EPIDEMIOLOGY OF AND RESISTANCE TO *WHEAT STREAK MOSAIC VIRUS* IN PERENNIAL WHEAT

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of LEIGH ANN HARRISON find it satisfactory and recommend that it be accepted.

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Abstract

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Perennial wheat has anticipated benefits as an alternative cropping system in eastern Washington, including reduced planting costs, improved wildlife habitat, reduced soil erosion and increased soil quality. *Wheat streak mosaic virus* (WSMV) is widespread and common in this region of the US. Though epidemics are rare in annual winter wheat, this research demonstrates that WSMV is a serious threat to perennial wheat since it persists through successive years of regrowth. Thirty-three perennial lines derived from *Thinopyrum* sp. were evaluated for WSMV resistance in a growth chamber. Lines 03JP011, 03JP031 and 03JP039 had reduced virus replication, as indicated by lower ELISA values and less severe symptoms, compared to a susceptible control. Lines 03JP031 and 03JP039 were tested for temperature-sensitive resistance at 25°C, but reactions did not differ from those at 22°C. Progeny of 03JP031 and 03JP039 were evaluated for segregation of resistance, and inheritance is complex. In field plots, six perennial lines, including 03JP039, and three annual wheat controls were planted at three locations for two consecutive years in eastern Washington. In first year perennial growth, WSMV was detected in 16% of rows of line 03JP039 by ELISA, but only 5% were symptomatic, as compared with susceptible annual wheat controls in which 65 to 94% of rows were symptomatic. In second year perennial growth, 56% of 03JP039 rows developed symptoms and

WSMV was detectable, and 100% of the five other perennial lines were symptomatic. Variable symptoms between first and second year growth may be explained by temperature sensitivity, susceptibility of initial perennial regrowth to expression of WSM symptoms, and/or heterogeneity within perennial wheat lines. WSMV natural infection was studied in bordering plots in two seasons; symptoms were observed in three perennial lines, and was not detected by ELISA in line 03JP039, nor were symptoms apparent. Compared with susceptible controls, perennial wheat had reduced virus replication in both controlled environment and field plots, especially line 03JP039. These results demonstrate that resistance to WSMV is present in perennial wheat and should be useful to plant breeders.

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INTRODUCTION

Eastern Washington State, USA is one of the most important wheat (*Triticum aestivum* L.) growing areas in the world. In the region known as the Palouse, winter wheat is the most common crop. Yields average 9.0 Mg ha^{-1} (170 bu ac^{-1}), and the majority of cultivars are grown for soft white flour end-use quality (Scheinost et al. 2001). These highly productive yields are attributed to the region's mild temperatures, 400-500 mm (16-20 in) average annual precipitation, and fertile soil (Scheinost et al. 2001). In the Palouse, winter wheat is typically grown in a three-year rotation with peas (*Pisum sativum* L.) and spring wheat or barley (*Hordeum vulgare* L.) (T. Murray 2004, personal communication).

The Palouse landscape is characterized by rolling hills with steep slopes ranging from 8 to 30%, and the majority of this land is currently used for wheat production. As a result, soil erosion is a serious problem that limits crop productivity and long-term sustainability (Papendick 1996). Current research at Washington State University is directed at developing a perennial wheat crop, which could serve as one solution to the problems associated with cultivation and tillage of the region's highly erodible soils (Jones et al. 2000). Along with serving as an alternative to conventional cropping systems, perennial wheat has several other anticipated benefits, such as reduced planting costs, improved wildlife habitat, and increased soil nutrient retention. As with the development of any new crop, an important consideration is the incorporation of resistance to potential disease threats. Sources of disease resistance within perennial hybrids to pathogens in the region must be identified and characterized. Addressing disease threats in perennial wheat is the focus of this thesis, and serves as one step toward attaining the ultimate goal of developing a vigorous, healthy perennial wheat crop.

The primary research objectives include the evaluation of perennial wheat germplasm lines for resistance to *Wheat streak mosaic virus* (WSMV) under controlled conditions, field evaluation of survival and yield potential in perennial wheat lines inoculated with WSMV, and epidemiology of WSMV under natural conditions in perennial and annual winter wheat. Although each objective is distinct, they are intertwined, and general conclusions are drawn and discussed from the research as a whole.

PERENNIAL WHEAT: AN ALTERNATIVE CROPPING SYSTEM

The primary objective of cereal growers is high yield. As such, many of the conventional practices used by growers have negative environmental consequences. Because of the steep topography of the landscape, the soil types, and other environmental conditions, the Palouse region of Washington is highly prone to soil erosion (Lindstrom et al. 1994). Conventional cultural practices, including annual tillage, expose the soil and result in erosion by wind and water (Low 1972). Between 25 and 75% of the cropland topsoil in the Palouse has been lost since the beginning of its cultivation over a century ago (USDA 1978). This area of Eastern Washington was recognized in the 1980 RCA (National Resources Inventory-Soil and Water Conservation Act) as having some of the most severe soil erosion in the United States (Batie 1983). Run-off from tilled cropland results in sediment pollution and diminished water quality of rivers and streams (Lal 1998). Soil particles released into the air during tillage or picked up by wind is the leading source of air pollution in Washington State (USDA-ERS 1996).

Soil erosion is a serious problem that limits crop productivity and long-term sustainability (Papendick, 1996). However, evidence of decreased productivity can be masked by growing improved wheat varieties and using inputs such as fertilizers and pesticides (Young et al. 1984).

Winter wheat yields have continued to increase at a slow rate over the last several decades, but the long-term quality of the soil and this cropping system is questionable (Jennings et al. 1990).

The soil erosion problem in the Palouse, and the Pacific Northwest in general, has been recognized for over a century. Among wheat growers, this awareness has led to an increase in the use of conservation tillage methods, with the greatest efforts beginning in 1970s (Carlson et al. 1994). Some of these control practices include no-till or minimum-till systems, chisel plowing, strip cropping, and contouring (Cook 1986); however, no-till methods are dependent on the intense use of herbicides and pesticides. Due to the expense of direct seeding equipment and reduced yields from weed and disease pressure during the transition from conventional to no-till, growers are reluctant to adopt this system (Papendick 1996).

Another important erosion control measure involves farmer participation in the government-implemented Conservation Reserve Program (CRP). The aim of this program is to reduce soil erosion and excess grain production, as well as to protect agricultural croplands. In the United States, several million acres of farmland are currently enrolled in the CRP, including Washington and other states in the Pacific Northwest. This land is often planted with perennial grasses (USDA-ERS 1996). The wild perennial plants provide a vegetation cover and structure to the soil that prevents soil erosion, increases soil water retention (Wood et al. 1991), restores nutrients to the soil (Gerbhart at al. 1994), and provides a habitat to grassland wildlife (Ryan et al. 1998).

Perennial wheat has the potential to serve as a sustainable alternative to conventional cropping system available to growers in eastern Washington State and potentially, all wheat-growing areas of the United States. At Washington State University (WSU), several hybrids have been made by crossing wild perennial grasses such as *Thinopyrum* sp. with annual winter

wheat, and tested in the field for the past 9 years (Scheinost et al. 2001). The harvestable perennial wheat crop has similar characteristics to commercial annual wheat, but regrows after each reproductive cycle. Multiple years of perennial wheat growth would have benefits similar to CRP, and would also provide a harvestable grain crop. Additional benefits include increased soil microbial activity (Blevins 1984), reduced soil erosion, increased soil nutrient and water retention, and improved wildlife habitat (Jones et al. 2004).

Plant breeders have been using wild perennial grass species to improve domesticated wheat production since the 1920s. The first successful crosses between *Triticum* x *Thinopyrum* were made in the Soviet Union in the 1920s (Tsitsin 1934, Tsitsin 1940). Efforts continued through the 1950s in the Soviet Union and the United States, but were abandoned due to low, unacceptable yields or poor end-use quality (Vinall and Hein 1937, Suneson and Pope 1946, Suneson, 1959, Jakubziner 1958, Tsitsin 1960). A pressing need to address erosion and environmental issues has lead to the re-emerging interest in perennial wheat in recent decades (Jackson 1980, Wagoner 1990, Cai et al. 1998, Jones et al. 2000).

The perennial wheat program at WSU has developed perennial wheat breeding lines from *Thinopyrum* x *Triticum* crosses. The *Triticum* genotypes in these crosses are soft white winter wheat cultivars adapted for production in the Pacific Northwest. Previous attempts were aimed at developing a hard red perennial wheat, but were restricted by strict end-use quality characteristics. Since soft white wheat has less strict requirements and is generally easier to breed than hard wheat quality (Jones and Cadle 1997), a soft white perennial wheat is desirable so it can be grown, harvested and sold along with other soft white wheat varieties. Currently, perennial wheat is not yet commercially grown in the United States.

When it is available, perennial wheat will have, along with ecological benefits, several economical benefits including less time contributed to planting, less petroleum usage, and lower labor costs. Compared to no-tillage, new planting equipment is not needed, since perennial wheat uses planting equipment developed for annual crops (Jones and Murray 2004). *Thinopyrum* species, which wheat breeders used to confer the perennial growth habit, is an important source of disease resistance (Friebe et al. 1996, Juahar and Peterson 1996); therefore, perennial wheat varieties will likely have resistance genes from these species, which should result in reduced pesticide and herbicide sprays, and a decrease in the cost of production. Planting perennial wheat will also free up CRP funds, so that other erodible lands can be accommodated in this program.

A perennial wheat crop has potential drawbacks to the grower. If the grower chooses to harvest the seed for market, perennial wheat currently has reduced grain yield as compared to a conventional annual wheat crop. In the most successful experiments, it yielded 70% of the annual wheat cultivars. As an explanation, annual plants disburse the majority of their energy into seed production, and perennial plants expend a larger portion of their energy into developing a vigorous root system and less into the seed (Gardner 1989). However, carbon allocation throughout the plant is controlled genetically, and so, it may be possible to produce a hybrid that has the genetic ability to allocate sufficient carbon to both the root system and the seed (Jackson and Jackson 1999). Yield and regrowth of a perennial crop are usually negatively correlated traits; however, simultaneous selection for these correlated traits can be achieved if compromises are allowed, such as intermediate grain yields (Cox et al. 2002b).

Another drawback to perennial wheat is greater risk of disease. Because the crop is managed as a no-till system, there is increased pressure from weeds and disease. Requirements

for weeding, fertilizing, and harvesting will be different from annual wheat; these requirements are not yet determined (Jones et al. 2000). Because the roots and crowns of perennial wheat persist in the soil for multiple years, there is an increased pressure from soil-borne fungal pathogens such as Cephalosporium stripe (*Cephalosporium gramineum*), eyespot (*Oculomacula yallundae* and *O. acuformis*), and other root pathogens, including take-all (*Gaeumannomyces graminis* var. *tritici*), and Pythium root rot (*Pythium ultimum)*. Previous research in perennial wheat (Cox et al. 2002a) explored the potential resistance in perennial wheat to eyespot, Cephalosporium stripe, and *Wheat streak mosaic virus* (WSMV). These fungal diseases are important in Pacific Northwest wheat production, but the impact of WSMV is largely unknown. Currently, no locally adapted annual wheat cultivars have resistance to WSMV. This virus is of specific interest to perennial wheat breeders because virus infections persist throughout the life of the plant, and thus, through successive years of perennial wheat growth. Wheat streak mosaic has the potential to cause severe damage to regrowth that is rarely observed in annual wheat in Washington State. A desirable goal of perennial wheat is to have at least three years of production, and this virus pathogen could cause severe damage if control measures are not taken.

Wheat streak mosaic virus

Wheat streak mosaic virus is an important pathogen in wheat that is widely distributed in the United States, Canada, Europe, the Middle East, North Africa, Australia, and Central, East, and Southeast Asia. It is an RNA virus with flexuous, filamentous particles *c.* 700 nm long and 15 nm in diameter (Brakke 1971). Wheat streak mosaic (WSM), was first described in Nebraska in 1922 (Wiese 1987) as "yellow mosaic." *Wheat streak mosaic virus* was first associated with WSM symptoms in 1937 (McKinney 1937). WSMV is a member of the Family *Potyviridae*, and

after phylogenetic studies, was designated as the type species of the Genus *Tritimovirus* (Stenger et al. 1998), which is genetically distinct from eriophyid mite-transmitted viruses in the genus *Rymovirus* (Hall et al. 1998, Stenger et al. 1998). The genus *Tritimovirus* includes WSMV and *Brome streak mosaic virus* (BrSMV) (Hall et al. 1998, Stenger et al. 1998). Along with wheat, its wide host range includes several members of the Family *Gramineae*: barley, corn, oats, rye, sorghum, pearl millet, and several wild perennial grasses (Slykhuis 1955, Staples and Allington 1956, Slykhuis 1963, Slykhuis 1965, Somsen and Sill 1970, Harvey and Seifers 1991, Seifers et al. 1996, Seifers et al. 1998). Outbreaks of WSMV have great potential for destruction in wheat crops, causing yield losses approaching 100% (Christian and Willis 1993, French and Stenger 2003). In the Great Plains region of the United States, this disease is most prevalent and causes significant crop losses in winter and spring wheat each year (Wiese 1987). Although WSMV epidemics occur rarely in the Pacific Northwest, the most recent in Washington State in 1994 caused severe crop loss (Gillespie et al. 1997). In addition to yield losses, WSMV also reduces the milling quality of the wheat grain (Finney and Sill 1963).

WSMV can cause significant damage to spring and winter wheat. Symptoms develop at temperatures above 10°C, and include mottling and chlorotic streaking in leaves, stunted growth, reduced tillering, and sterile or partially filled heads (Brakke 1971). This disease has received little attention in PNW because it rarely causes economic damage in annual wheat, and so resistance to WSMV is not present in wheat cultivars developed for this region. Perennial wheat may be more prone to severe damage by WSM if the pathogen persists in the subsequent year's growth and causes severe symptoms that are rarely seen in the first year.

Wheat streak mosaic virus is readily sap-transmissible (Martin 1978) and WSMV was recently shown to be seed transmitted in eight different wheat genotypes at a rate of 0.5% to

1.5% (Jones et al. 2005). The most important and predominate mode of WSMV dissemination is by the wheat curl mite, *Aceria tosichella* Keifer (formerly known as *A. tulipae)* (Slykhuis 1955). Besides serving as the vector, the mite causes distinct damage to the host, including leaf rolling and trapping (Weise 1987). The eriophyid mite acquires virus particles as nymphs within 15 minutes or more (Paliwal and Slykhuis 1967) of feeding on infected plant tissue, and remains viruliferous through molting and for at least 9 days when feeding on virus-immune plants (Staples and Allington 1956). The mites can transmit WSMV to a healthy host within 15 minutes of feeding. WSMV is not transovarially passed from adult mites to offspring (Slykhuis 1955). Virus particles have been observed in the alimentary canal, or digestive tract, of the wheat curl mite (Paliwal and Slykhuis 1967), but little research has been done to provide evidence for virus replication within the mite vector. Virus particles have been observed in the midgut, body cavity, and salivary glands of the mite *Eriophyes tulipae,* which suggests that the virus is circulative within the vector (Paliwal 1980).

For protection against desiccation, the mite vector often harbors at the leaf base, near the ligule (Painter and Schesser 1954) of the lower leaves of plants (Weise 1987). The mites are very small (~0.3 mm long) and are dispersed from host to host via wind. Once mite populations on a plant increase, the wingless individuals move to the tip of the host leaf and wait to be lifted and disseminated by wind currents. This reliance on the wind for dissemination usually results in initial infestations occurring on the field borders. The wheat curl mite has been reportedly trapped at a height of 46.3 m (152 feet) above and 2.4 to 3.2 km (1.5 to 2 miles) from the nearest wheat field (Pady 1955), which is an indication that this vector is capable of movement across long distances.

Behavior of the mite vector is a limiting factor to the spread of WSMV, since they are more likely to be active in warmer temperatures and sunny weather. Therefore, early fall plantings and late spring plantings expose the newly emerging plants to potential contact with mites carrying WSMV. The wheat curl mite can only survive for 4 to 6 days without green tissue (Slykhuis 1955), and so, an alternative host is needed for survival between harvest and planting of the next winter wheat crop. Wild perennial grasses serve as over-summering and over-wintering hosts for the wheat curl mite (Keifer 1952). These mites can be found considerable distances from the nearest wheat field (Painter 1954). Forms of conservation tillage (no-till or low-till) can also increase weedy hosts for survival of the mite vector (Wiese 1987). Movement of the wheat curl mite from wheat was thought to be associated with host senescence and deterioration (Nault and Styer 1969). However, the main influence on mite movement relates to the size of the source population and not the condition of the host plant (Thomas and Hein 2003).

Cross protection is a phenomenon that occurs between strains of a plant virus, and it has been well documented in WSMV (McKinney 1929, McKinney 1956, Hamilton 1980, Fraser 1985, Sherwood 1987, Hall et al. 2001a). This phenomenon, which was first observed in the 1920s (McKinney 1929), was demonstrated between mild and severe strains of WSMV (McKinney 1956). Cross protection was studied between two related WSMV isolates in a mixed infection and resulted in spatial division in the plant host (French and Stenger 2003). When two related strains were coinoculated, both were equally able to systemically infect the host. However, French and Stenger (2003) demonstrated that usually only one strain was established in a tiller, and the other strain was prevented from colonizing the tissue, most likely due to cross protection. One proposed outcome of cross protection is genetic isolation of closely related

WSMV populations in the field (Hall et al. 2001a). Cross protection has also been observed in other viruses that cause disease in cereals, including *Barley yellow dwarf virus* strains in soft red winter wheat (Perry et al. 2000).

Historically, severe epidemics of WSM do not frequently occur in cool climate wheat-growing areas such as the Pacific Northwest of the US. When epidemics occur that result in large yield loss, they are usually initiated by hail storms that shatter seed heads that are near maturity, resulting in volunteer winter wheat emergence just before harvest. Because wheat is the preferred host for WSMV and its vector, the volunteer wheat serves as an oversummering reservoir and the primary inoculum source (Staples and Allington 1956, Somsen and Sill 1970), supporting large populations of viruliferous mites. In early fall, mites move from volunteer wheat to the emerging fall-sown wheat, and WSMV infections occur when wheat is at its most susceptible growth stage. In comparison, young perennial wheat regrowth emerges at approximately the same time that volunteer wheat plants emerge, which suggests that perennial wheat can serve as a green bridge from season to season within the same field and to neighboring winter wheat fields. And so, in wheat-growing areas such as the Palouse, perennial wheat may be the only green host at the end of the summer season. Considering these patterns, large plantings of perennial wheat, without adequate resistance to WSMV, may increase the potential for epidemics.

WSM symptom severity is variable among environmental conditions, virus strains, and highly dependent on the plant host. Triticale (wheat x rye grass), as well as several wild grasses, may become infected with WSMV but do not express symptoms (Hendrich 2001). Some WSMV isolates in Europe do not infect oats or barley, and differences in RNA sequences between isolates from USA, Mexico, and Eurasia range from 10% to 20% (Choi et al. 2001,

Rabenstein et al 2002). However, WSMV genotypes in the USA share 97.6% nucleotide sequence identity (Chenault et al 1996). As early as 1956, different strains with mild and severe symptoms were documented (McKinney 1956). WSMV, like all other plant viruses, are dependent upon their host for replication. WSMV replicates within the host as the plant respires, which generally requires warmer temperatures and adequate sunlight. Temperature is the predominate factor that influences survival and replication of a virus strain (Hull 2002). Therefore, symptom expression may be prolonged if infection occurs in the cooler late autumn months or early summer months.

The wheat curl mite also transmits *Maize red stripe virus* (MRStV) (Skare et al. 2006), the causal agent of High Plains Disease (HPD) (Seifers et al. 1997). It produces mosaic patterns, leaf curling, general chlorosis, necrosis, and red striping in maize. HPD is quickly becoming an economically important problem in wheat and maize (*Zea mays* L.). In nature, MRStV is often found co-infected with WSMV (Marcon et al. 1997, Mahmood et al. 1998). MRStV is not mechanically transmissible, but is seed transmissible in sweet corn at a rate less than 0.01% (Forster et al. 2001).

At present, the most effective methods for control of WSM are destruction of the "green bridge" and alteration of planting date (Staples and Allington 1956, Wiese 1987). However, these practices may not be effective when winter temperatures are mild, wheat is planted in late spring, and abundant volunteer plants are present (Staples and Allington 1956). Because resistance to WSMV is not present in cultivars adapted to the PNW, disease control options are limited. Few safe and effective miticides are available to control the vector, and none are available for large-scale commercial use. Growers can avoid planting spring wheat directly adjacent to winter wheat to limit movement of the vector, and eliminate the green bridge.

However, the range of mite movement can span as far as 3.2 km (2 miles) (Pady 1955), and such a control option may not be feasible in intensive wheat-growing areas. Delaying seeding date for winter wheat reduces the window of mite vector activity in the fall and can serve as a control option. However, seeding dates are usually dictated by availability of soil moisture (Martin et al. 1984), and appropriate time for plant emergence and maturity is essential for winter hardiness. In Southcentral US, delaying seeding until October-November results in the less mature wheat that is more prone to early spring infection and development of severe symptoms by late summer (Hunger et al. 1992). The availability of host resistance to WSMV is a necessary option for control of WSM in perennial wheat.

RESISTANCE TO WSMV

Research in other areas of the United States has revealed that wild wheat relatives, including *Triticum, Agropyron, Aegilops, Elymus, Secale*, and "Agrotricum" hybrids are promising sources of resistance to WSMV (McKinney and Sando, 1951, Somsen and Sill 1970, Sharma et al. 1984, Stoddard and Lommel 1987). Among these grasses, *Agropyron* (now known as *Thinopyrum)* is the most promising and most consistent source of resistance. A resistance gene from *A. intermedium* (Host) Beauv, *Wsm1*, confers resistance to both WSMV and the wheat curl mite in *Agropyron* x wheat hybrids (Friebe et al. 1996). *A. elongatum* (Host) Beauv x wheat hybrids also were resistant to WSMV (Lay et al. 1971, Liang et al. 1979, Stoddard and Lommel 1987). The resistance gene was identified as *Cmc2* (Larson and Atkinson 1970).

Previous attempts to incorporate resistance from wheat relatives into a bread wheat has involved the transfer of alien genes by producing chromosome addition and substitution lines (Liang et al. 1979, Wells et al. 1982). However, addition of alien genes into wheat has yielded many undesirable characteristics, resulting in mixed effects on quality and agronomic traits

(Lukaszewski and Gustafson 1983). These observations were repeated using *Thinopyrum*-derived hybrids, specifically wheat x *Thinopyrum intermedium* (Lay et al. 1971, Wells et al. 1973, Liang et al. 1979, Wells et al. 1982) and wheat x *Agropyron elongatum* (Larson and Atkinson 1970, Sebesta et al. 1972, Martin et al. 1976).

Breeding for resistance to the wheat curl mite vector is another important option for controlling WSMV. Mite resistance has been identified in species of *Agropyron, Aegilops, Elymus,* and *Secale* (Somsen and Sill 1970, Martin et al. 1976, Thomas and Conner 1986, Stoddard and Lommel 1987, Friebe et al. 1996). *Agropyron intermedium* translocation lines had WCM resistance derived from the wheatgrass chromosome 6Ag (Conner et al. 1991).

Thinopyrum species have been used as parents in the perennial wheat breeding program at WSU because they carry resistance to diseases that are significant in the Pacific Northwest, including Cephalosporium stripe, barley yellow dwarf, eyespot, and wheat streak mosaic (Jones et al. 1995, Friebe et al. 1996, Juahar and Peterson 1996). Identifying sources of resistance to WSMV within perennial wheat is essential to the future plant breeding that will ultimately develop a commercially acceptable perennial wheat cultivar. Recently, Cox et al. (2002a) identified perennial wheat lines with resistance to WSM, Cephalosporium stripe, and eyespot. Eight lines were resistant to WSM, and one line expressed temperature-sensitive resistance. However, WSM resistance in perennial wheat lines has not been evaluated in the field. In this study, evaluation of perennial wheat germ plasm lines for WSMV resistance was determined under controlled conditions, and then under field conditions for two growing seasons. Knowledge gained from WSMV in perennial wheat, epidemiologically and agronomically, will reveal the importance of this disease and will be applicable to winter wheat cultivars grown in the Pacific Northwest.

MATERIALS AND METHODS

RESISTANCE TO WSMV UNDER CONTROLLED CONDITIONS

Perennial Germplasm Resistance Screening

Germplasm. Thirty-three F_3 perennial wheat germplasm lines developed by the Washington State University Winter Wheat Breeding Program were screened for resistance to WSMV under controlled conditions. These lines were derived from *Thinopyrum* sp., but the pedigree for individual lines is unknown. The parents of these lines include Spitzer (AT 3425), a *Thinopyrum ponticum* /Chinese Spring partial amphiploid, and a *Thinopyrum intermedium* (PI 206624)/ Chinese Spring partial amphiploid. The common backcross/topcross parent was Madsen (PI 511673). These lines appeared to be genetically stable and regrew in the field at least once. Germplasm parents XC14 and *Thinopyrum ponticum (*Barkworth & Dewey, 2n= 10x = 70) were also included. Montana-2 (MT-2; *Triticum durum x Th. intermedium*, 2n= 8x= 56) is a genetically stable perennial line and was used as a resistant control. Stephens winter wheat (CI 17596) is susceptible to WSMV and was used as the susceptible control.

Experimental design. The methods described by Cox et al. (2002a) were used to evaluate all lines for resistance to WSMV under growth chamber conditions. Seeds of each line were placed in a petri dish on moist filter paper and imbibed at 4ºC for 4 days to synchronize germination. Five seeds were sown per 8.9-cm-square pot filled with Sunshine Professional Growing Mix (Sun Gro Horticulture, Vancouver, BC). Pots were placed in a growth chamber at 22ºC with a 12-hour photoperiod and arranged in a randomized complete block design with four replicates. After emergence, plants were thinned to three per pot. Plants were fertilized with Osmocote (20-

20-20 N-P-K) every 2 weeks. This experiment was conducted three times: in January 2005, February 2005, and April 2005.

Virus inoculum and inoculation. WSMV was isolated from perennial wheat collected at the Spillman Agronomy Farm, Pullman, WA in August 2004, and maintained in Hill 81 (CI 17954) winter wheat in the greenhouse. This isolate was identified as WSMV based on symptom expression, serology, and RT-PCR. WSMV inoculum was prepared by shredding infected, symptomatic tissue in 0.01 M sodium phosphate buffer (1:10, w/v), pH 7.0, and diatomaceous earth (0.01 g/ml) with a blender. Within 1 hr of extraction, test plants were inoculated by rubbing leaves with a cheesecloth pad saturated with the inoculum. The second leaf was sandwiched with the thumb and forefinger and rubbed once from base to tip with light pressure (Martin 1978). Test plants were mechanically inoculated at the 2- to 3-leaf stage. To assure virus infection, the youngest leaf of each plant was inoculated 8 to 10 days after the first inoculation, using the same method. Stephens was used as the susceptible mock-inoculated control.

Disease evaluation. Eighteen days after the second inoculation, disease symptoms were scored on individual plants and averaged within pots. The symptom rating scale was developed from personal observations, and was similar to scales used previously (Cox 2002a), where: 0 = no WSMV symptoms; 1= light green to faint yellow mottling on the leaves; 2= yellow, elongated streaking, stunting, tillers slightly less erect than healthy; 3= severe yellow to white streaking, stunting, tillers much less erect than healthy; and 4= more than 75% chlorosis of the leaves, severe stunting, or death of the plant (Fig. 1).

Approximately 5 cm of the youngest leaf of the three plants in each pot was harvested and pooled for testing. Indirect enzyme-linked immunosorbent assay (indirect ELISA) was used

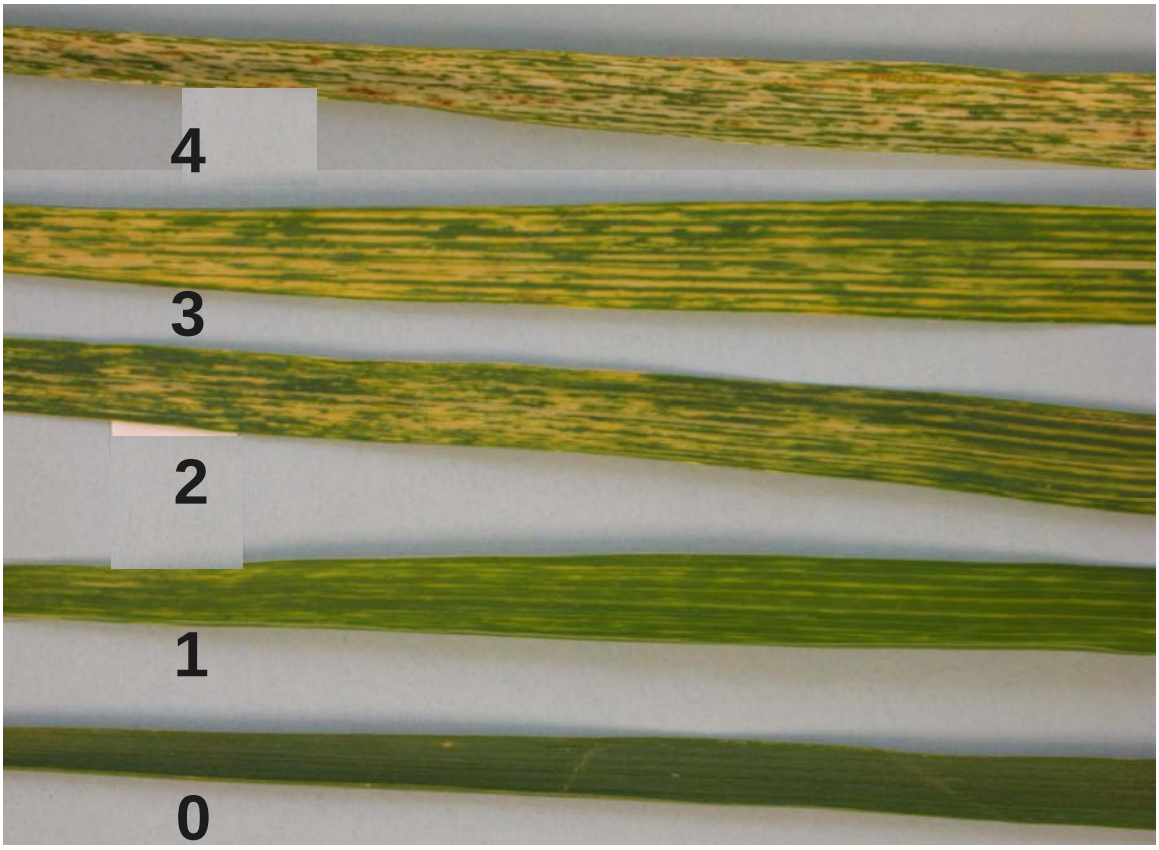

Figure 1. Symptom rating scale to assess Wheat streak mosaic symptom severity. These leaf symptoms were considered in conjunction with other symptoms, such as stunting.

to detect WSMV (Clark and Adams 1977). For perennial lines that appeared to be segregating based on visible phenotype (03JP012, 03JP014, 03JP017, 03JP020, 03JP024, 03JP038) and parent lines (XC14 and *Th. ponticum*), the three plants in each pot were tested separately to observe possible segregation for resistance.

Samples were frozen in liquid nitrogen, ground with a wood applicator stick in a 1.7 mL Eppendorf tube, and then 0.02M carbonate coating buffer, pH 9.5, was added (1:10 w/v). Aliquots of each sample (100 µl) were added to 96-well ELISA plates (Maxisorp™ surface, Nalge Nunc International, Rochester, NY) in duplicate wells. Plates were incubated at room temperature for 1 hr. Plate wells were completely filled and rinsed with Tris buffered saline-Tween (TBS-T; 8.7 g NaCl, 0.2 g KCl, 6.06 g 0.05M Tris base, 0.5 mL 0.05% Tween 20; pH 7.4) four times for 2 min each. One-hundred µl of anti-WSMV IgG (antiserum #9924 courtesy of T. Murray of Washington State University, Pullman, WA) (1.06 µg protein/ml) in V/C buffer (20 g Sigma Polyvinyl-Pyrrolidone, 2 g ovalbumin, 1 L TBS-T) was added and incubated for 1 hr at room temperature. The plate was washed as before and 100 µl of goat anti-rabbit conjugated to alkaline phosphatase (Sigma-Aldrich St. Louis, MI) diluted 1:2,000 in V/C buffer was added and incubated for 2 hrs at room temperature, or overnight at 4ºC to prevent desiccation of wells. After washing, p-nitrophenyl phosphate (p-NPP) (Sigma-Aldrich St. Louis, MI) (1 mg/ml in 1.0M diethanolamine buffer, pH 9.8) was added to each well (100 µl), incubated at room temperature for 30 min, and then optical density was measured with a Molecular Devices SpectaMax M_2 Microplate Reader (Molecular Devices, Sunnyvale, CA) at 405 nm. If necessary, colorimetric reaction was stopped by adding a drop of 3M NaOH to each well. Negative and positive wheat tissue controls and a carbonate buffer control were included in duplicate wells for each plate. ELISA samples were considered positive to WSMV if absorbance

readings were three times greater than the healthy control. The remaining tissue extract in eppendorf tubes was then stored at -20ºC.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was used to detect WSMV in samples that had borderline positive ELISA results. Samples were considered borderline if ELISA values were within ± 0.05 of the threefold value of the healthy tissue control. From the remaining tissue extract in ELISA carbonate buffer, a modified Dellaporta method was used to extract the total nucleic acids (Presting et al. 1995) as follows: to 0.5 ml Dellaporta buffer (10 mL 1M Tris, 10 mL 5M NaCl, 60 mL H_2O, 20 mL 0.25M EDTA, 70 µl β-ME), approximately 800 µl of tissue extract in carbonate buffer was added, samples were mixed with a wood applicator stick, and then 140 µl of 10% SDS (sodium dodecyl sulfate) was added to each sample and incubated at 65ºC for 15-20 min. Then, 250 µl of 8M KOAC was added, samples were mixed by inverting the tube, and placed on ice for 5-10 min. After centrifuging at 16,000 g for 5 min, 1 ml of supernatant was removed and added to a 1.7 mL Eppendorf tube with 0.6 ml of isopropanol. Samples were mixed by inversion and placed on ice for 5-10 min. Samples were centrifuged for 5 min at 16,000 g, the supernatant was discarded, and the pellet was washed with 70% ethanol. After centrifuging for 1 min at 16,000 g, the ethanol was discarded and the pellet was resuspended in 60 µl of sterile water.

WSMV specific primers (developed by Keri Druffel, Washington State University, Pullman, WA) were used to synthesize cDNA from the RNA virus genome, and then the PCR product. Primer sequences (5' to 3') were WS-ATG: ATG GCA TCA GGT TCT GGA GCA GCA; and WS-3P: GGG TAC CTC TCG TGG AGA AGT ACA CA. First strand synthesis (cDNA) reaction consisted of 4 µl of 5X first strand buffer (Invitrogen, Carlsbad, CA), 4 µl of 2.5 µM dNTPs (Invitrogen), 2 µl of 0.1 M DTT, 0.5 µl of 200 µM virus specific primer (WS-

3P), 0.25µl of RNase Out (Invitrogen), and 0.25 µl of SuperScript™ II Reverse Transcriptase (Invtirogen). The cDNA reaction was incubated at 42ºC for 1 hr. Then, 1 µl of the cDNA was used as template in a PCR reaction consisting of 2 µl of 10X reaction buffer, 1.2 µl of 2.5 µM of dNTPs, 0.8 µl of 50 mM $MgCl_2$, 0.6 µl of 20 µM of forward and reverse primers (WS-3P and WS-ATG), and 0.2 µl of Taq DNA polymerase (Invitrogen). PCR was performed in an iCycler™ Thermocycler (Bio-Rad, Hercules, CA). The program consisted of an initial denaturation at 72°C for 7 min, followed by 32 cycles of 94°C for 30 sec, 57°C for 20 sec, and 72°C for 50 sec, and 7 min at 72°C with an infinite hold at 4°C. The PCR product was amplified from the WSMV coat protein open reading frame and resulted in a band of 1025 base pairs. Aliquots (8 µl) of each PCR reaction was sized on a 1.5% agarose gel by electrophoresis and stained with ethidium bromide.

Data analysis. Because some of the perennial wheat lines were segregating for phenotype, an average of four replicates was used for each line. Repeatability of experimental procedures and subsequent data was determined by performing an F-test for homogeneity of variance on data from the two repeated experiments for both dependent variables (ELISA values and symptom rating). Because the variance between data sets was not significantly different, the results from the repeated experiments were combined for analysis. Symptom ratings were plotted against ELISA values and a linear regression analysis was performed using PROC REG in SAS (version 9.1, SAS Institute, Cary, NC). The combined results were then subjected to analysis of variance using PROC MIXED in SAS with a Dunnett's test for comparison of means to a control. The resistant control was MT-2 and lines were analyzed for statistical similarity (greater than $p=0.05$) as a measure of resistance. All statistical assumptions were satisfied, including data normality, equal variances, homogeneous variances, independence, lack of outliers, and model fit.

Segregation for WSMV Resistance

Two perennial wheat lines, 03JP031 and 03JP039, with variable phenotypic reactions to WSMV in greenhouse experiments were included in this study. These lines were F_3 *Thinopyrum* x *Triticum* hybrids. Stephens winter wheat (CI 17596) was the susceptible control and MT-2 was the resistant control. Seed germination was synchronized as previously described. One seed was sown per circular peat moss pad and placed in a tray. Trays were placed in a growth room at 22˚C until plants reached the 1- to 2-leaf stage and then seedlings were vernalized at 4˚C for 8 weeks. After vernalization, one seedling was sown per 8.9-cm-square pot filled with Sunshine Professional Growing Mix. Pots were placed in growth chambers at 22ºC with a 12-hr photoperiod and arranged in a randomized complete block design with three replicates. Plants were fertilized every 2 wks with Osmocote (20-20-20 N-P-K).

Two days after plants were moved to the growth chamber at 22ºC, plants (5- to 6-leaf stage) were mechanically inoculated with WSMV as previously described. The youngest leaf of each plant was then mechanically inoculated 8 days after the first inoculation. Eighteen days after the second inoculation, plants were visually rated for WSM symptom severity using a 0 to 4 scale, and plant tissue was harvested and tested by indirect-ELISA for virus detection as previously described.

Wheat plants were then moved to the greenhouse and allowed to reach physiological maturity, when seed was harvested from each plant (May 2006) and stored separately. Seed for progeny testing were selected and included seed from F_3 plants that showed symptoms and tested positive by ELISA and seed from 18 randomly selected symptomless F_3 plants that tested negative by ELISA. All borderline positive ELISA values were verified by RT-PCR for WSMV. Four seeds were taken from each of these plants and planted in a randomized complete block

design with four replicates. To insure that no WSMV seed transmission occurred, F_4 generation plants were sampled for ELISA at emergence (1-leaf stage). Each F_4 perennial wheat plant was then screened for resistance using the methods previously described.

Screening for Temperature-sensitive Resistance

Two perennial wheat lines, 03JP031 and 03JP039, with resistant phenotypes were evaluated for possible temperature-sensitive resistance to WSMV. These lines were negative for WSMV by ELISA in one or more of the previous experiments and had low average symptom ratings. Data on symptom expression were collected after 18 days from the third experiment and plants of lines 03JP031 and 03JP039 were placed in a growth chamber at 25°C. After 14 days, each plant was rated for WSMV infection based on visual symptoms and ELISA. Statistical analyses were not conducted due to the low number of experimental units (12 plants per line).

To observe possible temperature-sensitive resistance in perennial regrowth, plants grown for the segregation of disease resistance experiment were used. After harvest of first year growth, plants were placed in a growth chamber at 22°C for 7 days. The chamber was then raised to 32°C and plants were left for 8 days. Each plant was assessed for presence of WSMV based on symptoms and ELISA.

RESISTANCE TO WSMV IN FIELD PLOTS

Plant material. Six F_3 generation perennial wheat germplasm lines randomly selected from the 33 lines screened for resistance in the growth chamber were included in field experiments. In the 2004-05 plots, Eltan and Edwin winter wheat cultivars were included as susceptible controls, and Hill 81 winter wheat was included in the 2005-06 plots. Spitzer

(Agrotriticum no. 3425; wheat x *A. elongatum*), a perennial wheat, was included in the 2004-05 field plots as a resistant control, but adequate seed was not available for the 2005-06 plots.

Field plots. Field plots were established at three sites in eastern Washington to assess resistance to WSMV. In Fall 2004, field plots were planted at the WSU Spillman Agronomy Farm, Pullman, WA (high rainfall zone, 559 mm/yr), and at the WSU Central Ferry Research Station (intermediate rainfall zone, 432 mm/yr). In Fall 2005, a field plot was planted at the WSU Plant Pathology Research Farm in Pullman, WA. Central Ferry Station is an irrigated site, and the 30-yr average day temperature during the summer growing season (April-August) is 23°C. The Pullman sites are not irrigated, and the average temperature is 18ºC.

The same plot layout was used at each site with nine wheat lines represented in a split-plot, completely randomized block design with six blocks. Split-plot rows were approximately 1.2 m long at the Central Ferry and Spillman Farm plots, and 2.1 m long rows at the Plant Pathology plot. Row length within blocks was measured after harvest for final yield calculations.

All plots were planted with a head-row drill. In 2004, the Spillman Farm plot was sown on 23 September and the Central Ferry plot on 11 October. In Fall 2005, the WSU Plant Pathology Farm plot was sown on 25 September, and the annual winter wheat seed was sown by hand at Spillman Farm on 27 September and Central Ferry on 13 October. Rows were sown with 0.2 g seed per meter. Initial plant density was recorded in November 2004 and 2005 when plants were at 2- to 3-leaf growth stage.

At each field plot, one-half of each paired row was mechanically inoculated with WSMV using the rub method as previously described, and the other half was a noninoculated comparison. In 2004, the Spillman Farm plot was inoculated on 25 October and the Central

Ferry plot was inoculated on 12 November. In 2005, the Plant Pathology Farm plot was inoculated on 24 October. Because WSMV was not detected by ELISA in inoculated plants in Fall 2004 and early Spring 2005, these rows were mechanically inoculated a second time on 13 April 2005 at the Spillman Farm and Central Ferry plots, and on 4 April 2006 at Plant Pathology Farm plot, to insure infection. To protect against natural WSMV infection, 1.5 x 3.1 m wheat plots were planted surrounding the inoculated experiment. To assure that the observed growth in the 2005-06 season was second year perennial wheat growth, field trials were monitored for volunteer wheat and grassy weeds on a regular basis throughout the early autumn and spring months.

Evaluation of WSMV. At each location and date of data collection, plants were assessed for presence of WSMV by ELISA and visual symptom rating. Samples for ELISA testing were collected by randomly taking leaf tissue from five different plants per row. Inoculated and noninoculated rows were sampled separately. Harvested tissue was diluted 1:10 (w/v) in carbonate buffer and ELISA tests were conducted as described previously. Each row was also evaluated as a whole for presence or absence of WSM symptoms due to variation in virus symptom expression over the growing season.

At the Spillman Farm and Central Ferry 2004 field plots, leaf samples were collected randomly for ELISA tests at initial plant emergence in the fall (2-leaf stage), 15 days after the fall mechanical inoculation, 30 days after inoculation, and in early spring on 15 March 2005. Symptom ratings and leaf samples were collected for ELISA at 2 week intervals from 2 May to 28 July 2005. If a row was positive by ELISA, no further samples were collected for ELISA, but symptoms were still monitored. RT-PCR was used to detect WSMV on samples giving

borderline positive ELISA results. For both 2004-05 field plots, the 100 seed weight for inoculated and noninoculated rows was determined by measuring four subsamples per row.

At the Plant Pathology Farm, data for WSMV detection was collected using the methods as the 2004 trials. Leaf samples were randomly collected for ELISA at initial plant emergence in the fall (2-leaf stage), 15 days after the fall mechanical inoculation, and after winter on 8 March 2006. Samples for ELISA and symptom evaluations were collected at 2-week intervals from 18 April to 24 June. If a row was positive by ELISA, no further samples were collected for ELISA, but symptoms were still monitored.

After harvest of the first year growth, ELISA and symptom ratings for the second year of growth (regrowth) at Spillman Farm and Central Ferry were collected multiple times beginning 8 to 14 days after perennial wheat re-growth was observed in August 2005 and monitored until harvest in July/August 2006. At the Plant Pathology Farm, a one-time evaluation for WSMV in perennial wheat re-growth was collected 20 days after harvest of the first year of growth. All inoculated and noninoculated rows were evaluated for presence or absence of WSM symptoms, and 16 random leaf tissue samples were collected for ELISA to confirm visual assessments.

In July 2006, the presence of perennial wheat second year growth, its vigor, and presence of WSM symptoms were recorded. Reproductive tillers produced by each plant were counted at the time of harvest, and final plant survival was recorded and compared to initial plant density recorded at 2- to 3-leaf stage in November 2004. Perennial wheat regrowth was then assessed for WSMV severity using a 0 to 4 scale at soft to hard dough stage (Zadoks 85). The rating scale was based on WSM symptoms (Brakke 1971) and personal observation, where 0= no WSM symptoms; 1= yellow mottling and streaking on leaves, no evidence of plant height stunting and sterility of seed spikes; 2= yellow mottling and/or streaking on leaves, significant plant stunting,

production of viable seed spikes and/or spikes filled with shriveled seed; 3= yellow streaking on leaves, stunting of whole plant, presence of sterile seed spikes; and 4= yellow streaking on leaves, stunting of whole plant, and absence of seed spikes. Seed spikes were considered to be sterile if 50% or more of the spikes produced by the plant were not filled with seed. To assure sterility of seed spikes, plants were re-evaluated after maturity and kernel hardening.

At the time of rating, WSM symptoms on leaves were faded or masked by other environmental factors, so the leaf symptom ratings taken during the growing season were incorporated into the final regrowth symptom ratings.

Yield determination. Inoculated and noninoculated rows were harvested separately using a hand sickle and threshed with a Vogel stationary bundle thresher. In 2005, the Central Ferry field plot was harvested 21 July (both annual and perennial wheat lines), the annual wheat in the Spillman Farm plot was harvested on 12 August, and the perennial wheat was harvested on 25 August.

In 2006, annual wheat at the Central Ferry plot was removed and discarded on 12 July, and the perennial wheat was not harvested. The annual wheat at the Spillman Farm plot was harvested on 4 August and the perennial wheat was not harvested. The annual wheat at the Plant Pathology Farm plot was harvested on 4 August, and the perennial wheat was harvested on 24 August.

All wheat seed was threshed, weighed, and the overall yield determined. Yield reduction was calculated by subtracting the mean of the inoculated rows from the mean of the noninoculated rows, dividing by the mean of the noninoculated rows, and multiplying by 100. Test weights were determined and percent reduction was calculated similarly.

Data analysis. Analysis of variance was conducted for 100 seed weight collected for the 2004-2005 Central Ferry and Spillman Farm plots, and means for the inoculated and non-inoculated rows were compared using Fisher's LSD.

Analysis of variance was conducted for winter wheat yield and test weight from the Spillman Farm plot in 2006 and means for the inoculation treatment were compared within cultivars using PROC MIXED with a Tukey-Kramer adjustment in SAS. Analysis of variance was conducted for yield and test weight of perennial wheat and winter wheat cultivars from the Plant Pathology Farm plot using the same method. All statistical assumptions were satisfied.

ELISA values were not used as a measure of virus titer because leaves were sampled randomly and therefore, the same plants were not sampled consistently over time. Presence of WSMV was expressed as the percentage of inoculated rows with symptoms and rows positive for WSMV by ELISA. Values for the first year of growth are the combined results from multiple samples during the growing season, and values for the initial re-growth are results from a single sampling date 12-20 days after harvest of the first year growth.

To assess survival after 2 years of growth, WSM symptom ratings were plotted versus the number of reproductive tillers per perennial wheat plant. A linear regression analysis was performed using PROC REG in SAS.

EPIDEMIOLOGY OF WSMV

Field plots. Data were collected for the incidence, progression, and severity of WSM in a field plot over two growing seasons. Plots for this study were planted around the perimeter of the plots to evaluate perennial wheat for resistance. In 2004, 4-row plots 3.1 m long were planted with a plot drill. Two plots of each perennial line and Eltan and Edwin, and four plots of

Spitzer wheat were sown on 23 September at the Spillman Agronomy Farm, and 11 October at Central Ferry. Rows were sown with 0.3 g seed per meter. In 2005, plots 4.6 m long and 1.5 m wide with four rows were sown at the Plant Pathology Farm on 25 September. Initial plant density was recorded in November 2004 and 2005 when plants were at 2-to-3 leaf growth stage.

Evaluation of WSMV. After initial symptoms were observed, the presence or absence of WSM symptoms was assessed and leaf samples for ELISA were taken at 2 week intervals. In the 2004-05 plots, the sampling period extended from 2 May to 28 July. The same sampling and virus testing methods described previously were used, except that 10 random leaf samples were taken from the 4-row plots.

The purpose of these plots was to determine timing of initial infection, so the perennial wheat plots were tilled after harvest in July/August 2005 and the same perennial lines and winter wheat cultivars were planted in Fall 2005. The Spillman Farm plot was sown on 25 September, and the Central Ferry plot on 14 October. The perennial wheat seed used for the Fall 2005 plantings was obtained from the previous season's harvest from the Spillman Farm plot. Because Spitzer seed was not available, Hill 81 winter wheat was planted in its place.

Yield determination. Plots were harvested using a hand sickle and threshed with a Vogel stationary bundle thresher for both the 2004-05 and 2005-06 plots. In 2005, the Central Ferry plot was harvested on 21 July; at Spillman Farm, the annual wheat was harvested on 12 August and the perennial wheat was harvested on 25 August.

In 2006, the annual wheat at the Central Ferry plot was removed and discarded on 12 July, and the perennial wheat was harvested on 24 July; at the Spillman Farm plot, the annual wheat was harvested on 4 August and the perennial wheat was harvested on 25 August, and at the Plant Pathology Farm plot, the annual wheat was harvested on 4 August, and the perennial

wheat was harvested on 24 August. Seed yield, test weight, and 100 seed weight was not measured for this experiment.

Data analysis. Statistical analyses of the data was not conducted because only two replicates per line were monitored. Disease incidence as determined by ELISA and symptoms were recorded according to timing and orientation in the plot.

RESULTS

RESISTANCE TO WSMV UNDER CONTROLLED CONDITIONS

In the first experiment, all of the noninoculated Stephens control plants developed symptoms and were positive for WSMV by ELISA, but WSMV was detected in only half of the inoculated Stephens control plants. For this reason, experiment 1 was not included in subsequent analyses. In experiments 2 and 3, the noninoculated Stephens control did not develop symptoms, and the inoculated controls were positive for WSMV. Because samples from the three plants in each pot were pooled for testing by ELISA, results are expressed by pot in subsequent analyses.

Plants that tested positive by ELISA consistently had symptoms of WSM. Individual perennial lines differed in their reaction to WSMV in all experiments (Table 1). Symptoms severity ranged from light-yellow mottling to severe streaking and stunting (from 1 to 4 on the rating scale) but the majority of WSM symptoms were mild (ratings 1 and 2). In experiment 2, two lines, 03JP031 and 03JP039, were considered resistant, but all lines were susceptible in experiment 3 (Table 1).

An F-test was performed on the data from experiments 2 and 3 to test homogeneity of variance, and both the ELISA values and symptom ratings were not significantly different ($p= 0.469$ and $p= 0.146$, respectively). When data from experiments 2 and 3 were combined, none of the lines were resistant using standards defined by Cox (2002). Lines 03JP031 and 03JP039 had variable phenotypes; both were resistant in at least one, but not all experiments. Lines 03JP031, 03JP039, and 03JP011 had 75%, 62%, and 25% of pots, respectively, that were asymptomatic and negative by ELISA. The other 30 perennial wheat lines were completely susceptible to WSMV. Of these, 0 to 17% of pots had asymptomatic plants that were negative by ELISA.

Table 1. Phenotypic reaction of perennial wheat lines to mechanical inoculation of *Wheat streak mosaic virus* (WSMV) under controlled conditions.

Line	Exp. 1[a]	Exp. 2	Exp. 3	Exp. 4[b]	Phenotype[c]
Stephens (healthy susceptible)	4/4	0/4	0/4	0/12	--
Stephens (inoculated susceptible)	2/4	4/4	4/4	12/12	--
MT-2 (resistant control)	0/4	0/4	0/4	0/12	**R**
03JP031	0/4	0/4	2/4	3/12	**V**
03JP039	3/4	0/4	3/4	4/12	**V**
03JP004	3/4	4/4	4/4		**S**
03JP005	3/4	4/4	4/4		**S**
03JP006	3/4	3/4	4/4		**S**
03JP007	4/4	3/4	4/4		**S**
03JP008	4/4	4/4	4/4		**S**
03JP009	3/4	4/4	4/4		**S**
03JP010	4/4	4/4	4/4		**S**
03JP011	3/4	3/4	3/4		**S**
03JP012	10/12	11/12	11/12		**S**
03JP014	9/12	12/12	11/12		**S**
03JP016	4/4	4/4	4/4		**S**
03JP017	12/12	11/12	12/12		**S**
03JP019	4/4	4/4	4/4		**S**
03JP020	12/12	8/12	12/12		**S**
03JP022	4/4	3/4	4/4		**S**
03JP023	4/4	4/4	4/4		**S**
03JP024	12/12	11/12	12/12		**S**
03JP026	4/4	4/4	4/4		**S**
03JP027	4/4	4/4	4/4		**S**
03JP028	4/4	4/4	4/4		**S**
03JP029	4/4	4/4	4/4		**S**
03JP030	4/4	4/4	4/4		**S**
03JP032	4/4	4/4	4/4		**S**
03JP033	4/4	4/4	4/4		**S**
03JP034	4/4	4/4	4/4		**S**
03JP035	4/4	4/4	4/4		**S**
03JP036	4/4	4/4	4/4		**S**
03JP037	4/4	4/4	4/4		**S**
03JP038	11/12	11/12	11/12		**S**
XC14	11/12	10/12	11/12		**S**
2n=70	11/12	10/12	12/12		**S**

[a] Samples were positive to WSMV by Enzyme-Linked Immunosorbent Assay (ELISA) if absorbance readings were threefold greater than healthy check. Results were expressed by pot since 3 plant samples in each pot were pooled.

[b] Lines considered resistant for one or more experiments after experiment 3 remained in the growth chamber, and the temperature was raised from 22 to 25°C to detect temperature-sensitive resistance.

[c] A line was considered resistant (R) if all pots had negative ELISA readings in all experiments, variable (V) if all pots were negative for at least one experiment, or susceptible (S) if one or more pots were positive in all experiments.

A statistically significant positive linear relationship was observed ($p<0.001$) when the average symptom ratings were plotted versus ELISA absorbance values (Fig. 2). As ELISA values increased, the average symptom ratings also increased among the perennial wheat lines. The inoculated control (Stephens) had high ELISA values and high average symptom ratings as compared to the healthy control. The resistant (MT-2) and noninoculated controls had comparable low symptom ratings (average=0) and ELISA values, confirming the resistance of MT-2. The delineated box in Figure 2 was determined by analysis of each perennial line for statistical similarity (greater than $p=0.05$) to the resistant control using Dunnett's comparison of means to a resistant control. Lines 03JP011, 03JP031, and 03JP039, along with the healthy control, had ELISA values and symptom ratings statistically similar to the resistant control.

Average ELISA values and symptom ratings within and among individual lines varied (data not shown). Among perennial lines, ELISA values ranged from 0.22 to 0.80, and symptom ratings ranged from 0.33 to 1.42. Lines 03JP011, 03JP031, and 03JP039 had both ELISA values and symptom ratings that were not statistically different from the resistant control. Line 03JP008 had ELISA values that were not statistically different from the control, but symptom ratings were significantly greater ($p<0.05$). Line 03JP019 did not have statistically different symptom ratings from the resistant control, but ELISA values were significantly greater.

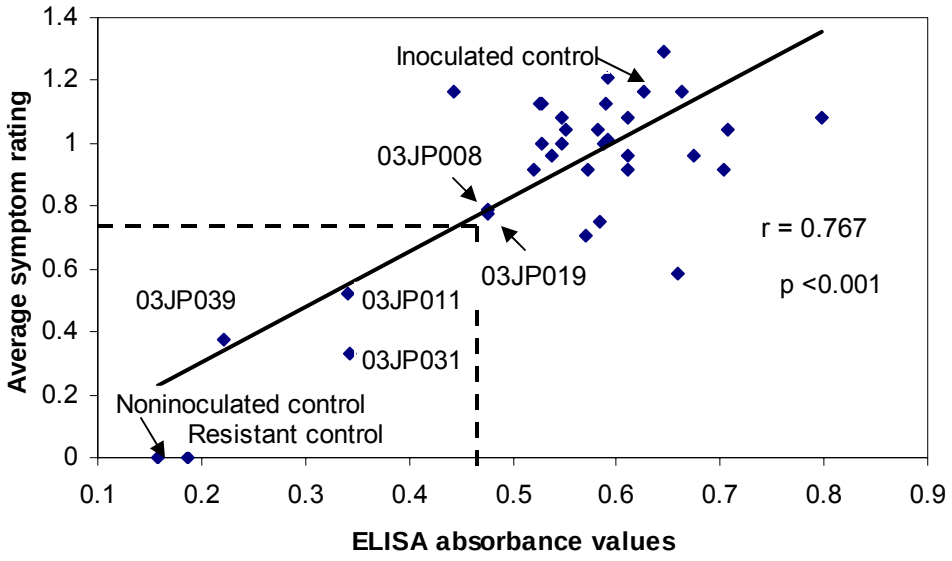

Figure 2. Reaction of 33 perennial wheat germplasm lines to *Wheat streak mosaic virus* in the growth chamber at 22°C. Each point represents the average of 4 replicates and two experiments. Points within the dotted line box are not statistically different from the resistant control for both Enzyme-Linked Immunosorbent Assay (ELISA) values and visual symptom rating variables, based on Dunnett's comparison of means to a resistant control. The R^2 value and significance value was determined by a linear regression analysis between the ELISA values and symptom ratings performed by SAS.

Lines 03JP031 and 03JP039 were considered resistant in at least one of the first three experiments (Table 1), so these lines were further evaluated for temperature-sensitive resistance. Line 03JP031 (Table 2) had a slight increase in the percentage of plants with WSMV and symptom ratings when the temperature was raised to 25ºC. Symptom ratings for line 03JP039 were the same, but the percentage of WSMV-infected plants decreased when the temperature was raised to 25ºC. There were no differences in reaction of the resistant control and the noninoculated control between these temperatures.

The F_3 and F_4 plants of 03JP031 and 03JP039 reacted differently to WSMV (Table 3). In both lines, at least one progeny plant from each susceptible parent plant was infected. Plant 3-2-5 was the only susceptible F_3 plant that had all susceptible F_4 plants; others had both resistant and susceptible progeny plants. In both lines, the F_4 had more severe symptoms than the F_3 and a greater percentage of infected plants. Symptom ratings of F_3 plants were not greater than 2, whereas some F_4 plants had ratings of 3 and 4. ELISA confirmed that WSMV was not transmitted by seed to F_4 plants. The progeny from the resistant and susceptible controls did not differ from the parents. Several samples had borderline positive ELISA values, so RT-PCR was performed (Fig. 3).

The F_3 perennial regrowth was screened for possible temperature-sensitive resistance at 32ºC (data not shown) although 15% of line 03JP031 plants and 17% of 03JP039 plants did not regrow. After 8 days, all regrowing plants were reassessed, and the presence of WSMV based on symptoms and ELISA was the same at 22ºC and 32ºC.

Table 2. Reaction of perennial wheat lines to *Wheat streak mosaic virus* (WSMV) in a growth chamber at 22°C and 25°C.

Line	22°C		25°C	
	(+) WSMV[a]	Symptom rating	(+) WSMV[a]	Symptom rating
Non-inoculated susceptible	0%	0	0%	0
Inoculated susceptible	100%	1.8	100%	1.1
MT-2	0%	0	0%	0
03JP031	17%	0.3	25%	0.4
03JP039	50%	0.5	33%	0.5

[a] Plants were considered positive for WSMV if Enzyme-Linked Immunosorbent Assay (ELISA) absorbance values were 3 fold greater than the healthy tissue control. Figures represent the mean of three plants and 4 replicates.

Table 3. Reaction of F_3 and F_4 plants of two perennial wheat lines to *Wheat streak mosaic virus* (WSMV) in controlled conditions.

Line	F_3			F_4	
	Plant #[a]	(+) ELISA[b]	Symptom rating	(+) ELISA[c]	Average symptom rating[d]
Stephens		+	3	4/4	2.25
03JP031					
	1-1-4	+	2	1/4	0.5
	1-1-6	-	0	0/4	0
	1-1-8	-	0	0/4	0
	2-1-1	-	0	1/4	0.5
	2-1-3	-	0	1/4	0.25
	2-1-6	-	0	0/4	0
	2-1-7	-	0	1/4	0
	3-1-4	-	0	2/4	0.25
	3-1-7	-	0	0/4	0
	3-1-8	-	0	3/4	1.75
03JP039					
	1-2-1	+	1	3/4	1
	1-2-3	-	0	0/4	0
	1-2-4	-	0	0/4	0
	1-2-5	-	0	0/4	0
	1-2-6	+	2	2/4	1
	1-2-8	-	0	0/4	0
	2-2-2	-	0	0/4	0
	2-2-4	-	0	1/4	0.25
	3-2-2	-	0	0/4	0
	3-2-4	-	0	1/4	0.25
	3-2-5	+	2	4/4	3.25
	3-2-7	-	0	0/4	0
MT-2		-	0	0/4	0

[a] From the F_3, 10 plants of 03JP031 and 12 of 03JP039 were evaluated for WSMV. Enzyme-Linked Immunosorbent Assay (ELISA) and symptom rating were performed once on individual plants.

[b] A (+) indicates detection of WSMV by ELISA, and (-) indicates no WSMV detection.

[c] Fractions indicate the number of progeny plants positive for WSMV by ELISA out of the four that were tested.

[d] Average symptom ratings given by the four progeny plants from each F_3 plant.

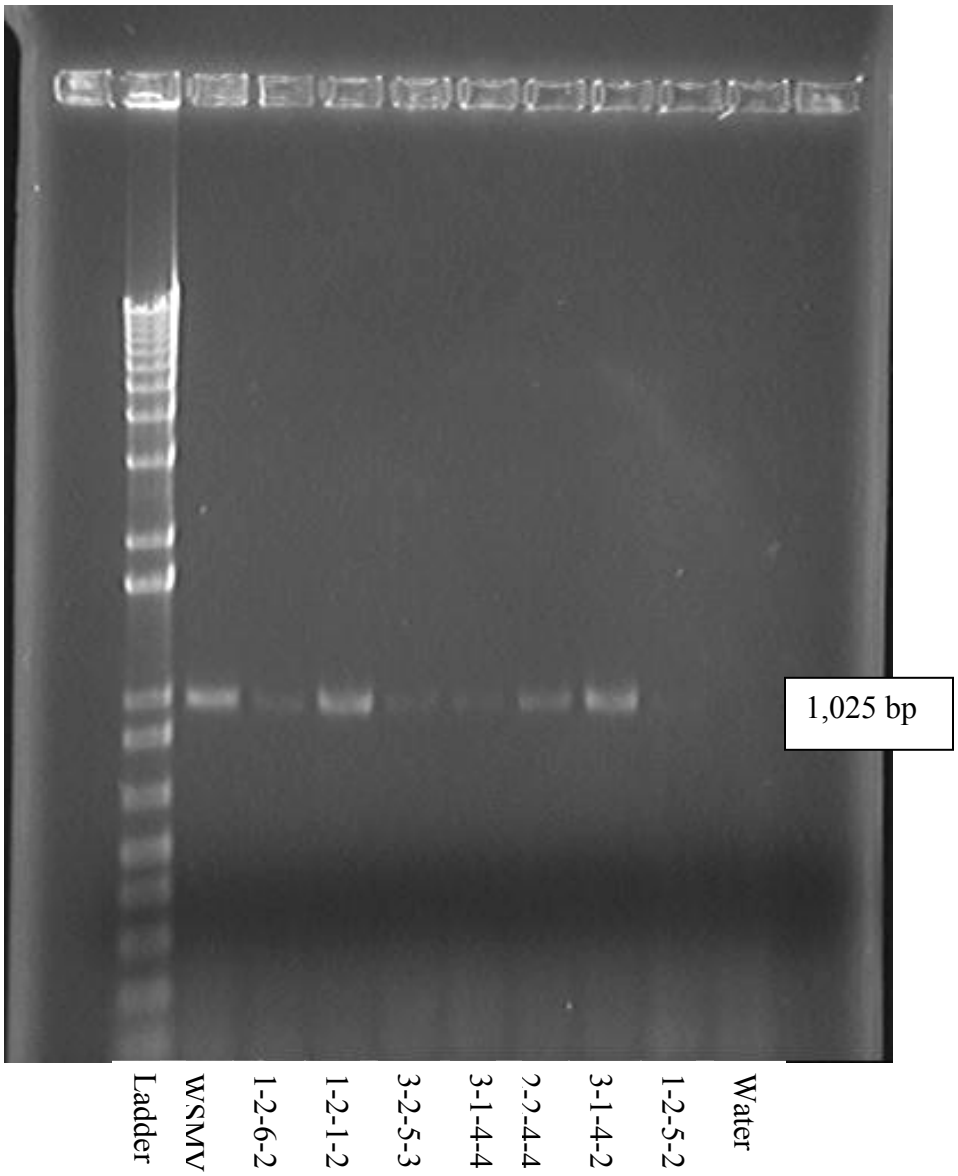

Figure 3. Reverse Transcriptase-PCR for *Wheat streak mosaic virus*, used to confirm presence of the virus in samples from the segregation for resistance experiment (F_4). Lane 1= 1 Kb DNA ladder; 2= positive control; 3= 03JP039 block 1 plant #6; 4= 03JP039 block 1 plant #1; 5= 03JP039 block 3 plant #5; 6= 03JP031 block 3 plant #4; 7= 03JP039 block 2 plant #4; 8= 03JP031 block 3 plant #4; 9= 03JP039 block 1 plant #5; and 10= water control.

RESISTANCE TO WSMV IN THE FIELD

WSMV was detected in the inoculated rows in spring 2005 at the Spillman Agronomy Farm and Central Ferry plots. In early summer 2005, WSMV was detected in the noninoculated rows, indicating that natural spread occurred. Because the extent of exposure in these rows to natural infection is unknown and not quantified, analyses for resistance in the field included only the inoculated rows. The noninoculated rows were used in yield and test weight comparisons with the inoculated rows.

Spillman Farm Plot

ELISA values and symptom ratings for the first year of perennial wheat growth represent the combined results from multiple sampling dates (Table 4). Both susceptible controls, Eltan and Edwin, expressed symptoms when infection occurred and both had a high percentage of symptomatic rows, with 83.3% and 40%, respectively. In comparison, all perennial lines had a lower percentage of rows positive by ELISA, except for line 03JP024, which was equal to Edwin. For all lines except 03JP028, the percentage of symptomatic rows was less than the percentage of rows in which WSMV was detected by ELISA. No WSM symptoms were observed in lines 03JP012, 03JP035, and 03JP039.

The first year growth was harvested on 25 August 2005; regrowth was observed in some rows 4 days before harvest and in all rows by 10 days after harvest. The symptom rating and ELISA results expressed as initial regrowth were collected only from inoculated rows, 16 days after harvest (Table 4). Results were confirmed by ELISA tests conducted in October 2005 and spring 2006. In all perennial regrowth, symptoms were present when WSMV was detected by ELISA. All rows of 03JP012, 03JP024, 03JP028, 03JP032 and 03JP035 were symptomatic, and line

Table 4. Detection of *Wheat streak mosaic virus* (WSMV) during 2004-05 and 2005-06 by symptoms and Enzyme-Linked Immunosorbent Assay (ELISA) in mechanically inoculated rows.

Line	Spillman Farm[a]				Central Ferry				Plant Pathology Farm			
	First Year Growth		Initial Regrowth		First Year Growth		Initial Regrowth		First Year Growth		Initial Regrowth	
	ELISA[b]	Symptoms[b]	ELISA	Symptoms	ELISA	Symptoms	ELISA	Symptoms	ELISA	Symptoms	ELISA	Symptoms
03JP012	16.7	0	100	100	83.3	33.3	100	100	50.0	33.3	100	100
03JP024	40.0	20.0	100	100	50.0	16.7	100	100	16.7	0	100	100
03JP028	16.7	16.7	100	100	66.7	33.3	100	100	83.3	66.7	100	100
03JP032	33.3	16.7	100	100	50.0	33.3	100	100	66.7	33.3	100	100
03JP035	40.0	0	100	100	50.0	0	100	100	83.3	50	100	100
03JP039	16.7	0	50.0	50.0	0	0	66.7	66.7	33.3	16.7	50.0	50.0
Eltan[c]	83.3	83.3	--	--	100	100	--	--	100	100	--	--
Edwin[c]	40.0	40.0	--	--	50.0	50.0	--	--	100	100	--	--
Hill 81[cd]	--	--	--	--	--	--	--	--	83.3	83.3	--	--

[a] Spillman Farm and Central Ferry field plots were planted in 2004 and the Plant Pathology Farm plot was planted in 2005. For the 1st year growth, each value represents the combined results from all samplings taken during that season's growth, and the values for initial regrowth represent a one-time sampling 12-20 days after harvest of the 1st year's crop. WSMV assessments were conducted by row (2 m long).

[b] Figures for ELISA and symptoms were calculated as the percentage of mechanically inoculated rows that were positive for WSMV.

[c] Eltan, Edwin, and Hill 81 are winter wheat cultivars and do not have a second year of growth, as indicated by (--) in initial regrowth columns.

[d] Hill 81 was included in the 2005-06 growing season, but not included in 2004-05.

03JP039 had 50% infected rows. At harvest in August 2006, survival ranged from 18.3 to 51.7% and line 03JP028 had the greatest survival, when compared with initial plant density (Table 5).

A significant negative linear correlation was observed ($p=<0.001$) between final reproductive tiller count and perennial regrowth symptom ratings (Fig. 4). Plants with 0 rating were not infected by WSMV; however, a wide range of reproductive tillers (3-30) was observed. Overlapping confidence intervals among means indicates distinct differences between mild and severe symptom ratings. Plants with a rating of 1 had a similar number of reproductive tillers compared to healthy plants. The Spillman Farm plot had 1% of surviving plants with a rating of 4 and 34% with a rating of 0. In comparison, Central Ferry had more severe symptoms, with 24% of surviving plants having a rating of 4 and 19% with a rating of 0.

No significant difference was observed between the inoculated and noninoculated rows among the perennial lines for 100 seed weight (data not shown). Due to the low plant population size, perennial wheat seed yield or test weight was not measured.

Although yield of the inoculated Eltan and Edwin rows was 22.2 and 21.9% respectively less than the noninoculated rows, the difference was not significant (Table 6). There was a small reduction in test weight for Eltan and Edwin. Yield and test weight of inoculated rows of Hill 81 had small reductions but the differences were not significant.

Central Ferry

The susceptible controls Eltan and Edwin had 100% and 50%, respectively, infected and symptomatic rows. Perennial lines 03JP024, 03JP032, 03JP035, and 03JP039 had an equal or lower percentage of infected rows, and 03JP012 and 03JP028 had a higher percentage of infected rows than the susceptible controls. For all perennial lines, the percentage of symptomatic rows

Table 5. Initial and final plant population counts for perennial wheat lines, and percent survival at Spillman Farm and Central Ferry plots from Fall 2004 to Summer 2006.

Line	Spillman Farm			Central Ferry		
	Initial[a]	Final[b]	% Survival	Initial	Final	% Survival
03JP012	93	17	18.3	90	9	10.0
03JP024	96	25	26.0	129	5	3.9
03JP028	87	45	51.7	90	30	33.3
03JP032	75	33	44.0	126	11	8.7
03JP035	84	23	27.4	96	20	20.8
03JP039	63	21	33.3	81	13	16.0

[a] Initial = Plant emergence count taken 4 weeks after planting in fall 2004 when plants were at 2- to 3-leaf growth stage. Counts were conducted in 1 m of row in 6 replicates.

[b] Final = Final plant count in summer 2006 at harvest of second year growth. All plants were counted in individual rows at the soft- to hard-dough ripening stage.

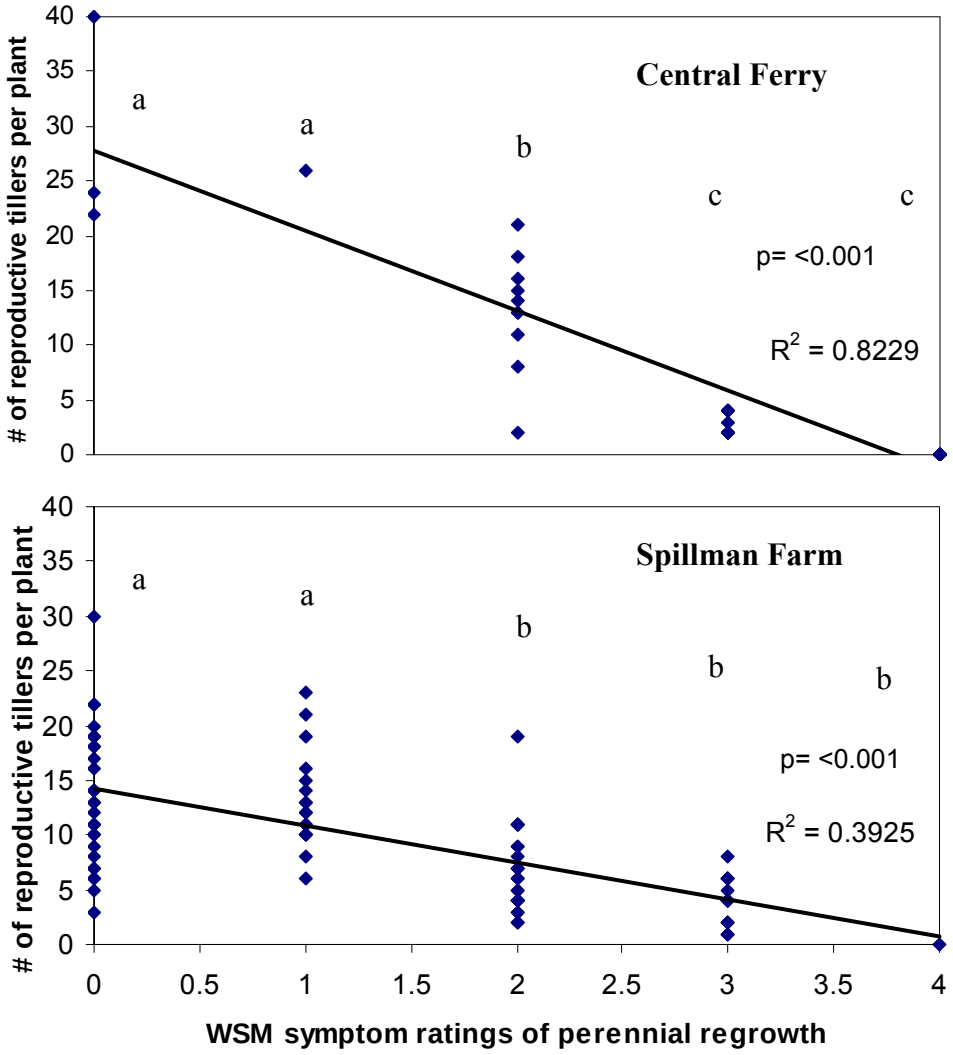

Figure 4. Wheat streak mosaic symptom ratings versus number of reproductive tillers per perennial plant. Each point represents one perennial wheat plant in its second year of growth that was exposed to natural infection by *Wheat streak mosaic virus.* Symptom rating means followed by the same letter are not significantly different based on analysis of variance with a Tukey's comparison of means at $p < 0.05$. A linear regression analysis was performed using PROC REG in SAS.

Table 6. Mean and range of seed yield and test weight of winter wheat varieties, and percent reduction as a result of WSMV inoculation in the Spillman Farm field plot, 2005-06.

Source	Yield, kg ha^{-1}			Test Weight, kg m^{-3}		
	Range	Mean [a]	% Reduction	Range	Mean [a]	% Reduction
Eltan						
Noninoculated	5,420-11,815	8,154.2		493-517	505.7	
Inoculated	4,898-8,663	6,862.9	22.2%	478-511	493.9	2.3%
Edwin						
Noninoculated	3,693-6,908	5,551.6		494-521	506.8	
Inoculated	3,851-5,199	4,568.9	21.9%	502-511	506.2	0.1%
Hill 81						
Noninoculated	5,727-10,483	7,863.1		492-512	504.0	
Inoculated	7,146-8,136	7,647.5	3.5%	480-512	495.9	1.6%

[a] No significant statistical difference between yield and test weight, as determined by analyses of variance.

was less than the rows in which WSMV was detected by ELISA. No WSM symptoms were observed in lines 03JP035 and 03JP039.

Perennial wheat regrowth was observed as early as 6 days after harvest on 21 July 2005 and in all rows by 14 days after harvest. The results, expressed as initial regrowth, were collected 12 days after harvest (Table 4) and confirmed by ELISA conducted in October 2005 and spring 2006. In all perennial regrowth rows, symptoms were present when WSMV was detected by ELISA. Line 03JP039 initial regrowth had 66.7% of rows expressing symptoms, compared to 100% in all other perennial lines.

At harvest in July 2006, survival ranged from 3.9 to 33.3%, and line 03JP028 had the greatest survival compared to initial plant density (Table 5).

A significant negative linear correlation was observed ($p=<0.001$) between final reproductive tiller count and perennial regrowth symptom ratings (Fig. 4). Only three plants were asymptomatic and not infected; number of reproductive tillers ranged from 22 to 40. Overlapping confidence intervals amongst means indicates distinct differences between mild and severe symptom ratings. Plants with a rating of 1 had a similar number of reproductive tillers as the healthy plants.

There was no significant difference between the inoculated and noninoculated rows among the perennial lines for 100 seed weight (data not shown). The yield and test weight was not measured in 2005 or 2006.

Plant Pathology Farm

Presence of WSMV was evaluated by ELISA and symptom ratings for the first year of perennial wheat growth, and values represent the combined results from multiple sampling dates (Table 4). The susceptible controls Eltan and Edwin had 100% infected and symptomatic rows,

and Hill 81 had 83.3% infected rows. Perennial lines 03JP024 and 03JP039 had 16.7 and 23.3%, respectively infected rows, and 03JP012, 03JP028, 03JP032 and 03JP035 had 50 to 83% infected rows. For all perennial lines, the percentage of symptomatic rows was less than the rows in which WSMV was detected by ELISA. No WSM symptoms were observed in line 03JP024.

Perennial wheat regrowth of all lines was observed 5 days before harvest on 24 August 2006, and WSM symptoms were first observed in regrowth 11 days after harvest (Figs. 5a, 5c). The results expressed as initial re-growth were collected 20 days after harvest (Table 4). WSM symptoms were observed in 58.3% of 03JP039 inoculated rows, compared with 100% symptoms in all other perennial lines. Symptoms of wheat curl mite infestations were also observed throughout the plot (Figs. 5b, 5d).

Yield of Edwin was significantly less in inoculated than noninoculated rows ($p= 0.0203$), but the test weight was not significantly different (Table 7). There were no significant differences in yield or test weight for the inoculated and noninoculated rows of Hill 81, Eltan and the perennial lines. In all winter wheat varieties, the yield and test weight from the non-inoculated rows were higher than the inoculated rows. In four of the six perennial wheat lines, yield of the inoculated rows was higher than the noninoculated rows; however, none of these differences were statistically significant.

Figure 5. Wheat streak mosaic and wheat curl mite symptoms observed in perennial wheat second year growth at the Plant Pathology Farm, 20 days after harvest in 2006. **A, C:** Wheat streak mosaic in young perennial wheat regrowth. **B, D:** Symptoms caused by wheat curl mite infestation.

Table 7. Mean and range of seed yield and test weight of perennial wheat and winter wheat lines, and the percent reduction as a result of *Wheat streak mosaic virus* in the Plant Pathology Farm plot, 2005-06.

Source	Yield, kg ha^{-1}			Test Weight, kg m^{-3}		
	Range	Mean	% Reduction	Range	Mean	% Reduction
03JP012						
Noninoculated	1,361- 2,720	1,989.4		400- 441	423.9	
Inoculated	1,607- 3,477	2,317.5	-18.9%	432- 460	442.3	-4.5%
03JP024						
Noninoculated	1,408- 4,588	2,525.9		416- 473	446.5	
Inoculated	1,278- 5,105	2,905.7	-11.5%	429- 483	463.7	-4.1%
03JP028						
Noninoculated	2,023- 3,204	2,737.9		406- 448	430.2	
Inoculated	2,166- 3,805	2,750.1	-3.4%	411- 451	428.5	0.3%
03JP032						
Noninoculated	1,433- 2,891	2,061.0		402- 441	430.1	
Inoculated	1,371- 2,756	1,870.9	4.2%	408- 446	426.1	0.8%
03JP035						
Noninoculated	1,456- 3,144	2,139.4		355- 467	428.8	
Inoculated	1,186- 3,263	2,147.5	-10.3%	410- 457	436.7	-2.6%
03JP039						
Noninoculated	874- 1,505	1,234.8		400- 430	408.6	
Inoculated	823- 1,954	1,573.2	-26.4%	356- 445	407.6	0.2%
Hill 81						
Noninoculated	6,308- 10,143	8,209.5		493- 526	515.4	
Inoculated	5,960- 10,056	8,008.8	2.3%	499- 515	509.8	1.1%
Eltan						
Noninoculated	7,128- 12,079	9,263.6		501- 533	523.1	
Inoculated	6,387- 10,907	8,567.4	7.5%	508- 534	516.4	1.2%
Edwin						
Noninoculated	4,228- 9,586	6,616.3		522- 533	528.1	
Inoculated	3,293- 7,516	5,716.1	13.3% [a]	516- 535	524.3	0.7%

[a] Significant difference ($p \leq 0.05$) between treatments, determined by analysis of variance with a Tukey-Kramer comparison of means.

EPIDEMIOLOGY OF WSMV

Natural infection by WSMV occurred in all plots and growing seasons. The locations of WSMV infection within plots and detection date are illustrated for the 2004-05 and 2005-06 field trials (Figs. 6 and 7). The amount of natural infection varied between the growing seasons. In 2004-05, WSMV was detected in 38% of the perennial wheat plots when Spillman Farm and Central Ferry results were combined. In 2005-06, infection was detected in 11% of perennial plots, after combining results from Spillman Farm, Central Ferry, and Plant Pathology Farm. Opposite results were detected in the winter wheat plots; WSMV was identified in 35% of winter wheat plots in 2004-05 and 57% of plots in 2005-06.

2004-05

WSMV was not detected before May 2005 (Table 8). WSMV was first detected on 2 May 2005 at both Central Ferry and Spillman Farm in the susceptible controls. In all cases, susceptible annual wheat that was positive for WSMV by ELISA also had WSM symptoms. Spitzer seed had a low germination rate; therefore, no resistant control was present in the field plots.

Infection was inconsistent among perennial lines. For example, line 03JP012 was infected at Spillman Farm, but not at Central Ferry. In line 03JP024, WSMV was observed on 17 May at Central Ferry, but not until 28 June at Spillman Farm, and natural infection was not detected in lines 03JP032 and 03JP039 at either plot. Perennial lines also had variable symptom expression; in line 03JP035, WSMV was detected by ELISA at Spillman Farm and Central Ferry, but no symptoms were observed during the growing season. Line 03JP012 was symptomatic at the time of WSMV detection at the Spillman Farm plot. When a perennial line expressed symptoms at an early sampling date, symptoms were observed at all subsequent

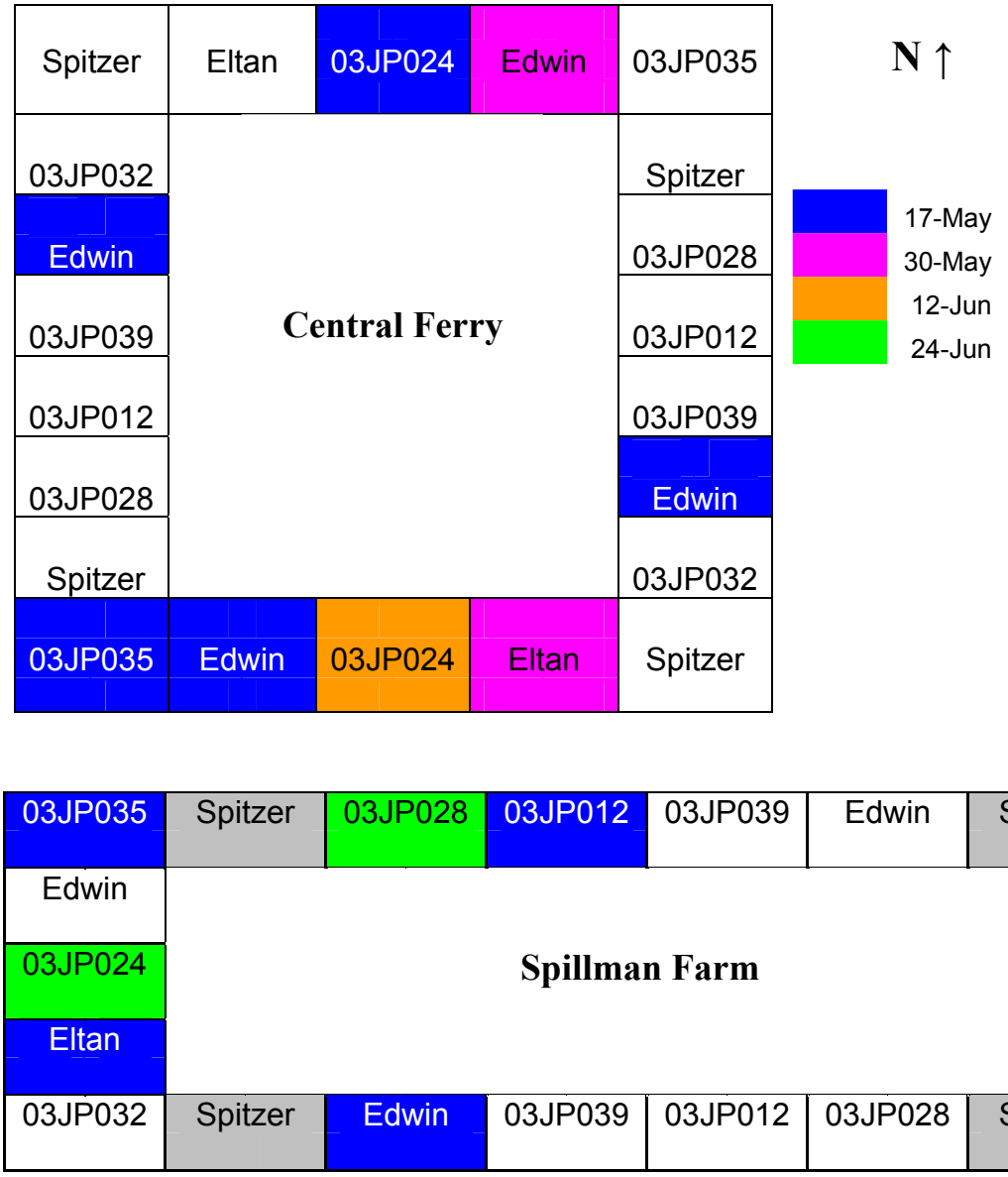

Figure 6. Central Ferry and Spillman Farm field plots: Timing of *Wheat streak mosaic virus* detection by Enzyme-Linked Immunosorbent Assay (ELISA) during the 2004-05 season. Each color represents an individual sampling date.

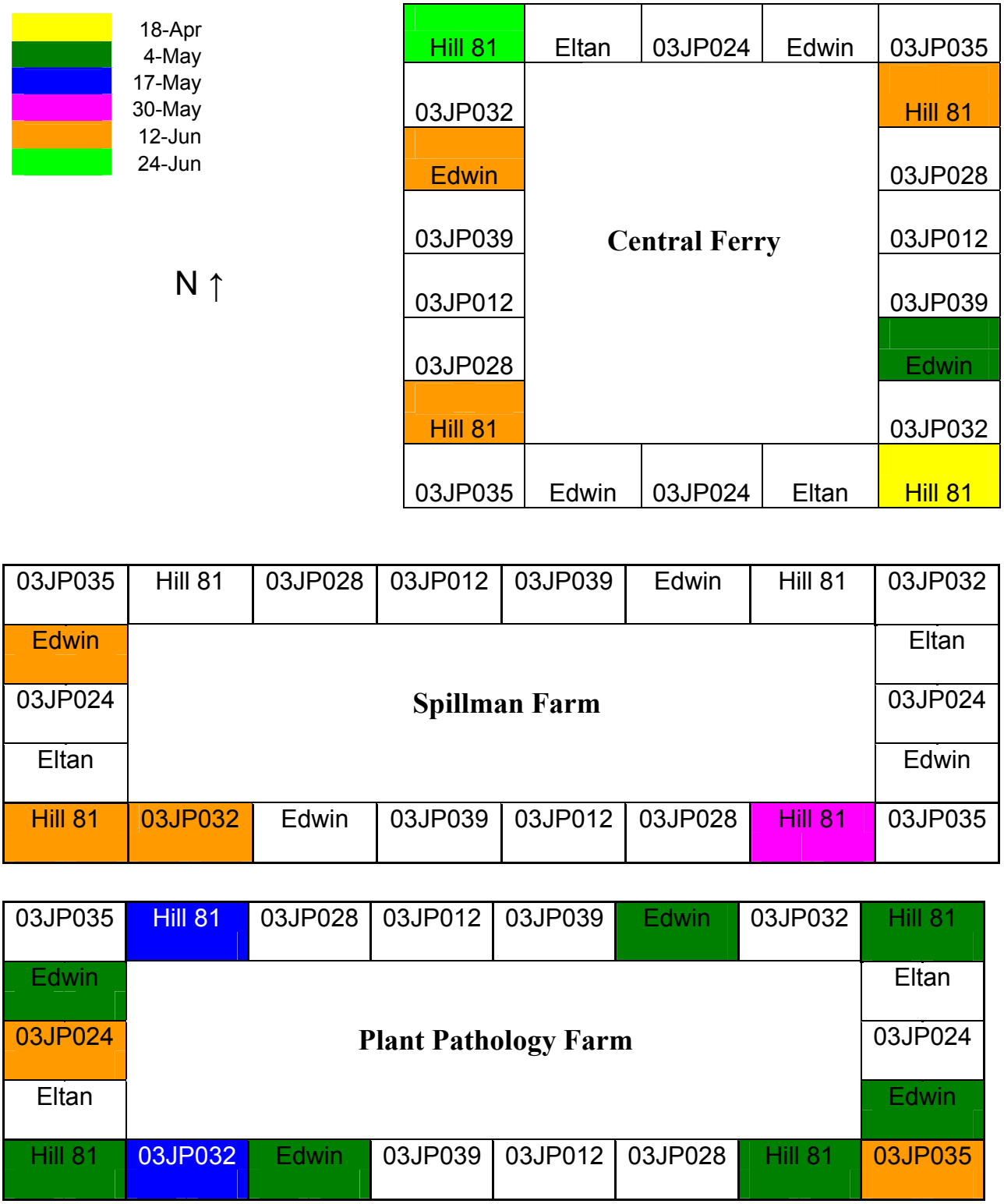

Figure 7. Central Ferry, Spillman Farm, and Plant Pathology Farm field plots: Timing of *Wheat streak mosaic virus* detection by Enzyme-Linked Immunosorbent Assay (ELISA) in the bordering plots during the 2005-06 season. Each color represents an individual sampling date.

Table 8. Timing of natural *Wheat streak mosaic virus* (WSMV) infection during the 2004-05 season in perennial wheat and winter wheat controls at the Spillman Agronomy Farm and Central Ferry Research Station. WSMV was detected by Enzyme-Linked Immunosorbent Assay (ELISA) only (x), and by ELISA and symptoms (▲).

Sampling Date	Central Ferry								Spillman Farm							
	03JP012	03JP024	03JP028	03JP032	03JP035	03JP039	Eltan	Edwin	03JP012	03JP024	03JP028	03JP032	03JP035	03JP039	Eltan	Edwin
11/8/2004																
11/23/2004																
12/15/2004																
3/13/2005																
5/2/2005								▲							▲	
5/17/2005		X			x			▲	▲				x		▲	▲
6/3/2005		X			x		▲	▲	▲				x		▲	▲
6/16/2005		▲			x		▲	▲	▲				x		▲	▲
6/28/2005		▲			x		▲	▲	▲	▲	x		x		▲	▲

sampling dates. Only line 03JP024 in at Central Ferry was symptomless and tested positive to WSMV by ELISA; it expressed symptoms later in the growing season (Table 8).

No obvious pattern of infection was observed in timing of infection across the Central Ferry plot (Fig. 6). Most WSM was detected in the winter wheat cultivars in the first two sampling dates and on each side of the plot. Because these cultivars are susceptible, early detection indicates inoculum was present in all bordering rows. At Spillman Farm, all early infection occurred on the northwestern side of the plot, which was adjacent to a perennial wheat nursery.

2005-06

WSMV infection was first detected on 18 April 2006, when it was found at the Central Ferry plot (Table 9) in Hill 81. Natural infection in perennial wheat was limited and symptoms were only expressed in line 03JP035 at the Plant Pathology Farm. No virus was detected in lines 03JP012, 03JP028, and 03JP039 at any of the field locations. WSMV was not detected in Eltan plots at any of the three field plots. All susceptible annual wheat that tested positive to WSMV by ELISA also had WSM symptoms. Both perennial and annual wheat at the Plant Pathology Farm plot had the most natural infection and expression of symptoms, compared with the Spillman Farm and Central Ferry plots.

At Spillman Farm, most of the natural infection was limited to the northwest side of the plot; at Central Ferry, no pattern of infection was observed (Fig. 7).

Table 9. Timing of natural *Wheat streak mosaic virus* (WSMV) infection during the 2005-06 season in perennial wheat lines and winter wheat controls at the Central Ferry Research Station, the Spillman Agronomy Farm, and the Plant Pathology Farm. WSMV was detected in the leaves by Enzyme-Linked Immunosorbent Assay (ELISA) only (x), and by ELISA and symptoms (▲).

Sampling Date	Central Ferry									Spillman Farm									Plant Pathology Farm								
	03JP012	03JP024	03JP028	03JP032	03JP035	03JP039	Hill 81	Eltan	Edwin	03JP012	03JP024	03JP028	03JP032	03JP035	03JP039	Hill 81	Eltan	Edwin	03JP012	03JP024	03JP028	03JP032	03JP035	03JP039	Hill 81	Eltan	Edwin
11/9/2005																											
3/8/2006																											
4/18/2006							▲																				
5/4/2006							▲		▲																▲		▲
5/17/2006							▲		▲													x			▲		▲
5/30/2006							▲		▲							▲						x			▲		▲
6/12/2006							▲		▲					x		▲		▲		x		x	▲		▲		▲
6/24/2006							▲		▲					x		▲		▲		x		x	▲		▲		▲

DISCUSSION

RESISTANCE TO WSMV UNDER CONTROLLED CONDITIONS

Of the 33 *Thinopyrum*-derived perennial wheat lines inoculated with for *Wheat streak mosaic virus* in the growth chamber, three exhibited resistance to WSMV. Lines 03JP031 and 03JP039 were resistant in at least one of the experiments and had lower average symptom ratings and ELISA values than the susceptible control. Upon further statistical analysis, line 03JP011 was also identified as having some resistance to WSMV. Presence of resistance in these lines is supported by other work with *Thinopyrum* sp. (Lay et al. 1971, Liang et al. 1979, Stoddard and Lommel 1987, Friebe et al. 1996).

Lines 03JP011, 03JP031, and 03JP039 had reduced virus replication, as indicated by lower ELISA values and less severe symptoms, as compared to the susceptible control and other perennial lines. These were the only perennial lines to have both ELISA values and average symptom ratings comparable to the resistant control (Fig. 1). However, two additional lines could be considered resistant when either ELISA values or symptom ratings are compared to the resistant control. Line 03JP008 had comparable ELISA values to the resistant control, but more severe symptoms; 03JP019 had statistically comparable symptom ratings but greater ELISA values. These additional two perennial lines show some resistance to WSMV and are a promising source of resistance to WSMV.

There was a significant linear relationship between WSM symptom development and virus detection by ELISA in the perennial lines. This correlation reinforces the validity of using ELISA as a measure of virus content and has been used in previous work in virus diseases (Skaria et al. 1985, Strausbaugh et al. 2003). Confounding factors in a controlled growth chamber condition are minimal and precautions were taken to reduce variability between

experiments; the time of seed germination, plant growth stage, time of inoculation and disease rating, temperature, and other environmental conditions were synchronized. In ELISA tests, the tissue weight to buffer volume was consistent (1:10) for all samples, and therefore, ELISA values can be used as a semi-quantitative measure of virus content and resistance, especially when described in conjunction with symptom severity.

The average symptom ratings were relatively low, ranging from 0 in the resistant control to an average of 1.3, which indicates mild symptoms in all perennial lines. Symptom ratings and ELISA samples were taken 18 days post inoculation (dpi), and WSMV is expected to become systemic in the plant within 8 to 10 dpi (Martin 1978). It is unknown if WSMV titer changes over time in a perennial wheat host and consequently, symptom severity changes over time. So, the long-term effects of WSMV inoculation under controlled conditions are unknown, since yield, test weight, and end-use quality were not determined in the perennial lines.

According to the parameters outlined in Cox et al. (2002a), a perennial wheat line was considered resistant to WSMV if all inoculated plants in each repeated experiment were negative by ELISA and expressed no symptoms. By these standards, none of the 33 perennial wheat lines in this study had resistance to WSMV comparable to MT-2 (Table 1), even though the 33 perennial wheat lines in this experiment have the same pedigrees as the lines screened by Cox. This divergence can be explained by several possibilities. One possibility is the current perennial lines were F_3, and segregation was occurring within these populations for morphological characteristics, such as seed color and spike morphology, so segregation for resistance to WSMV may also be occurring in these populations. Another possibility is difference in virulence of WSMV isolates. The WSMV strain used by Cox (GenBank accession

no. AF034415) was collected in Washington in 1997, and the isolate used in these screening experiments was collected in 2004.

If the lines are segregating for resistance, the initial sampling and testing methods used in the screening experiments may not represent the true percentage of resistant plants within a perennial line. Because the three plant samples taken from each pot were bulked as one sample for ELISA testing, it is unknown whether one or all of the individual plants supported the same level of WSMV replication. However, each plant per pot was individually rated for symptoms. All perennial lines had both symptomatic and asymptomatic plants in all experiments, which suggests that segregation for resistance is occurring at various degrees in all the perennial lines.

Segregation for resistance to WSMV in these lines was suspected, so further screening was conducted in progeny of lines 03JP031 and 03JP039 (Table 3). The gene or genes that confer resistance to WSMV within these populations is unknown. In both lines, at least one progeny plant from each susceptible parent plant was infected, and only one susceptible parent had all susceptible progeny plants. Several resistant parent plants had progeny that were susceptible. These results suggest that inheritance of WSMV resistance in these lines is complex.

Lines 03JP031 and 03JP039 showed evidence for resistance to WSMV with less than 50% of plants testing positive for the virus, so these lines were further evaluated for possible temperature-sensitive resistance. Line 03JP031 (Table 2) had a slight increase in the percentage of plants with WSMV detected by ELISA and average symptom ratings, and line 03JP039 had a slight decrease when the temperature was raised to 25ºC. The resistance in these lines was not significantly affected when the temperature was raised to 25°C. However, the population of test plants in this experiment was not large enough to conclusively identify temperature-sensitive

resistance. To gain a more complete understanding of this phenomenon in these perennial lines, future work must be conducted, repeating the experiment with more replications and a greater range of temperature increases. Furthermore, because the specific *Thinopyrum sp.* parent included in the cross with *Triticum aestivum* is unknown, any level of resistance within these perennial lines is difficult to define through the preliminary resistance screenings performed here. Resistance derived from *Th. intermedium* is temperature-sensitive and not expressed at high temperatures (Pfannensteil and Niblett 1978, Seifers et al. 1995). However, Pfannensteil and Niblett (1978) found that resistance derived from *Th. ponticum* was not temperature-sensitive. Because the source of resistance from *Thinopyrum* is unknown within these perennial germplasm lines, confirming results from this previous work cannot be concluded without molecular characterization of genome composition.

RESISTANCE TO WSMV IN THE FIELD

Previous to this research, the effects of WSMV on perennial wheat in the field were unknown. Research conducted by Cox (2002a) and in this study has identified sources of resistance to WSMV, but only under growth chamber conditions. Though this experiment was not directly representative of naturally-occurring infections, it was designed to evaluate resistance against the virus, not the vector.

Because WSMV was not detected during the fall and early spring months, plots were mechanically inoculated a second time to insure infection. Mechanical inoculation was used to synchronize timing of infection. The split-plot layout was designed as a controlled comparison to measure the effect of WSMV on perennial wheat in the field and identify potential sources of resistance. However, natural infection occurred in the noninoculated rows in all three field plots, even though exterior plots were planted around the perimeter to intercept vector infestation and

reduce virus infection. Conclusive comparisons were not possible for the 2004-05 season results. As an alternative approach, Spitzer, which is resistant to WSMV (Cox 2002a), was planted in the field as a comparison for the perennial lines with unknown resistance phenotypes. However, the seed had very low germination, and so no resistant wheat lines were present in the field plots. By the beginning of the second year of perennial growth, no healthy plants were identified to serve as controls for comparison, and therefore, the effect of WSMV infection on perennial wheat vigor could not be determined conclusively through yield or test weight.

Susceptible annual wheat cultivars were included in the field plots, and their contrasting reactions to WSMV inoculation, observed by symptom development and positive ELISA results, in comparison to the perennial wheat lines were apparent. The first year perennial wheat growth indicates WSMV infection but low symptom development, especially 03JP039. In annual wheat, WSMV infection was always associated with symptom expression; in perennial wheat lines, the percentage of plants expressing symptoms was always less than the percentage of infected plants. Some varieties of spring, wheat or perennial wheat may express less severe symptoms due to presence of tolerance genes and be considered resistant (Baley 1999). Tolerance is defined as a plant that appears susceptible, but the damage caused by the disease is less than that of a fully susceptible cultivar (Browning 1974). Specifically for plant viruses, Matthews (1991) defined tolerance as the ability of the pathogen to infect and replicate in protoplasts, showing little or no apparent effect on the plant, and/or giving rise to latent infection. All the perennial lines were able to tolerate virus replication at titers detected by ELISA. The symptoms in first year perennial growth were predominately mild, and sterile spikes were never observed.

Another explanation for detection of WSMV in asymptomatic plants is “symptom recovery.” For some plant viruses, an infected host can recover from initial systemic symptoms,

and virus particles can be detected in symptomless leaves (Wingard 1928, Cadman and Harrison 1959, Al-Kaff and Covey 1995). So, perennial plants were monitored for symptoms during the growing season, but once symptoms were observed, they were present at all subsequent dates. Chlorotic streaking was observed most commonly; stunting was not usually observed. Nonetheless, reduction in plant size is the most common symptom caused by plant viruses (Hull 2002). Perennial wheat may react to WSMV with slight stunting and be mistaken for a latently infected or asymptomatic plant.

Eight days after harvest of the first year growth in 2005, 50% of 03JP039 plants and 100% of the five other perennial lines developed symptoms and virus was detectable by ELISA in the regrowth. At the time, day temperatures rose above 32°C for several consecutive days, and so, the resistance observed in 03JP039 may be temperature-sensitive (Seifers et al. 1995), and only expressed in the regrowth. Whether this resistance is temperature-sensitive or is less effective due to other factors is not known, but the expression of resistance appears variable based on these data. WSMV resistance in this line may be more appropriately called tolerance, since WSMV infection occurred but the virus replicated at relatively low levels.

Temperature-sensitive resistance was not observed in the perennial wheat lines under controlled conditions. It was neither observed when first year perennial growth was moved from 22 to 25ºC, nor when perennial regrowth plants were placed at 32ºC. Therefore, alternative explanations for the observed reaction in the second year growth in the field must be explored. WSMV infection was detected in most of the second-year perennial wheat plants at both field plots, but the timing of infection is unclear. Because perennial wheat has variable expression of symptoms in the first year of growth, this characteristic is unreliable as evidence of infection. Other wheat-grass hybrids, such as triticale (wheat x cereal rye cross), become infected but do

not express WSM symptoms (Hendrich 2001). ELISA is a reliable serological test for presence of WSMV, but the virus titer could have been at a level too low to be detectable at the time of sampling. Therefore, infection could have occurred during the first growing season and was not detectable until the second season. Alternatively, the young perennial regrowth may be comparable to the first year growth as a seedling, which is most susceptible to WSMV infection. The perennial wheat could have been healthy prior to harvest but became infected soon after it began regrowing.

Considering the patterns of WSM observed in the PNW, large plantings of perennial wheat without effective resistance to WSMV could serve as a reservoir of inoculum resulting in more frequent epidemics. Historically, severe epidemics of WMV do not occur frequently in cool climate wheat-growing areas such as the PNW. When epidemics occur that result in high yield losses, they are usually initiated by hail storms that shatter seed heads near maturity, resulting in a flush of volunteer winter wheat just before harvest. Because wheat is the preferred host for WSMV and its vector, volunteer wheat is an important oversummering reservoir that can support large populations of viruliferous mites. In early fall, mites then move from the volunteer wheat to the young, emerging fall-sown wheat, and WSMV infections occur in high numbers to wheat at its most susceptible growth stage.

In comparison, young perennial wheat regrowth emerges at approximately the same time as volunteer wheat plants, which indicates that perennial wheat could serve as a green bridge from season to season within the same field and to neighboring winter wheat fields. Because perennial regrowth can begin before harvest of the previous year's crop (personal communication, S. S. Jones), green wheat tissue is almost continually present in a perennial field, attracting mites that are moving from neighboring wheat fields. Thus, perennial wheat

may be the only green host still growing at the end of the summer season. Because of epidemiology of this virus pathogen, perennial wheat must have resistance to WSMV at both adult and young regrowth stages of its life.

Natural infection unexpectedly occurred within the noninoculated rows. The mechanically inoculated and plants within each field plot most likely served as a close inoculum source to the neighboring noninoculated rows. Mites that infested the interior rows could have easily become viruliferous by feeding on inoculated plants, then moved to adjacent noninoculated plants and spread WSMV. These factors could explain why almost complete virus infection occurred throughout the interior rows of both Spillman Farm and Central Ferry plots by the beginning of the second year of growth.

Vigor of perennial wheat second year growth was assessed as the number of reproductive tillers, and compared among perennial lines, and healthy versus infected plants. Due to the low number of plants surviving two full growing seasons, ELISA tests, symptom ratings, and spike counts were performed on an individual plant basis. At both Spillman Farm and Central Ferry plots, a negative linear correlation was observed between the number of reproductive tillers and symptom severity. The number of tillers on healthy plants was significantly higher than on plants with severe symptoms. These results confirm the detrimental effects of WSMV infection on perennial wheat vigor and productivity.

The perennial regrowth at both Spillman and Central Ferry field locations had very low percentages of plants surviving from initial emergence to the second year harvest. Even though initial plant density was similar at the two field plots, Central Ferry had a 44% reduction as compared to Spillman Farm. In general, Central Ferry had more severe symptoms than Spillman Farm, with 24% of surviving plants rating 4 and 19% rating 0. These results may be explained

by the higher temperatures and longer growing season at Central Ferry, which are conducive for wheat curl mite dispersal and WSMV infection and replication.

WSMV was detected in almost 100% of rows in all blocks in both field plots, so it is suspected as a significant contributing factor to the observed plant loss. Several other factors could have also contributed including: nutrient or water stress, winter kill, genomic instability, other diseases, high temperatures, or loss of energy from premature production of spikes in fall months before vernalization. WSMV infection could have produced synergistic effects with one or more of these other factors. Notably, recent work has demonstrated that WSMV inhibits root growth in hard red winter wheat and thereby reduces water uptake efficiency (Price et al. 2006). Root damage, in combination with drought stress, can have a devastating effects on wheat and could shorten the lifespan of perennial wheat. Preliminary research (Jones and Murray 2004) has shown that carbohydrate partitioning in the crown and roots of *Thinopyrum elongatum* increases during senescence, presumably to insure regrowth in future years. If WSMV infection interferes with the root system, it could be a factor in reducing regrowth. WSMV was observed in wheat roots 12 days post-inoculation (Choi et al. 2000). These observations are expected because long-distance transport of plant viruses occurs through vascular tissue. After initial inoculation, virus particles travel to the roots, followed by systemic infection (Samuel 1934). Physiologically, the impact of WSMV on wheat roots is unknown.

At the Plant Pathology Farm plot, the inoculated rows of perennial wheat had higher yields than the noninoculated rows and may be explained by cross protection between WSMV strains. All perennial lines, except for 03JP032, had higher average yields when mechanically inoculated with WSMV; however, none of the differences were statistically significant. The entire field plot was exposed to natural infection, as confirmed by ELISA results in

noninoculated rows. So, yield losses in the noninoculated rows may have been due to a naturally occurring WSMV strain. The plot was sown in late fall when temperatures are not conducive for mite movement, so natural exposure probably occurred in spring. The initial mechanical inoculation took place in October 2005, so there was adequate time for prior virus replication and establishment. The isolate used for WSMV inoculum was collected in summer 2004 at Spillman Farm and may be a less virulent or attenuated strain that cross protected the perennial plants against naturally occurring strains in summer 2006. Cross protection has been well-documented between WSMV strains (McKinney 1929, McKinney 1956, Hamilton 1980, Fraser 1985, Sherwood 1987, Hall et al. 2001a). Based on symptomology, evidence suggests that the Spillman Farm isolate may be less virulent. In greenhouse studies, other isolates of WSMV collected in eastern Washington expressed more severe symptoms compared to the Spillman Farm isolate. For example, an isolate from Pasco, WA (Table 11, #11) was inoculated to seedlings that expressed severe symptoms within ten days, and all were dead within thirty.

Another explanation for these yield differences is that the inoculum isolate was maintained in greenhouse plants for 15 months, with at least 7 inoculation passages. The effect of several consecutive inoculation transfers was observed in greenhouse studies. The Spillman Farm inoculum isolate was removed from long-term storage, which had gone through one inoculation passage, and was used to inoculate seedlings. The greenhouse maintained source was inoculated to seedlings on the same day, and symptom expression was delayed and mild compared to the long-term storage inoculum. For many RNA viruses, defective interfering (DI)-like RNA populations can arise after multiple passages (Adam et al. 1983, Ismail and Milner 1988). Such DI-like RNAs can interfere with normal virus replication and reduce symptom production by the parent virus (Hull 2002). Hall et al. (2001b) demonstrated that variation in

WSMV sequence can increase after multiple passages, and speculated that these mutants serve as replication templates, then becoming established into the population. Possibly, DI-like RNAs derived from the WSMV genome were introduced by inoculation, and interfered with replication of any naturally infecting isolates in the field. However, these explanations are not complete, since an obvious host-pathogen interaction is observed. The higher yields in inoculated rows were only observed in perennial wheat lines, but not in winter wheat. Similarly, winter wheat yields for the 2005-06 Spillman Farm plot were higher in the noninoculated rows.

EPIDEMIOLOGY OF WSMV

The objective of this experiment was to gain a better understanding of the timing and pattern of WSMV infection in conventional annual wheat as well as perennial wheat. Plots of perennial and annual wheat were planted around field plots during the 2004-05 and 2005-06 field seasons to serve as a sentinel for natural infection.

The results attained by ELISA to detect WSMV in the surrounding plots were valid. Although 10 leaf samples were taken from each plot, they were bulked as one sample. Therefore, if virus particles were present in one of the 10 plants, this testing method could have diluted its concentration to a level not detectable by ELISA. However, samples with borderline positive ELISA values were tested by RT-PCR, which is sensitive to low concentrations of virus. The detection of WSMV was not completely reliant on results by ELISA; these tests were also coupled with symptom assessment. Other tools were used to validate result by ELISA, but to prevent this discrepancy, individual plants could have been tested.

In the 2005-06 season (Fig. 7), WSM in the Central Ferry plot was limited and no obvious pattern of infection was observed. The earliest infection was detected at the southeast

corner of the plot. The majority of natural infection at Spillman Farm was limited to the northwest side of the plot. A perennial wheat bulk nursery was growing directly adjacent to that side of the plot. This nursery may have served as a source of inoculum and allowing a short-distance movement of vectors to the field plot. Because wind gusts in the Palouse typically come from the southwest, a direct, primary mite infestation may have occurred by this mode. Slykhuis (1955) noted that the severity of WSMV infections and pattern of spread was greatest in the direction of the prevailing winds.

The amount of natural infection varied between the two growing seasons. In the two 2004-05 field plots, WSMV was detected at a higher percentage in the perennial wheat plots than plots the following season. However, virus detection in the susceptible winter wheat plots was greater in 2005-06. Overall, the majority of the annual wheat plots were infected with WSMV in both growing seasons. Infection in the susceptible controls indicates that inoculum was not a limiting factor, and WSM disease incidence was not an isolated event in time. In 2004-05, 35% of the susceptible wheat plots were infected, whereas the inoculum was more prevalent during 2005-06, with 57% of infected susceptible plots. So, compared to 2004-05, the lower percent detection of WSMV in the perennial wheat during 2005-06 was unexpected.

CONCLUSIONS

This research illustrates that *Wheat streak mosaic virus* is widespread and common in eastern Washington. In three consecutive years, WSMV was detected in multiple counties throughout eastern Washington (Table 11). WSMV poses a serious threat to annual wheat production in this area, as well as perennial wheat. The development of perennial wheat is a worthwhile endeavor, and the threat of WSMV to its perennial vigor is now apparent.

The ultimate goal of this research was to determine the effects of WSMV on perennial wheat growth, and to identify potential sources of resistance. Experimentation was carried out in a controlled growth chamber environment, as well as in field trials planted at multiple locations and in multiple growing seasons. Three perennial lines appeared to have resistance to WSMV in growth chamber studies, based on reduced virus replication and less severe symptoms, compared to the susceptible control. One of these resistant lines, 03JP039, was also planted in field plots, and exhibited a similar reaction to WSMV in the first year of growth. The six perennial lines had variable symptom development in the first year, but all developed symptoms in the second year of perennial wheat growth at both field plots. The presence of symptoms in regrowth may be the result of temperatures above 32°C as regrowth was beginning and/or the presence of temperature-sensitive resistance in perennial wheat. However, temperature-sensitive resistance was not conclusively demonstrated in growth chamber studies, so this conclusion cannot be made with these current results. Another explanation is heterogeneity in perennial lines for resistance to WSMV. Greenhouse studies suggest that resistance to WSMV in lines 03JP031 and 03JP039 is heterozygous and the method of inheritance is unknown. For most plant viruses, incompletely dominant and recessive alleles allow spread of the virus pathogen, but multiplication is reduced and symptom expression is inhibited (Fraser 1992). Specifically, the

mode of resistance heritability of perennial wheat may allow systemic WSMV infection that is asymptomatic.

In the USA, WSMV infections that occur in the fall generally lead to more severe WSM symptoms than spring infections. When considering disease pressure to a wheat-growing region as a whole, not just perennial wheat, volunteer wheat in the summer is important because of its role as a "green bridge" for pests: the take-all pathogen, leaf and stripe rust fungi, *Barley yellow dwarf virus*, Hessian fly, and *Wheat streak mosaic virus* (Staples and Allington 1956, Somsen and Sill 1970, Bowden et al. 1991). Volunteer wheat seedlings that emerge before harvest of the previous year's crop is the greatest attractant for these pests. However, severe epidemics of WSM have occurred in the USA when little volunteer wheat is present (Willis 1981). Because regrowth of perennial wheat can emerge several days before harvest of the previous year's crop, the timing of regrowth is comparable to volunteer wheat emergence and therefore, can potentially contribute to the same disease pressures. To avoid serving as a WSMV reservoir, adequate resistance is necessary.

Between 2004-06, the WSM symptoms in the field were mild in both the winter wheat varieties and first year perennial wheat growth. Severe symptoms were only observed in perennial second year growth. The nature of this phenomenon is unknown, but may be attributed to temperature sensitive resistance, or initial regrowth susceptibility and strong WSM disease pressure after harvest.

The nature of resistance conferred from *Thinopyrum* spp. to perennial wheat may be described as tolerance, since WSMV infection occurs but replication occurs at a low level. In the field, infected perennial wheat plants appear healthy, but are harboring the pathogen and therefore serving as an inoculum source for surrounding fields. In both field plots, all perennial

lines expressed symptoms in regrowth, and therefore, the resistance observed in the first year growth was no longer expressed. The reasons for this observation is unknown, but may be explained by temperature sensitivity, drought conditions, or a combination of multiple factors. Several issues are yet to be addressed concerning *Wheat streak mosaic virus* and how it affects perennial wheat production in the PNW. The most pressing issue, however, is the necessity to identify and characterize resistance in perennial wheat, so that it may be effectively incorporated into stable perennial wheat populations.

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APPENDIX 1

Distribution of WSMV in eastern Washington

Between August 2004 and July 2006, WSMV was detected in plant sampled in eight counties in eastern Washington state (Table 11). The virus was detectable by ELISA from samples collected between the months of April to October. Eight different plant hosts were infected, all in the Family *Gramineae.*

Samples were gathered from multiple sources, as listed in the following table:

Courtesy of Keith Pike, Washington State University: #1-3, 6-10

Courtesy of Tim Paulitz, Washington State University: #17, 18

Courtesy of Ken Eastwell, Washington State University: #5

Collected by local farmers: #11

Other samples were personally collected in experimental field plots.

Table 11. *Wheat streak mosaic virus* detected by ELISA in multiple graminaceous hosts in eastern Washington during 2004-06.

	Host	Date	Location
1	Winter wheat	Jun-04	Franklin Co, SR260 & Buehler Rd
2	green foxtail	Aug-04	Franklin Co, Sagemore & N Glade Rd
3	sweet corn	Aug-04	Yakima Co, Satus area, Newland Rd
4	perennial wheat regrowth	Aug-04	Whitman Co, Spillman Farm
5	sweet corn	Sept-04	Benton Co, near Prosser
6	crested wheatgrass	Oct-04	Whitman Co, Hwy 27 & Mader Rd
7	orchard grass	Oct-04	Whitman Co, Hwy 27
8	sweet corn	Oct-04	Spokane Co, Hwy 27 & Irving Rd
9	wild grass	Oct-04	Whitman Co, Hwy 27 & Mader Rd
10	fescue grass	Oct-04	Asotin Co, Fields Spring St Pk
11	spring wheat	Apr-05	Franklin Co, 10mi N of Pasco
12	winter wheat	May-05	Garfield Co, Central Ferry Station
13	perennial wheat	May-05	Garfield Co, Central Ferry Station
14	winter wheat	May-05	Whitman Co, Spillman Farm
15	perennial wheat	May-05	Whitman Co, Spillman Farm
16	perennial wheat	May-05	Adams Co, near Ritzville
17	spring wheat	Jun-05	Whitman Co, near Thornton
18	winter wheat	Jun-05	Whitman Co, RJ Cook Farm
19	perennial wheat regrowth	Aug-05	Garfield Co, Central Ferry Station
20	perennial wheat regrowth	Sept-05	Whitman Co, Spillman Farm
21	winter wheat	Apr-06	Garfield Co, Central Ferry Station
22	winter wheat	May-06	Whitman Co, Spillman Farm
23	perennial wheat	May-06	Whitman Co, Plant Pathology Farm
24	winter wheat	May-06	Whitman Co, Plant Pathology Farm
25	sweet corn	Jul-06	Garfield Co, Central Ferry Station
26	perennial wheat regrowth	Sept-06	Whitman Co, Plant Pathology Farm