouse for at least 5 months for observations. At this time, I did not have a population of mites not carrying the causal agent to use in parallel control transmission tests. Leaf subsamples and whole mites were fixed and processed for ultrathin sectioning.

Samples of the field collected mites were placed in 70% ethanol and sent to Everet Linquist at the Biosystemic Research Institute, Ottawa, Canada for identification.

<u>Diapausal Mite Eggs Collected from Fields</u>. Parallel transmission tests were conducted with mites that were initially hatched from their diapausal white eggs and then given access to diseased or healthy leaf tissue before exposing to several different healthy plant species. From this approach, a plant host range for the mites and the assumed causal agent would be determined, and the transovarial transmission of the causal agent clarified.

Diapausal white eggs of <u>Petrobia</u> <u>latens</u> were collected on 16 October 1986 from stubble barley fields that had plants affected by the disease during the growing seasons of 1984, 1985, and 1986. The white eggs were easily found on rocks lying on the soil surface. Although the eggs were visible with the unaided eye, a 10x hand lens was used for additional egg identification and confirmation. The rocks were placed in large plastic garbage bags and stored at room temperature in the laboratory.

The diapausal egg of P. latens will not hatch without moisture. Therefore, the egg-laden rocks were placed into glass containers of appropriate size, moistened with distilled water, and covered with a wet paper towel. The containers were sealed with plastic Saran Wrap for 5 days to maintain a high moisture content. The plastic top was replaced with a fine nylon mesh screen (Nitex, 115 micron opening) and detached healthy or diseased barley leaves were placed at least twice a day in the containers for 9 consecutive days. The room temperature was between 22-27°C and metal-halide lamps (40,000 lux) provided a 12 hr./day photoperiod. After 9 days of detached leaf exposure, the mites were removed from the containers with an aspirator, placed in a small glass vial, and then gently tapped onto the soil surface in which healthy Dicktoo barley seedlings (1-leaf stage) were growing. All of the indicator test seedlings were initially sown in greenhouse soil and germinated in a disease-free growth chamber before transplanting 4-5 seedlings to 15 cm diameter plastic pots. White pebbles previously washed in Clorox bleach were scattered on the soil surface to provide for a drier environment and a possible egg laying surface for the mites. The plants, mites, and original field rocks were all enclosed in two separate 45 cm square cages. The cage was constructed of a plexiglass floor, two opposing plexiglass sides, a monofilament nylon top (115 microns opening), and two nylon sides. Velcro strips were sewed and glued to the front side for easy access into and out of the cage. Mites that had been exposed to the detached diseased leaves and those exposed to healthy leaves were maintained in cage-D and cage-H, respectively.

The indicator test plants were exposed to the mites and their offspring for 21 days under a 12 hr./day photoperiod with metal-halide lamps with temperatures between 22-27°C. Water and fertilizer were applied as needed by sub-irrigation. After two weeks of mite exposure, the first and/or third leaf (leaves) were removed from a subsample of plants exhibiting mite damage and/or virus-like symptoms. The leaf subsamples from cage-D were processed for both ultrathin sectioning and leaf dips, while those leaves removed from cage-H were processed for only ultrathin sectioning; leaves from healthy control plants which were not exposed to mites were simultaneously processed. The procedures for preparing ultrathin sections and leaf dips are previously described (Chapter I, page 4).

After 21 days of mite exposure, visible mites were aspirated from the plants and the field rocks removed. The plants were removed from the cage and placed inside a fumigation hood for 2 hours with Vapona (dichlorvas). Each plant was then transplanted into a 20 cm diameter plastic pot in greenhouse soil. The plants were grown under a 16 hr./day photoperiod with metal-halide lamps and natural daylight at temperatures between 15-25°C in the day and 10-15°C in the night. These plants were under daily observations for eight more weeks.

Unless specifically stated, the following groups of plants cycled into and out of each cage were treated in the same manner as the first described group of plants.

A second group of indicator barley seedlings (emergent stage) was cycled into and out of cages-H and -D in 15 and 19 days, respectively. Different plant species were then introduced into cage-H during the

third cycle of mite exposure, which included carrot (Daucus sp., 'Nantes Coreless,' 1-leaf stage) and onion (Allium sp., 'White Pearl,' 1-leaf stage) indicator test seedlings. The mite population and carrots and onions were observed for 44 days, at which time a fourth group of plants was cycled into cage-H; this fourth cycle included barley seedlings introduced in the 1-leaf stage. The caged carrot and onion plants and their healthy controls were subsampled and processed for electron microscopy.

Diapausal eggs were also hatched from soil clods collected 4 November 1986 near Noble Fork, Canada; the eggs were removed from a site which had been in recrop barley for three consecutive years provided by B. Byers, Agriculture Canada, Research Station, Lethbridge, Canada. The eggs were stored at 0.5°C until 1 December 1986, at which time they were shipped to Bozeman and were then stored in the refrigerator for 36 days. To initiate egg hatching, the soil clods were distributed on glass plates, moistened with distilled water, and covered with wet paper towels and Saran wrap for 4 days inside a 45 cm square cage designated 'cage-C.' After the removal of the wet paper towels, detached healthy Dicktoo barley leaves were added to the soil thrice a day for 6 consecutive days. The mites were then distributed among healthy Dicktoo barley seedlings (emergent stage) by placing the infested detached leaves on the soil surface of the pots. After 19 days of mite exposure, the plants were removed and replaced with three sequential sets of indicator barley plants, each cycled in and out of the cage every 21 days. All the plants had the mites removed with an aspirator, and then were fumigated, transplanted, and grown under

greenhouse conditions as previously described. Leaf samples from infested plants in sets I and II were processed for ultrathin sectioning as described for those plants in cages-D and -H.

## Results and Discussion

### Mechanical Transmission Tests

No diagnostic symptoms of the disease in question (or other symptoms) were visible on any of the healthy indicator test seedlings that had been mechanically inoculated with the different buffers, additives, and dilutions of leaf sap from affected leaves (Table 3, Appendix). The buffers and additives chosen were based upon several of those that had been used successfully in transmitting enveloped virus particles to the dicotyledonous plants (14,26,47). Buffers most effective for virion stability and infectivity during purification procedures for rhabdoviruses were tested (37,44,54). Phosphate buffers are the most commonly used buffers for mechanical inoculation studies (95), while organic solvents are avoided for enveloped viruses because they rapidly destroy the membrane of the intact virus particle. Black (6) found that the buffer should be near neutrality for rhabdovirus stability. Early studies on the stability of potato yellow dwarf virus (rhabdovirus) indicated that glycine, magnesium chloride, and sucrose helped retain the infectiousness of the virus (7). Abrasives such as carborundum and Celite were used to create wounds on the plant surface for virus entry into the plant and are nearly universally employed in mechanical inoculation procedures.

The inability to transmit the causal agent to plants by mechanical inoculation techniques was particularly disappointing in that other studies involving the isolation and purification of the virus-like particles were dependent on field material available only during the growing season. However, it was not surprising that the mechanical transmissibility of the causal agent would be difficult, if not impossible, since to date no membrane enveloped plant virus or mycoplasma have been transmitted by mechanical inoculation to a species in the Gramineae (Poaceae) (45).

Seed Transmission Tests

Symptomatic plants were not visually detected in the 1,076 seedlings produced from a subsample of the 1982 harvest from the diseased Morex field near Conrad.

From the leaf subsamples of tagged field diseased plants producing the seed for seed transmission studies in 1983, 8 out of 10 Norstar wheat plants, 10 out of 10 Morex barley plants, and only 3 out of 10 Fortuna wheat plants had the unique associated virus-like particles (VLPs) in ultrathin sections. The Norstar wheat plants not having the VLPs had wheat streak mosaic virus (WSMV) detected by immunosorbent electron microscopy (ISEM), but only the unique VLPs were found in ultrathin sections. The Fortuna wheat leaf samples surprisingly had 6 out of 10 plants infected with rhabdovirus-like particles confined to the phloem cells. WSMV was not detected in any of the 10 Fortuna wheat plants by ISEM or in ultrathin sections; no virus particles were found in one Fortuna wheat plant by either assay method.

The rhabdovirus-like particles found in the Fortuna wheat phloem cells closely resembled those of wheat striate mosaic virus (WStMV) in morphology. Based upon micrographs of these particles, R. Timian confirmed that the particles were in fact a rhabdovirus and probably WStMV. R. I. Hamilton reported the presence of WStMV in Montana in 1963 (38). Therefore, the rhabdovirus infecting the Fortuna wheat plants may have been WStMV.

In contrast, wheat streak mosaic virus is endemic in North Central Montana (10) and, therefore, was not to be considered unusual when it was found in one of the Morex barley and one of the Norstar wheat field plants tagged for seed transmission tests.

The seed harvested from the tagged symptomatic field plants of Morex barley, Fortuna wheat, and Norstar wheat in 1983 also did not produce symptomatic plants (Table 4, Appendix). Ultrathin sections from the leaves of these symptomless plants derived from the seed of diseased barley plants did not contain the associated VLPs or any detectable virus particles when viewed with the electron microscope. The relatively low number of seeds produced by the field diseased Fortuna wheat plants could be attributed to the high incidence of infecting rhabdovirus (i.e., WStMV induces the production of shrivelled seeds or none at all).

The seed transmission tests provided good evidence that the causal agent of the virus-like disease is not seed-borne in Morex barley, Fortuna wheat, and Norstar wheat when seed produced from diseased field plants is sown under greenhouse conditions.

Soil Transmission Tests

Barley seeds sown in autoclaved or non-autoclaved soil previously removed from a diseased field in 1983 did not produce plants displaying symptoms. A total of 150 Morex and 51 Klages barley seedlings were grown in the autoclaved field soil, while 278 Morex and 65 Klages barley seedlings were grown in the non-autoclaved field soil; the control greenhouse soil had 105 healthy Morex barley plants.

In addition, none of the 60 Morex plants sown in soil obtained in 1984 from the roots of diseased barley plants developed symptoms.

Barley and wheat plants growing in all three of the methyl bromide fumigated plots developed diagnostic symptoms of the virus-like disease. Ultrathin sections of a few leaf samples also contained the unique VLPs. Although the fumigated plots were sown 23 May, germination did not occur until July due to lack of moisture, and symptoms were not visible until 17 July. It was difficult to obtain incidence of infection in these plots due to the additional masking effect of plant stunting and yellowing caused by severe drought. That the soil in the plots was in fact sterile before the seed was introduced was suggested by the lack of volunteer barley and wheat, weedy grasses, and thistle growing within the plots during the growing season. The plots were surrounded by these plants and the volunteer barley and wheat were affected with the virus-like disease. In addition, 91 out of 100 Morex seedlings, of which the seeds were sown between two plots, had diagnostic symptoms.

The probability of the causal agent originating within the sterilized plots was low, thereby implying that the causal agent had to have moved into the plots and infected the plants. Since most soil inhabiting nematodes and fungi advance or move slowly in undisturbed soil (40), the probability that they moved rapidly into the plots and vectored the causal agent to plants is slight. In addition, soil-borne nematodes and fungi usually have a preference for high soil moisture, and these plots were under extreme drought conditions.

## Interaction Between Healthy and Diseased Plants under Greenhouse Conditions

Transplanted Field Sown Diseased Plants, 1984. A total of 586 diseased plants removed from the fields did not induce symptoms on 1,631 healthy indicator test plants to which they had been exposed for approximately two months in the greenhouse. The transplants included spring sown barley, *S. viridis*, and *E. crusgalli*. Two aphid species, *R. maidis* and *M. avenae*, which were originally on the field transplants, colonized the healthy indicator test plants. Later, *R. padi* and a web-forming spider mite colonized both the diseased field plants and the indicator test plants as greenhouse contaminants. Therefore, it was evident that these three aphid species and the web-forming spider mite did not transmit the causal agent from diseased transplants to healthy plants under greenhouse conditions. In addition, healthy and diseased plants growing in the same container did not provide a means for transmission.

Trap Plants, Greenhouse Sown Plants Exposed to Fields of Diseased Plants in 1986. At the time that the 24 pots of trap plants were removed from a diseased barley field, 99% of the 136 barley plants had symptoms and the plants were infested with a spider mite species, *Petrobia latens* Müller. No other insect or mite species were visible on these plants. By subirrigating and not fumigating the plants, the mites were allowed to stay on the plants while growing in the greenhouse. As the indicator test seedlings were randomly placed among the trap plants in the greenhouse, mites infested the new seedlings. Twenty-seven (8 pots) out of a total of 122 (20 pots) indicator test seedlings showed symptoms of virus-like barley disease. Although only 22% of the introduced barley plants developed symptoms, this was the first time that transmission of the causal agent was demonstrated in a greenhouse environment. The presence and association of *P. latens* with the diseased plants implicated the mite as a possible vector.

## Trap Seedlings

1985, Conrad Site. On two dates in 1985, potted trap plants were exposed to a Lew wheat field; the field wheat plants had an estimated 0.5% incidence of infection by July (Table 5, Appendix). However, no symptoms were visible on the field plants (2-leaf stage) when the first set of trap plants was initially placed in the field on 19 May. Sixty-two out of a total of 1,024 (6%) barley plants developed symptoms in the first set of trap pots. The diseased trap plants were confined to eight out of 51 pots of barley. All but one of the diseased trap plants were near each other on the site, suggesting that during this

time period of exposure the disease was localized. The unique VLPs associated with the disease were found in ultrathin sections of leaf samples that were removed from an affected plant in each of the eight pots. Leaf samples from five plants not showing symptoms did not have the VLPs.

Disease spread to the second set of trap plants occurred at a higher incidence and in a more scattered distribution than the first set. In addition to plants becoming diseased on the same eight 'holes' as those of the first set of plants, apparent secondary disease spread occurred in trap plants growing in 14 other pots. These pots of plants were distributed on all but one cluster. Ninety out of a total of 438 (20%) barley plants showed symptoms. None of a total of 81 aldicarb-treated barley plants in five pots and 51 wheat plants in five pots got the disease (Table 5, Appendix).

1985, Valier Site. The last set of trap plants exposed to the disease in 1985 obtained a relatively high incidence of infection (Table 6, Appendix). The trap plants were distributed on three contiguous recrop barley fields (Klages-A, Clark-B, and Klages-C) for nearly three weeks. All three fields had at least 50% incidence of infection. Trap plants in 12 pots placed on the Clark-B field were destroyed by herbicide 'drift' (Round-up, glyphosate) after the producer sprayed ditch weeds. By combining the data for all the trap plants, the results showed that 221 barley plants out of a total of 441 (50%) became affected with the disease. Only one out of 50 caged barley plants and five out of 109 aldicarb-treated barley plants developed symptoms. Hence, of the total 282 barley plants that were

not protected by cages or by an insecticide, 215 (76%) of the plants became diseased. Thirteen percent of the trap wheat plants (9 out of 70 plants) developed symptoms.

1986, Conrad Site. Detailed results for the occurrence of disease spread from a summer-fallow site, a Lew wheat field, and a Piroline barley field to containerized trap plants are summarized in Tables 7, 8, and 9, respectively, in the Appendix.

The Lew spring wheat crop plants were at the 2-leaf stage and showed no symptoms when the first set of trap plants were placed on the field on 23 May 1986. After the allocated four weeks of field exposure, only barley plants in four pots of the 20 pots developed the virus-like disease; this included 14 affected barley plants out of a total of 62 barley trap pots (23%). When the second set of trap plants were placed on the site, the wheat crop had an estimated one percent incidence of infection. However, only 11% of these barley trap plants (9 out of 74) developed symptoms. No disease spread occurred in the last set of trap plants exposed in the later part of July to the wheat field.

The contiguous eastern boundary of the Lew wheat field and a Norstar winter wheat field was next to a summer-fallow site in 1986. This site had been in dryland Piroline barley in 1985 with a 100% incidence of infection of the virus-like disease. Clusters '1', '2', and '3' contained pots of plants adjacent to the Norstar winter wheat, and clusters '3' and '4' contained pots of plants next to the Lew spring wheat (Table 7, Appendix). Disease movement took place only in those trap plants of the first set that were placed on the clusters

from 23 May to 18 June. The incidence of infection was quite high at 80% (44 out of a total of 55 barley plants). Grasshoppers ate all the seedlings placed in the clusters '1', '2', and '3' during the second cycle of trap plants, and in clusters '4' and '5' of the third set of plants. Those seedlings that did survive the last two cycles had a considerable amount of grasshopper damage. Perhaps disease spread would have occurred in the second and third sets of plants had the leaves not been destroyed by grasshoppers.

The first set of trap plants exposed to the field of Piroline barley in 1986 developed symptoms on 97% of the 103 barley trap plants; none of the 7 wheat trap plants were affected. The Piroline crop plants were at the 2,3-leaf stage with no visible symptoms when this first set of trap plants were initially placed on the site. Later, when the other two sets of trap plants were cycled into and out of the site, nearly all of the crop plants were affected. However, these last two sets of trap plants exposed between 18 June-16 July and 16-31 July resulted in relative low levels of affected plants, decreasing to 5 and 18%, respectively. No wheat plants were affected.

<u>1986, Valier Site</u>. The results for disease spread into trap plants on the Valier site in 1986 were impressive in that nearly all the trap barley plants developed symptoms in the first three sets of plants cycled into and out of the Klages fields 'A' and 'B' in May and June (Tables 10 and 11, respectively, in the Appendix). Trap wheat plants were also affected, though at a considerably lower incidence. The succeeding sets of plants exposed to fields 'A', 'B', and 'D' in July did not attain the previous high levels of infection (Tables 10,

11, and 12, respectively, in the Appendix). Trap plant survival in field 'B' was poor during the fourth cycle (2-16 July) due to grasshopper damage. Therefore, to avoid the grasshoppers during the fifth set of trap plant exposure, 16 pots of plants previously specified for field 'B' and 16 other pots of plants specified for the summer-fallow field on the Conrad site, were placed instead on field 'D'.

Symptomology. In 1985, most of the trap plants did not develop visible symptoms until one to two weeks after the initial two weeks of exposure to the fields of diseased plants. After returning the trap plants to the greenhouse, it was possible to observe the progression of symptomatic changes occurring on a plant. The first detectable leaf discoloration consisted of a very faint, light green mottle distributed randomly on the leaf; symptoms nearly always occurred in plants no younger than the 5-leaf stage. Light green parallel dashes developed into streaks, which sometimes coalesced into stripes. As time progressed, the light green gradually changed to a yellow color, resulting in an overall yellow-streaked leaf. Prominent discolored bands were rarely produced on these plants, whereas leaf banding was a diagnostic feature exhibited by diseased field plants (Chapter II, page 38).

In contrast, the first two sets of trap plants that were exposed to diseased fields in May and June of 1986 contained plants that had diagnostic streaks and stripes within only two weeks of initial field exposure; several of the plants later developed parallel bands on leaf surfaces.

Summary. At the time these tests were conducted in 1985 and 1986, the most important aspect and concern involved the question of disease spread to healthy, greenhouse sown trap plants which were exposed for a period of time on or near a diseased site and then returned to the greenhouse for observation. Once it was established that disease movement did in fact occur, then questions applicable to how the causal agent of the disease moved from plant to plant could be addressed. Indeed, movement of the causal agent from diseased plants to healthy seedlings did occur. The trap plants had the highest incidence of infection when they were exposed to diseased fields during the months of May and June in 1985 and 1986. If the field had a high percentage of diseased plants, then disease spread to the trap plants was greater. The barley cultivars used as trap plants were all susceptible, whereas the wheat cultivars were usually resistant to the disease. However, as the incidence of infection of barley trap plants increased, the probability that a wheat plant would become diseased increased. The plants which had previously been treated with aldicarb or enclosed within a screened cage had a negligible incidence of infection relative to the other unprotected trap plants.

No insects or mites had colonized or were found on the trap plants in 1985, with the exception of grasshoppers and a few aphids in the last set of plants. Therefore, at that time, it was assumed that the vector of the causal agent was an active, mobile arthropod which did not readily colonize barley plants. However, in 1986 trap plants from the first two sets cycled into and out of diseased fields not only had over 90% incidence of infection, but also were heavily infested with

the brown wheat mite. This apparent association between the mites and the high level of diseased trap plants led to several mite transmission studies conducted in the laboratory and greenhouse in 1986 and 1987.

## Transmission Tests Using Insects and Mites

Transmission Tests Using Laboratory Aphids. The three laboratory aphid species did not transmit the casual agent from detached leaves with the virus-like disease to indicator test seedlings. Included in the test were groups of 6 Morex barley, 5 TA-I (*Triticum* x *Agropyron*), and 5 Coast-Black oat plants exposed separately to each of the species *R. padi*, *R. maidis*, or *S. avenae*.

It was demonstrated that the 'detached' leaf method did work in transmitting a known virus to healthy plants. *M. avenae* was allowed to feed on field collected Klages barley infected with barley yellow dwarf virus (BYDV). The aphids then transmitted BYDV to 10 out of 13 healthy indicator test plants (Black-Coast oats). Nonviruliferous aphids did not transmit a symptom inducing agent.

Based upon these tests and the negative results obtained for disease movement that occurred when the three aphid species (biotypes native to Montana) colonized plants with the virus-like disease and healthy plants growing next to one another (page 65), it was concluded that these aphid species were not the vector responsible for transmitting the causal agent of the new virus-like disease.

Transmission Tests Using Field Collected Leafhoppers. Subsamples of leafhoppers collected from the Conrad site in July and August, 1983

were identified as *Balclutha neglecta* (Delong and Davidson), *Psammotettix alienus* (Dahlbom), *Psammotettix lividellus* (Zett.), *Hebecephalus occidentalis* (Beamer), *Macrosteles fascifrons* (Stal), and *Dicraneura probicarneola* (Stal). The majority of these leafhoppers were from Morex barley plants which had the virus-like disease. No symptoms indicative of the virus-like disease were expressed on the healthy indicator test plants that were previously exposed to the leafhoppers and their progeny collected in July, August, and October 1983 (Table 13, Appendix). However, an unidentified leafhopper and two of its progeny did transmit a mycoplasma-like organism (MLOs) to two Morex barley seedlings. These plants were yellow and stunted, and ultrathin sections of leaf samples revealed MLOs in the phloem cells when viewed with the electron microscope.

Leafhopper collections conducted from mid-May through mid-August, 1984 on the Conrad site consisted of the following 13 species: *Macrosteles fascifrons* (Stal), *Dicraneura carneola* (Stal), *Amblysellus grex* (Oman), *Auridius auratus* (Gillette and Baker), *Psammotettix lividellus* (Zett.), *Psammotettix alienus* (Dahlbom), *Sorhoanus uhleri* (Oman), *Athysanella attenuata* Baker, *Athysanella acuticauda* Baker, *Endria inimica* (Say), *Hebecephalus rostratus* (B. and T.), *Balclutha neglecta* (Del. and D.), and *Athysanella occidentalis* Baker. The most prevalent species included *A. auratus*, *M. fascifrons*, *P. alienus*, and *P. lividellus*.

Nearly all of these leafhoppers used for identification and in transmission tests (Table 14, Appendix) originated from a pasture of Kentucky bluegrass, *Poa pratensis*, near affected barley and wheat

fields. The numbers of leafhoppers found in the wheat and barley fields were too few to carry out transmission tests.

As in 1983, no symptoms diagnostic to the virus-like disease were found on the indicator tests plants previously exposed to the leafhoppers.

In 1985, virtually no leafhoppers were swept into the nets from barley fields with the virus-like disease until late July, and then only a few leafhoppers were caught per 100 sweeps. Therefore, as in 1984, most of the leafhoppers used in the 1985 transmission studies originated from the pasture on the Conrad site.

An estimated 100 leafhoppers collected on 19 May 1985 and exposed to 126 healthy indicator test seedlings, and about 100 other leafhoppers exposed to two cycles of 134 healthy indicator test seedlings, did not transmit a disease inducing agent. In addition, the leafhoppers which were allowed to feed on field transplanted diseased barley plants for 5 days did not subsequently transmit the assumed causal agent or another agent to healthy indicator test seedlings.

Transmission Tests Using Laboratory Reared Leafhoppers. About 200 painted leafhoppers, E. inimica, failed to transmit the assumed causal agent from 6 diseased barley plants transplanted from the field to 189 healthy indicator test plants. They had been exposed to transplanted diseased barley plants for 6 days, after which they were allowed to feed on four sets of sequential healthy indicator test plants for eight, six, 14, and 10 days, respectively.

Parallel transmission tests conducted with E. inimica feeding on infected WStMV leaves, followed by exposure to healthy test plants, resulted in 22 infected WStMV Crosby durum wheat plants and none of the 11 Fortuna plants. These leafhoppers and their progeny were continually reared on infected plants and continued to transmit WStMV to healthy Crosby durum wheat seedlings but failed to transmit WStMV to the 29 healthy Morex seedlings employed in these studies. The work with WStMV and its efficient vector, E. inimica, was, in part, conducted to prove that the manual techniques to which the leafhoppers were subjected were non-injurious and that the environmental conditions were conducive for the transmission of a specific virus.

Approximately 500 aster yellow leafhoppers, M. fascifrons, failed to transmit the assumed causal agent to 50 healthy indicator test seedlings after they had been exposed to 12 field transplants of diseased Morex barley plants.

Initially, leafhoppers were thought to be prime candidates as the vector involved in spreading the new virus-like disease of barley. To date, leafhoppers and planthoppers are the only known vectors responsible in transmitting enveloped viruses to the small grains (Gramineae) (45). Thus, correlating this information with the abundance of leafhoppers found on diseased barley in 1983, greater emphasis was initially placed upon leafhopper transmission tests than with other arthropods visible on the field sites. Furthermore, three of the species that predominated the field and pasture leafhopper populations were known to transmit enveloped viruses or mycoplasma-like organisms in other areas of the world. P. striatus (L.) and P. alienus (Dhlb.)

transmit (Russian) winter wheat mosaic virus (a rhabdovirus) to the small grains in Eastern Europe (96). M. fascifrons is a vector of both the mycoplasma of aster yellows and oat blue dwarf virus (a non-enveloped icosahedral virus) in the small grains (3,4). Although E. inimica is an efficient vector for WStMV (81,84), no E. inimica leafhoppers were found on the Conrad site in 1983 at the time when a wheat field had plants infected with a rhabdovirus thought to be WStMV. A few species of E. inimica were collected in 1984 on the same site, but no rhabdovirus particles were detected in field plants. Therefore, it is quite possible that, in fact, P. alienus and/or P. striatus were vectoring the rhabdovirus to the infected field Fortuna plants in 1983.

Now, based upon 1) extensive tests using leafhoppers collected from diseased barley and pasture grass, 2) transmission tests involving laboratory derived and reared M. fascifrons and E. inimica, and, most importantly, 3) the paucity of leafhoppers found in heavily infected barley fields throughout the growing seasons of 1983, 1984, and 1985, it was concluded that leafhoppers were not involved with the epidemiology of this new disease.

Transmission Tests Using Field Collected Thrips. Greater numbers of thrips were observed in barley fields than in contiguous wheat fields. Limothrips denticornis Haliday, the barley thrips, was the predominant thrips species and was found in Lew spring wheat and Klages and Piroline barley fields. Anapothrips obscurus bv achypterous Müller and Thrips tabaci Lindeman were identified from Klages and Piroline

fields, whereas Frankliniella tritici (Fitch) and F. fusca Hinds were identified in relatively low numbers from Lew wheat fields.

No symptoms indicative of the disease were expressed on 220 indicator test plants previously exposed to thrips. Ultrathin sections which were cut randomly through 16 whole thrips that had been removed from diseased barley plants did not reveal the associated VLPs within thrips tissue.

After eliminating the leafhopper and aphid species used in the previously discussed transmission tests as being probable vectors for the causal agent of this new disease, other possibilities were sought. Thrips and grasshoppers appeared to be the two dominant species found in barley and wheat crops throughout the growing season of 1985; very few aphids and leafhoppers were observed, and then only in mid-May and July. Thrips numbers have been reported elsewhere to dramatically increase in extremely dry weather (42), which corresponded to the dry growing season in 1984 and 1985 encountered in Pondera County.

Presently, thrips are known to vector only two viruses, tomato spotted wilt virus and tobacco ringspot virus. T. tabaci (78), F. schultzei (Trybom) (78), F. occidentalis Pergande (34) and F. fusca (78,79) are the only four species that have been shown to act as the vector for tomato spotted wilt virus. T. tabaci also may transmit tobacco ringspot virus (5,59).

Transmission Tests Using Brown Wheat Mites

'Mass transfer' transmission studies: The results for those mites, Petrobia latens, collected from fields of diseased barley plants

on 7 June and 1 August 1986 and subsequently exposed to caged healthy indicator barley seedlings 'en masse' gave evidence that, indeed, the mite had a role with the new virus-like disease of barley (Table 15, Appendix). Thirty-four percent of the 26 barley plants developed symptoms within three weeks after initial mite exposure. Kearney (43%) and Ellis barleys (38%) had a greater incidence of infection than Tennessee Winter barley (25%). However, further testing with the mites and larger numbers of each cultivar are required for determining susceptibility differences among cultivars. Ultrathin sections from three leaves of an affected Kearney barley plant revealed the unique VLPs associated with the disease. However, of the 16 mites which were randomly thin sectioned, no VLPs were detected.

Single indicator test seedlings: The results obtained from varying the mites inoculation access time from 12 to 168 hours to caged single indicator test seedlings showed that at least 24 hours of mite exposure was required for some plants to become affected (Table 16, Appendix). The numbers of diseased plants did not significantly increase with increasing the time for mite exposure. Only Ellis barley, and not Kearney barley seedlings became diseased. This does not necessarily make sense, in that Kearney seedlings developed the disease in the 'en masse' transmission tests (Table 15); and Kearney seedlings used as trap plants also developed diagnostic symptoms (Tables 5-12, Appendix). Another inconsistency was detected between those mites that were collected 3 July and subsequently exposed to test plants which produced 13 diseased Ellis plants out of a total of 76 (17%), while those mites that were collected 18 July produced

5 diseased Ellis plants out of a total of 102 plants (5%). Even though both sets of mites were removed from the same field, the infectivity of the mite population could have changed with time. In addition, the mortality of the mites varied from plant to plant during the inoculation access time period allowed. Often, moisture formed within the inner sides of the cages and destroyed some of the mites. Also, it was impossible to know if and when each of the ten mites exposed per plant actually fed upon the plant; mite feeding marks were visible on most of the plants. Therefore, before definitive statements can be made about the time requirements of mite inoculations for the induction of diseased plants, replicated transmission tests should be conducted using different types of mite confinement and including reduced inoculation access time below 12 hours.

Symptoms were visible about two weeks after initial exposure to the mites. Ultrathin sections from the six affected plants that were sampled contained the unique VLPs associated with the disease; sections from two healthy control plants did not have the VLPs.

<u>Mites derived from field collected diapausal eggs</u>: Indicator test seedlings that were exposed to populations of mites which were initially derived from diapausal eggs developed symptoms that were diagnostic to the virus-like disease (Tables 17, 18, 19, Appendix). The first mite generations in cage-H and cage-C had not been exposed to diseased leaves, while those mites in cage-D were allowed to feed on detached diseased leaves for nine days. However, plants in all three cages became diseased. The results for each cage of mites and its corresponding sets of indicator test plants are discussed further.

Cage-D: After hatching from the diapausal eggs, the larvae and subsequent mite stages were exposed to detached diseased leaves up to nine days before allowing them to infest barley seedlings in two sequential sets of plants for about two weeks each (Table 17, Appendix). The first set of 25 indicator test seedlings were initially exposed to about 60 active mites on 23 December 1986. Several seedlings had symptoms by 1 January 1987, ten symptomatic seedlings by 4 January, 16 seedlings by 10 January, and all 25 seedlings had symptoms by 24 January 1987, or within four weeks of the initial mite exposure.

The leaf samples that were processed for electron microscopy when the plants from cage-D in the first set were about two weeks old and still infected with the mites gave some interesting results. All of the leaves except the healthy control seedlings were physically damaged by the mites. All of the leaves displaying virus-like symptoms contained the virus-like particles (VLPs), and those leaves just having the apparent mite damage did not contain the VLPs. Even though the first leaf was exposed and infested by the mites, it never developed the virus-like symptoms and never contained the VLPs. Yet, if the third leaf of the same plant had virus-like symptoms, VLPs were found in the symptomatic leaf.

Those infested plants that did not display virus-like symptoms at two weeks of mite exposure later developed symptoms. However, the later the onset of symptom development, the younger the first symptomatic leaf.

The number of mites had increased to about 400 mites in the first three weeks and were then introduced to a new set of 24 test seedlings

at the emergent stage. In response to the high numbers of mites feeding upon the young first leaves, three plants died and the remaining 21 plants had pale yellow, necrotic, stunted, and dried cotyledons. The subsequent leaves also had much mite feeding damage, but were not as stunted or necrotic. Nineteen of these plants developed symptoms of the disease.

Cage-H: Approximately 120 active mites which had been hatched from diapausal eggs and reared on detached healthy barley leaves were subsequently introduced to the first in a series of four sets of barley test seedlings (Table 18, Appendix). A few symptomatic plants were visible on 1 January, six diseased plants on 4 January, 11 diseased plants on 10 January, and all 25 plants were diseased by 24 January 1987. Although the first generation of mites which had hatched from the diapausal eggs had never been exposed to a diseased plant, the mites appeared to have transmitted the assumed causal agent to previously healthy seedlings.

The plants that were sampled for the presence of VLPs in their leaf tissue by ultrathin sectioning had VLPs only in the diagnostic symptomatic leaves. All six of those symptomatic plants had VLPs, whereas the four plants without symptoms did not contain the VLPs. However, after one more week of mite exposure and within four weeks of initial mite exposure, all of the indicator test plants developed virus-like symptoms on younger leaves other than the first leaf.

The second set of indicator test seedlings had 18 out of 22 plants develop symptoms; two of the original 24 test plants apparently died due to the large number of mites feeding on the first leaf at the

emergent stage. The mite numbers dropped from about 500 to 200. These remaining mites increased to an estimated 1,500 mites when they were exposed to carrot and onion seedlings for six weeks. Even though considerable mite feeding damage was imposed upon both of these plant species, none of the plants developed the virus-like symptoms, and ultrathin sections from two plants of each species did not contain the VLPs. This relatively large mite population was, in turn, allowed to feed on a new set of 24 test barley seedlings at the 1-leaf stage for three weeks. However, only nine seedlings eventually developed symptoms, and these were not visible until at least two weeks of mite exposure.

Cage-C: The original 60 mites that were hatched from diapausal eggs found near Lethbridge, Canada and their progeny also induced virus-like symptoms on indicator test seedlings (Table 19, Appendix). The barley plants of the four sets that were cycled into and out of the cage containing these mites developed symptoms on 18, 24, 24, and 18 seedlings out of a total of 21, 24, 24, and 24 seedlings, respectively. Three plants died in the first set due to the effect of mite feeding upon emergent stage seedlings; the other sets of barley were all introduced to the cages at the 1-leaf stage. The mite numbers increased from 60 to 224 mites between 15 January and 23 February 1987, and then dropped to a low of 50 and 19 mites on 15 March and 4 April, respectively. Although the incidence of infection was consistently high, the incidence was less when the mite population was relatively low.

Four of the symptomatic plants and two of the non-symptomatic plants in cage-C were sampled for the presence of VLPs by ultrathin sectioning. Comparable results were obtained from Cages D, H, and C; all the symptomatic leaves contained the VLPs, while the non-symptomatic leaves did not have the VLPs; the two healthy control plants also did not have the VLPs (Table 1).

Symptomology. In order to discuss and define the symptoms induced by the assumed causal agent of the disease, it was necessary to distinguish between the symptoms caused by mite feeding and those induced by the causal agent. Presently, the only method of transmitting the assumed agent is to expose healthy plants to the brown wheat mite. To date, a population of mites free of the assumed causal agent has not been available for parallel transmission studies. However, plants and/or leaves which did not develop virus-like symptoms, but were infested with mites, were tentatively used to distinguish symptom differences.

The literature on the feeding damage of wheat and barley plants caused by Petrobia latens was generally in agreement (9,22,33,50,87). All active stages of the mites feed on the abaxial and adaxial surfaces of the leaf, sheath, and spikes. Their piercing-sucking mouthparts consist of two acerate stylets which produce deep slit-like punctures through the tissue and suck sap from the cells. It is not clear if the stylets of P. latens penetrate through or between the epidermal and mesophyll layers of cells. The first visual effect of mite feeding is a stippled appearance on the infested section of a leaf, which may turn a pale yellow to bronze color if heavily infested. The leaf then may

Table 1. Results of two electron microscopic assays used to determine the presence of unique VLPs in leaves of barley seedlings infested with Petrobia latens.

| Seedling no. | Leaf no. | Appearance of leaf[a] | Occurrence or non-occurrence of VLPs in | |
|---|---|---|---|---|
| | | | Ultrathin sections[b] | Leaf-dips[c] |
| 1 | 1 | - | - | - |
| | 3 | + | + | + |
| 2 | 1 | - | - | - |
| | 3 | - | - | - |
| 3 | 1 | - | - | - |
| | 3 | + | + | + |
| 4 | 1 | - | - | - |
| | 3 | + | + | + |
| 5 | 3 | + | + | + |
| 6 | 3 | - | - | - |
| 7 | 3 | + | + | + |
| 8 | 3 | - | - | - |
| 9 | 3 | + | + | + |
| 10 | 3 | - | - | - |
| 12 (Healthy) | 1 | - | - | - |
| | 3 | - | - | - |

[a] (+) = leaf having virus-like symptoms; (-) = leaf having no virus-like symptoms but mite feeding marks (except on control leaves).

[b] (+) = presence and (-) = absence of the virus-like particles (VLPs) detected in ultrathin sections with the electron microscope.

[c] (+) = presence and (-) = absence of the virus-like particles (VLPs) detected on grids previously exposed to sap of ground leaves.

dry from the tip backwards. The mites do not seem to directly damage the roots, but if they are persistent in large numbers, then stunting and a reduction of tillering may occur. The plants may also appear wilted, even if sufficient water is available. Only two published articles, specifically on P. latens infestations of barley in India (82,83), state that damage first occurs on the lower leaves of seedlings and then "travels up as whitish minute spots."

The symptoms induced by P. latens described in published articles were comparable to some of the symptoms that were observed in the laboratory and/or greenhouse on mite infested seedlings. The first leaves of barley plants that were exposed to mites usually had trails of silver to whitish dots; if heavily infested, the leaf developed a silvery overcast. Occasionally, the first true leaf developd a light green to brownish band parallel to the veins. However, if the leaves had just emerged from the seed and were subsequently exposed to large numbers of mites, the leaf would become pale yellow and turn necrotic from the tip downward. Several seedlings were in fact destroyed by such mite infestation at the emergent-leaf stage. The next one to two leaves that emerged were similarly affected until the mite numbers dropped, at which time the plant showed some recovery. The mites were usually distributed randomly on the sheath, ligule, and more often on the leaf surfaces. Mites would also aggregate on sections of leaves that had been physically damaged by human intervention, or on leaves that were parallel to and near the soil surface. Often mites fed on the leaf margins, resulting in a whitish-yellow outline, or the leaf tip frequently had relatively high numbers of mites centered on it.

However, within six days of initial mite exposure, the seedlings were usually at the 3-leaf stage and the first noticeable symptoms of light green to yellow dashes and streaks were usually displayed on the second and/or third leaves. The dashes and streaks later turned necrotic or coalesced into necrotic bands only on those leaves that had been infested with mites.

After infested plants were fumigated, transplanted, and subsequently allowed to grow and tiller under greenhouse conditions, the newly emerging leaves displayed virus-like symptoms. These leaves had a light green mottled discoloration, and then developed light green dashes, streaks, and stripes which turned yellow with time. Areas of leaf necrosis and banding rarely developed on those leaves that had never been exposed to the mites. Plants that had been adversely affected and appeared sickly during mite exposure produced new vigorously growing leaves with streaks and stripes.

A few of the diseased plants were smaller in height and had fewer tillers than the healthy control plants; the stunted plants did not require much water. Therefore, the only striking difference between the healthy controls and those plants subjected to mite feeding was the yellow streaks and stripes of the diseased plants.

Thus, it appears that these diseased plants were, in fact, affected with something other than the mechanical injury of mite feeding and that the mites were transmitting a substance to healthy seedlings which was further translocated to the growing point of the plant.

It is possible that _P. latens_ could have a substance in its saliva which is injected into plant cells before or during feeding. Spider mites have well-developed salivary glands (62) containing salivary secretions of mucous material, glycoproteins, and liquid material. _Eotetranychus uncatus_ Garman, another spider mite, is suspected of injecting a toxic material through its saliva into _Bauhinia variegata_ L., causing burning of the leaves (53). The toxic effect of saliva injected into almond by _Tetranychus pacificus_ results in defoliation (2). Similarly, infestations of _P. latens_ on wheat and barley resulted in scorched leaves and the defoliation of lower leaves. However, leaves do not usually become scorched or contain necrotic streaks after the mite population is reduced or removed from the plants. Therefore, it appears that scorched leaves and/or necrotic tissue on the barley plants may be directly attributed to the feeding damage of _P. latens_. It is not known if this is due to mechanical damage or a toxin. _Petrobia latens_, as such, is not known to inject toxic substances into a plant or induce a plant to produce toxic material. Chemical analysis of saliva and isolation of a toxic substance and injection of the substance into a healthy plant must be accomplished to confirm the presence of a toxin or toxic inducing substance.

Nevertheless, diseased barley plants previously exposed to _P. latens_ continued to produce symptomatic new leaves. Though the symptoms were less severe due to less necrotic tissue, the light green to yellow dashes, streaks, and stripes persisted throughout the life of the plant. The degree of severity varied from leaf to leaf and from

plant to plant, and usually the symptom severity was less in older plants and in cooler temperatures.

Thus, it is possible that P. latens transmits the VLPs through its saliva into the plant cells prior to feeding or as it is feeding. Salivary glands in some of the efficient vectors of plant viruses are known to contain specific sites for viral attachment and replication (1,17,89). These particles are then released into the saliva and into the plant tissue as the vector feeds. Therefore, ultrastructural studies of P. latens should be further pursued to establish if, in fact, the mite acts as another host for the VLPs.

Possible plant species acting as non-host for the assumed causal agent, but as a good plant host for the mites, were tentatively shown to be carrots and onion cultivars. The mite population was shown to increase substantially on these two introduced plant species. Mite feeding damage resulting in scorched and dried leaves on the onion and carrot seedlings was evident. However, no virus-like symptoms were ever displayed on these plants, and no VLPs were detected in the leaf tissue. Only 36% of the barley seedlings that were subsequently exposed to the mites that had been reared on the onion and carrot plants displayed diagnostic symptoms. Therefore, carrot and onion plants appeared to be nonhosts for the disease, but hosts for the mite.

Brown Wheat Mite, a Probable Vector? The literature concerning the brown wheat mite, Petrobia latens, is scant, with the species first described in 1776 in Denmark (63). It is now nearly worldwide in distribution and has been reported in Europe, Africa, North America, Australia, India, Eqypt, Japan, and China. Petrobia latens is in the

class Arachnida; the subclass Acari; the order Acariformes; the sub-order Actinedida; the family Tetranychidae; the subfamily Bryobiinae; and the tribe Petrobiini.

Studies on the biology of P. latens under field and/or laboratory conditions have been limited and incomplete (15,18,20,33,50). All the mites are females and reproduce parthenogenetically, with one male mite found in China (55). They have eight distinct stages of growth and maturation, which include the egg, larva, two sequential nymphal stages, and the adult; quiescent (chrysalis) stages occur after the larval and each of the nymphal stages. It may take as little as 7 days or as long as 20 days to become an adult from the larval stage, depending on the environment. The adult mite may live up to a month before it becomes moribund and dies. The mite produces two types of eggs. A red, spherical active egg hatches in about 10 days or will become nonviable. In contrast, a white diapausal egg must have at least two months of dormancy before it may hatch, and then only if moisture is present and the temperature is relatively warm. An adult mite only lays one type of egg, either about 30 diapausal eggs or 90 active eggs. Both kinds of eggs are found on solid substrates such as rocks, soil clods, and small sticks, rather than on host plants. The majority of the active eggs are laid and hatch early in the crop growing season, which may result in high populations of mite infestations on seedlings. Once the crop has matured, the mite numbers drop because the white diapausal eggs are then predominantly produced.

The mites are said to have a preference for monocotyledonous plants, and will infest dicotyledonous plants only as a secondary host

(71,93). Their method for dispersal is not clear. They do run rapidly or jump off a plant if disturbed. It has been observed that when they leave a host plant to lay eggs, they may not return to the same plant (46). Some reports suggest that the mites move in air currents, but this appears to be speculation and has not been documented.

The occurrence of brown wheat mite epidemics appears to be the result of recurring drought conditions. Ideally, snow covered ground and/or spring rains (northern hemisphere) provide enough moisture to hatch the diapausal overwintering eggs. Subsequent warm, dry environmental conditions and a source of plant food enhance mite development and increase the population of the mites to high levels.

During the 1950's and 1960's, P. latens was found to have infested wheat and barley crops in drought stricken regions of the western United States. Indeed, the brown wheat mite was first reported in Montana in the fall of 1960 on winter wheat seedlings in Cascade County (77). The following year it was reported to have occurred in wheat fields in the area bounded by Chinook, Lewistown, Great Falls, and Cut Bank. The triangle area again contained infested wheat fields in 1964. Recent infestations had not been observed in Montana until 1985, and then in wheat fields in the northeastern region, and in wheat and barley fields of the north central area of the state.

Actual yield reductions directly attributed to the brown wheat mite in wheat and barley fields were difficult to determine in previous studies in the United States (22,23) and in India (83) due to the accompanying drought conditions.

Although brown wheat mite infestations appeared sporadically, they have never been associated with a virus-like disease. Other species of mites in the Tetranychidae have been implicated in the transmission of plant viruses (64). Wallace and Sinha (94) reported *Tetranychus* sp. infestations on barley, rye, and wheat in Manitoba during the summer of 1961; it was suggested that the mite was transmitting a virus to wheat plants, and that the symptoms occurring on the barley and rye were due to the toxic effect of mite feeding. However, further reports on this research could not be found by the author.

Schulz (80) reported that potato virus Y (PVY) was transmitted by *Tetranychus urtica* (L.) to potatoes, but these results were not repeatable in other research facilities (32,65). Similarly, tobacco ringspot virus was reported to be transmitted by *Tetranychus* sp. under laboratory conditions, but later other researchers (36,66) were unable to repeat this.

Based upon the above contradictions, it is not surprising to find skepticism concerning a new report of a spider mite transmitting a virus. However, in the same suborder, the Eryophyidae mites are very efficient vectors of plant viruses (85,86). More recently, *Brevipalpus phoenicis* (Geijskes), a false spider mite belonging to the Tenuipalpidae family and the same suborder as the spider mites, has been found to transmit coffee ringspot virus (11,13). Interestingly, this virus is thought to be a rhabdovirus and, therefore, somewhat similar to the VLPs found in the diseased barley tissue (12).

Control of the brown wheat mite in crops may involve a combination of cultural management and chemical application. Late sowing, deep

ploughing, and crop rotation give an effective control for the mite population in China (15). In South Africa (87), miticides are applied when 30 or more mites per plant are found, the plants are actively growing and are not dry, patches of yellow to brown areas of wheat plants are noticeable, and no rain is predicted. The miticide is reapplied in two to three weeks if the mite numbers persist. However, since a pathogenic transmitted disease has never been reported or associated with the brown wheat mite, it was not essential for producers to control the initial early appearance of the mites occurring at low levels.

# DISCUSSION AND CONCLUSIONS

The stated objectives pertaining to the virus-like disease of barley have, in part, been met. The assumed causal agent and its vector have been determined on the basis of transmission experiments resulting in diseased plants and the presence of the associated virus-like particles (VLPs) in the tissue. The disease and its geographical distribution, prevalence, and natural host range have been examined.

The geographical distribution of the disease has been centered in North Central Montana and in nearby areas of Canada. It has increased both in incidence within fields and to other fields and regions. It is not clear if the disease was in the area before 1982 with negligible incidences or if it is a new disease spreading in a southerly and northerly direction from the original site of identification.

To date, the natural host range includes barley, wheat, and three annual grasses. However, this appears to be a disease of barley, and not wheat. All barley cultivars examined in the field were susceptible to the disease, including the spring barley cultivars sown by growers and the winter barley cultivars used as trap plants. Wheat is affected, and only in low incidences, if it is adjacent to diseased barley fields and/or the field had previously contained diseased barley.

The causal agent of the disease spreads from plant to plant in association with high numbers of mites in diseased barley during the

early part of the growing season. Laboratory and greenhouse experiments confirmed the transmission of the causal agent to healthy plants by the brown wheat mite, *Petrobia latens*. The vernacular name of the mite implies that the mite is a pest of wheat. This is true, and, in fact, brown wheat mite epidemics on wheat plants are emphasized in the literature. Only two reports of brown wheat mite infestations solely on barley have been published (82,83). Such studies occurred in India and include the only report on yield reduction in barley caused by the mites. Two reports from the United States have incidentally involved barley infested fields (18,52). Interestingly, Cox reports that the number of mites found in barley fields was at least three times that of comparable wheat fields. The majority of the other reports on brown wheat mite infestations, in general, state that the numbers are highest in wheat fields.

Thus, it is not apparent from the literature if the mite has a preference for wheat or barley. It is possible that biotypes within the mite species exist, requiring different plant hosts. In addition, the VLPs may have a definite preference for barley plants.

The sudden appearance of the mites and the disease may be explained by favorable environmental conditions. The spring weather patterns in North Central Montana had been highly conducive for mite development and expansion from 1983 through 1986. Snow cover and April showers provided moisture and, thus, stimulated the diapausal eggs to hatch and infect the emerging barley seedlings. The ensuing drought conditions experienced during the months of May and June most likely favored increased mite populations. Moreover, based upon laboratory

transmission tests, it appears that the diapausal eggs carry the causal agent and, therefore, may act as the overwintering host in the epidemiology of the disease. This is further substantiated by the relatively early onset of symptomatic plants distributed over entire fields by June.

Recrop barley fields were usually found to have a higher number of mites and greater incidence of disease relative to fields that had previously been in wheat or summer-fallow. Also, the incidence of disease was less in fields sown in March and the first part of April.

Field observations and laboratory studies have indicated that the mites survive best under dry conditions, and the host symptoms appear to be best expressed under high light intensities and warm temperatures.

The causal agent has been tentatively identified as a virus-like particle. Indirect evidence is based upon the association and presence of structures of definite morphology in diseased tissue, and never in healthy tissue. The submicroscopic size of the structures was found to be similar to virus particles, and, more specifically, the overall shape and morphology of the structures resembled the plant rhabdoviruses. Unlike the rhabdoviruses, the VLPs consisted of variable length which reached lengths up to an extraordinary 4,000 nm _in situ_. The apparent particle maturation appeared to take place in the cytoplasm and within cisternae of the endoplasmic reticulum. Similar maturation events occur with some of the rhabdoviruses and tomato spotted wilt viruses. Purification and subsequent physicochemical

analysis of the VLPs will give conclusive evidence as to the true nature of the VLPs found in diseased barley tissue.

This new disease of barley is a disease-complex in which the plant host (barley), the assumed causal agent (virus-like particles), and the vector (*P. latens*) all must interact with each other in specific ways under environmental conditions conducive for producing diseased plants at epidemic levels. Moreover, the morphological uniqueness of these VLPs, combined with the first time association of *P. latens* vectoring a plant pathogen, truly introduces a new plant disease. However, further mite transmission tests and physicochemical tests of the VLPs must be conducted to approach fulfilling Koch's postulates and thereby establish the etiology of this barley disease.

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# APPENDIX

Table 2. Survey information obtained from barley producers in 1985 concerning the new virus-like disease in fields of irrigated and nonirrigated barley.

| Location | Seeding date | Barley cultivar | Hectares | Disease incidence (% diseased) | Hl/Ha |
|---|---|---|---|---|---|
| Sunburst | | | | | |
| Nonirrigated | 15 April | Moravian III | 9 | 0.1 | 11.0 |
| | 15 April | Moravian III | 10 | 0.1 | 31.5 |
| | 15 April | Moravian III | 8 | 0.1 | 27.0 |
| | 22 April | Horsford | 62 | 30 | 13.5 |
| | 28 April | Horsford | 43 | 60 | 13.5 |
| Irrigated | 8-10 April | Klages | not given | 1 | 25.0 |
| Valier | | | | | |
| Irrigated | 27 April | Klages | 8 | 100 | 50 |
| | 17 April | Clark | 16 | >50 | 45 |
| | 17 April | Clark | 14 | >50 | 54 |
| | 27 April | Klages | 14 | >75 | 45 |
| | 27 April | Klages | 10 | >75 | 54 |
| | 27 April | Klages | 53 | 100 | 60 |
| Conrad | | | | | |
| Nonirrigated | 15 April | Klages | 8 | 30 | 14 |
| | 22 April | Piroline | 14 | >90 | 11 |
| Irrigated | 15 April | Piroline | 61 | 5 | 60 |
| | 25 April | Klages | 23 | >90 | 32 |

Table 2. Continued.

| Location | Seeding date | Barley cultivar | Hectares | Disease incidence (% diseased) | Hl/Ha |
|---|---|---|---|---|---|
| Choteau | | | | | |
| Irrigated | 16 April | Klages | 40 | 5(70)[b] | 68 |
| | 16 April | Klages | 20 | 5(70)[c] | 68 |

[a] All of the fields were surveyed at least once by the author, except the Choteau fields were not checked; instead, the information was based solely on testimonials of the producer and extension personnel.

[b] The diseased plants found in the 40 ha field were mainly confined to a circular 2 ha area, which had an estimated 70% incidence of infection; the remaining plants in the field had about 5% incidence.

[c] The diseased plants found in the 20 ha field were mainly confined to a 1.7 ha patch with 70% incidence of infection; the remaining plants in the field had about 5% incidence.

Table 3. Buffers, inocula, and indicator test plants used for mechanical transmission tests.

| Buffer | Source[a] of leaf inoculum (barley) | Inoculum preparation % leaf sap $\frac{\text{Leaf tissue (g)}}{\text{Buffer (ml)}} \times 100\%$ | Indicator test species | No. seedlings[b] inoculated with diseased tissue | No. seedlings[c] inoculated with healthy tissue |
|---|---|---|---|---|---|
| 1. 0.3 M glycine ($NH_2CH_2COOH$) + 0.1 M magnesium chloride ($MgCl_2$), pH 6.98 | Morex | 2 | Morex (barley) | 7 | 7 |
| | Morex | 4 | Morex | 11 | 9 |
| | Morex | 8 | Morex | 9 | 9 |
| | Morex | 10 | Morex | 8 | 10 |
| | Morex | 14 | Morex | 10 | 9 |
| | Morex | 16 | Morex | 10 | 9 |
| | Morex | 20 | Morex | 11 | 8 |
| | Morex | 30 | Morex | 10 | 9 |
| 2. 0.1 M glycine ($NH_2CH_2COOH$) + 0.1% 2-mercapto-ethanol, pH 8.0 | Ellis | 50 | Ellis (barley) | 103 | 53 |
| | Ellis | 20 | Ellis | 175 | 51 |
| 3. 0.04 M sodium sulfite ($Na_2SO_3$), pH 9.37 | Ellis | 50 | Ellis | 51 | 22 |
| | Ellis | 25 | Ellis | 48 | 14 |
| | Ellis | 17 | Ellis | 46 | 17 |
| 4. 0.04 M sodium sulfite + 0.2% Celite 545 (diatomaceous earth), pH 9.0 | Ellis | 50 | Ellis | 12 | 13 |

Table 3. Continued.

| Buffer | Source[a] of leaf inoculum (barley) | Inoculum preparation % leaf sap $\frac{\text{Leaf tissue (g)}}{\text{Buffer (ml)}} \times 100\%$ | Indicator test species | No. seedlings[b] inoculated with diseased tissue | No. seedlings[c] inoculated with healthy tissue |
|---|---|---|---|---|---|
| 5. 0.05 M phosphate ($NaK_2HPO_4$) + 0.1% 2-mercapto-ethanol, pH 7.2 | Klages | 20 | Klages (barley) | 61 | 52 |
| | Clark | 20 | Clark (barley) | 44 | 35 |
| | Klages[d] | 20 | Klages | 122 | 99 |
| | Clark[d] | 20 | Clark | 109 | 126 |
| 6. 0.05 M Tris-HCl ($(HOCH_2)_3CNH_2$) + HCl), pH 7.2 | Morex | 20 | Morex | 6 | 6 |
| | Morex | 20 | Michigan amber (wheat) | 4 | 6 |
| | Morex | 20 | TA-I (_Triticum_ X _Agropyron_) | 13 | 5 |
| | Morex | 20 | Coast Black Oat | 14 | 6 |
| | Morex | 20 | Perennial Ryegrass | 24 | 24 |
| 7. 0.05 M Tris-HCl + 0.01 M $MgCl_2$, pH 7.2 | Alpine | 20 | Alpine (barley) | 41 | 22 |
| | Alpine | 10 | Alpine | 44 | 14 |
| 8. Distilled water ($H_2O$), pH 7.2 | Morex | 20 | Morex | 36 | 8 |
| 9. 0.2 M Sodium Citrate[e] ($(HOC(COONa)CH_2COONa)_2 \cdot 2H_2O$) + 10% sucrose ($C_{12}H_{22}O_{11}$), pH 7.55 + 545 Celite pad | Alpine | 25 | Alpine | 107 | 86 |
| | Alpine | 12.5 | Alpine | 59 | 34 |
| | Alpine | ?[f] | Alpine | 52 | 7 |

Table 3. Continued.

[a] Fresh leaf tissue was ground with cold buffer in mortar and pestle.

[b] Number of indicator test seedlings dusted with carborundum and inoculated with diseased leaf suspension by a cotton swab.

[c] Number of indicator test seedlings dusted with carborundum and inoculated with healthy test suspension by a cotton swab.

[d] Fresh leaf tissue was initially ground in liquid nitrogen before further grinding in buffer.

[e] The buffer, sucrose and tissue were homogenized at a low speed, centrifuged at 5,000-*g*, before filtering through a Celite pad.

[f] Six ml of inoculum were removed from a 10-40% sucrose density gradient (0.2 M Na Citrate) after it was subjected to 22,000-*g* for 25 minutes. The inoculum was at an estimated 25% sucrose density zone.

Table 4. Results of greenhouse seed transmission tests with seed collected in 1983 from plants in a field that had the new virus-like disease near Conrad, Montana.

| Cultivar | No. seed[a] parent plants | No. progeny[b] plants | No. control[c] plants | No. progeny[d] plants with symptoms | No. progeny[e] leaf samples | No. leaf[f] samples with VLPs |
|---|---|---|---|---|---|---|
| Norstar (wheat) | 72 | 13,576 | 94 | 0 | 11/3 | 0 |
| Fortuna (wheat) | 98 | 7,914 | 156 | 0 | 15/3 | 0 |
| Morex (barley) | 98 | 19,021 | 312 | 0 | 28/4 | 0 |

[a] The number of field plants having symptoms of the new virus-like disease that were used as seed sources in greenhouse seed transmission tests. Ten field plants of each cultivar were sampled for positive diagnosis of the disease.

[b] The number of greenhouse plants produced from seed of diseased field plants.

[c] The number of control plants produced from certified seed in the greenhouse.

[d] The number of greenhouse plants produced from seed of diseased parents displaying symptoms.

[e] The numerator is the number of leaf samples taken randomly from greenhouse plants produced from the seed of field diseased plants; the denominator is the number of control leaf samples taken randomly from greenhouse plants produced by certified seed. These leaf samples were processed for ultrathin sectioning.

[f] The number of greenhouse leaf samples with virus-like particles in ultrathin sections.

Table 5. The response of healthy trap plants exposed to a field of diseased Lew spring wheat plants on the Conrad site in 1985.

| | | Exposure period | | | |
|---|---|---|---|---|---|
| | | 19 May - 1 June[b] | | 1 June - 18 June[c] | |
| Location[a] Cluster | Pot no. | Cultivar[d] | No. plants affected[e] / Total no. of plants | Cultivar | No. plants affected / Total no. of plants |
| 1 | 1 | Kearney[f+] | 17/17 | Clark | 5/5 |
| | 2 | Wong[f+] | 2/2 | Alpine | 8/8 |
| | 3 | Dicktoo[f+] | 7/7 | Ellis | 2/3 |
| | 4 | Kearney[f+] | 1/12 | Alpine | 4/4 |
| 2 | 5 | Morex[f+] | 28/28 | Clark | 6/9 |
| | 6 | Dicktoo[f+] | 5/10 | Dicktoo | 2/7 |
| | 7 | Morex[f-] | 0/31 | Clark | 3/8 |
| | 8 | Morex | 0/11 | Ellis | 3/8 |
| | 9 | Kearney[f-] | 0/12 | Kearney | 11/11 |
| | 9a | -- | -- | Alpine | 12/12 |
| | 10 | Minter (wheat) | 0/20 | Norstar (wheat) | 0/8 |
| 3 | 11 | Fortuna (wheat) | 9/24 | Norstar (wheat) | 0/11 |
| | 12 | Clark[f+] | 1/23 | Clark | 2/10 |
| | 13 | Kearney | 0/9 | Kearney | 0/7 |
| | 14 | Minter (wheat) | 0/25 | Alpine | 2/6 |

Table 5. Continued

| Location[a] Cluster | Pot no. | Exposure period: 19 May - 1 June[b] Cultivar[d] | No. plants affected[e] / Total no. of plants | Exposure period: 1 June - 18 June[c] Cultivar | No. plants affected / Total no. of plants |
|---|---|---|---|---|---|
| 3 | 15 | Dicktoo | 0/19 | Dicktoo | 8/8 |
| | 16 | Minter (wheat) | 0/20 | Reno | 0/0 |
| 4 | 17 | Dicktoo | 0/7 | Dicktoo | 0/6 |
| | 18 | Kearney | 0/34 | Kearney | 0/6 |
| | 19 | Morex | 0/65 | Morex | 0/5 |
| | 20 | Morex | 0/16 | Clark | 0/6 |
| 5 | 21 | Dicktoo | 0/21 | Dicktoo | 2/10 |
| | 22 | Morex | 0/29 | Morex | 1/5 |
| | 23 | Fortuna (wheat) | 0/26 | Morex | 2/10 |
| | 24 | Kearney | 0/14 | Kearney | 0/6 |
| | 25 | Clark | 0/17 | Clark | 0/1 |
| | 26 | Minter (wheat) | 0/18 | Norstar (wheat) | 0/9 |
| 6 | 27 | Morex | 0/22 | Ellis | 0/8 |
| | 28 | Morex | 0/30 | Morex | 0/11 |
| | 29 | Morex[f-] | 0/20 | Tenn. Wt. | 0/8 |
| | 30 | Morex[f-] | 0/16 | Morex[g] | 0/20 |

Table 5. Continued

| Location[a] Cluster | Pot no. | Exposure period: 19 May - 1 June[b] | | Exposure period: 1 June - 18 June[c] | |
|---|---|---|---|---|---|
| | | Cultivar[d] | No. plants affected[e] / Total no. of plants | Cultivar | No. plants affected / Total no. of plants |
| 6 | 31 | Morex | 0/23 | Morex | 2/15 |
| | 32 | Morex | 0/16 | Morex | 0/9 |
| | 33 | Morex | 0/15 | Morex | 0/11 |
| | 34 | Morex[f-] | 0/22 | Morex[g] | 0/15 |
| | 35 | Wong | 0/7 | Reno | 0/2 |
| | 36 | Tenn. Winter | 0/50 | Tenn. Wt. | 0/6 |
| | 37 | Morex | 0/20 | Morex | 0/15 |
| | 38 | Morex | 0/26 | Tenn. Wt. | 0/9 |
| | 39 | Morex | 0/22 | Morex[g] | 0/20 |
| | 40 | Morex | 0/32 | Morex[g] | 0/16 |
| 7 | 41 | Tenn. Winter | 0/38 | Tenn. Wt. | 0/10 |
| | 42 | Kearney | 0/41 | Kearney | 0/7 |
| | 43 | Clark[f+] | 1/21 | Clark | 5/7 |
| | 44 | Minter (wheat) | 0/24 | Norstar (wheat) | 0/13 |
| | 45 | Morex | 0/19 | Morex | 1/8 |
| | 46 | Clark | 0/22 | Clark | 6/7 |

Table 5. Continued

| Location[a] Cluster | Pot no. | Exposure period: 19 May - 1 June[b] Cultivar[d] | No. plants affected[e] / Total no. of plants | Exposure period: 1 June - 18 June[c] Cultivar | No. plants affected / Total no. of plants |
|---|---|---|---|---|---|
| 8 | 47 | Dicktoo | 0/26 | Dicktoo | 0/8 |
| | 48 | Kearney | 0/7 | Alpine | 0/4 |
| | 49 | Dicktoo | 0/18 | Morex | 0/15 |
| | 50 | Clark | 0/23 | Tenn. Wt. | 1/4 |
| | 51 | Morex | 0/20 | Clark | 0/8 |
| | 52 | Dicktoo | 0/18 | Morex[g] | 0/10 |
| | 53 | Kearney | 0/10 | Reno | 0/3 |
| | 54 | Kearney | 0/15 | Morex | 2/11 |
| | 55 | Kearney | 0/8 | Norstar (wheat) | 0/10 |
| | 56 | Kearney | 0/13 | Reno | 0/10 |
| | 57 | Clark | 0/20 | Morex | 0/10 |
| Totals | | Barley and wheat | 62/1,181 | Barley and wheat | 90/489 |
| | | Barley | 62/1,024 | Barley | 90/438 |
| | | Wheat | 0/157 | Wheat | 0/51 |

Table 5. Continued

[a] Individual pots were randomly distributed on an estimated 35 meters (north-south) x 10.5 meters (east-west) area near the edge of a field sown into Lew spring wheat; the pots were in eight distinct clusters and each pot of seedlings was given a number according to its position.

[b] Fifty-seven pots were placed on the site from 19 May to 1 June and then returned to the greenhouse for observation.

[c] The pots that were removed from the site on 1 June were replaced with 58 pots of seedlings; these seedlings were then moved back to the greenhouse 18 June for symptom observation.

[d] All of the trap seedlings were barley cultivars except where indicated as being wheat; underlined cultivars became affected.

[e] The number of plants affected per pot is equal to the numerator, and the total number of plants per pot is equal to the denominator.

$f^{(+) \text{ or } (-)}$ Ultrathin sections were processed from leaf samples and viewed under the electron microscope for the unique virus-like particles (VLPs); $f^+$ equals presence of VLPs and $f^-$ equals no VLPs.

[g] The Morex trap seedlings were treated with aldicarb (Temik) in the greenhouse before being placed on the field site.

Table 6. The response of healthy trap plants exposed to diseased barley plants in three fields on the Valier site from 17 June to 9 July 1985.

| Diseased field[a] | Trap Plant Cultivar[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Alpine | Clark | Dicktoo | Ellis | Kearney | Klages | Morex | Reno | Tenn.Wt. | Norstar |
| Klages-A | 9/9[c] | | 6/6 | 9/9 | 3/3 | 0/15[e] | 0/10[e] | 2/2 | 6/6 | 2/7 |
| (flood irrigated) | 8/9 | | 6/6 | 11/11 | 8/8 | 0/9[d] | 0/9[e] | 1/1 | 5/6 | 1/9 |
| | 11/11 | | | 7/9 | 6/6 | 0/10[e] | 0/10[e] | 2/2 | | 0/10 |
| | | | | | 6/6 | 0/13[e] | 0/10[e] | | | |
| | | | | | | 1/10[e] | 0/9[e] | | | |
| | | | | | | 2/11 | 0/10[d] | | | |
| | | | | | | 12/14 | 0/10[d] | | | |
| | | | | | | 0/1 | 1/10[d] | | | |
| | | | | | | 4/12 | 0/10[d] | | | |
| | | | | | | 1/13 | 0/10[d] | | | |
| | | | | | | | 0/10 | | | |
| | | | | | | | 0/9 | | | |
| | | | | | | | 1/10 | | | |
| | | | | | | | 6/7 | | | |
| | | | | | | | 10/10 | | | |

Table 6. Continued.

| Diseased field[a] | Trap Plant Cultivar[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Alpine | Clark | Dicktoo | Ellis | Kearney | Klages | Morex | Reno | Tenn.Wt. | Norstar |
| Clark-B | 2/2 | 2/2[e] | | 3/3 | 3/3 | | | | 8/8 | 3/6 |
| (pivot-irrigated) | | 2/2[e] | | | 2/3 | | | | | |
| Klages-C | 7/7 | 6/6 | 5/5 | | 7/7 | | | 4/4 | 7/7 | 2/9 |
| (pivot-irrigated) | | | 4/4 | | 5/5 | | | 1/1 | | 1/7 |
| | | | | | 5/5 | | | | | 0/6 |
| | | | | | 4/5 | | | | | 0/11 |
| | | | | | | | | | | 0/5 |
| Total 230/511 | 37/38 | 10/10 | 21/21 | 30/32 | 49/51 | 20/108 | 18/144 | 10/10 | 26/27 | 9/70 |

[a] The potted indicator seedlings were randomly placed near the southern boundary of each of the three adjacent diseased barley fields.

[b] The cultivars used for the trap plants were all barley unless denoted as wheat.

[c] The numerator is equal to the number of diseased trap seedlings per pot and the denominator is equal to the total number of trap seedlings per pot.

[d] The seedlings were enclosed with a cloth cage when they were exposed to the field.

[e] The seedlings were treated with aldicarb (Temik) at the 2-leaf stage before they were exposed to the field.

Table 7. The response of healthy trap plants exposed to diseased Lew spring wheat plants in a field on the Conrad site in 1986.

| Cluster[b] | No.[c] | Cultivar[d] | Exposure period: 23 May - 18 June[a] (Four weeks) No. plants affected[e] / Total no. plants | Exposure period: 18 June-16 July (Four weeks) No. plants affected / Total no. plants | Exposure period: 16 July-31 July (Two weeks) No. plants affected / Total no. plants | Total |
|---|---|---|---|---|---|---|
| 1 | 1 | Ellis | 0/3 | 0/4 | 0/3 | 0/10 |
| | 2 | Dicktoo | 0/4 | 0/3 | 0/6 | 0/13 |
| | 3 | Norstar (wheat) | 0/1 | 0/13 | 0/10 | 0/24 |
| | 4 | Kearney | 1/2 +[f] | 0/13 | 0/4 | 1/9 |
| 2 | 5 | Kearney | 0/5 | 0/3 | 0/7 | 0/15 |
| | 6 | Ellis | 0/0 | 0/9 | 0/9 | 0/18 |
| | 7 | Norstar (wheat) | 0/0 | 0/13 | 0/12 | 0/25 |
| | 8 | Dicktoo | 0/2 | 4/6 + | 0/15 | 4/13 |
| 3 | 9 | Dicktoo | 0/4 | 0/2 | 0/10 | 0/16 |
| | 10 | Ellis | 0/5 | 0/2 | 0/11 | 0/18 |
| | 11 | Kearney | 0/3 | 0/3 | 0/5 | 0/12 |
| | 12 | Norstar (wheat) | 0/2 | 0/15 | 0/12 | 0/29 |
| 4 | 13 | Kearney | 2/6 + | 0/2 | 0/7 | 2/15 |
| | 14 | Dicktoo | 5/5 + | 2/5 + | 0/8 | 7/18 |
| | 15 | Norstar (wheat) | 0/3 | 0/12 | 0/13 | 0/28 |
| | 16 | Ellis | 6/7 + | 3/16 + | 0/8 | 9/31 |
| 5 | 17 | Norstar (wheat) | 0/0 | 0/14 | 0/11 | 0/25 |
| | 18 | Ellis | 0/5 | 0/7 | 0/8 | 0/20 |
| | 19 | Kearney | 0/7 | 0/5 | 0/12 | 0/24 |
| | 20 | Dicktoo | 0/4 | 0/3 | 0/5 | 0/12 |

Table 7. Continued.

| Cluster[b] | No.[c] | Cultivar[d] | Exposure period: 23 May - 18 June[a] (Four weeks) No. plants affected[e] / Total no. plants | Exposure period: 18 June-16 July (Four weeks) No. plants affected / Total no. plants | Exposure period: 16 July-31 July (Two weeks) No. plants affected / Total no. plants | Total |
|---|---|---|---|---|---|---|
| Total | | | 14/68 | 9/141 | 0/166 | 23/375 |
| Percent barley affected | | | 23% | 6% | 0% | |
| Percent wheat affected | | | 0% | 0% | 0% | |

[a] Fresh trap seedlings were cycled into and out of the wheat field on the dates depicted; the first two cycles of trap plants were exposed to the field for four weeks, and the last cycle was exposed for two weeks before returning to the greenhouse.

[b] The pots of trap seedlings were placed in clusters of four pots, each pot placed at the corner of a square, with about 60 cm between ports.

[c] The pots of trap seedlings were placed in a hole so that the rim of the pot was even with the soil surface of the field; each hole which trap seedlings were cycled into and out of was given a specific number (No.).

[d] All of the trap seedlings were barly cultivars except those of Norstar winter wheat; a designated hole number was always replaced with trap plants of the same cultivar.

[e] The numerator equals the total number of diseased plants and the denominator equals the total number of trap plants within a pot.

[f] The plus (+) sign designates pots of diseased seedlings.

Table 8. The response of healthy trap plants exposed to a summer-fallow field on the Conrad site in 1986.

| Cluster[b] | No.[c] | Cultivar[d] | Exposure period: 23 May - 18 June[a] | 18 June-16 July | 16 July-31 July | Total |
|---|---|---|---|---|---|---|
| | | | No. plants affected[e] / Total no. plants | No. plants affected / Total no. plants | No. plants affected / Total no. plants | |
| 1 | 1 | Norstar (wheat) | 0/0 | 0/0 | | 0/0 |
| | 2 | Dicktoo | 0/0 | 0/0 | | 0/0 |
| | 3 | Ellis | 0/0 | 0/0 | | 0/0 |
| | 4 | Kearney | 0/3 | 0/0 | | 0/3 |
| 2 | 5 | Norstar | 0/0 | 0/0 | | 0/0 |
| | 6 | Dicktoo | 2/2 +[f] | 0/0 | | 2/2 |
| | 7 | Ellis | 8/8 + | 0/0 | | 8/8 |
| | 8 | Kearney | 7/7 + | 0/0 | | 7/7 |
| 3 | 9 | Norstar | 0/0 | 0/0 | | 0/0 |
| | 10 | Dicktoo | 0/2 | 0/0 | | 0/2 |
| | 11 | Ellis | 2/2 + | 0/0 | | 2/2 |
| | 12 | Kearney | 0/0 | 0/0 | | 0/0 |
| 4 | 13 | Norstar | 0/0 | 0/5 | 0/0 | 0/5 |
| | 14 | Dicktoo | 5/5 + | 0/1 | 0/0 | 5/6 |
| | 15 | Ellis | 2/4 + | 0/15 | 0/0 | 2/19 |
| | 16 | Kearney | 3/3 | 0/2 | 0/0 | 3/5 |
| 5 | 17 | Norstar | 0/0 | 0/13 | | 0/13 |
| | 18 | Dicktoo | 5/5 + | 0/0 | 0/0 | 5/5 |
| | 19 | Ellis | 7/7 + | 0/1 | 0/0 | 7/8 |
| | 20 | Kearney | 3/7 | 0/0 | 0/0 | 3/7 |

Table 8. Continued.

| Cluster[b] | No.[c] | Cultivar[d] | Exposure period: 23 May - 18 June[a] No. plants affected / Total no. plants[e] | Exposure period: 18 June-16 July No. plants affected / Total no. plants | Exposure period: 16 July-31 July No. plants affected / Total no. plants | Total |
|---|---|---|---|---|---|---|
| Total | | | 44/55 | 0/37 | 0/0 | 44/92 |
| Percent barley affected | | | 80% | 0% | 0% | |
| Percent wheat affected | | | 0% | 0% | 0% | |

[a] Fresh trap seedlings were cycled into and out of the wheat field on the dates depicted; the first two cycles of trap plants were exposed to the field for four weeks, and the last cycle was exposed for two weeks before returning to the greenhouse.

[b] The pots of trap seedlings were placed in clusters of four pots, each pot placed at the corner of a square, with about 60 cm between ports.

[c] The pots of trap seedlings were placed in a hole so that the rim of the pot was even with the soil surface of the field; each hole which trap seedlings were cycled into and out of was given a specific number (No.).

[d] All of the trap seedlings were barley cultivars except those of Norstar winter wheat; a designated hole number was always replaced with trap plants of the same cultivar.

[e] The numerator equals the total number of diseased plants and the denominator equals the total number of trap plants within a pot.

[f] The plus (+) sign designates pots of diseased seedlings.

Table 9. The response of healthy trap plants exposed to a field of diseased Piroline barley plants on the Conrad site in 1986.

| | | | Exposure period | | | |
|---|---|---|---|---|---|---|
| | | | 23 May - 18 June[a] | 18 June-16 July | 16 July-31 July | |
| Cluster[b] | No.[c] | Cultivar[d] | No. plants[e] affected / Total no. plants | No. plants affected / Total no. plants | No. plants affected / Total no. plants | Total |
| 1 | 1 | Norstar (wheat) | 0/1 | 0/10 | 0/0 | 0/11 |
| | 2 | Dicktoo | 4/4 +[f] | 0/1 | 0/2 | 4/7 |
| | 3 | Kearney | 7/7 | 0/8 | 0/0 | 7/15 |
| | 4 | Ellis | 4/6 | 0/2 | 0/5 | 4/13 |
| 2 | 5 | Norstar | 0/1 | 0/14 | 0/0 | 0/15 |
| | 6 | Ellis | 9/9 + | 0/1 | 0/0 | 9/10 |
| | 7 | Dicktoo | 7/7 + | 0/1 | 0/0 | 7/8 |
| | 8 | Kearney | 9/10 + | 0/4 | 0/0 | 9/14 |
| 3 | 9 | Norstar | 0/1 | 0/5 | 0/0 | 0/6 |
| | 10 | Dicktoo | 4/4 + | 0/7 | 0/0 | 4/11 |
| | 11 | Ellis | 0/0 | 0/0 | 0/0 | 0/0 |
| | 12 | Kearney | 6/6 + | 0/2 | 0/0 | 6/8 |
| 4 | 13 | Norstar | 0/1 | 0/11 | 0/7 | 0/19 |
| | 14 | Dicktoo | 8/8 + | 0/4 | 4/9 + | 12/21 |
| | 15 | Ellis | 8/8 + | 0/0 | 0/2 | 8/10 |
| | 16 | Kearney | 10/10 + | 1/5 + | 0/4 | 11/19 |
| 5 | 17 | Norstar | 0/3 | 0/3 | | 0/6 |
| | 18 | Dicktoo | 4/4 + | 0/1 | 0/0 | 4/5 |
| | 19 | Ellis | 7/7 + | 0/2 | 0/0 | 7/9 |
| | 20 | Kearney | 5/5 + | 1/6 + | 0/0 | 6/11 |
| | 21 | Minter (wheat) | 8/8 + | -- | -- | 8/8 |

Table 9. Continued.

| Cluster[b] | No.[c] | Cultivar[d] | Exposure period: 23 May - 18 June[a] — No. plants affected / Total no. plants[e] | 18 June-16 July — No. plants affected / Total no. plants | 16 July-31 July — No. plants affected / Total no. plants | Total |
|---|---|---|---|---|---|---|
| Total | | | 100/110 | 2/87 | 4/29 | 106/226 |
| Percent barley affected | | | 97% | 5% | 18% | |
| Percent wheat affected | | | 53% | 0% | 0% | |

[a] Fresh trap seedlings were cycled into and out of the barley (Piroline) field on the dates depicted; the first two cycles of trap plants were exposed to the field for four weeks, and the last cycle was exposed for two weeks before returning to the greenhouse.

[b] The pots of trap seedlings were placed in clusters of four pots, each pot placed at the corner of a square, with about 60 cm between ports.

[c] The pots of trap seedlings were placed in a hole so that the rim of the pot was even with the soil surface of the field; each hole which trap seedlings were cycled into and out of was given a specific number (No.).

[d] All of the trap seedlings were barley cultivars except those of Norstar and Minter winter wheat; a designated hole number was always replaced with trap plants of the same cultivar.

[e] The numerator equals the total number of diseased plants and the denominator equals the total number of trap plants within a pot.

[f] The plus (+) sign designates pots of diseased seedlings.

Table 10. The response of healthy trap plants exposed to a field of diseased Klages barley on the Valier site in 1986: Field A.

| Cluster[b] | No.[c] | Cultivar[d] | Cycle no.[a] and exposure period | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | | 1<br>24 May-7 June | 2<br>7-19 June | 3<br>19 June-2 July | 4<br>2-16 July | 5<br>16-31 July | |
| 1 | 1 | Alpine | 13/13[e] +[f] | 8/8 + | 7/7 + | 1/8 + | 0/10 | 29/46 |
| | 2 | Dicktoo | 1/1 + | 4/4 + | 5/5 + | 0/0 | 0/8 | 10/18 |
| | 3 | Ellis | 13/13 + | 13/13 + | 3/3 + | 2/5 + | 0/7 | 31/41 |
| | 4 | Kearney | 8/8 + | 7/7 + | 1/2 + | 0/1 | 0/8 | 16/26 |
| | 5 | Minter (wheat) | 8/14 + | 0/11 | 0/7 | 0/8 | 0/0 | 8/40 |
| | 6 | Norstar (wheat) | 0/2 | 0/2 | 0/7 | 0/0 | 0/0 | 0/11 |
| | 7 | Reno | 4/4 + | 4/7 + | 3/3 + | 3/5 + | 0/9 | 14/28 |
| | 8 | Tenn. Wt. | 9/11 + | 13/13 + | 9/9 + | 0/3 | 0/9 | 31/45 |
| 2 | 9 | Alpine | 8/8 + | 10/10 + | 0/6 | 0/11 | 0/10 | 18/45 |
| | 10 | Dicktoo | 5/5 + | 4/4 + | 0/2 | 0/4 | 0/0 | 9/15 |
| | 11 | Ellis | 14/14 + | 6/6 + | 1/8 + | 0/8 | 0/0 | 21/36 |
| | 12 | Kearney | 7/7 + | 6/6 + | 7/7 + | 0/8 | 0/4 | 20/32 |
| | 13 | Minter (wheat) | 8/8 + | 1/10 + | 0/11 | 0/8 | 0/3 | 9/40 |
| | 14 | Norstar (wheat) | 0/5 | 0/2 | 0/15 | 0/7 | 0/0 | 0/29 |
| | 15 | Tenn. Wt. | 9/9 + | 11/11 + | 2/9 + | 0/5 | 0/8 | 22/42 |
| | 16 | Reno | 3/3 + | 5/5 + | 4/4 + | 0/3 | 3/4 + | 15/19 |
| 2 | 17 | Alpine | 8/8 + | 11/11 + | 5/11 + | 2/9 + | 2/10 + | 28/49 |
| | 18 | Dicktoo | 2/2 + | 8/8 + | 1/1 + | 0/5 | 0/2 | 11/18 |
| | 191 | Ellis | 8/8 + | 5/6 + | 7/10 + | 0/9 | 0/9 | 20/42 |
| | 20 | Kearney | 8/8 + | 6/7 + | 1/4 + | 0/6 | 0/7 | 15/32 |
| | 21 | Minter (wheat) | 4/8 + | 0/9 + | 2/16 + | 0/9 | 1/11 | 7/53 |
| | 22 | Norstar (wheat) | 0/1 | 1/4 | 0/10 | 0/8 | 0/11 | 1/34 |
| | 23 | Tenn. Wt. | 9/9 + | 11/11 + | 5/12 + | 0/8 | 0/8 | 25/48 |
| | 24 | Reno | 5/5 + | 3/3 + | 5/5 + | 0/5 | 2/11 + | 15/29 |

Table 10. Continued.

| Cluster[b] | No.[c] | Cultivar[d] | Cycle no.[a] and exposure period | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| | | | 24 May-7 June | 7-19 June | 19 June-2 July | 2-16 July | 16-31 July | |
| Total | | | 154/174 | 137/178 | 68/174 | 8/143 | 8/149 | 375/818 |
| Percent of barley affected | | | 99% | 96% | 62% | 8% | 6% | |
| Percent of wheat affected | | | 67% | 5% | 3% | 0% | 4% | |

[a] Five sets of potted trap plants were cycled into and out of a diseased barley field at the designated dates.

[b] Twenty-four pots of trap plants were divided equally into three distinct clusters; each cluster consisted of two rows of trap seedlings with about 1.8 meter between rows and about 1 meter between pots of trap seedlings within a row.

[c] Each pot of trap plants was placed into a hole dug between two rows of field barley; each hole was given a specific number.

[d] The trap plants consisted of six barley and two wheat cultivars: each pot of trap seedlings had a specific cultivar and that cultivar always replaced an identical cultivar.

[e] The numerator is equal to the number of affected plants per pot and the denominator is equal to the total number of plants per pot.

[f] A plus sign (+) designates pots of affected plants.

Table 11. The response of healthy trap plants exposed to a field of diseased Klages barley on the Valier site in 1986: Field B.

| | | | Cycle no.[a] and exposure period | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| Cluster[b] | No.[c] | Cultivar[d] | 24 May-7 June | 7-19 June | 19 June-2 July | 2-16 July | 16-31 July | Total |
| 1 | 25 | Alpine | 7/7[e] +[f] | 10/11 + | 0/3 | 0/0 | -- | 17/21 |
| | 26 | Dicktoo | 2/2 + | 7/7 + | 0/0 | 0/0 | -- | 9/9 |
| | 27 | Ellis | 2/6 + | 8/8 + | 1/6 | 0/0 | -- | 11/20 |
| | 28 | Kearney | 8/8 + | 7/7 + | 0/1 | 0/0 | -- | 15/16 |
| | 29 | Minter (wheat) | 3/12 + | 0/12 | 0/9 | 0/11 | -- | 3/44 |
| | 30 | Norstar (wheat) | 1/3 + | 0/1 | 0/7 | 0/0 | -- | 1/11 |
| | 31 | Tenn. Wt. | 4/4 + | 5/5 + | 0/1 | 0/0 | -- | 9/10 |
| | 32 | Reno | 8/8 + | 3/3 + | 0/1 | 1/2 | -- | 12/14 |
| 2 | 33 | Alpine | 9/9 + | 14/14 + | 4/4 + | 2/4 + | 6/11 + | 35/42 |
| | 34 | Dicktoo | 4/4 + | 6/6 + | 0/0 | 0/0 | 0/0 | 10/10 |
| | 35 | Ellis | 8/8 + | 8/8 + | 5/5 + | 0/0 | 2/6 + | 23/27 |
| | 36 | Kearney | 7/7 + | 4/4 + | 1/3 + | 0/0 | 1/14 + | 13/18 |
| | 37 | Minter | 1/12 + | 5/7 + | 0/4 | 0/0 | -- | 6/23 |
| | 38 | Norstar | 0/5 | 1/2 + | 0/0 | 0/10 | -- | 1/17 |
| | 39 | Reno | 5/5 + | 6/6 + | 3/4 + | 0/0 | -- | 14/15 |
| | 40 | Tenn. Wt. | 6/6 + | 11/11 + | 3/5 + | 0/0 | -- | 20/22 |
| 2 | 41 | Alpine | 7/7 + | 15/15 + | 4/4 + | 7/7 | -- | 33/33 |
| | 42 | Dicktoo | 4/4 + | 5/5 + | 0/2 | 0/0 | -- | 9/11 |
| | 43 | Ellis | 0/6 | 11/11 + | 2/14 + | 4/6 + | -- | 17/37 |
| | 44 | Kearney | 9/9 + | 6/6 + | 2/9 + | 4/6 + | -- | 21/30 |
| | 45 | Minter | 3/9 + | 0/8 | 0/6 | 0/0 | -- | 3/23 |
| | 46 | Norstar | 0/3 | 0/2 | 0/9 | 0/0 | -- | 0/14 |
| | 47 | Reno | 0/4 | 4/4 + | 0/1 | 0/0 | -- | 4/9 |
| | 48 | Tenn. Wt. | 7/7 + | 7/9 + | 0/3 | 0/0 | -- | 14/19 |

Table 11. Continued.

| Cluster[b] | No.[c] | Cultivar[d] | Cycle no.[a] and exposure period 1 24 May-7 June | 2 7-19 June | 3 19 June-2 July | 4 2-16 July | 5 16-31 July | Total |
|---|---|---|---|---|---|---|---|---|
| Total | | | 105/155 | 143/172 | 25/101 | 18/46 | 9/21 | 300/495 |
| Percent of barley affected | | | 87% | 99% | 38% | 72% | 43% | |
| Percent of wheat affected | | | 18% | 18% | 0% | 0% | -- | |

[a] Between 24 May and 16 July, four sets of potted trap plants were cycled into and out of a diseased barley field during designated dates; only four pots of plants were used in the fifth set.

[b] Twenty-four pots of trap plants were divided equally into three distinct clusters; each cluster consisted of two rows of potted trap seedlings which were about 1.8 meters between rows and about 1.2 meters between pots within a row. Cluster '1' represented a site that was double-sown on 15 April. Cluster '2' represented a site that was sown on the same date as '1'. Cluster '3' was a site that was sown later on 7 May.

[c] Each pot of trap plants was placed into a hole dug between two rows of field barley; each hole was given a specific number.

[d] The trap plants consisted of six barley and two wheat cultivars: each pot of trap seedlings had a specific cultivar and that cultivar always replaced an identical cultivar.

[e] The numerator is equal to the number of affected plants per pot, and the denominator is equal to the total number of plants per pot.

[f] A plus sign (+) designates pots of affected plants.

Table 12. The response of healthy trap plants exposed to a field of diseased Klages barley on the Valier site 17-31 July 1986[a]: Field D.

| Cultivar[b] | Cluster 1[c] No. affected plants[e] / No. total plants | Total | Cluster 2[d] No. affected plants / No. Total Plants | Total | Total (1+2) |
|---|---|---|---|---|---|
| Alpine | 5/3, 8/8 | 11/11 | | | 11/11 |
| Dicktoo | 3/9, 1/7 | 4/16 | 4/4, 3/8, 6/6 | 13/18 | 17/34 |
| Ellis | 8/10, 2/11, 2/11 | 12/32 | 3/8, 1/2, 5/5 | 9/15 | 21/47 |
| Kearney | 4/9 | 4/9 | 2/4, 10/10, 0/9, 0/0 | 12/23 | 16/32 |
| Minter (wheat) | 1/11, 4/12, 0/7 | 5/30 | | | 5/30 |
| Norstar (wheat) | 0/12, 0/11, 0/11 | 0/34 | 1/10, 0/9, 0/0 | 1/19 | 1/53 |
| Reno | 0/0, 2/5 | 2/5 | | | 2/5 |
| Tennessee Winter | 1/16, 2/9, 0/11 | 3/36 | | | 3/36 |
| Total | | 41/173 | | 35/75 | 76/248 |

[a] Trap seedlings were exposed to a recrop Klages field from 17-31 July, after which they were returned to the greenhouse; Field D was sprayed with parathion on 20 July 1986.

[b] Each pot of trap plants contained a barley cultivar except those with Minter or Norstar wheat plants.

[c] Cluster '1' consisted of potted trap plants placed on the soil surface between two rows of relatively tall, headed Klages plants that were still green with some disease.

[d] Cluster '2' consisted of potted trap plants placed on the soil surface between two rows of stunted, diseased Klages plants that had a high incidence of brown wheat mites.

[e] The numerator is equal to the number of affected plants, and the denominator is equal to the total number of plants per pot.

Table 13. Leafhoppers collected on the Conrad site in 1983 and exposed to caged healthy indicator test plants under greenhouse conditions.

| 9-11 August, 1983[a] | | 2-7 August, 1983 | | 9 October 1983 | |
|---|---|---|---|---|---|
| No. plant[b] Cultivar | No.[c] Leafhoppers | No. plant Cultivar | No. Leafhoppers | No. plant Cultivar | No. Leafhoppers |
| 4/Fortuna (wheat) | 5 | 3/Norstar (wheat) | 12 | 9/Morex (barley | 7 |
| 6/Fortuna | 6 | 6/Norstar | 15 | 12/Morex | 13 |
| 7/Fortuna | 3 | 7/Norstar | 17 | 11/Morex | 9 |
| | | 3/Norstar | 18 | 10/Morex | 18 |
| 20/Red Oats | 15 | 3/Norstar | 16 | 12/Morex | 11 |
| 8/Red Oats | 1 | 6/Norstar | 20 | 10/Morex | 24 |
| | | 7/Norstar | 15 | 14/Morex | 10 |
| 11/Norstar (wheat) | 10 | 5/Norstar | 21 | 5/Morex | 10 |
| 9/Norstar | 10 | | | 8/Morex | 3 |
| 20/Norstar | 10 | 3/Morex (barley | 15 | | |
| | | 2/Morex | 20 | | |
| 13/Morex (barley) | 2 | 5/Morex | 16 | | |
| 4/Morex | 10 | 4/Morex | 15 | | |
| 9/Morex | 1 | 3/Morex | 16 | | |
| 9/Morex | 2 | 4/Morex | 10 | | |
| 1/Morex | 3 | 3/Morex | 17 | | |
| | | 3/Morex | 20 | | |
| | | 3/Morex | 12 | | |
| 121 plants (all cultivars) | 78 | 70 plants (all cultivars) | 275 | 91 plants (all cultivars) | 105 |

[a] Date the leafhoppers were collected from field soil.

[b] Number of plants per cage/cultivar of plant.

[c] Number of leafhoppers exposed to caged plants.

Table 14. Leafhoppers collected in a pasture on the Conrad site on 18 May 1984 and exposed to caged barley plants under greenhouse conditions.

| Barley Cultivar | | | | | | | |
|---|---|---|---|---|---|---|---|
| Morex | | Tennessee Winter | | Kearney | | Dicktoo | |
| No.[a] plants | No.[b] leafhoppers | No. plants | No. leafhoppers | No. plants | No. leafhoppers | No. plants | No. leafhoppers |
| 9 | 12 | 8 | 30 | 4 | 30 | 4 | 13 |
| 9 | 30 | 8 | 10 | | | | |
| 7 | 15 | 7 | 8 | | | | |
| 8 | 20 | 6 | 5 | | | | |
| 8 | 10 | 15 | 10 | | | | |
| 8 | 10 | 20 | 15 | | | | |
| 6 | 15 | | | | | | |
| 7 | 15 | | | | | | |
| 7 | 25 | | | | | | |
| 8 | 10 | | | | | | |
| 8 | 90 | | | | | | |
| 17 | 15 | | | | | | |
| 102 Total | 267 Total | 64 Total | 78 Total | 4 Total | 30 Total | 4 Total | 13 Total |

[a] Number of indicator test plants per cage.

[b] Number of leafhoppers placed on indicator test plants.

Table 15. The response of healthy barley seedlings exposed to field collected *Petrobia latens* in 1986.

| 8,9 June-2 August[a] | | 8,9 June-2August | | 8,9 June-2 August | | 2 August-25 August | |
|---|---|---|---|---|---|---|---|
| Cage-A 250 mites[b] | | Cage-B 100 mites | | Cage-C 50 mites | | Cage-D 65 mites | |
| Kearney[c] | Tennessee Winter[c] | Kearney | Tennessee Winter | Kearney | Tennessee Winter | Ellis | |
| 2/6[d] | 3/6 | 3/4 | 0/5 | 3/6 | 0/5 | 2/4 | |
| 3/4 | 4/5 | 2/6 | 1/4 | | | 1/4 | |
| 1/6 | 1/5 | 3/5 | | | | 1/4 | |
| 0/6 | 0/4 | | | | | 2/4 | |
| 3/4 | 0/6 | | | | | | |
| 9/26 | 8/26 | 8/15 | 1/9 | 3/6 | 0/5 | 6/16 | Total |

[a] Time of mite exposure to plants for each designated cage.

[b] The initial number of field mites exposed to plants; these mites were collected from a diseased Klages barley field.

[c] The barley cultivar used as the healthy barley test plant.

[d] The numerator is equal to the number of diseased plants per pot, and the denominator is equal to the total number of plants per pot.

Table 16. Response of single indicator test plants exposed to field collected _Petrobia latens_ for varying periods in 1986.

| Test plant | Exposure time[a] 168 hrs.[b] | 144 hrs.[b] | 120 hrs.[c] | 96 hrs.[c] | 72 hrs.[c] | 48 hrs.[c] | 24 hrs.[c] | 12 hrs.[b] |
|---|---|---|---|---|---|---|---|---|
| Ellis | 2/45[d] | 3/21 | 3/12 | 3/8 | 2/8 | 0/10 | 2/9 | 0/23 |
| Kearney | 0/5 | 0/3 | -- | 0/3 | 0/2 | 0/12 | 0/12 | 0/5 |
| Total | 2/50 | 3/24 | 3/12 | 3/11 | 2/10 | 0/22 | 2/21 | 0/28 |

[a] Ten mites were exposed to each individually caged plant for the specified time prior to treatment of each plant with Vapona and/or Malathion treatment.

[b] The mites were collected 18 July 1986 from diseased Klages plants in a barley field on the Valier site; these mites were placed on healthy indicator test plants on 19,20 July and exposed to the mites for 12 hrs., 144 hrs., 168 hrs. before treatment with Vapona and Malathion.

[c] The mites were also collected from the Valier site on 3 July 1986 and placed on indicator test plants 4,5 July for 120 hrs, 96 hrs., 72, hrs. 48 hrs, or 24 hrs. before treatment with Malathion and Vapona.

[d] The numerator is equal to the number of affected caged single indicator test seedlings and the denominator is equal to the total number of caged single indicator test seedlings; caged healthy control plants not exposed to mites did not become affected.

Table 17. The response of sequential sets of healthy test seedlings exposed to _Petrobia latens_ in Cage-D during 1986 and 1987.

| | Indicator Set | |
|---|---|---|
| | I | II |
| Exposure time[a] | 23 Dec.-15 Jan. | 15 Jan.-3 Feb. |
| Quantity of mites used[b] | 60-400 mites | 400-200 mites |
| Test plant cultivar | 'Dicktoo' barley | 'Dicktoo' barley |
| No. of plants that became diseased[c] | 25/25 | 19/21 |

[a] Time test plants were exposed to mites.

[b] The first number is equal to the approximate mite numbers at the onset of plant exposure and the last number is equal to the number of mites on the plants at the end of plant exposure. The original 60 mites had been exposed to detached diseased leaves.

[c] The numerator is equal to the number of diseased plants and the denominator is equal to the total number of indicator test plants.

Table 18. The response of sequential sets of healthy test seedlings exposed to <u>Petrobia latens</u> in Cage-H during 1986 and 1987.

| | Indicator Set | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Exposure time[a] | 23 Dec.-15 Jan. | 15-30 Jan. | 30 Jan.-15 March | 15 March-4 April |
| Quantity of Mites used[b] | 120-500 mites | 500-200 mites | 200-1,500 mites | 1,500-32 mites |
| Test plant cultivar | 'Dicktoo' barley | 'Dicktoo' barley | 'Nantes Coreless' carrot | 'Dicktoo'barley |
| No. of plants that became diseased[c] | 25/25 | 18/22 | 0/15 | 9/24 |
| Test plant cultivar | | | 'White Pearl' onion | |
| No. of plants that became diseased[c] | | | 0/7 | |

[a] Time test plants were exposed to mites.

[b] The first number is equal to the approximate mite numbers at the onset of plant exposure, and the last number is equal to the number of mites on the plants at the end of plant exposure. The original 120 mites had been exposed to healthy detached leaves.

[c] The numerator is equal to the number of diseased plants and the denominator is equal to the total number of indicator test plants.

Table 19. The response of sequential sets of healthy test seedlings exposed to Petrobia latens in Cage-C during 1987.

| | Indicator Set | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Exposure time[a] | 15 Jan.-3 Feb. | 3-23 Feb. | 23 Feb.-15 March | 15 March-4 April |
| Quantity of mites used[b] | 60-200 mites | 200-224 mites | 224-50 mites | 50-19 mites |
| Test plant cultivar | 'Dicktoo' barley | 'Dicktoo' barley | 'Dicktoo' barley | 'Dicktoo' barley |
| No. of plants that became diseased[c] | 18/24 | 21/24 | 24/24 | 18/24 |

[a] Time test plants were exposed to mites.

[b] The first number is equal to the approximate number of mites at the onset of mite exposure, and the last number is equal to the number of mites on the plants at the end of the plant exposure. The original 60 mites had been exposed to healthy detached leaves.

[c] The numerator is equal to the number of diseased plants and the denominator is equal to the total number of indicator test plants.

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