SEROLOGICAL INVESTIGATIONS OF THE INTERACTION BETWEEN WHEAT SOILBORNE MOSAIC VIRUS AND HARD RED WINTER WHEAT

By

CHARLA RAE ARMITAGE

Bachelor of Science
The Pennsylvania State University
University Park, Pennsylvania
1979

Master of Science
Colorado State University
Fort Collins, Colorado
1984

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Thesis Approved:

Robert M. Hungerford
Thesis Adviser

John L. Sherwood

Albert O. Russell

L. L. Singleton

Alvin F. Krueger

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

During 1976-1980, Oklahoma planted a yearly average of 3 million hectares of wheat. From this planting, 2.5 million hectares were harvested with a yearly average grain yield of 2595 L/ha. Oklahoma also produced 672 Kg dry matter/ha in forage yield which was used for grazing cattle November through March 15. Approximately 1.5 million stocker cattle were grazed on wheat pastures each year (Tweeten 1982).

Wheat soilborne mosaic virus (WSBMV) was first reported in Oklahoma in 1952 in the Ponca City area (Wadsworth and Young 1953). The virus is now found in all the major wheat growing counties in Oklahoma but the disease remains most severe along the Chikaskia and Salt Fork Rivers (Williams 1986). Reductions in yield due to WSBM of 32 to 61% and reductions in test weight of 0 to 3% have been reported (Wadsworth and Young 1953).

Hunger, et al. (1989) conducted evaluations for two seasons by growing wheat in a location with no history of the disease and in a location with a history of severe WSBM. Grain yield reductions ranged from 31.6% (Mustang in 1985-86) to 69.4% (Triumph 64 in 1984-85). Resistant and susceptible cultivars had average yield decreases of 40.2%

(sd=10.97) and 54.3% (sd=10.66) over the two years. Reductions in thousand kernel weight (TKW) ranged from 0.8 to 18.8%.

The use of resistant cultivars is the most practical method to control WSBM. Visual assessment of wheat is the most commonly used method to evaluate resistance to wheat soilborne mosaic (WSBM), but other factors may mimic symptoms of WSBM. This mimicry of symptoms presents a need to confirm visual assessments in programs breeding for resistance to WSBM.

Enzyme-linked immunosorbent assay (ELISA) has been used in conjunction with visual assessment to verify the presence of WSBMV. The objectives of these studies were: 1) to determine the optimum time to sample wheat for evaluation of resistance to WSBM by ELISA, 2) to determine the superior method of storing leaf tissue prior to ELISA, and 3) use symptomology, ELISA, and polyacrylamide gel electrophoresis (PAGE) to examine the relationships between capsid production, virion concentration, and the expression of resistance by hard red winter wheat to WSBM.

CHAPTER II

LITERATURE REVIEW

Introduction

Wheat soilborne mosaic was first reported in soft red winter wheat in Madison County, IL, during April 1919 by G. R. Lyman in a report on "take-all" in the United States of America (Johnson et al. 1924). Farmers in the area believed they had observed the disease in wheat for many years. The disease was originally called "take-all" because of similarity to Australasian take-all (McKinney 1923). Another name was "footrot" because the decay that often occurred in the base of the culms of infected plants in Illinois and Indiana resembled European footrot. After the decay at the base of the culms was found to be secondary, the name "rosette disease of wheat" was used to describe the characteristic stunting and proliferation of tillers that occurred in spring in infected plants (McKinney 1923). Intercellular bodies formed in plants infected with the rosette disease resembled those found in plants with a leaf mottling disease. Rosetting was never found without leaf mottling so McKinney, et al. (1923) suggested that the rosette disease might be a severe case of the mosaic-like

leaf mottle disease. The most current name for this disease has been proposed by The American Phytopathological Society: wheat soilborne mosaic (WSBM: Hansing 1985).

Susceptibility to WSBM is found in all cereal species of the tribe Hordeae (McKinney 1930). Also, the disease and its causal agent appear to be spreading. East of the Mississippi, the disease was reported in Florida in 1974 (Kucharek and Walker 1974). Only the yellow strain has been reported on the Great Plains (Sill 1958). WSBM was reported in north-central Oklahoma in 1952, (Wadsworth and Young 1953) in Texas in 1984, (Toler 1984) and also has been reported in Argentina, Brazil, Egypt, Italy, Japan (Wiese 1977) and China (Xu, et al. 1984).

The Virus

The discovery of inclusion bodies in the cells of infected plants led to the identification of a viral causal agent. McKinney et al. (1923) suggested the rosette disease of wheat might be a severe case of mosaic-like leaf mottling of wheat. McKinney (1948) later found that some "strains" (cultigens) of wheat did not rosette when infected by a rosetting strain of the virus, and some virus isolates did not cause rosetting when infecting a host with rosetting capability. The rosetting strain was designated the "green strain" because infected lamina and sheaths were often dark blue-green. The non-rosetting strain was designated the

"yellow strain" because infected wheat lamina and sheaths exhibited a pale green or yellow mottling (McKinney 1930). A green strain and a yellow strain are also found in Japan. Only the Japanese strains infect tobacco and only the American strains infect spinach (Sill 1958, Tsuchizaki, et al. 1973, Wada and Fukano 1937). None of the WSBMV isolates found west of the Mississippi River caused rosetting when inoculated to rosette-susceptible wheat (Sill 1958). Mckinney et al. (1944) renamed the viral strains "mosaic-rosette virus" (Marmor tritici var. typicum McK.) and "yellow-mosaic virus" (M. tritici var. fulvum McK.). The common name for the virus proposed by The American Phytopathological Society is "wheat soilborne mosaic virus" (WSBMV: Hansing 1985).

WSBMV consists of two separate particles (virions) with single-stranded RNA and a single species of capsid protein to produce two rigid, rods. Both rods are 20 nm wide, have flush ends, and are morphologically similar to tobacco mosaic, tobacco rattle, and barley stripe mosaic viruses (Brakke 1971). One rod is 300 nm long and controls infectivity and viral concentration. The second rod is 110-160 nm long and controls rod length, serotype, and inclusion body type (Tsuchizaki, et al. 1975). Both rods are necessary for infection, (Shirako and Brakke 1984a). No serological differences have been reported between the short and long particles. The Japanese strains and the American

strains have antigens in common and complementation between virions of the two strains occurs (Tsuchizaki, et al. 1975). The sedimentation coefficients are 219S for the long rod and 177S-159S for the short rod (Brakke 1977). WSBMV is thermally inactivated in sap after 10 min at 60-65 °C. The dilution end point is 10^{-2}-10^{-3}, viruliferous sap remains infective at 15 °C for three months, and the virus remained infective after being stored 11 years in desiccated wheat leaves (McKinney et al. 1965). The virus also has been shown to survive on montmorillonite-containing kaolin and bentonite clays for up to 8 months when kept at 10 - 15 °C. (Miyamoto 1959).

An apparent lack of stability is the reason for the range in size of the short virion. The long particles, 281-300 nm are designated 1L while particles ranging 138-160 nm are designated 0.5L, 111-137 nm are 0.4L, and 92-110 nm are 0.35L. The 0.4L particle is formed by a spontaneous deletion mutation of the 0.5L particle, and the 0.35L particles appear to be formed by spontaneous deletions of both the 0.5L and the 0.4L particles (Shirako and Brakke 1984a-b, Hsu and Brakke 1985a). A positive correlation was found between disease severity and the amount of virions smaller than 0.5L (Shirako and Brakke 1984a). The 0.5L particle is dominant in early spring but the shorter particles become dominant as spring progresses (Shirako and Brakke 1984b). Variation in disease between fields can not

be explained by variation in short virion sizes nor the propensity for deletion mutations (Hsu and Brakke 1985b). Cell-free translation of the 1L virion (RNA I) with rabbit reticulocyte lysates results in proteins of 180K, 152K, 135K, 80K, and 45K molecular weights. The maximum molecular weight protein possible from an RNA this size (6700 nucleotide residues) is 228K, thus, no read-through proteins are being produced. RNA from the short virion (RNA II) has no genome-linked proteins nor a cap structure at the 5' end (Shirako and Brakke 1984a). Cell-free translation products of the 0.5L RNA have molecular weights of 90K, 28K, and 19.7K. Cell-free translation products of the 0.4L RNA have molecular weights of 66K, 28K, and 19.7K while products of the 0.35L RNA are 55K, 28K, and 19.7K in molecular weight (Hsu and Brakke 1985c). Thus, all three lengths of RNA II code for a 19.7K protein that is thought to be the coat protein (Hsu and Brakke 1985c, Shirako and Brakke 1984a, b). Immunoglobulin G (IgG) against WSBMV reacts with all products of RNA II, but none of the products of RNA I. Hsu and Brakke (1985c) conclude that the 19.7K sequence is included within the 28K, 55K, 66K, and 90K products. The RNA from 0.5L and 0.35L show heterogeneity at the 5' end, (Hsu and Brakke 1985a) while the reading frames for the 90K, 66K, and 55K products end at the same place near the 3' end of RNA II. Thus, Hsu and Brakke (1985c) concluded that the spontaneous deletions occurred at the 3' end of RNA II.

McKinney, et al. (1923) found intracellular inclusion bodies (also called "X-bodies") associated with WSBM. Crystalline aggregates of virions in parallel arrangement were found in cells infected with Japanese isolates of WSBMV but not in cells infected with American isolates (Hiboyuki, et al. 1974). Tsuchizaki, et al. (1973) reported a correlation between particle length of the short virion and type of inclusion body formed. The 0.5L virion was associated with type A inclusion bodies with clear margins and vacuoles attributed to the green strain of WSBM. The 0.35L virion was associated with the small type B inclusion bodies with rough margins attributed with the yellow strain of WSBM. The 0.4L virion was associated with the irregular type M inclusion bodies attributed to composite infections by both WSBM strains (Wada and Fukano 1937). Hsu and Brakke (1985c) suggest that the 90K, 66K, and 55K products for the three RNA IIs are the source of variation in inclusion body type. However, these inclusion bodies have been classified by light microscopy and types of structures identified in this manner have not been shown to correlate well with the types of fine structures observed by electron microscopy (Hiboyuki, et al. 1974).

WSBMV and tobacco mosaic virus (TMV) was thought to be related because they cross-react in microprecipitin, and in Ouchterlony agar double-diffusion serological tests. Also, infectivity of TMV on Pinto bean is reduced by 80% by the

addition of WSBMV (Powell 1976). Thus, Gibbs (1977) included WSBMV in the tobamovirus group. However, hybridization was not found to occur between WSBMV cDNA and TMV RNA (Hsu and Brakke 1985). Shirako and Brakke (1984a) propose a new virus group, the fungus-borne rod-shaped virus group, or "furoviruses". Criteria for furoviruses are: 1) the virus genome be divided, 2) the viral particles be rigid, hollow rods, and 3) the virus be transmitted by a plasmodiophoraceous fungus. Other furoviruses are beet yellow vein virus, Nicotiana velutina mosaic virus, peanut clumping virus, potato mop top and Hypochoeris mosaic virus.

The Vector

The soilborne plasmodiophoraceous fungus, Polymyxa graminis Ledingham, was first suggested as a vector for WSBMV by Linford and McKinney (1954) because of the association between fungal-infected roots and viral-infected plants. Fungal zoospores from infected plants were shown to transmit the virus to healthy plants. Rao (1968) obtained successful transmission of WSBMV from powdered roots with a treatment of soil extracts or kinetin in distilled water. Powdered roots were incubated at 28 °C for two months prior to a pre-inoculation incubation at 20 °C. Non-viruliferous isolates of P. graminis were made viruliferous by parasitizing wheat infected with WSBMV, while viruliferous isolates were made non-viruliferous by maintenance on

Trifolium incarnatum (Canova 1966). However, non-viruliferous zoospores of P. graminis acquired little if any virus from exposure to purified virus. Neither repeated low speed centrifugation of zoospores nor treating the zoospores and resting spores with antisera, acid, or alkali eliminated viral transmission. These results suggest that virions are either attached to the surface of zoospores in such a way as to make the virions impervious to inactivation by antisera, acid or alkali, or are carried within the zoospore (Rao and Brakke 1969, Campbell 1979). However, Langenberg and Giunchedi (1982) used electron microscopy to observe the association between virus and vector, and, although the virus was seen in close contact with the plasmodia of P. graminis, the fungal cytoplasmic contents were so densely stained that the virus was not seen inside zoospores, plasmodia, zoosporangia or cystosori. Most contact between virions and vector was an end-on attachment of virions to the outer membrane of the plasmodium, however Langenberg and Giunchedi conceded that this arrangement may have been an artifact of fixation.

Plasmodia in the epidermal and cortical cells develop into zoosporangia or cystosori. Cystogenous plasmodia are amoeboid and lack an outer membrane. Mature cystosori cleave into cysts which are 4-7 μm in diameter with a hyaline inner wall and a yellowish-brown to dark brown, smooth outer wall. Cysts, or resting spores, are the

survival structures of the fungus and germinate to produce primary zoospores which directly penetrate host root hairs and other epidermal cells (Ledingham 1939, Karling 1968, Rao 1968). Infection occurs most frequently at a soil-depth of 3 cm but never below 15 cm (Ikata and Kawai 1938). Zoosporangia may become as large as 40 x 200 μm and are lobed, tubular and somewhat "zigzag" shaped. At maturity, zoosporangia are enveloped by thin membranes or walls. Zoosporangia produce one or more long exit tubes through which motile, secondary zoospores emerge. These biflagellate zoospores average 4.2 μm in dia. (Barr 1979, Ledingham 1939, Karling 1968). Mature zoosporangia appear at 4-6 days after inoculation but do not become abundant until 8-9 days after inoculation. At this time, infected plants yield infectious root washing although visible symptoms are not yet expressed (Rao 1968). Ledingham (1939) reported that secondary zoospores swam actively for 2-3 hr then lost their flagella and became amoeboid before eventually infecting host cells. Satisfactory methods to examine fungal structures using lactophenol with acid fuchsin have been developed (Rao 1968).

Roots of wheat, barley, rye, hairy bromegrass, pigweed and lambsquarters are parasitized by P. graminis (Barr 1979) but WSBMV symptoms have been reported only on the grasses (Wiese 1977). The 10 min thermal death point for cystosori

is 45-50 °C, and for zoospores, 30-35 °C (Brakke and Estes 1967).

Inoculation

WSBMV is not seed transmitted (Brakke 1971). Soil debris collected in spring and summer is not as infective as soil debris collected in autumn and winter (Brakke and Rao 1967, Brakke and Estes 1967). Soaking soil debris or infected roots increases transmission of WSBMV. The best buffer for zoospore release and virus transmission is 0.01 M potassium phosphate, pH 7.6 (Brakke and Estes 1967). Source plants may be of any age and are better sources of inoculum if maintained in soil/sand (1:1 v/v) rather than vermiculite (Brakke and Rao 1967). Conditions for soaking the inoculum source for optimum release of zoospores are 20 min at 25 °C (Brakke, et al. 1965). This water can then be used to flood flats of seedlings (Bockus and Niblett 1984, Hunger and Sherwood 1985a) or to soak seedling roots before transplanting into soil (Brakke et al. 1965, Rao 1969). The temperature and time for soaking seedling roots for optimum infection is 24 hr at 5 - 10 °C (Brakke et al. 1965).

Sap from foliage showing symptoms of WSBM and expressed into a potassium phosphate or sodium sulfate buffer can be used as inoculum. Inoculum may be wiped onto roots or foliage with a pad of cheese cloth and an abrasive like carborundum, corundum, or diatomaceous earth (McKinney

1948, McKinney, et al. 1957, Rao and Brakke 1970, Shirako and Brakke 1984a-b, Hsu and Brakke 1985a-c). However, relative to other mosaic viruses, transmission of WSBMV in this manner is difficult. Sap solutions may be applied to foliage with an artist's airbrush resulting in viral transmission (Pring and Gumpf 1970). Maize streak virus is not successfully transmitted mechanically except through electro-endosmosis (von Wechmar and Polson 1980). Perhaps electro-endosmosis would also be effective with WSBMV. WSBMV/P. graminis cultures may be maintained in dried and powdered roots (Rao 1968), or in live plants through serial transfer through root washings (Brakke and Rao 1967, Hunger and Sherwood 1985a).

Infection

WSBMV is presumed to be released into host roots following infection of seedling roots by P. graminis during cool, wet periods primarily in the autumn (Brakke and Estes 1967, Rao and Brakke 1969). In the field, the virus may be found in the roots within 2-3 weeks after planting. Although mottling of the leaves may become visible in susceptible cultivars as early as November, symptoms generally are not seen until spring (Brakke, et al 1965). Late-maturing susceptible cultivars appear to recover from infection in late spring (McKinney 1923, Eversmeyer, et al. 1983).

The yellow strain of Japan produced the most severe mosaic at 15 °C, moderately severe mosaic at 10 °C, inconspicuous mosaic at 20 °C, and imperceptible mosaic at 25 °C (Ikata and Kawai 1938). Using an isolate of American yellow strain from Nebraska in the greenhouse, a mild mosaic was shown to develop at 20-25 °C in two weeks after inoculation. At 15 °C, symptoms were more severe but did not develope until three weeks following inoculation (Brakke, et al. 1965). Eversmeyer et al. (1983) theorized that temperatures below 15 °C favor viral increase while temperatures above 15 °C favor wheat growth.

Both grain and forage yields may be reduced as much as 50% in hard red winter wheat (Wadsworth and Young 1953, Young and Williams 1981, Campbell, et al. 1975), and grain losses of 80.74% have been reported for soft red winter wheat (Bever and Pendleton 1954), and 70% for some Italian durum cultivars (Vallega and Rubies-Autonell 1985). WSBM reduces grain yield by reducing the number of kernels, kernel weight, test weight, plant height, and increasing weediness in the field (Campbell, et al. 1975, Vallega and Rubies-Autonell 1985). Finney and Sill (1963) reported that WSBMV did not significantly alter the mixing properties or protein quality of flour produced from the grain of diseased plants. However, protein quantity, ash content, and water absorption increased.

Control

Late-seeding may lower disease severity but it also increases vulnerability to other diseases and to winterkill (Johnson, et al. 1924). Infectious soils lost infectivity after 10 min at 60 °C (Johnson 1942), or after treatment with formaldehyde, chloropicrin, carbon disulfide, dichloropropene-dichloropropane, ethyl alcohol or by autoclaving. Toluene did not reduce infectivity of soil (McKinney, et al. 1957). Fumigation with methyl bromide and chloropicrin increased wheat yields (Eversmeyer et al. 1983), and application of nitrogen reduced the severity of visual symptoms and improved yields (Williams and Young 1976). Crop rotation aids in disease control (Wiese 1977). However, attempts to control WSBM by cultural practices have been inefficient or impractical, (Williams and Young 1976, Modawi, et al. 1982). Thus, resistant cultivars has been the recommended method of control (McKinney 1923, Johnson, et al. 1924, Williams and Young 1976, Modawi, et al. 1982).

Resistance

Assessment for resistance. Originally, non-rosetting cultivars were considered resistant, (Webb, et al. 1923) but more recently, disease resistance is indicated by a reduction in the incidence and severity of symptoms (McKinney 1930, Campbell, et al. 1975). However, comparable

populations of cystosori of P. graminis are formed in roots of field resistant and susceptible cultivars (Larsen, et al. 1985), and both types of cultivars are susceptible to WSBM when mechanically inoculated (Mckinney 1948, Larsen, et al. 1985). Larsen, et al. (1985) reported finding the earliest onset of visual symptoms and the greatest amount of stunting in field resistant cultivars that have been mechanically inoculated. Single, dominant genes for resistance to WSBM have been reported (Dubey, et al. 1970, Modawi, et al. 1982, Merkle and Smith 1983) but no qualitative resistance (situ Kegler and Meyer: 1987) has been reported.

Campbell, et al. (1975) reported that yield loss due to WSBMV could be determined by the formula:

$$L_x = (X_n - X_i) - (\bar{R}_n - \bar{R}_i)$$

where X_n is the yield of susceptible cv. X in a disease-free soil, X_i is the yield of cv. X in an WSBMV infested soil, R_n is the average yield of all resistant cultivars in the disease-free soil, and R_i is the average yield of all resistant cultivars in WSBMV infested soil.

Visual assessment of disease symptoms is frequently confounded by various factors. Plants in the greenhouse and growth chambers exhibit misleading chlorotic streaking (Brakke, et al. 1965, Hunger and Sherwood 1985a). Cultigens normally vary in growth habits including relative heights and color. Necrosis and stunting can be due to P. graminis,

(Teakle, 1969) other pathogens, drought and frost damage. Cultigens susceptible to WSBM are often also susceptible to winterkill (Campbell, et al. 1975). This mimicry of visual symptoms of WSBM by factors other than WSBMV presents a need to confirm visual assessments in programs breeding for resistance to WSBM.

Other methods of assessing WSBM have been proposed. Aerial photographs using Kodachrome and Ektachrome films have been used for surveying the disease. However, aerial infrared photography does not allow for differentiation between levels of disease severity (Young and Williams 1981). Assessment with transmission electron microscopy is considered unsuitable because the scarcity of virions found in leaves which exhibit WSBM symptoms leads to inconsistent results. Extractions and purifications of the virions for relative concentration determinations are possible but tedious (Hunger and Sherwood 1985a).

Enzyme-linked immunosorbent assay (ELISA) has been used in conjunction with visual assessment to verify the presence of WSBMV, but during late spring ELISA values (absorbance at 405 nm) from resistant cultivars increase to levels comparable to ELISA values in susceptible cultivars (Hunger and Sherwood 1985b). ELISA readings did not correlate well with yield reductions in the same field trial (Hunger and Sherwood 1989). This may be due to ELISA measuring capsid protein which may be production without virion assemblage.

For evaluations of resistance, plant samples are taken at various times during the growing season. If these samples are analyzed at the time of each sampling, reagents age or are replaced and laboratory environmental conditions vary with each analysis. These variations could result in differences in ELISA readings that are unrelated to treatment effects. In order to reduce statistical error of this nature, all plant samples frequently are stored and analyzed at the same time. However, duration of storage then becomes an experimental variable. For example, Adams (1978) demonstrated a decline in plum pox virus antigen during the first 4 of 13 months of storage at -14 °C. Torrance and Dolby, (1984) found reductions in virus antigen titres of prunus necrotic ringspot, prune dwarf virus and apple mosaic in leaves stored for 12 weeks at -20 °C. Furthermore, virus antigen titres did not decrease proportionally with duration of storage. Decline of detectable antigen of lettuce mosaic virus in lettuce, cucumber mosaic virus in marrow (Ward et al. 1987), potato leafroll virus and potato virus A, S, X, and Y in potato (Singh and Somerville 1983) depended, in part, upon temperature of storage and physical condition of the sample. Singh and Somerville also found that the common procedure of storing leaves in plastic bags at -20 °C resulted in 50% and greater losses in virus antigen after 4 days as compared to fresh leaves. Dehydration also occurs in leaf tissue frozen

for extended periods of time. Thus, leaf samples collected and frozen at the end of a growing season contain a higher percentage of moisture than samples collected at the beginning of the season. This difference in water weight can affect the resulting ELISA values because tissue samples are standardized by weight. These variations in ELISA values may not appreciably affect a qualitative evaluation but could render a quantitative evaluation meaningless.

Sources of Resistance. McKinney (1930) demonstrated that all cereal species, including eight Triticum spp. were susceptible to WSBM. However, potential sources of resistance were observed in every species tested. Some cultivars were less susceptible than others and none showed homozygosity for this trait. The spring habit wheats were susceptible only when planted in the fall. Miyake (1938) reported that resistance to both the green and the yellow strains of WSBMV in Japan was due to a single, dominant allele. Nakagawa, et al. (1959) found three genes for resistance to both green mosaic and yellow mosaic of Japan in cvs. Norin 45, Kinki 54, and Norin 61. Genes H and M determine susceptibility while A inhibits H. Shaalan, et al. (1966) reported finding 2 genes for resistance to the WSBMV strain in Kansas in cv. Ottawa. The alleles for resistance demonstrated partial dominance over the alleles for susceptibility. Dubey, et al. (1970) suggested the

presence of one gene with three alleles for host resistance to the U.S.A. strains. The allele imparting susceptibility to both mosaic and rosetting (r^{mr}) was recessive to the other alleles. The allele imparting resistance to both mosaic and rosetting (R^{mr}) was dominant over the other two alleles. The allele imparting resistance to rosetting with susceptibility to mosaic (r^{m}) was recessive to R^{mr} but dominant over r^{mr}. Modawi, et al. (1982) reported finding a single, dominant resistance gene in cvs. Shawnee, Oasis and in developmental lines KS73148 and KS73256. Vallega and Rubies-Autonell (1985) reported WSBM of Triticum durum in Central Italy. The older cultivars of Italy were resistant to WSBM, but many of the more recently developed, higher-yielding, semidwarf types were extremely susceptible.

Williams (1986) reported the following hard red winter wheat cultivars as resistant to WSBM: cvs. Newton, Rocky, Plainsman V, Chanute, Pronto, and Satanta. Cvs. Centurk '78 and Century 2148 were reported as tolerant. Willis and Brooks (1988) reported the following wheat cultivars as resistant to WSBM: cvs. Abilene, Arkan, Caldwell, Carson, DeLange 7837, DeLange 7846, Dodge, GB 2148, Hart, Hawk, KS83 1374, Mesa, McNair 1003, Mustang, Newton, Norkan, Pioneer 2154, Pioneer 2157, Pioneer 2165, Pioneer 2172, Plainsman V, Stallion, Tam 108, Thunderbird, Trailblazer, Victory, and Wrangler.

CHAPTER III

STORAGE OF WHEAT FOLIAGE PRIOR TO ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF WHEAT SOILBORNE MOSAIC VIRUS

C. R. Armitage, R. M. Hunger, J. L. Sherwood, and D. L. Weeks

Graduate Research Assistant, Associate Professor, Associate Professor, Department of Plant Pathology, and Professor, Department of Statistics, Oklahoma State University, Stillwater, OK 74078-9947, USA.

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ABSTRACT

Plant samples, collected at various times during a growing season, are frequently stored prior to evaluating resistance to wheat soilborne mosaic virus (WSBMV) by enzyme-linked immunosorbent assay (ELISA). Leaves of winter wheat cvs. Sage and Vona, showing symptoms of WSBMV infection, were cut in half along the midrib. Each half was either: 1) refrigerated at 4 °C, 2) frozen at -20 °C, 3) frozen at -70 °C, or 4) desiccated with $CaCl_2$. Relative virus antigen titres were evaluated for individual leaf

halves by ELISA. ELISA absorbance means from desiccated leaf halves were consistently higher than absorbance means from corresponding leaf halves that had been frozen. This distinction suggests that virus antigen decreases during freezing but is retained during chemical desiccation. All 4 methods of storage were found to be suitable for short-term storage prior to qualitative evaluations by ELISA, but chemical desiccation was the superior method for long-term storage and for storage of foliar samples prior to quantitative evaluations by ELISA.

Enzyme-linked immunosorbent assay (ELISA) is used to evaluate host resistance to wheat soilborne mosaic virus (WSBMV: Hunger and Sherwood 1985). For evaluations of resistance, plant samples are taken at various times during the growing season. When samples are analyzed at the time of each sampling, evaluation and environmental conditions in the laboratory vary with each analysis. These alterations could result in disparities in ELISA readings that are unrelated to treatment effects. In order to reduce statistical error due to these variables, all plant samples are frequently stored and analyzed at the same time. However, duration of storage then becomes an experimental variable. For example, Adams (1978) demonstrated a decline in plum pox virus antigen during the first 4 of 13 months of storage at -14 °C. Torrance and Dolby (1984) found

reductions in virus antigen titres of prunus necrotic ringspot virus, prune dwarf virus and apple mosaic virus in leaves stored for 12 weeks at -20 °C. Furthermore, virus antigen titres did not decrease proportionally with duration of storage. Decline of detectable antigen of lettuce mosaic virus in lettuce, cucumber mosaic virus in marrow (Ward et al. 1987), and potato leafroll virus, potato virus A, S, X, and Y in potato (Singh and Somerville 1983) was partially dependent upon temperature of storage and physical condition of the sample. Singh and Somerville also found that the common procedure of storing leaves in plastic bags at -20 °C resulted in losses of 50% (or greater) of the amount of virus antigen after 4 days as compared with fresh leaves. Dehydration also occurs in leaf tissue frozen for extended periods of time. Thus, leaf samples collected and frozen at the end of a growing season contain a higher percentage of moisture than samples collected at the beginning of the season. This change in water weight can affect the resulting ELISA values because tissue samples are standardized by weight. These inconsistencies in ELISA values may not appreciably affect a qualitative evaluation but could render a quantitative evaluation meaningless.

A common method of storage in which virus infectivity is maintained is chemical desiccation of leaf material. WSBMV remained infective after being stored 11 years in desiccated wheat leaves (McKinney et al. 1965). If

infectivity is maintained, then reactions to ELISA should be maintained. The present study assessed chemical desiccation as a method of storing winter wheat foliage naturally infected with WSBMV prior to evaluation with ELISA.

MATERIALS AND METHODS

Plant tissue. Hard red winter wheat (Triticum aestivum L.) cvs. Sage and Vona are susceptible to WSBMV and were planted at 5 g per 3.0 m row, in 5 row plots at a location with a history of severe WSBMV infestation near Stillwater, Oklahoma. Several times from January to March, 1987 and 1988, leaves showing visual symptoms of WSBMV were collected from these field plots. Individual leaves were cut in half along the midrib and each half was individually weighed. Only leaves with a fresh weight difference of less than 12 mg (1987) and 5 mg (1988) between the resulting halves were used. Each leaf half was either: 1) dried at room temperature (20-25 °C) in paper coin envelopes in racks in a desiccator with $CaCl_2$, 2) refrigerated at 4 °C in small medicine cups for 3-5 days, 3) frozen in small medicine cups in a frost-free freezer at -20 °C, or 4) frozen in small medicine cups at -70 °C (1988 only). Each experiment consisted of a pair of storage treatments on typically 8 or 16 leaves. Medicine cups for each experiment-treatment group were wrapped in polyethylene before storage. Leaf halves were stored a minimum of 3 days (the minimum time required for desiccation). When one set of leaf halves were

refrigerated, neither set of leaf halves were stored no longer than 5 days to avoid fungal growth and decay. Desiccated leaf halves were reweighed to determine a dry weight for the sample and percentage weight loss.

Crude sap extracts were produced by grinding each leaf half with a mortar and pestle and 1 ml of sample buffer (phosphate buffered saline with 0.05% Tween 20 and 2% polyvinyl pyrrolidone) and then adding sample buffer to produce a final 1:100 (w/v) dilution. To correct for dehydration during storage, fresh weights were used for the refrigerated and frozen samples. Fresh weights were also used for desiccated leaf halves in some experiments (DESF), while dry weights were used for the other desiccated leaf halves (DESD).

Blind checks of the storage treatments were performed. One person cut and weighed leaves. A second person then labelled the leaf halves in such a manner that matching both halves of a leaf during analysis would be impossible without the key. Rematching of leaf halves was performed during the numerical analyses that followed the ELISA.

ELISA procedures. Rabbit polyclonal antiserum to WSBMV was prepared as previously reported (Bahrani et al. 1988). ELISA was performed using standard flat bottom plates by the double antibody sandwich as described previously (Bahrani et al. 1988) except samples were applied at 100 µl per well because of the limited amount of sap extracted from single

leaf halves. In all assays, 5 wells per leaf half were used on each of 2 ELISA plates providing a total of 10 wells (and 10 ELISA values) per leaf half.

In 1987, the mean absorbance of 12 wells per plate with sap extracted from virus-free leaves of cv. Sage raised in a glasshouse were used as the negative check to determine background absorbance for each plate. Another four wells contained sap from cv. Sage that was known to contain high titres of virus antigen as a positive check. The alkaline phosphatase labeled IgG conjugate was used at a 1:200 dilution. Absorbance values were measured at 405 nm with a EIA READER (Model EL-307, Bio-Tek Instruments, Inc., Laboratory Division, Burlington, VT 05401, U.S.A.). The positive check wells were providing absorbance readings of 1.600 at 2-4 min after the addition of the substrate. Reactions were then stopped by the addition of 50 μl of 5 M NaOH to each well in the plate resulting in ELISA values approaching the 2.000 limit of the ELISA reader. In 1988, 16 negative check wells were used with the samples being used as positive check. A conjugate dilution of 1:1600 was used, and plates were incubated 17 min after the addition of substrate prior to adding the NaOH.

RESULTS AND CONCLUSIONS

All four methods of storage were found to be suitable for qualitative evaluation as indicated by moderate to high ELISA means obtained for each storage treatment within

paired experiments. ELISA absorbance means obtained when both leaf halves were stored in the same manner (data not shown) were not found to differ significantly ($P<0.05$). Thus, variation between leaf halves did not influence resulting means when leaf halves were stored differently. Absorbance means and the difference between mean pairs are given in Table 1. Mean differences for treatment pairs are illustrated in Fig. 1.

Changes in reagents, laboratory conditions, and maturity of host plants at time of sampling resulted in significant ($P<0.05$) diversity in mean differences between paired experiments, (Table 1; Experiments 1a-c, 4a-b, 6a-b, 7a-b, 8a-b). This diversity illustrates the need to analyze all samples at the same time. However, this inconsistency was a matter of degree and treatment effects within paired experiments remained.

ELISA absorbances were significantly lower ($P<0.05$) for leaf halves frozen at -20 °C, than for corresponding leaf halves stored by any other method (Table 1; Experiments 1a-c, 5a-7b and Fig. 1). These distinctions in treatment effect supported previous findings in other disease systems (Singh and Somerville 1983, and Ward *et al*. 1987). Singh and Somerville (1983) suggested that membrane disruption by ice crystals which formed during slow freezing at -20 C resulted in release of host enzymes that degrade virus antigen. Although the same, if not greater, damage probably

occurred during quick freezing at -70 °C (Levitt 1980a), host enzymes apparently did not accomplish similar amounts of degradation.

Use of dry weights when preparing desiccated leaf tissue for ELISA (DESD) consistently provided significantly greater ($P<0.05$) ELISA means than the use of fresh weights (DESF). (Table 1; Experiments 4a-b and Fig. 1). This is due to the concentration of virus antigen that occurs during desiccation, i.e. 0.1 g of fresh tissue would yield less virus than 0.1 g of the same tissue when desiccated because 67% of the fresh weight was water.

ELISA means for desiccated leaf halves (DESD and DESF) were significantly higher ($P<0.05$) than the ELISA means for corresponding frozen leaf halves (Table 1; Experiments 1a-c, 2a-c, 5a-b and 9). Although membrane and protein integrity is affected by desiccation (Levitt 1980b), these data suggest that host enzymes that degrade virus antigen during and/or following freezing are not released during desiccation. This hypothesis is further supported by the lack of appreciable differences between DESF ELISA means and CHIL ELISA means (Table 1; Experiments 9a-b). Variation in the data for Experiment 8b was apparently low enough that the ELISA mean 1.468 was statistically different from 1.413. However, this is not an appreciable difference in most ELISA data.

In a healthy plant cell, hydrophilic regions of plant proteins interact with the ionic water molecules and ions in the cytosol such that the hydrophilic regions remain exposed and hydrophobic regions are buried within the folds of the protein structure or within the hydrophobic region of cell membranes. During dehydration, changes occur in the plant cytosol that cause plant membranous proteins to separate from the membranes and causes these and other proteins to lose their integrity. The proteins essentially are turned inside-out as hydrophobic regions become exposed and hydrophilic regions aggregate internally. These proteins are often denatured permanently and aggregate by the formation of sulphur-sulphur bonds (Levitt 1980b).

Intact capsids of tobacco mosaic virus are thought to be stabilized by hydrophobic bonds more than by electrovalent or other types of bonds (Gibbs and Harrison 1976). Possibly the same is true of WSBMV. However, the capsid does not appear to lose integrity as a plant protein does but instead appears to remain intact during dehydration. Proteins of drought-hardened, frost-hardened and drought-tolerant plants are modified to reduce or avoid damage due to dehydration (Levitt 1980a and b). Since WSBMV retains infectivity and serological properties after desiccation, perhaps the capsid proteins are similarly modified.

There are some special considerations to using chemical desiccation: 1) The loss in water weight should be accounted for, i.e. obtain and use fresh weights or desiccate all samples before analysis. 2) Samples should be reasonably small and well spaced in the desiccator to allow aeration. Otherwise, the centres of large samples or tight bundles of samples may start to decay before desiccation. Leaf halves stored at -20 °C in a frost-free freezer started to smell of decay after 2-3 months, and after 6 months for whole leaves. However, leaf halves were stored in a desiccator at room temperature (20-25 °C) for 18 months without showing signs of decay or fungal growth. 3) Frozen samples must be assayed immediately upon removal from the freezer because the lysing of host membranes that occurs during freezing results in an eventual release of enzymes that degrade virus antigen. These enzymes do not appear to be released during desiccation. Therefore, virus antigen titres in desiccated leaf tissue should be stable indefinitely.

Refrigeration at 4 °C, freezing at -20 °C or -70 °C, and chemical desiccation at room temperature were all found to be suitable methods for short-term (0-5 days) storage of leaf tissue infected with WSBMV prior to analysis by ELISA. Freezing leaf tissue and chemical desiccation are suitable for medium-term (0-3 months) storage but chemical desiccation is a superior method for long-term storage. All

4 methods of storage are suitable for qualitative evaluation by ELISA but chemical desiccation is a superior method for storage of leaf tissue prior to quantitative evaluation by ELISA.

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Table 1. Comparisons of enzyme-liked immunosorbent assay (ELISA) absorbance (A_{405}nm) means from paired experiments on leaves of winter wheat cv. Sage and Vona infected with wheat soilborne mosaic virus.

EXPERIMENT	TREATMENT PAIR[w]	N[x]	ELISA VALUE MEANS[y]	T1-T2[z]
#1a	T1=DESD	6	1.708 *	
	T2=FR20		1.406	0.302 ab
#1b	T1=DESD	7	1.758 *	
	T2=FR20		1.584	0.174 a
#1c	T1=DESD	8	1.945 *	
	T2=FR20		1.393	0.551 b
#2a	T1=DESD	8	0.908 *	
	T2=FR70		0.565	0.343 a
#2b	T1=DESD	8	1.649 *	
	T2=FR70		1.206	0.443 a
#2c	T1=DESD	8	1.727 *	
	T2=FR70		1.312	0.415 a
#3a	T1=DESD	12	1.618 *	
	T2=CHIL		1.318	0.300 a
#3b	T1=DESD	8	0.883 *	
	T2=CHIL		0.568	0.315 a
#4a	T1=DESD	8	1.417 *	
	T2=DESF		1.054	0.363 a
#4b	T1=DESD	8	0.835 *	
	T2=DESF		0.735	0.100 b
#5a	T1=DESF	8	1.692 *	
	T2=FR20		1.081	0.610 a
#5b	T1=DESF	8	1.666 *	
	T2=FR20		0.962	0.704 a
#6a	T1=CHIL	8	2.097 *	
	T2=FR20		1.671	0.426 a
#6b	T1=CHIL	8	1.543 *	
	T2=FR20		1.294	0.249 b
#7a	T1=FR70	8	0.872 *	
	T2=FR20		0.699	0.173 a
#7b	T1=FR70	8	1.590 *	
	T2=FR20		1.473	0.117 b
#8a	T1=DESF	8	1.225	
	T2=CHIL		1.104	0.121 a
#8b	T1=CHIL	8	1.468 *	
	T2=DESF		1.413	0.055 b
#9	T1=DESF	7	1.550 *	
	T2=FR70		1.364	0.186
#10	T1=CHIL	8	1.674 *	
	T2=FR70		1.332	0.343

[w] Leaves were cut in half along the midrib, and each half was treated in one of the following ways: DESD -- Leaf halves were desiccated and dry weights used for dilution with buffer (w/v) for ELISA, FR20 -- Leaf halves stored at -20 °C; dilution based on fresh weights, FR70 -- Leaf halves stored at -70 °C; dilution based on fresh weights, CHIL -- Leaf halves stored at 4 °C; dilution based on fresh weights, or DESF -- Leaf halves desiccated; dilution based on fresh weights.

[x] Number of leaves tested.

[y] * denotes a significant difference (Fisher's LSD, P=0.05) for the within-experiment comparison.

[z] Means followed by the same letter are not significantly different (Fisher's LSD, P=0.05) for comparisons of experiments within a treatment pair. (ex. Experiment 1b is significantly different from Experiment 1c within the DESD/FR20 treatment pair.)

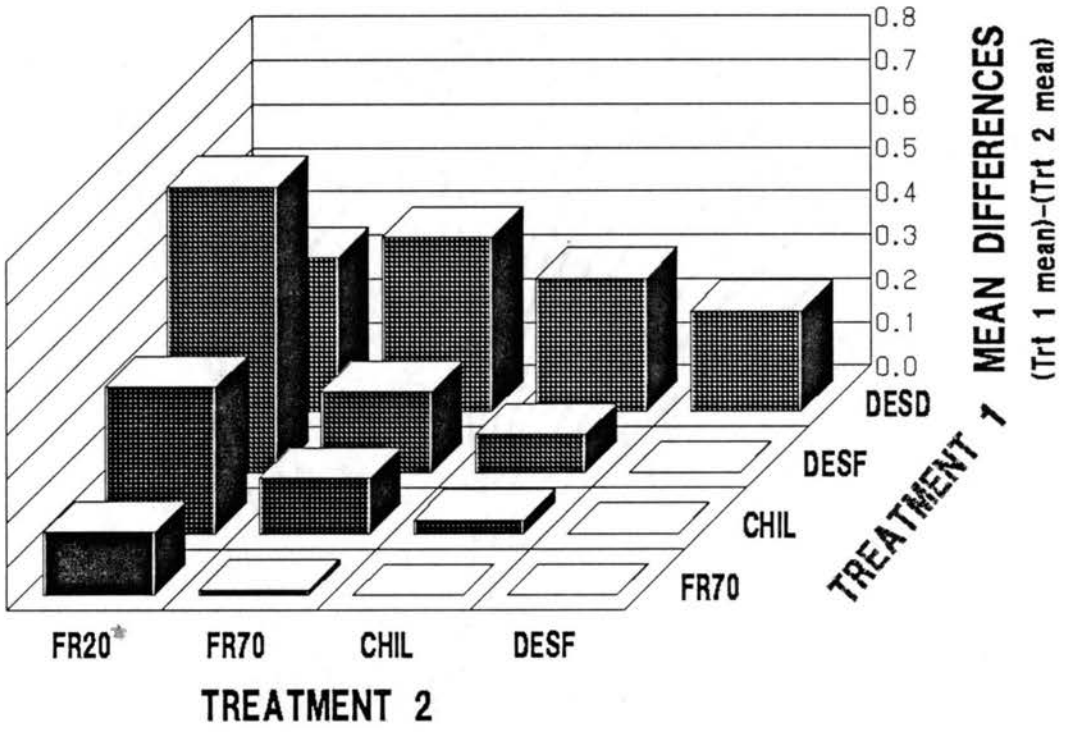

Figure 1. Differences for ELISA absorbance (A_{405}nm) means from paired experiments on leaf halves of winter wheat cvs. Sage and Vona infected with WSBMV.

* Leaves were cut in half along the midrib, and each half was treated in one of the following ways: DESD -- Leaf halves were desiccated and dry weights used for dilution with buffer (w/v) for ELISA, FR20 -- Leaf halves stored at -20 °C; dilution based on fresh weights, FR70 -- Leaf halves stored at -70 °C; dilution based on fresh weights, CHIL -- Leaf halves stored at 4 °C; dilution based on fresh weights, or DESF -- Leaf halves desiccated; dilution based on fresh weights.

CHAPTER IV

RELATIONSHIP BETWEEN ONTOLOGICAL DEVELOPMENT OF HARD RED WINTER WHEAT AND EXPRESSION OF RESISTANCE TO WHEAT SOILBORNE MOSAIC VIRUS.

C. R. ARMITAGE, R. M. HUNGER, J. L. SHERWOOD, and D. L. WEEKS.

Graduate Research Assistant, Associate Professor, Associate Professor, Department of Plant Pathology, and Professor, Department of Statistics, Oklahoma State University, Stillwater, OK 74078-9947, USA

ABSTRACT

Armitage, C. R., Hunger, R. M., Sherwood, J. L., and Weeks, D. L. 1989. Relationship between ontological development of hard red winter wheat and expression of resistance to wheat soilborne mosaic virus. Plant Disease 73:

Expression of resistance to wheat soilborne mosaic (WSBM) in field plots of susceptible cvs. Sage and Vona, and resistant cvs. Newton and Hawk was evaluated by polyacrylamide gel electrophoresis, enzyme-linked immunosorbent assay (ELISA), and visual assessment. All three evaluations showed that cvs. Newton and Hawk became infected by wheat soilborne mosaic virus (WSBMV), however, virus concentration and disease development differed between susceptible and resistant cultivars. Symptoms were

strongest for all cultivars during early jointing in February and March. Symptoms waned from April through senescence in June. High ELISA values (A_{405}nm) were obtained from susceptible cvs. Sage and Vona from February through May. However, the highest ELISA values (A_{405}nm) were not obtained from resistant cvs. Newton and Hawk until late in the growing season, April and May, respectively. The highest virus concentrations obtained from cvs. Hawk, Sage and Vona were found in April, and from cv. Newton in May. The cv. Vona responded similarly when maintained in a growth chamber at 20/15 C with a 11/13 hr photoperiod. However, the late-season rise in ELISA values (A_{405}nm) in Newton were better related to tiller maturity than to sampling date.

Winter wheat (Triticum aestivum L.) is thought to be inoculated with wheat soilborne mosaic virus (WSBMV) following infection of seedling roots by the fungal vector Polymyxa graminis Ledingham during cool, wet periods primarily in the autumn (Brakke and Estes 1967, Rao and Brakke 1969). A mottling of the leaves is visible in susceptible cultivars as early as November, but generally is not seen until spring (Brakke, et al 1965). Disease development appears to be favored by early spring temperatures below 15 - 17 C, and late-maturing susceptible cultivars recover from disease symptoms in late spring (Eversmeyer, et al. 1983).

Visual assessment of wheat is the most commonly used method to evaluate resistance to wheat soilborne mosaic (WSBM), but other factors may mimic macroscopic symptoms of WSBM. Fertility and other viruses can induce chlorosis similar to the mosaic associated with WSBM, (Brakke, et al. 1965; Hunger and Sherwood, 1985a), and P. graminis may cause stunting (Teakle, 1969). This mimicry of symptoms presents a need to confirm visual assessments in programs breeding for resistance to WSBM.

Enzyme-linked immunosorbent assay (ELISA) has been used in conjunction with visual assessment to verify the presence of WSBMV. However, during late spring, ELISA values (absorbance at 405 nm) from resistant cultivars often increase to levels comparable to ELISA values in susceptible cultivars (Hunger and Sherwood, 1985b). Thus, we previously determined that, in north central Oklahoma, February is the optimum month to sample wheat for evaluation of resistance to WSBM with ELISA (Armitage, et al. 1988). The objective of this study was to use symptomology, ELISA, and polyacrylamide gel electrophoresis (PAGE) to examine the relationships between visual symptoms, capsid production, and virion concentration, in order to better understand the nature of resistance by hard red winter wheat to WSBM.

MATERIALS AND METHODS

Field. Four replicate field plots of two hard red winter wheat cultivars resistant to WSBM (cvs. Hawk and Newton) and two susceptible cultivars (cvs. Vona and Sage) were planted in a locale with a history of severe WSBM. Each plot consisted of five 3 m rows, solid seeded with 100-150 kernels per row. Plots were assessed six times during 1988, (Feb. 14, March 1 and 24, April 8 and 21, and May 6, 1988) on a scale of 0=no mosaic or stunting, 1=mild mosaic and little or no stunting, 2=moderate mosaic and stunting, and 3=severe mosaic and stunting.

Leaves were collected at random from the second and fourth rows of each plot twelve times, commencing in Nov. 1987 and ending in May 1988. From each sample, 5 g subsamples of 5-8 cm leaf segments were taken. These leaf segments were passed through a Leaf Squeezer (Piedmont Machine & Tool Inc., Box 109, Six Mile, SC 29682) and expressed sap was rinsed into 50 ml grinding buffer (0.5 M sodium borate, pH 9, with 0.001 M EDTA, 1:10 w/v dilution) to produce a stock sap solution.

Virus Extractions. Aliquots of 21 ml were removed from each stock sap solution for virus extraction. Virus extraction was performed as previously described (Hunger and Sherwood 1985a). These viral extracts were stored at -20 C until run against standards in PAGE to determine the relative absorbance (A_{595}nm) and estimate virus concentration

as previously described (Hunger and Sherwood 1985a). Six samples and four standards (20, 10, 5, and 2.5 μg virus/well) were applied in 100 μl aliquots to each gel. The twelve samples of a cultivar/replicated plot combination were run concurrently, each sample being run on two gels. Samples were randomly distributed among the wells of a gel. Mean absorbances (A_{595}nm) were plotted against virus concentrations for the standards. This plot was used to estimate virus concentration for the samples.

ELISA Procedures. The remaining stock sap solutions were stored at -20 C until all sampling had been completed. All sap samples were analyzed concurrently by ELISA. Aliquots of stock sap solution were diluted (1:10 v/v) with sample buffer, (phosphate buffered saline with 2% polyvinyl-pyrrolidone and 0.05% Tween 20), applied in 200 μl aliquots to five wells on each of two ELISA plates.

Rabbit polyclonal antiserum was prepared to WSBMV and ELISA was performed using standard flat bottom plates by the direct double antibody sandwich procedure described previously (Bahrani, et al. 1988). Alkaline phosphatase labeled IgG conjugate was used at a 1:1600 dilution. The reaction was stopped by the addition of 50 μl aliquots of 5 M NaOH 30 min after the addition of substrate. Absorbance values were measured at 405 nm with an EIA READER (Model EL-307, Bio-Tek Instruments, Inc., Laboratory Division, Burlington, VT 05401, U.S.A.). Sixteen wells on each ELISA

plate contained aliquots of sap extracted from virus-free leaves of cv. Blue Jacket, and the mean absorbance from these wells was used as a negative check to correct for background absorbance for each plate.

Growth chamber. Seed of cvs. Newton and Vona were soaked in a 0.26% sodium hypochlorite solution for 15 min on a reciprocal shaker at room temperature. Seeds were rinsed 3 times with double distilled water, placed on filter paper in polyethylene petri dishes, saturated with double distilled water, and allowed to germinate on a bench top under laboratory conditions. Germinated seeds were then planted into a clay loam soil collected in September from an area with a history of severe WSBM. Seeds were separated by standard Monarch plant bands (5.6 x 3.8 x 3.8 cm) in wooden flats (51 x 38 cm). Four flats of each cultivar were planted and maintained in a Conviron Plant Growth Chamber - PGW36 under fluorescent and incandescent lights (180 μE M^{-2} sec^{-1} at plant level) at 15/10 C (11/13 hr day/night). Photoperiod remained constant throughout the experiment. Seedlings were trimmed to 5 cm to enhance foliar infection (Rao and Brakke 1969) after reaching growth stage 4 on the Feekes' Scale (Large 1954). When the seedlings reached growth stage 5, temperatures were reduced to 5 C for vernalization. After six weeks, seedlings were transplanted into a 1:1:1 (v/v/v) peat-sand-soil mix in glazed 3.785 L clay pots, 3 plants to a pot, 44 pots per cultivar. Potted

plants were returned to the growth chamber and kept at 5 C for three days after transplanting, then raised to 7/5 C (day/night) for three days, 10/7 C for eight days, 15/10 C for six days, and 20/15 C for the duration of the experiment. Negative checks were treated similarly except for being planted in a steamed mixture of soil-peat-sand (1:1:1, v/v/v) and maintained in separate drainage pans throughout the experiment to avoid infection by WSBMV.

Leaves were collected at random from seedlings of each flat before vernalization and again during the day of transplanting. Samples were collected six additional times commencing one week after growth chambers were set at 20/15 C and ending at senescence. Four pots of each cultivar were sampled from each growth chamber, three with infested soil and one check. The youngest two or three leaves were collected from tillers of similar maturity. On the last three sampling dates a wide range of maturities existed between tillers within individual pots, so tillers were sampled according to maturity. Samples from plants maintained in growth chambers were processed, stored, and analyzed on the same weight-to-volume basis as samples collected from the field.

RESULTS AND DISCUSSION

Field. Frequently, symptoms were not uniform between plants within replicated plots. Differences in visual assessments, ELISA values (A_{405}), and virus concentrations

were sometimes significant ($P<0.05$) among replicated plots of the same cultivar (data not shown). These inconsistencies suggest that natural infection was not uniform among replicated plots within a field nor among plants within individual plots. This lack of uniformity of infection may have been due to non-uniform irrigation, drainage, or inoculum densities across the field although efforts were made to prevent these from being factors in the experiment. Infection of foliage within plants was also inconsistent. For example, within samples collected concurrently, the highest ELISA values were sometimes obtained from the youngest leaves, second youngest, or the third youngest leaves of different plants (data not included). Due to this non-uniformity which was observed over several growing seasons, foliage samples of 6 - 10 g per 6 row-meters consisting of random leaves were collected for evaluation.

All three evaluations showed that resistant cvs. Newton and Hawk became infected by WSBMV, however, virus concentration and disease development differed between susceptible and resistant cultivars (Table 2). Virus concentrations, ELISA values (A_{405}nm), and visual assessments were often significantly different ($P<0.05$) for cvs. Hawk and Newton, and for cvs. Sage and Vona (Table 2). Symptoms were most pronounced during jointing in March. High ELISA values (A_{405}nm) were obtained from susceptible cvs. Sage and

Vona during February but these values remained high into May after the mosaic had partially faded. The highest ELISA values (A_{405}nm) obtained from cvs. Newton and Hawk were in April and May, respectively. The highest virus concentrations were found in April for cvs. Newton, Sage and Vona and in May for cv. Hawk (Table 2). Multivariant analyses of variance were performed for ELISA and PAGE data by sampling dates. Although the late-season rise in viral antigen measured by ELISA in resistant cultivars might have been due, in part, to the rise in pelletable virions, the partial correlation coefficients for these data were low, -0.043319 (0.19%) for cv. Hawk and -0.061453 (0.38%) for cv. Newton. The partial correlation coefficients for the susceptible cultivars in field plots were also low, 0.079768 (0.69%) for cv. Sage and -0.056826 (0.32%) for cv. Vona. Capsid protein production is apparently favored during more of the growing season than is viral assemblage. Although ELISA detects the presence of WSBMV, it is not necessarily a good indicator virion concentration.

Growth chamber. No late-season rise in ELISA values was found in resistant cv. Newton when data were averaged by sampling date (Table 3). However, when ELISA values were averaged by maturity of the tillers in each sample, Newton showed a late-season rise (Table 4). Changes in photoperiod and the high temperatures that occur in the field late in the growing season were not present in the growth chamber

and, therefore, do not account for the late-season rise in ELISA values. The late-season rise in ELISA values and virus concentration in disease resistant cultivars in field plots (Table 2) may be related to a reduced rate of viral activity and/or assemblage (Hunger, et al. 1989) but this explanation does not fit the pattern of ELISA values and virion concentrations found in the growth chamber (Tables 3 and 4). Virus concentrations were moderately high in Newton prior to and immediately following cold treatment, dropped during jointing, and rose substantially during and/or following anthesis. Thus, the late-season rise in ELISA values observed in resistant cultivars may be dependant upon changes in host physiology associated with maturation and senescence.

Eversmeyer et al (1983) theorized that fading of the mosaic symptom of WSBM which occurs in late spring is due to the rise in temperatures that also occurs at this time. Brakke, et al (1965) reported that seedlings maintained in a controlled environment developed pronounced leaf symptoms at 15 C, less pronounced leaf symptoms at 20 and 25 C, and faint, transient leaf symptoms in few plants at 30 C. Yet, in our study mosaic faded almost completely in Vona and completely in Newton during jointing in the growth chamber set at 20 C and constant photoperiod. This suggests that the disappearance of foliar symptoms may not entirely result from the change in photoperiod or high temperatures that

occur in late spring and summer. Chlorophyll content in wheat increases as plants approach "sexual maturity" then falls rapidly after heading (Whyte 1948). Perhaps this natural increase in chlorophyll content partially or completely masks the mosaic of WSBM. Further work is indicated to more fully explain the interaction between changes in host physiology associated with vernalization, maturation and senescence, and symptom expression, virus replication and virus assembly.

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Table 2. Comparisons of virus concentration means, ELISA absorbances, and visual assessments for foliar samples of winter wheat collected during the 1987-88 growing season from a locale with a history of severe WSBM.

	VIRUS CONCENTRATION MEANS (μg Virus/g Fresh Leaf Tissue)[u]						ELISA VALUE MEANS[v]						VISUAL ASSESSMENT MEANS[w]					
	RESISTANT CULTIVARS			SUSCEPTIBLE CULTIVARS			RESISTANT CULTIVARS			SUSCEPTIBLE CULTIVARS			RESISTANT CULTIVARS			SUSCEPTIBLE CULTIVARS		
DATE	HAWK		NEWTON	SAGE		VONA	HAWK		NEWTON	SAGE		VONA	HAWK		NEWTON	SAGE		VONA
1987, Nov. 13	0.383	*[x]	0.327	0.591		0.415	0.018		0.023	0.027	*	0.019	--[z]		--	--		--
1987, Dec. 10	3.392	*	4.862	8.970	*	2.086	0.043		0.032	** 1.796	*	0.874	--		--	--		--
1987, Dec. 30	4.234	*	7.199	3.028	*	4.648	-0.004		-0.001	** 1.535	*	1.051	--		--	--		--
1988, Jan. 17	2.525	*	9.020	**[y] 8.267	*	10.746	0.005		-0.004	** 1.837	*	1.437	--		--	--		--
1988, Jan. 30	2.111	*	8.392	** 32.430	*	15.996	0.017	*	0.129	** 2.093	*	1.791	--		--	--		--
1988, Feb. 13	4.950		6.319	** 25.347	*	10.296	0.068		0.040	** 1.775	*	1.402	0.16	*	0.42	** 1.58	*	1.16
1988, Feb. 27	2.474	*	7.249	** 12.812	*	19.429	0.105	*	0.341	** 1.970	*	1.829	--		--	--		--
1988, Mar. 10	4.171	*	10.465	** 19.475	*	36.437	0.156	*	0.424	** 2.107		2.135	0.75	*	1.00	** 2.08		2.08
1988, Mar. 24	3.920	*	10.050	** 19.118	*	24.554	0.272	*	0.412	** 2.079	*	1.998	0.58	*	0.92	** 2.25		2.25
1988, Apr. 8	5.302	*	8.505	** 46.922	*	21.634	0.293		0.301	** 1.962	*	1.884	0.50	*	0.58	** 1.66		1.67
1988, Apr. 21	5.113	*	20.299	** 38.161	*	43.986	0.348	*	0.614	** 2.115		2.079	0.24		0.33	** 1.33		1.24
1988, May 6	7.299	*	11.212	** 30.581	*	38.130	0.895	*	0.566	** 1.975		2.026	0.33	*	0.66	** 1.66	*	1.24

[u] Leaves were collected at random from the second and fourth rows in each of four, 5-row plots for each cultivar. Sap from 5 g foliage subsamples was expressed into 50 ml 0.5 M sodium borate with 0.001 M EDTA to produce 1:10 (w/v) dilution stock sap solutions. Aliquots of 21 ml stock sap solution were used for virus extractions. Virus extracts were stored at -20 °C until assayed by acrylamide gel electrophoresis. Four standards (20, 10, 5, and 2.5 μg virus/well) were run on each gel and mean absorbances (A_{595}nm) were plotted against virus concentration for the standards. This plot was used to determine the mean virus concentration for each sample.

[v] Stock sap solution was stored at -20 °C until all sampling was completed, thawed and assayed by ELISA. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates for each of four replicated field plots.

[w] Visual assessment means are averages from four replicated field plots, rated with a scale of 0=no mosaic or stunting, 1=mild mosaic and little or no stunting, 2=moderate mosaic and stunting, and 3=severe mosaic and stunting.

[x] * Means from the two cultivars are significantly different (Fisher's LSD P=0.05).

[y] ** Means for the resistant cultivars are significantly different from the means for the susceptible cultivars (Fisher's LSD P>0.05).

[z] Visual assessment was not made.

Table 3. WSBMV virion concentrations and ELISA values for foliar samples of resistant cv. Newton and susceptible cv. Vona.

	NEWTON		VONA	
DATE	VIRUS[v]	ELISA[w]	VIRUS	ELISA
Jan. 10[x]	3.530	0.355	7.689	1.216
March 14[y]	4.849	0.139	31.004	1.548
April 10[z]	0.352	-0.003	13.961	0.869
April 24	0.504	-0.008	3.631	1.055
May 8	0.654	0.120	4.724	0.761
May 15	3.669	0.322	37.913	1.181
May 24	1.860	0.125	52.203	1.020
June 9	0.716	0.134	6.332	1.079

[v] Foliar samples of leaves of similar age from tillers of similar maturity were collected, and sap was expressed into 0.5 M sodium borate with 0.001 M EDTA to produce a 1:10 (w/v) dilution stock solution. Aliquots of 21 ml stock sap solution were collected for virus extractions. Virus extracts were stored at -20 °C until assayed by acrylamide gel electrophoresis. Four standards (20, 10, 5, and 2.5 μg virus per well) were run on each gel and mean absorbances (A_{595}nm) were plotted against virus concentration for the standards. This plot was used to determine the mean virus concentration for each sample.

[w] Stock sap solutions were stored at -20 °C until all sampling was completed, thawed and assayed by ELISA. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates.

[x] Germinated seeds were planted in soil from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. Sampling one this date was just prior to six weeks at 4 °C.

[y] Sampling on this date followed six weeks at 4 °C and corresponded to the transplanting of seedlings to 3.785 L pots, 3 seedlings per pot.

[z] Potted plants were kept at 5 °C for three days after transplanting, then raised to 7/5 °C (day/night) for three days, 10/7 °C for eight days, 15/10 °C for six days, and 20/15 °C for the duration of the experiment. Data means are averages for 3 pots per cultivar. Sampling this date is following 1 week at 20/15 °C day/night, when plants were jointing.

Table 4. Means of ELISA absorbances averaged by maturity of tillers in foliar samples from resistant cv. Newton and susceptible cv. Vona.

TILLER MATURITY OF SAMPLE[w]	NEWTON	VONA
5 (before vernalization)[x]	0.355	1.216
5 (after vernalization)[y]	0.139	1.548
6 - 7[z]	-0.003	0.869
7 - 9	-0.008	1.055
9 - 10.5	0.102	0.958
10 - 11.1	0.228	1.033

[w] Foliar samples of leaves of similar age were collected from tillers of similar maturity according to Feekes' Scale, and sap was expressed into 0.5 M sodium borate with 0.001 M EDTA to produce a 1:10 (w/v) dilution stock solution. Stock sap solutions were stored at -20 °C until all sampling was completed, thawed and assayed by ELISA. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates.

[x] Germinated seeds were planted in soil from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. This sampling was just prior to six weeks at 4 °C. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates for each sample.

[y] Sampling on this date followed six weeks at 4 °C and corresponded to the transplanting of seedlings to 3.785 L pots, 3 seedlings per pot.

[z] Growth chambers were kept at 5 °C for three days after transplanting, then raised to 7/5 °C (day/night) for three days, 10/7 °C for eight days, 15/10 °C for six days, and 20/15 °C for the duration of the experiment. Data means are averages for 3 pots per cultivar and growth chamber. Sampling this date is following 1 week at 20/15 °C day/night, when plants were jointing.

CHAPTER V

SUMMARY AND CONCLUSIONS

Resistance to wheat soilborne mosaic (WSBM) is typically measured by rating the severity of mottling and stunting which occurs following natural infection. Mimicry of these symptoms by other viruses and physiological conditions has resulted in the need for an additional method of evaluation to verify visual ratings. Enzyme-linked immunosorbent assay (ELISA) is used as such an assessment.

Foliar samples collected at various times during a growing season are frequently stored prior to evaluating resistance to WSBM by ELISA. Leaves of winter wheat cvs. Sage and Vona, showing symptoms of WSBM, were cut in half along the midrib. Each half was either: 1) refrigerated at 4 °C, 2) frozen in a frost-free freezer at -20 °C, 3) frozen at -70 °C, or 4) desiccated with $CaCl_2$. Relative virus antigen titers were determined for individual leaf halves by ELISA. Means of ELISA absorbances (A_{405}nm) from desiccated leaf halves were consistently higher than absorbance means from corresponding leaf halves that had been frozen. This suggests that virus antigen decreases during freezing but is retained during chemical desiccation. All four methods of storage were suitable for short-term storage prior to qualitative evaluations by ELISA, but chemical desiccation

was the superior method for long-term storage and for storage of foliar samples prior to quantitative evaluations by ELISA.

Qualitative resistance to WSBM has not been reported. Cultivars that are considered to be resistant under conditions which favor natural inoculation show mottling and stunting later than cultivars considered to be susceptible. Mottling and stunting remain less severe in resistant plants than in susceptible plants while incidence of infection is also repressed. A common assumption is that visual symptoms correspond to virus activity and/or virion concentration. However, virus antigen as measured by ELISA, frequently increases in resistant cultivars while mottling is fading. Sometimes this increase in virus antigen is equivalent to the levels found in susceptible cultivars. This late-season rise in virus antigen has also been observed in a growth chamber under constant temperature and photoperiod.

Ideally, several grams of foliage are collected from replicated field plots several times during January through March to evaluate host resistance to WSBM by ELISA. However, a wheat breeder testing F2 or F3 plants may have limited tissue to sacrifice for ELISA. Furthermore, testing large numbers of lines is not conducive to repeated samplings and assays. To facilitate evaluations, the optimum month to collect foliar samples for ELISA was determined. ELISA value (A_{405}nm) curves were established for

winter wheat cvs. Hawk and Newton (resistant to WSBM) and cvs. Sage and Vona (susceptible to WSBM) and analyzed for three growing seasons. Field plots of cv. Vona did not show consistent infection as late as January 30 in the 1987-88 growing season. However, ELISA values for cv. Newton sometimes approached those of susceptible cultivars in March. During February, ELISA values were consistently higher in the susceptible cultivars than in the resistant cultivars. Based on these data, February was identified as the optimum month for sampling foliar tissue in north central Oklahoma for evaluation of resistance to WSBM by ELISA. Thus, researchers who are comparing cultivars for selection purposes for resistance to WSBM using ELISA should sample wheat foliage in February and chemically desiccate samples prior to storage so as to maximize observed differences in susceptible and resistant cultivars.

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APPENDIXES

APPENDIX A

EFFECTS OF WHEAT SOILBORNE MOSAIC ON HARD RED WINTER WHEAT CULTIVARS

R. M. HUNGER, C. R. ARMITAGE, and J. L. SHERWOOD:

Associate Professor, Graduate Research Assistant and Associate Professor, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947

ABSTRACT

Hunger, R. M., Armitage, C. R., and Sherwood, J. L. 1989. Reaction of hard red winter wheat to wheat soilborne mosaic virus. Plant Disease 73:

The effects of wheat soilborne mosaic (WSBM) on 13 hard red winter wheats was evaluated using visual disease severity assessment, the enzyme linked immunosorbent assay (ELISA), and determination of virion concentration. Evaluations were conducted for two seasons by growing the wheats in a location with no history of the disease and in a location with a history of severe WSBM. Resistant cultivars (Hawk, Newton, Mustang, Plainsman V, and Tam 108) consistently demonstrated lower disease severity and lesser reductions in height, grain yield, and thousand kernel weight (TKW) than susceptible cultivars (Chisholm, Sage, Tam 101, Vona, Danne, Payne, Tam 105, and Triumph 64). The cultivar Mustang demonstrated the least reduction in number

of tillers (8.3%), height (4.2%), grain yield (31.6%), and TKW (0.8%). ELISA was useful to insure the presence of the virus, and results suggest that some mechanism(s) inhibits or slows capsid protein production and virion accumulation or production in cultivars resistant to WSBM.

Wheat soilborne mosaic virus (WSBMV), which causes a serious disease on wheat, is considered to be vectored by the soilborne fungus Polymyxa graminis (Estes and Brakke 1966, Rao and Brakke 1969). Wheat soilborne mosaic (WSBM) was first reported in the United States in the eastern soft red winter wheat growing areas (McKinney 1937), but since has become one of the major virus diseases of hard red winter wheat in the Plains States.

The first record of WSBM in Oklahoma was in 1952, and reductions in yield of 32 to 61% and reductions in test weight of 0 to 3% were reported (Wadsworth and Young 1953). Subsequent studies have investigated the effects of WSBMV on various aspects of winter, spring, and durum wheat production. Bever and Pendleton (1954) reported reductions of grain yield ranging from 0 to 85% from 25 winter wheats cultivated in both a location with a history of WSBM and a location with no previous history of the disease. Roane, Starling, and McKinney (1954) reported yields of 49 and 13.5 bu/acre by 'Atlas 50' in areas without and with a history of WSBM, respectively. Kucharek and Walker (1974) reported

that yield losses varied from 42 to 52.5% in infected areas of commercial fields in Florida, and Palmer and Brakke (1975) attributed reduced yields from WSBM ranging from 6.2 to 44.4% (average=20%) in 11 of 13 fields observed over 3 years. More comprehensive studies conducted during the 1970's examined the effects of WSBMV on several aspects of wheat production. Campbell, et al. (1975) reported reductions in grain yield, tiller number, kernel weight, test weight and plant height in a study conducted at four locations over three years. Environment significantly affected all characters studied and environment X variety interactions were significant. A subsequent study by Nykaza, et al. (1979) examined these same parameters over 5 years using five near-isogenic lines and the two wheat cultivars (cvs) Centurk and Eagle. Reductions in grain yield, kernel weight, tiller number, test weight, and plant height averaged 22.0, 11.8, 11.8, 3.4, and 4.7%, respectively, and losses due to WSBM varied considerably from season to season and by location. Eversmeyer, et al. (1983) compared winter wheats cultivated in areas showing slight or severe WSBM symptoms in Kansas in 1973, and reported increases of 6.6 to 87.9%, -4.6 to 25.5% and 5.3 to 50.4% in grain yield, thousand kernel weight, and heads/m^2, respectively from wheats in the area with slight symptoms. Vallega and Rubies-Autonell (1985) examined the reaction of durum wheats in Italy to WSBMV and reported grain yield

losses as high as 70% in the more susceptible cvs. Test weight, plant height, and weediness also were affected with grain number per square meter rather than kernel weight accounting for most of the yield reduction.

The results from all of these studies indicate that WSBMV affects yield and growth parameters of wheat. However, no studies have been published which report the effects of WSBMV on cvs. of hard red winter wheats currently being grown in the Central and Southern Plains States and which have been released since the mid-1970s. Also, no studies have been reported that address the use of enzyme-linked immunosorbent assay (ELISA) and isolation of pelletable virions to monitor reaction of wheat cvs. to WSBMV in the field, although these techniques have been used in growth chamber and greenhouse studies of WSBMV (Hunger and Sherwood 1985). Development of ELISA (to monitor capsid protein production) and isolation of virions (to monitor virus production) should facilitate studies elucidating mechanisms of resistance to WSBMV and the extent of replication once the virus has entered the plant. Therefore, this study was initiated to determine the effect(s) of WSBMV on selected hard red winter wheats adapted to the Central and Southern Plains, and to evaluate the reaction of these cvs. to WSBMV using symptomatology, ELISA, and virion concentration.

MATERIALS AND METHODS

Experiment location and design. Trials were conducted at the Plant Pathology Department Experimental Farm west of Stillwater, OK during 1984-85 and 1985-86. Plots were planted in two locations approximately 150 m apart and situated so neither area drained into the other. One location had a consistent history of severe WSBM (Norge loam) and the other location had no previous history of the disease (Easpur loam). Soil tests for pH, nitrogen, phosphorous and potassium were conducted each year in each location. Preplant fertilization and/or liming were used to provide appropriate pH and N-P-K for wheat production in north central Oklahoma and to result in comparable fertility for the two locations. Additional nitrogen (38.1 kg/ha, in the form of ammonium nitrate) was topdressed onto each area in March or early April of each season. Glean (chlorosulfuron, E. I. duPont deNemours and Co., 9.46 g in 18.9 L per 0.405 ha) was applied in the fall to control weeds, and Bayleton (triadimefon, Mobay Chemical, 113.5 g in 14.2 L per 0.405 ha) was used during the spring as needed to maintain a low incidence of foliar fungal diseases. Plots were planted during September in each season and irrigated with 2.5 to 5.1 cm of water as coleoptiles were emerging through the soil.

Ten hard red winter wheat (HRWW) cvs. (Hawk, Newton, Chisholm, Sage, Tam 101, Vona, Danne, Payne, Tam 105, and

Triumph 64) were tested in 1984-85. Twenty kernels of each cv. were planted into each of two, 5 m rows replicated 5 times in each location in early September 1984. Early in March 1985 replications were thinned to 10 plants, or contained 7-9 plants as a result of poor emergence. Nine HRWW cvs. were evaluated in 1985-86. Six (Hawk, Newton, Chisholm, Sage, Tam 101, and Vona) had been evaluated in 1984-85, and three (Mustang, Plainsman V, and Tam 108) were evaluated for the first time. Agronomic practices and treatment of plots in 1985-86 were the same as in 1984-85; however, in 1985-86 plot design differed. Plots in each location consisted of three, 30.05 m rows, solid planted, with 4 replications per cv.

Disease assessment. In 1984-85, individual plants (considered subsamples for each replication) were assessed for disease reaction on 3-14-85, 3-28-85, and 4-15-85 using a scale of 0=no mosaic or stunting symptoms present, 1=slight mosaic present and slight stunting may or may not be present, 2=moderate mosaic with some stunting, and 3=severe mosaic and stunting present. A disease severity index (DSI) was calculated for each cv. in each area using the formula of Sherwood and Hagedorn (1958). In 1985-86, severity of WSBM in the middle row of each replication was determined on 3-20-86 using the same scale as described for 1984-85; however, the entire row rather than individual plants were evaluated and no DSI was calculated.

In 1984-85, foliage for evaluation by ELISA and isolation of pelletable virions was collected from each plant after each visual assessment, combined by replication and stored at -20 C until evaluations could be conducted. Collections were made sufficiently early in the season so that no flag or flag minus-1 leaves were collected. Following the ELISA evaluation of foliage by replication, foliage was combined from replications of each cv. in each location and evaluated again by ELISA. In 1984-85, ELISA values obtained from averaging replications resulted in the same information regarding the presence or absence of WSBMV capsid protein as did analyzing the combined samples of 5 replications. Thus, samples for analysis by ELISA in 1985-86 were obtained by combining foliage collected from the outer two rows of each replication 9 times starting in November 1985 and ending in May 1986 and reserving the middle row to collect data pertaining to grain yield. ELISA was conducted in both years using a direct sandwich ELISA with polyclonal antibodies as previously described (Bahrani, et al. 1988). Isolation and determination of concentration of pelletable virions by electrophoresis was conducted as previously reported (Hunger and Sherwood 1985) using the cvs. Hawk, Newton, Sage, and Tam 101 from the 1984-85 season.

Grain yield and thousand kernel weight were obtained for each replication in 1984-85 by combining grain harvested

from individual plants and using the mean for the value of the replication. Results were analyzed using an unpaired t-test to compare the grain yield and thousand kernel weight from the same cv. grown in the two different locations. Regression analysis was conducted to determine the usefulness of visual assessment of symptoms (disease severity index) and ELISA for predicting the effect of WSBMV on yield. For this analysis, data from the ten plots (5 from each location) were used for each cv, and analysis determining coefficients of determination were obtained for each assessment date. In 1985-86, the middle row (not sampled for ELISA) was used to gather data pertaining to tiller count, plant height, grain yield, and thousand kernel weight. These parameters were analyzed using an unpaired t-test as described for 1984-85.

RESULTS AND DISCUSSION

Symptoms indicative of WSBM were uniformly observed on plants in both years in the location with a history of severe WSBM. Non-uniform symptoms of WSBM were observed in the location with no history of the disease which resulted in considerable statistical variation; however, disease severity in plots in the location with no history of the disease were consistently lower (especially for the resistant cultivars) than in the plots in the location with a history of severe WSBM (Table 5 and 6). Grain yield was consistently greater in the location with no history of WSBM

(Table 5 and 6) with yield reductions ranging from 31.6% (Mustang in 1985-86) to 69.4% (Triumph 64 in 1984-85). Resistant and susceptible cvs. had average yield decreases of 40.2 (sd=10.97) and 54.3% (sd=10.66) over the two years. Reductions in thousand kernel weight (TKW) ranged from 0.8 to 18.8% with 2 cvs. (Hawk and Chisholm) showing increases (Table 5 and 6). Plant height and tiller counts also were consistently greater in the location with no disease history (Table 6), but due to variation between replications, only the values for plant height were consistently significantly different. Hawk, Newton and Mustang demonstrated the least yield reduction (x=34.3% sd=2.44), and Mustang demonstrated the least reduction in tillers (8.3%), height (4.2%), grain yield (31.6%), and TKW (0.8) of all cvs. evaluated.

Regression analysis of data from 1984-85 to determine the relationship between visual assessment of symptoms (DSI) with grain yield and ELISA with grain yield revealed that visual assessment was the best indicator of the effect of WSBMV on yield. Coefficients of determination (r^2) between DSI and yield for the susceptible cvs. were significant at 3-14-85 (P=0.01), and at 3-28-85 and 4-15-85 (P=0.05). However, no significant r^2 values were obtained with the resistant cvs. at any assessment date. Coefficients of determination between ELISA and yield were significant (P=0.05) only with the susceptible cvs. at the first assessment date. Occasionally high ELISA values were

obtained from resistant cvs. that showed low disease severity [e.g. Hawk and Newton in 1984-85 (Table 5)]. This may be explained by the sensitivity of ELISA to detect virus capsid protein. Foliage from one or a few plants in these resistant cvs. may have been infected with WSBMV. Thus, visual assessment would indicate a low severity, but there would be sufficient virus capsid in the foliage sample to result in a high ELISA value. This helps explain the poor correlation between ELISA and yield observed in the regression analysis. Thus, we feel that visual assessment of symptoms used in conjunction with ELISA to assure presence of the virus in plants is the best approach to ascertaining the effect of WSBMV on yield and to identify resistant germplasm.

ELISA values from the location with no history of WSBM in 1984-85 initially were lower than comparable values from the location with a history of severe WSBM, and ELISA values obtained from resistant cvs. initially were much lower than values in susceptible cvs. (Table 5). By the final assessment on 4-15-85, ELISA values of all cvs. were comparable. No virus particles were obtained from the resistant cvs. (Hawk and Newton) growing in the location with no history of WSBM (Table 5), although virions were obtained from Hawk and Newton growing in the location with a history of severe WSBM. ELISA values obtained during 1985-86 were more sporadic over a longer period of time than

those obtained in 1984-85. No ELISA values were considered positive from resistant cvs. planted in the location with no history of WSBM until the final reading of the season on 5-11-85 (Table 7). Positive ELISA values from resistant cvs. in the location with a history of severe WSBM were obtained as early as 1-19-86. In contrast, positive ELISA values were obtained from susceptible cvs. as early as 11-24-84 and 1-19-85 from the locations of severe history and no history of WSBM, respectively (Table 7). These results from two seasons indicate that WSBMV capsid protein and virions are produced in resistant wheat cvs, but at a lower concentration and/or at a reduced rate as compared to susceptible cvs. In this and in a previous study (Hunger and Sherwood 1985) amounts of virus obtained from resistant cvs. never equalled amounts obtained from susceptible cvs. indicating that virus accumulation may be inhibited or replication may proceed at a slower rate in resistant cvs. Larsen, et al. (1985) reported greater sensitivity to foliar inoculation with WSBMV by cvs. field resistant to WSBM, and thus, felt that tolerance or resistance to the virus at the cellular level were not possible mechanisms of the plant resistance. Our results indicate that some mechanism is present in resistant cvs. which slows capsid protein and virus accumulation. This could result from a reduction in some aspect of the replicative cycle of the virus, although

further work is indicated to ascertain more fully the mechanisms of resistance to WSBMV.

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Table 5. Reaction of ten winter wheats to WSBM in 1985 in a location with no prior history of the disease (a) and in an adjacent location with a history of severe WSBM (b).

		Assessment of reaction to wheat soilborne mosaic										
		3-14-85			3-28-85			4-15-85			Yield[y]	TKW[z]
	Cultivar[u]	DSI[v]	ELISA[w]	µg/gfw[x]	DSI	ELISA	µg/gfw	DSI[v]	ELISA[w]	µg/gfw[x]	(g)	(g)
a	Hawk (R)	0.7	0.058	0.0	0.0	0.311	0.0	0.7	1.180	0.0	102*	27
b	Hawk (R)	6.7*	0.413	2.1	4.0	0.724	7.8	4.0*	0.826	0.8	67	32*
a	Newton (R)	0.0	0.102	0.0	0.0	0.440	0.0	0.0	1.400	0.0	73*	22
b	Newton (R)	8.7*	0.505	9.2	12.0	0.967	28.1	3.9	1.290	2.8	49	22
a	Chisholm (S)	44.0	1.126	---	18.6	1.238	---	20.0	1.846	---	74*	28
b	Chisholm (S)	62.7	1.367	---	39.3*	1.345	---	40.0*	1.673	---	37	27
a	Sage (S)	41.6	0.999	60.3	33.3	1.330	36.5	24.7	1.356	29.5	57*	27
b	Sage (S)	78.6	1.730	72.7	64.9	1.511	89.0	48.3*	1.512	51.5	28	25
a	Tam 101 (S)	44.0	0.924	72.2	32.6	1.207	78.3	24.6	1.230	47.0	46	29
b	Tam 101 (S)	63.3	1.501	74.2	44.3	1.648	100.1	43.3*	1.589	50.5	28	26
a	Vona (S)	16.7	0.387	---	15.3	0.974	---	12.6	1.013	---	68*	23*
b	Vona (S)	80.7*	1.449	---	58.0*	1.477	---	45.9*	1.342	---	23	19
a	Danne (S)	32.7	0.681	---	22.0	1.332	---	12.7	1.390	---	73*	29
b	Danne (S)	75.0*	1.156	---	40.6	1.161	---	40.0*	1.305	---	27	25
a	Payne (S)	16.7	0.845	---	14.6	0.919	---	8.5	1.163	---	116*	32*
b	Payne (S)	76.6*	1.444	---	42.6*	1.508	---	39.3*	1.953	---	55	26
a	Tam 105 (S)	29.9	0.741	---	22.5	1.096	---	13.4	1.716	---	61*	25*
b	Tam 105 (S)	76.7*	1.323	---	55.8*	1.359	---	39.4*	1.771	---	31	22
a	Triumph 64 (S)	32.8	0.495	---	17.3	1.159	---	17.6	1.409	---	85*	32
b	Triumph 64 (S)	81.3*	1.378	---	58.7*	1.428	---	42.2*	1.418	---	26	26

[u] The first listing of each pair of cultivars represents results from the location with no prior history of WSBM. The second listing represents results from the location with a history of severe WSBM. Letter in parenthesis denotes R for resistant and S for susceptible.

[v] DSI (disease severity index): $\Sigma = \frac{\text{class x no. plants in class x 100}}{\text{total no. plants X 3}}$, where 0=no mosaic or stunting symptoms present, 1=slight mosaic, slight stunting may or may not be present, 2=mosaic moderate with some stunting, and 3=severe mosaic and stunting present. Values presented are the means of 7-10 individual plants rated in each of five replications. Asterisks indicate significant difference (P=0.05) as determined by an unpaired t-test.

[w] ELISA values presented are the means obtained from foliar samples collected from 5 replications with 2 readings/replication.

[x] Micrograms of sedimentable virions per gram fresh weight of foliar samples of cvs. Hawk, Newton, Sage, and Tam 101 only.

[y] Yield is the average amount of grain collected from 7-10 plants from each of 5 replications. Asterisks indicate significant difference (P=0.05) as determined by an unpaired t-test.

[z] Thousand kernel weight determined by weighing 500 kernel samples from each of 5 replications and multiplying by 2. Asterisks indicate significant difference (P=0.05) as determined by an unpaired t-test.

Table 6. Disease severity (DS), tiller count, height, yield, and thousand kernel weight (TKW) of 9 hard red winter wheats planted in locations with no prior history of WSBM (a) and with a history of severe WSBM (b).

	Cultivar[u]	DS[v] (3-20-86)	Tiller count[w] (per 0.3 m of row)	Height[x] (cm)	Yield[y] (g)	TKW[z] (g)
a	Hawk (R)	0.3	133*	78.5*	351.3	25.8*
b	Hawk (R)	0.8	105	70.0	230.0	22.9
a	Newton (R)	0.0	129	83.0*	327.0*	24.5
b	Newton (R)	1.0*	84	71.3	202.5	23.6
a	Chisholm (S)	0.8	121	74.3*	427.3	27.4
b	Chisholm (S)	1.8*	87	64.8	265.8	29.2
a	Sage (S)	1.0	105	80.0*	257.5*	24.3*
b	Sage (S)	2.5*	87	66.5	137.3	20.3
a	Tam 101 (S)	1.0	105	75.5*	325.5	29.9*
b	Tam 101 (S)	2.3	74	58.8	132.8	26.7
a	Vona (S)	0.5	120*	76.5*	493.8*	23.6*
b	Vona (S)	2.3*	78	64.3	163.0	21.4
a	Mustang (R)	1.5	96	72.0	309.0*	26.5
b	Mustang (R)	1.8	88	69.0	211.3	26.7
a	Plainsman V (R)	0.5	92	71.0*	451.8*	24.5
b	Plainsman V (R)	1.3	63	62.0	173.8	24.1
a	Tam 108 (R)	0.0	119	82.5*	444.8*	25.6*
b	Tam 108 (R)	1.0	99	71.3	229.0	21.9

[u] The first listing of each pair of cultivars represents results from the location with no prior history of WSBM. The second listing represents results from the location with a history of severe WSBM. Letter in parenthesis denotes R for resistant and S for susceptible.

[v] Disease severity obtained from averaging the ratings of four replicate plots each consisting of 1, 3.05 m row, where 0=no mosaic or stunting symptoms present, 1=slight mosaic, slight stunting may or may not be present, 2=moderate mosaic with some stunting, and 3=severe mosaic and stunting present. Asterisks indicate significant (P=0.05) differences as determined by an unpaired t-test.

[w] Number of tillers was determined by counting the number of tillers with fertile heads in 0.305 m of each row in each of 4 replications. Asterisks indicate significant (P=0.05) differences as determined by an unpaired t-test.

[x] Height was determined by measuring from the ground to the base of ears several times within each of 4 replicates. The average of these values was used for each replication. Asterisks indicate significant (P=0.05) differences as determined by an unpaired t-test.

[y] Yield is the average amount of grain collected from 4, 3.05 m rows. Asterisks indicate significant (P=0.05) differences as determined by an unpaired t-test.

[z] Thousand kernel weight determined by weighing 500 kernel samples from each of four replications and multiplying by 2. Asterisks indicate significant (P=0.05) differences as determined by an unpaired t-test.

Table 7. Detection of WSBMV capsid protein by ELISA in 9 hard red winter wheats during 1985-86 in a location with no history of the disease (a) and in a location with a history of severe WSBM (b).

		Date of assessment by ELISA[y]								
		1985		1986						
	Cultivar[z]	11-24	12-22	1-19	2-16	3-2	3-16	3-30	4-20	5-11
a	Hawk (R)	-0.030	0.019	-0.010	-0.005	0.002	0.013	-0.043	0.046	0.215
b	Hawk (R)	-0.004	0.012	0.026	-0.005	0.013	0.040	0.021	0.109	0.368
a	Newton (R)	-0.005	-0.013	0.004	-0.022	0.022	0.012	-0.012	-0.002	0.151
b	Newton (R)	-0.007	0.008	0.297	0.009	0.054	0.504	-0.014	-0.003	0.217
a	Chisholm (S)	-0.045	0.061	2.080	0.262	0.174	0.628	0.681	0.902	0.062
b	Chisholm (S)	1.175	0.234	2.415	1.015	1.310	1.124	1.373	1.643	0.679
a	Sage (S)	-0.040	-0.001	0.775	0.208	0.018	0.525	0.454	0.424	0.078
b	Sage (S)	1.728	0.226	1.871	0.889	1.268	0.696	0.646	0.549	0.826
a	Tam 101 (S)	-0.002	0.026	0.954	1.018	0.761	0.253	0.879	0.025	0.485
b	Tam 101 (S)	1.374	1.595	2.168	1.220	0.883	1.463	0.889	1.089	0.914
a	Vona (S)	-0.082	0.143	-0.015	-0.052	-0.005	0.008	0.564	0.012	0.016
b	Vona (S)	0.783	1.268	1.446	1.047	0.702	0.778	0.656	0.471	0.651
a	Mustang (R)	-0.039	0.008	-0.021	-0.030	0.002	-0.014	0.055	-0.007	0.085
b	Mustang (R)	-0.006	0.023	0.048	0.615	0.309	0.460	0.666	0.008	0.503
a	Plainsman V (R)	-0.020	-0.025	-0.019	0.002	0.000	0.033	0.042	0.043	0.032
b	Plainsman V (R)	-0.008	-0.023	0.070	0.489	0.692	0.031	0.232	0.027	0.332
a	Tam 108 (R)	-0.030	0.007	-0.048	-0.005	0.046	0.015	-0.046	0.085	0.076
b	Tam 108 (R)	-0.035	0.156	0.415	0.436	0.298	0.128	-0.048	0.271	0.562

[y] ELISA values are the average of 7 ELISA readings obtained from a composite foliar sample collected from 4 replicate plots in each of the two locations.

[z] The first listing of each pair of cultivars represents results from the location with no prior history of WSBM. The second listing represents results from the location with a history of servere WSBM. Letter in parenthesis denotes R for resistant and S for susceptible.

APPENDIX B

OPTIMUM MONTH FOR SAMPLING WINTER WHEAT FOLIAGE FOR EVALUATION OF RESISTANCE TO WHEAT SOILBORNE MOSAIC VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY.

Enzyme-linked immunosorbent assay (ELISA) is used in conjunction with visual assessment to confirm the presence of wheat soilborne mosaic virus (WSBMV) in evaluation of host resistance. Confirmation is necessary because fertility, other viruses, and other parameters can induce similar chlorotic mottling, especially in greenhouse plants. Ideally, several grams of foliage are taken from replicated field plots several times during January through March. However, breeding for resistance to WSBMV frequently involves sampling from head rows or from individual plants. Sampling for ELISA is destructive and so the evaluation may be limited to a single sampling. The purpose of this study is to identify the optimum month for making this sampling.

MATERIALS AND METHODS

For three years, four replicated field plots of hard red winter wheat cultivars resistant to WSBM, Hawk and Newton, or susceptible to WSBM, Vona and Sage, were planted in soil with a history of severe wheat soilborne mosaic virus. Sampling commenced in autumn (October 1986, and

November 1985 and 1987) and ended in May. Sampling techniques varied slightly between growing season. Leaf samples were stored in "Glad Zip-lock Freezer Bags" at -20 °C. From each sample, 5 g subsamples of 5-8 cm leaf segments were taken and homogenized in phosphate buffered saline with 0.05% Tween 20 and 2% polyvinyl-pyrrolidone. Aliquots of 200 μl were applied to 7-10 wells divided between two standard flat bottom ELISA plates. ELISA was performed using the direct double antibody sandwich procedure as previously described (Bahrani et al. 1988). Absorbance values were measured at 405 nm with an EIA READER (Model EL-307, Bio-Tek Instruments, Inc., Laboratory Division, Burlington, VT 05401, U.S.A.).

RESULTS AND CONCLUSIONS

Means for ELISA absorbance values (A_{405}nm) from cvs. Hawk, Newton, Sage and Vona at each sampling date are demonstrated in Fig. 2-4. Mean ELISA values for individual replicated field plots are demonstrated for January 30, 1987 (Fig. 5), February 13, 1988 (Fig. 6) and March 24, 1988 (Fig. 7).

The highest mean ELISA values for cvs. Sage and Vona were found in January during the 1985-86 growing season (Fig. 2), and in March or April during the 1986-87 and 1987-88 growing seasons (Figs. 3 and 4). WSBMV was detected by ELISA in one of four field plots of cv. Vona sampled on

January 30, 1987 (Fig. 5). Thus, and evaluation based upon an ELISA of a single sampling of new cultigen with a phenotype similar to cv. Vona, taken in January may result in a resistant rating when another parameter is the reason for the low ELISA values. High ELISA values were obtained from two plots of cv. Newton on March 24, 1988 (Fig. 7). Thus, a single evaluation of a cultigen similar to cv. Newton based on samples taken in March may result in a susceptible rating and removal from the breeding program when the cultigen contains quantitative resistance.

WSBMV was detected more consistently in cvs. Sage and Vona during February than during January. WSBMV was occasionally detected in cvs. Hawk or Newton in February but the mean ELISA values were consistently lower than those of cvs. Sage and Vona (Fig. 6). Thus, the risk of false ratings based on a single ELISA is lowest during February. For this reason, we recommend that foliar sampling for quantitative resistance to WSBM in north-central Oklahoma occur during February.

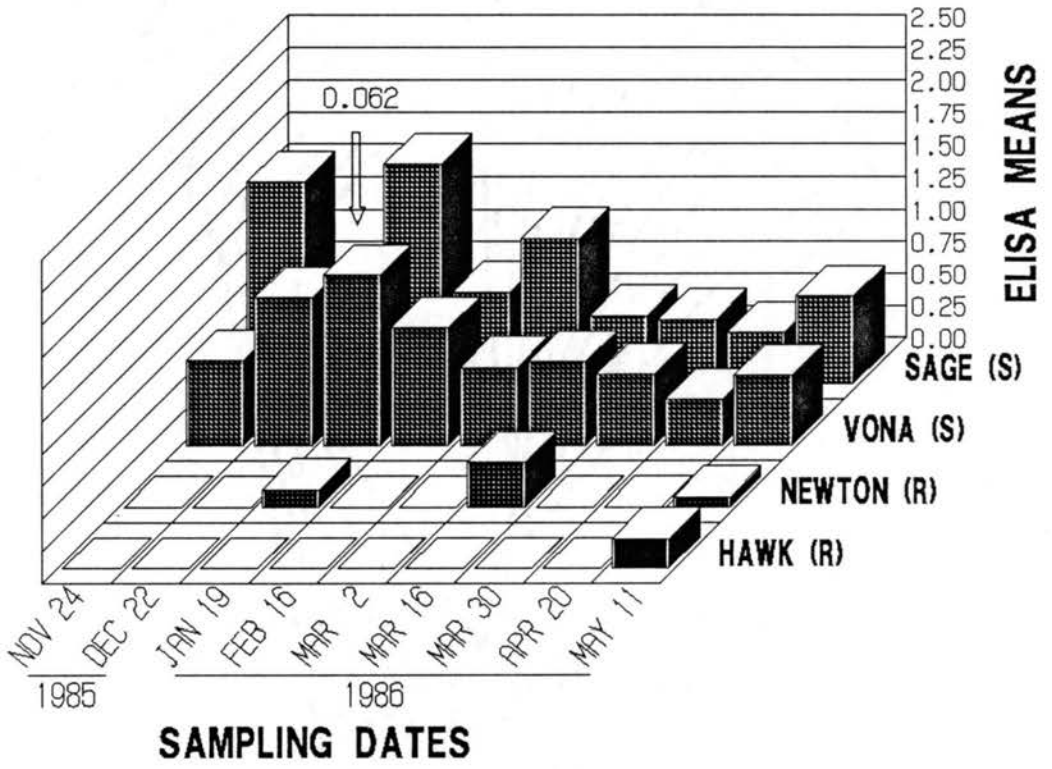

Figure 2. ELISA absorbance means of foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on nine dates during the 1985-86 growing season.

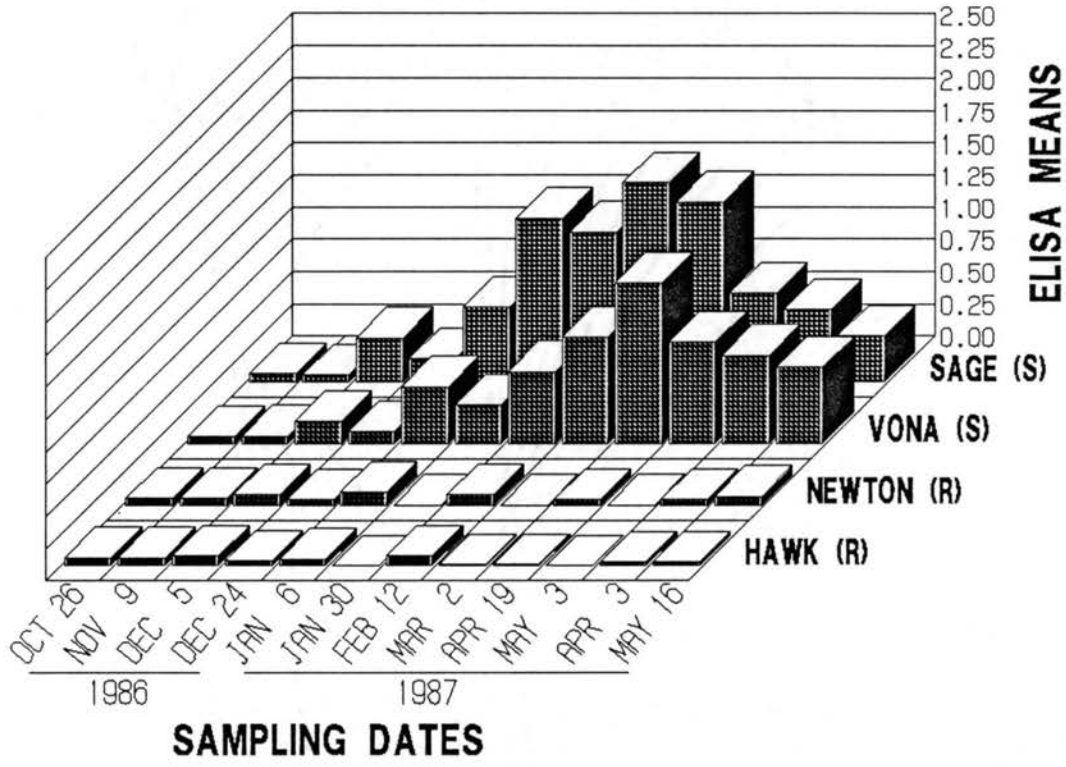

Figure 3. ELISA absorbance means of foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on twelve dates during the 1986-87 growing season.

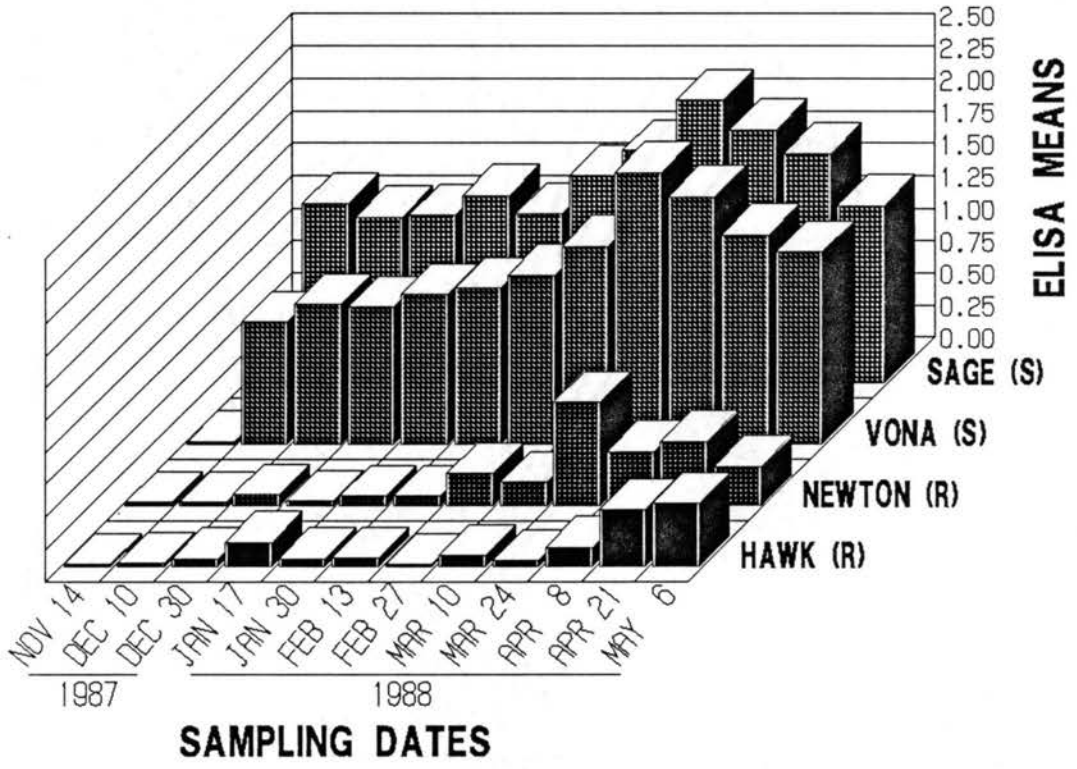

Figure 4. ELISA absorbance means of foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on twelve dates during the 1987-88 growing season.

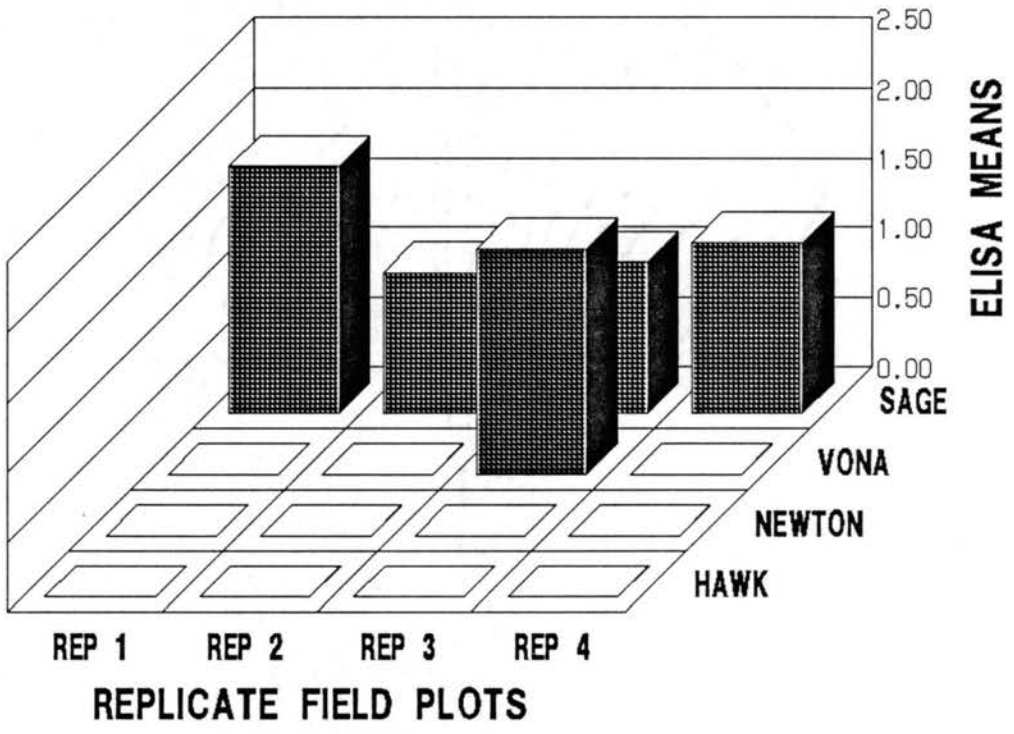

Figure 5. ELISA absorbance means for foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on January 30, 1987.

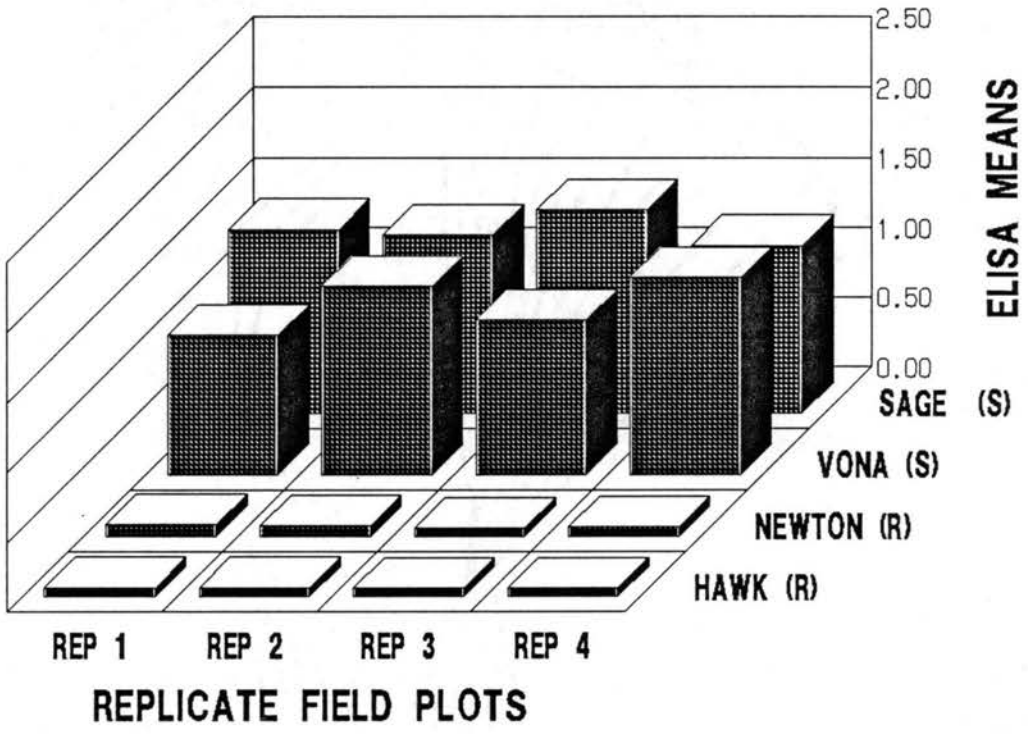

Figure 6. ELISA absorbance means for foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on February 13, 1988.

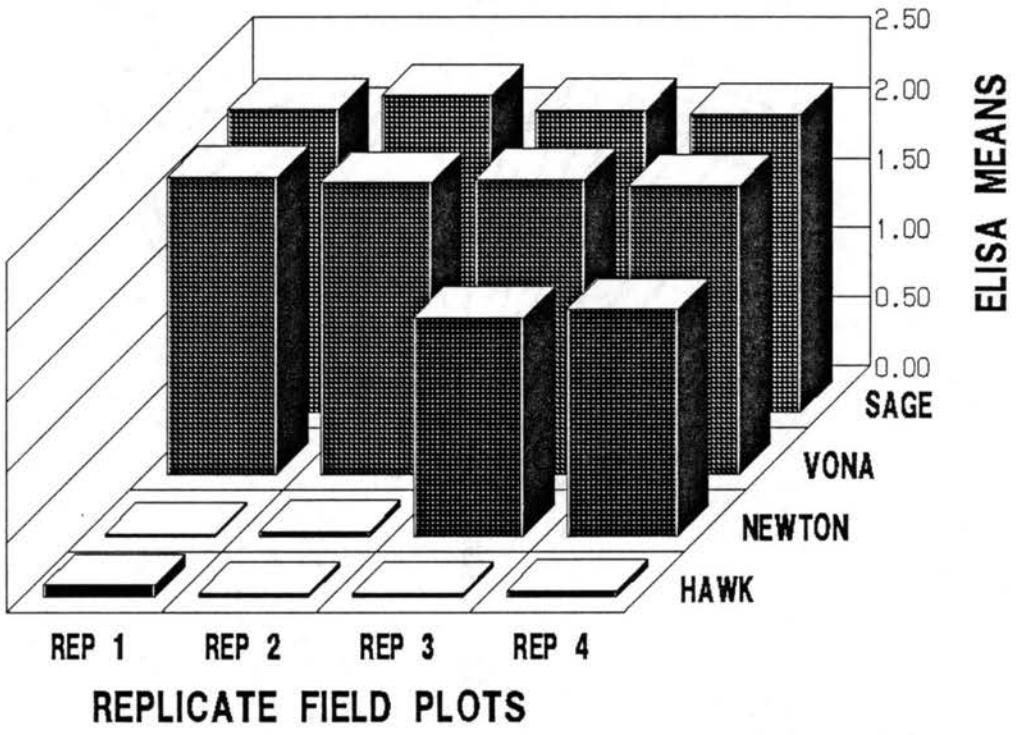

Figure 7. ELISA absorbance means for foliar samples of two hard red winter wheat cultivars resistant (R) to WSBM and two susceptible (S) cultivars, collected on March 24, 1988.

APPENDIX C

SUGGESTIONS FOR FURTHER RESEARCH

One of the most difficult aspects of graduating is leaving the research project to which you've devoted several years of your life when there are questions still unanswered and hypotheses still untested. In this section, I have attempted to provide some of my thoughts and hypotheses based on the literature, and on personal observation, for possible examination in the future.

RESEARCH PROJECT I

THE EFFECT OF VERNALIZATION OF HARD RED WINTER WHEAT ON THE EXPRESSION OF RESISTANCE TO WHEAT SOILBORNE MOSAIC VIRUS

Two growth chambers were used in the experiment described in Chapter IV. The data from one of the growth chambers (#1 in Tables 8 and 9) was not presented because of a mechanical malfunction resulting in a three day heat shock at 19/12 °C when the chamber was set at 7/5 °C. ELISA values from both growth chambers were similiar for cv. Vona (Fig. 8). However, when ELISA values were averaged by sampling date, cv. Newton showed a late-season rise as seen in the field only in Chamber #1 (Table 8, Fig. 8). Only when ELISA values were averaged by tiller maturity was a late-season rise shown in both growth chambers (Table 9, Fig. 9). Plants in Chamber #1 did not mature as fast as plants in Chamber #2 and a few of these plants had not headed by the end of the experiment. Consequently, the heat shock, occurring so soon after vernalization, probably caused partial devernalization of the plants in Chamber #1 and may account for the divergences in ELISA values and virus concentrations demonstrated in cv. Newton in the two growth chambers. Dissimilarities in net vernalization may

contribute to the yearly variability in disease response in the field as well. The effect of net vernalization on ELISA values and symptom expression is probably limited to disease resistant cultivars of winter wheat because susceptibility is known to occur in spring wheat (McKinney 1930). Although the heat shock may have effected the virus itself, this is unlikely because ELISA values for cv. Vona in the two growth chambers differed very little (Table 8, Fig. 8).

The initial growth chamber experiment needs to be repeated. Lower temperatures might be pursued since the threshold suggested by Eversmeyer et. al. (1983) is 17 °C. The possible effect of devernalisation upon disease expression and virus replication also needs to be further investigated.

Table 8. WSBMV virion concentrations and ELISA absorbances for foliar samples of resistant cv. Newton and susceptible cv. Vona maintained in two growth chambers.

	NEWTON				VONA			
	GROWTH CHAMBER #1		GROWTH CHAMBER #2		GROWTH CHAMBER #1		GROWTH CHAMBER #2	
DATE	VIRUS[v]	ELISA[w]	VIRUS	ELISA	VIRUS	ELISA	VIRUS	ELISA
Jan. 10[x]	--	--	3.530	0.355	--	--	7.689	1.216
March 14[y]	--	--	4.849	0.139	--	--	31.004	1.548
April 10[z]	0.176	0.013	0.352	-0.003	14.148	1.115	13.961	0.869
April 24	2.123	0.325	0.504	-0.008	9.208	1.256	3.631	1.055
May 8	1.068	0.299	0.654	0.120	4.460	0.925	4.724	0.761
May 15	0.352	-0.012	3.669	0.322	17.534	0.789	37.913	1.181
May 24	9.659	0.492	1.860	0.125	71.899	1.131	52.203	1.020
June 9	2.551	0.840	0.716	0.134	6.759	1.192	6.332	1.079

[v] Foliar samples of leaves of similar age from tillers of similar maturity were collected, and sap was expressed into 0.5 M sodium borate with 0.001 M EDTA to produce a 1:10 (w/v) dilution stock solution. Aliquots of 21 ml stock sap solution were collected for virus extractions. Virus extracts were stored at -20 °C until assayed by acrylamide gel electrophoresis. Four standards (20, 10, 5, and 2.5 μg virus per well) were run on each gel and mean absorbances (A_{595}nm) were plotted against virus concentration for the standards. This plot was used to determine the mean virus concentration for each sample.

[w] Stock sap solutions were stored at -20 °C until all sampling was completed, thawed and assayed by ELISA. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates.

[x] Germinated seeds were planted in soil from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. Sampling one this date was just prior to six weeks at 4 °C.

[y] Sampling on this date followed six weeks at 4 °C and corresponded to the transplanting of seedlings to 3.785 L pots, 3 seedlings per pot.

[z] Growth chambers were kept at 5 °C for three days after transplanting, then raised to 7/5 °C (day/night) for three days, 10/7 °C for eight days, 15/10 °C for six days, and 20/15 °C for the duration of the experiment. Data means are averages for 3 pots per cultivar and growth chamber. Sampling this date is following 1 week at 20/15 °C day/night, when plants were jointing.

Table 9. Means of ELISA absorbances averaged by maturity of tillers in the samples from resistant cv. Newton and susceptible cv. Vona maintained in two growth chambers.

TILLER MATURITY OF SAMPLE[w]	NEWTON		VONA	
	CHAMBER 1	CHAMBER 2	CHAMBER 1	CHAMBER 2
5 (before vernalization)[x]	--	0.355	--	1.216
5 (after vernalization)[y]	--	0.139	--	1.548
6 - 7[z]	-0.013	-0.003	1.115	0.869
7 - 9	0.319	-0.008	1.250	1.055
9 - 10.5	0.329	0.102	0.974	0.958
10 - 11.1	0.789	0.228	1.158	1.033

[w] Range of tiller maturities according to Feekes' Scale within a sample.

[x] Germinated seeds were planted in soil from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. This sampling was just prior to six weeks at 4 °C. Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates for each sample.

[y] Sampling on this date followed six weeks at 4 °C and corresponded to the transplanting of seedlings to 3.785 L pots, 3 seedlings per pot.

[z] Growth chambers were kept at 5 °C for three days after transplanting, then raised to 7/5 °C (day/night) for three days, 10/7 °C for eight days, 15/10 °C for six days, and 20/15 °C for the duration of the experiment. Data means are averages for 3 pots per cultivar and growth chamber. Sampling this date is following 1 week at 20/15 °C day/night, when plants were jointing.

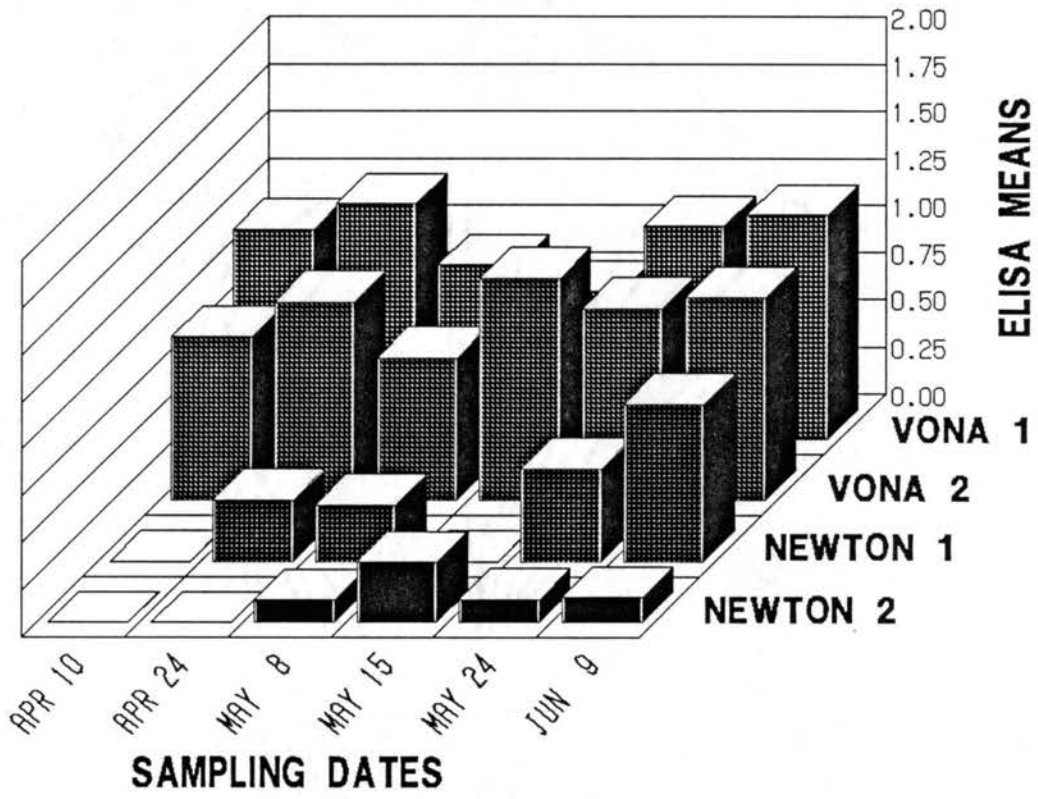

Figure 8. ELISA absorbances for foliar samples of resistant cv. Newton and susceptible cv. Vona maintained in two growth chambers.

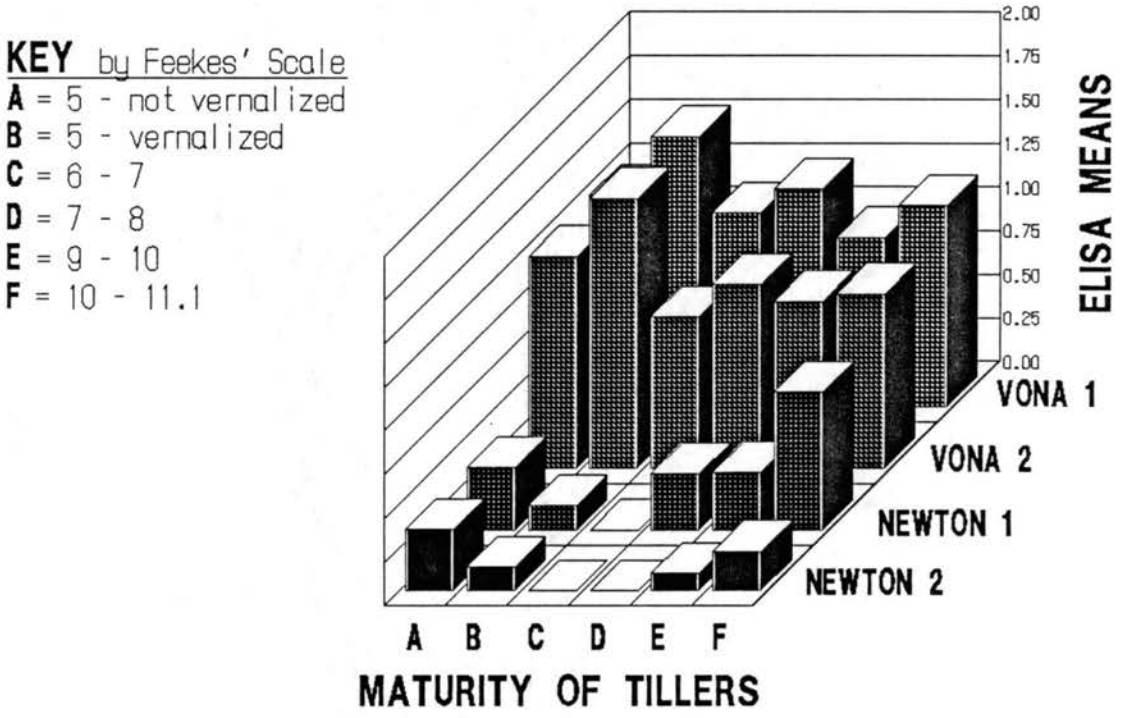

Figure 9. ELISA absorbances averaged by maturity of tillers in the samples from resistant cv. Newton and susceptible cv. Vona maintained in two growth chambers.

RESEARCH PROJECT II

MOST PROBABLE NUMBER ESTIMATION WITH WHEAT SOILBORNE MOSAIC VIRUS.

An important concept in epidemiology is "inoculum potential" which may be defined as "the capacity of a pathogen population to infect a population of fully susceptible host plants under conditions optimum for infection" (Michell 1979 situ Pfender, et al. 1981). Inoculum potential of soilborne pathogens may be estimated by the most probable number (MPN) technique. Optimal conditions for infection are determined. A concentration series of infested soil diluted with disinfested soil is produced. Host plants are grown in set amounts of each soil concentration and assayed for infection. The proportion of pots with infected plants at each soil concentration is used in the MPN formula to determine the inoculum potential of the original soil (Pfender, et al. 1981, Wilson and Trinick 1982).

Wheat soilborne mosaic virus (WSBMV) is vectored by zoospores of Polymyxa graminis. Infective units, i. e. the number of virions/zoospore and viruliferous zoospores/seedling, has not been reported. However, the MPN technique would permit quantitative comparisons between different soils or different soil treatments. Thus, the

object of this study was to determine if MPN could be used with wheat soilborne mosaic (WSBM).

MATERIALS AND METHODS

Soil was collected in October from an area with a history of severe WSBM (SD), and an area with a history of mild WSBM (MD), sifted through #8 and #20 mesh screens to remove rocks and large clumps, and mixed with autoclaved fine sand to produce 100%, 50%, 25%, 10%, 1%, and 0.1% soil/sand (by weight) dilutions. Fifteen standard Monarch plant bands (5.6 x 3.8 x 3.8 cm) were filled with 100 g soil/sand mix for each dilution and wedged into a 2 inch plastic pot. Seeds of cv. Vona (susceptible to WSBM) were soaked in a 0.26% sodium hypochlorite solution for 15 min on a reciprocal shaker at room temperature. Seeds were rinsed 3 times with double distilled water, placed on filter paper in polyethylene petri dishes, saturated with double distilled water, and allowed to germinate on a bench top under laboratory conditions. Nine germinated seeds were placed (3 x 3) on each soil mix in plants bands and covered with 5 g autoclaved fine sand. Pots were maintained in a growth chamber at a 10/14 hr day/night cycle at 24/20 °C until emergence of the coleoptile to enhance seedling establishment and release of zoospores, then at 13/10 °C to enhance infection and symptom development (Brakke et al. 1965). Pots were bottom-watered with tap water on alternate

days. After the sixth week, 18 ml of 20/20/20 N-P-K fertilizer was applied to each pot, then watering continued as before. At eleven weeks, plants were evaluated visually and by ELISA.

RESULTS AND CONCLUSIONS

The SD soil was infective down to the 0.1% level (Table 10) while the MD soil was not shown to be infective. The MPN for the SD soil was 3 infective units/100 g soil.

Table 10. The ratio of the number of plants showing symptoms of WSBM over the total number of plants per pot at six soil/sand dilutions.

POTS	100%	50%	25%	10%	1%	0.1%
#1	7/9	8/9	7/9	1/9	0/9	0/9
#2	6/9	6/9	3/9	2/9	0/9	0/9
#3	6/9	8/9	6/9	0/9	0/9	0/9
#4	6/8	9/9	1/9	2/9	0/8	0/9
#5	7/8	6/9	5/9	2/9	0/9	0/9
Infested Total Pots	5/5	5/5	5/5	4/5	0/5	0/5

The conditions used for this study were not optimum for infection by WSBMV. A 9/15 hr photoperiod might have been more effective (Rao and Brakke 1970). The plants were in poor health as the nutrients in the soil/sand mix were depleted, and improved with fertilization. Because the amount of nutrients available vary with the amount of soil in each mix, another source of nutrients should be applied

on a regular basis to standardize the effects of nutrients on disease. The amount of water received by the plants in each pot should also be better regulated to minimize pot-to-pot variability. Much room for investigation remains in this project.

RESEARCH PROJECT III

INDUCTION OF RESISTANCE TO WHEAT SOILBORNE MOSAIC (WSBM) IN HARD RED WINTER WHEAT BY *POLYMYXA GRAMINIS*.

Polymyxa graminis is generally assumed to vector WSBMV (Brakke *et al*. 1965, Estes and Brakke 1966). Comparable infections by *P. graminis* are found in roots of both susceptible cultivars and cultivars traditionally considered to be disease resistant (Larsen *et al*. 1985), yet, these resistant cultivars develop severe symptoms when mechanically inoculated with WSBMV in the absence of *P. graminis* (Larsen *et al*. 1985, McKinney 1948).

In north central Oklahoma, the 1986-87 growing season was more wet than the 1985-86 growing season by 30.5 cm. Most of this precipitation fell during the last week in September and the first week in October after winter wheat in experimental plots had emerged. Resistant cultivars were healthier during the second growing season than the first (C. R. Armitage, personal observation) as might be expected since water can be a limiting factor in Great Plains agriculture, i.e. the extra water might have resulted in plants better able to overcome viral activity or the effects of infection. However, disease symptoms were more severe

in the susceptible cultivars during the second growing season than the first (C. R. Armitage, personal observation). In this case, the extra water seems to have aided germination and mobility of viruliferous zoospores resulting in a higher initial inoculum. This pair of observations, along with the susceptibility of Newton when mechanically inoculated, suggested that P. graminis induced resistance in some cultivars. Precedence exists for this hypothesis. Kassanis and Macfarlane (1965) reported evidence for a similar induction by Olpidium brassicae to tobacco necrosis virus in cress.

MATERIALS AND METHODS

Soil was collected in October from an area with a history of severe WSBMV, sifted through #8 and #20 mesh screens to remove rocks and large clumps, and mixed with autoclaved fine sand to produce 50%, 25% and 10% soil/sand (by weight) dilutions. Fifteen standard Monarch plant bands (5.6 x 3.8 x 3.8 cm) were filled with 100 g soil/sand mix for each dilution and wedged into a 2 inch plastic pot. Seed of cvs. Newton and Vona were soaked in a 0.26% sodium hypochlorite solution for 15 min on a reciprocal shaker at room temperature. Seeds were rinsed 3 times with double distilled water, placed on filter paper in polyethylene petri dishes, saturated with double distilled water, and allowed to germinate on a bench top under laboratory

conditions. Nine germinated seeds of cv. Newton (resistant) were placed (3 x 3) on each soil mix in plants bands and covered with 5 g autoclaved fine sand. Fifteen bands with 50% soil/sand were planted with Vona as a check. For each cultivar, five pots of each soil/sand dilution were watered, via a pipet, with 18 ml, 12 ml, or 6 ml tap water, and weighed. Pots were maintained in a growth chamber at a 10/14 hr day/night cycle at 24/20 °C until emergence of the coleoptile, then at 13/10 °C. Pots were brought up to weight by the addition of tap water on alternate days. An extra 6 ml of water was added to the 6 ml treatment pots four days after planting first because of problems with seedling establishment. After the third week, 18 ml of 20/20/20 N-P-K fertilizer was applied to each pot, then watering continued as before. Plants were evaluated visually and by ELISA.

RESULTS AND DISCUSSION

At the moisture levels used, the highest disease incidences and severities were generally found for cv. Newton with the most diluted soil mix (Table 11). For cv. Vona, high soil content favors high disease incidence and severity (see Appendix C:II). At any particular soil content used, the highest disease incidence and severity was found at the highest moisture level in cv. Vona but generally at the lowest moisture level in cv. Newton (Table

11). This suggests that soil moisture and soil concentration may influence development of WSBM. Presumably, the greater the soil content, the greater the P. graminis content. Likewise, the greater the soil moisture, the greater the P. graminis mobility and inoculum load. An interaction between these two variables is likely and suggested by the moderate rating for cv. Newton at 50% soil/12 ml and the high ELISA value at 25% soil/6 ml. However, these results, along with evidence given in the introduction, suggest that P. graminis may induce disease resistance in cv. Newton.

FURTHER CONSIDERATIONS

WSBMV is thought to be released into host roots following infection of seedling roots by the vector, Polymyxa graminis L. during cool, wet periods primarily in the autumn (Brakke and Estes 1967, Rao and Brakke 1969). Cystosori are the probably primary inoculum when soil and soil debris are the virus source (Estes and Brakke 1966). Soil debris collected in spring and summer is not as infective as soil debris collected in autumn and winter (Brakke and Rao 1967, Brakke and Estes 1967). Rao (1968) obtained successful transmission of WSBMV from powdered roots with a treatment of soil extracts or kinetin in distilled water. Powdered roots were incubated at 28 °C for two months prior to a pre-inoculation incubation at 20 °C.

It seems probable that P. graminis goes dormant in the spring as wheat plants mature and developing heads become an increasingly dominant nutrient sink.

If we accept the hypotheses that P. graminis induces resistance in resistant cultivars, and that the fungus is going dormant in the spring, then it is possible that this induction is reduced or ended as the fungus goes dormant in the spring. Thus, the effect on WSBM symptom expression by changes of host physiology during maturation and senescence may not be directly on viral replication but on the vector and its effect on resistance expression. Detailed studies of P. graminis populations in wheat roots during maturation and study of disease progress in mechanically inoculated plants are needed to investigate these hypotheses.

Table 11. Ratios of pots containing plants infected with WSBMV and ELISA absorbances for susceptible cv. Vona and resistant cv. Newton[v].

		WATER TREATMENT[w]								
	SOIL	18 ml			12 ml			6 ml		
CULTIVAR	CONCENTRATION	PWS[x]	PWA[y]	ELISA[z]	PWS	PWA	ELISA	PWS	PWA	ELISA
VONA	50%	5/5	5/5	0.823	4/5	3/5	0.469	0/5	0/5	-0.103
NEWTON	50%	0/5	0/5	-0.031	1/5	3/5	0.054	0/5	0/5	-0.102
	25%	0/5	1/5	0.004	1/5	0/5	-0.028	3/5	4/5	0.151
	10%	0/5	2/5	0.004	2/5	3/5	0.044	3/5	5/5	0.092

[v] Soil from an area with a history of severe WSBMV, was mixed with autoclaved fine sand to produce 50%, 25% and 10% soil/sand (by weight) dilutions. Fifteen standard Monarch plant bands (5.6 x 3.8 x 3.8 cm) were filled with 100 g soil/sand mix for each dilution and wedged into a 5 cm plastic pot. Nine germinated seeds of Newton were placed (3 x 3) on the soil mix in the plants bands and covered with 5 g autoclaved fine sand. Fifteen bands with 50% soil/sand were planted with Vona as a check.

[w] For each cultivar, five pots of each soil/sand dilution were watered by 18 ml, 12 ml, or 6 ml tap water and weighed. Pots were maintained at 24/20 °C until emergence then reduced to 13/10 °C at a 10/14 (day/night) in a growth chamber. On alternate days pots were brought up to weight by the addition of tap water.

[x] Three weeks after symptoms were first visible, plants were evaluated visually, defoliated, and the foliar samples were analyzed by ELISA. Ratio of the number of pots containing one or more plants showing visual symptoms of infection by WSBMV (i.e. pots with symptoms) over the total number of pots.

[y] Ratio of the number of pots containing one or more plants showing the presence of WSBMV antigen by ELISA (i.e. pots with antigen) over the total number of pots.

[z] Means of ELISA absorbances (A_{405}nm) are from five wells on each of two ELISA plates.

RESEARCH PROJECT IV

EFFECT OF ATTACHMENT BY WHEAT SOILBORNE MOSAIC VIRUS PARTICLES TO *POLYMYXA GRAMINIS* ZOOSPORES ON VIRION UNCOATING.

The first step in uncoating of rod-shaped viruses appears to be closely associated with end-on attachment to a lipid. In mechanical inoculation studies of tobacco mosaic virus, translation of the exposed viral RNA appears to follow attachment to host lipids. The product of this early translation appears to be involved in the remaining step(s) in viral uncoating (Kiho 1970, Shaw 1969). However, certain rod-shaped viruses demonstrate similar end-on attachment to the membranous surface of plasmodiophorid zoospores. These zoospores, in turn, enter host cells, exposing the surface-bound virions to host ribosomes and translation system. Thus *in vivo* (and, presumably, *in vitro*) translation of the uncoating product is possible if uncoating is initiated by attachment to the zoospore.

Langenberg and Giunchedi (1982) used electron microscopy to observe the association between wheat soilborne mosaic virus (WSBMV) and vector *Polymyxa graminis*. Although the virus was seen in close contact with the plasmodia of *P. graminis*, the virus was not seen inside zoospores, plasmodia, zoosporangia or cystosori, perhaps

because the fungal cytoplasmic contents were densely stained. Most contact between virions and vector was an end-on attachment of virions to the outer membrane of the plasmodium, however Langenberg and Giunchedi allowed that this arrangement may have been an artifact of fixation.

The hypothesis that uncoating of WSBMV initiates with attachment to P. graminis may be tested in two ways. Place viruliferous zoospores in a cell-free translation system, for example, the rabbit reticulocyte lysate system used by Shirako and Brakke (1984a) and look for: 1) formation of viral polysomes on the zoospore surface, and 2) production of the same products found by Shirako and Brakke (1984a).

VITA

Charla Rae Armitage

Candidate for the Degree of

Doctor of Philosophy

Thesis: SEROLOGICAL INVESTIGATIONS OF THE INTERACTION BETWEEN WHEAT SOILBORNE MOSAIC VIRUS AND HARD RED WINTER WHEAT

Major Field: Plant Pathology

Biographical:

Personal Data: Born in New Kennsington, Pennsylvania, June 10, 1957, first-borne child and daughter of Charles S. and Frances A. Armitage.

Education: Graduated from Elderton High School, Elderton, Pennsylvania, in May 1975; received Bachelor of Science Degree in Biology from The Pennsylvania State University at University Park in May, 1979; received Masters of Science Degree from Colorado State University at Fort Collins in May, 1984; complete requirements for the Doctor of Philosophy Degree at Oklahoma State University at Stillwater in July, 1989.

Professional Affiliations: The American Phytopathological Society; Graduate Student Council; Graduate Student Representative to the Graduate Faculty Council.

Professional Experience: Graduate Research Assistant, Department of Plant Pathology, Oklahoma State University, January, 1984, to July, 1989; Graduate Teaching Assistant, Department of Plant Pathology, Oklahoma State University, August to December 1985, and August to December, 1988; Graduate Teaching Assistant, Department of Plant Pathology, Colorado State University, January 1980 to January 1984; Plant Disease Diagnostician, Department of Plant Pathology, Colorado State University, May to August, 1982; Maintenance Assistant, Department of Plant Pathology, Colorado State University, May to December, 1980.