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# Dynamics of wheat spindle streak mosaic bymovirus in winter wheat

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**Abstract**

The dynamics of wheat spindle streak mosaic bymovirus in winter wheat were studied during two crop cycles in a field site with a history of high virus incidence. Individual plants of two susceptible cultivars were sampled from autumn to spring and the presence of virus antigen in roots and leaves was determined by ELISA. Virus incidence was higher in cv. Frankenmuth than in cv. Augusta. During year one, incidence of viral antigen in roots remained very low for four months after sowing, and did not reach maximum levels until the following spring. During year two, incidence of viral antigen in roots rose to maximum levels in autumn, only three months after sowing. These results strongly suggested that root infection occurred in spring as well as in autumn. In both cultivars and in both years, we detected the virus in roots one month prior to its detection in leaves, suggesting that virus moves slowly from roots into leaves. Maximum incidence of virus in leaves occurred in spring of both years, coinciding with the period of symptom development. Typical symptoms (yellow streaks, spindles, and mosaic) were observed in year two, whereas only mild mosaic was observed in year one. Virus antigen was detected in nonsymptomatic leaves from two months after sowing through crop senescence. Because antigen could be detected in roots throughout the crop cycle, and zoosporangia and cystosori of the fungal vector could be detected one and two months, respectively, after sowing, it is possible that wheat spindle streak mosaic bymovirus is acquired and/or spread by the vector during the majority of the crop cycle.

## Introduction

Wheat spindle streak mosaic is a prevalent viral disease of winter wheat (*Triticum aestivum* L.) in North America that can cause yield losses exceeding 22% in susceptible cultivars (Brakke et al., 1982; Cunfer et al., 1988; Jackson et al., 1975; Lommel et al., 1982; Miller et al., 1992; Slykhuis and Polack 1969; Wiese et al., 1970; Williams et al., 1975). The causal agent, wheat spindle streak mosaic virus (WSSMV), is a member of the genus Bymovirus of the family Potyviridae and its transmission from plant to plant has been associated with infection by *Polymyxa graminis* Led. (Slykhuis and Barr, 1978). The epidemiology of diseases caused by bymoviruses has not been studied in detail due to the difficulty of experimentation with these viruses and their vectors (Adams, 1990). The bymoviruses are not readily transmitted mechanically (Slykhuis, 1975a), have low infectivity in purified preparations (Usugi and Saito, 1976), and are often found in mixtures with furoviruses (Lommel et al., 1986). The vector is an obligate parasite of roots, is difficult to observe in roots (hyaline except for the resting spore stage), and inhabits single cortical or epidermal cells (Ledingham, 1939).

By assessing initial field infection in Ontario, Canada via induction of symptoms in a growth chamber, Slykhuis concluded that transmission of WSSMV occurred when soil temperatures were between 5 and 15 °C and that the principal period of transmission was during October, although spring transmission did occur, but at low rates (Slykhuis, 1970; Slykhuis, 1975b). Furthermore, Slykhuis concluded that plants infected in autumn developed symptoms the following

spring. And the few plants infected in spring would not develop symptoms, perhaps due to a lack of prolonged temperatures between 5 and 15 °C. In growth chamber studies, Slykhuis and Barr (1978) determined the optimum temperature range for development of the vector, *P. graminis*, in wheat roots to be 15 to 22 °C, the optimum temperature for transmission of virus to be 15 °C, and the optimum temperature for development of virus (i.e. symptoms) to be 10 °C. Wiese and Hooper (1971) found that plant vernalization increased symptom severity as well as the proportion of plants infected. Other studies on wheat spindle streak mosaic in Georgia (Cunfer et al., 1988), Pennsylvania (Nguyen and Pfeifer, 1980), and New York (Miller et al., 1992) centered on host resistance and yield loss assessment, and indirectly supported the earlier conclusions that infections occur in primarily autumn, symptom expression occurs in spring following vernalization, and symptom development ceases after the average temperature exceeds 15 °C (Slykhuis 1970; Slykhuis 1975b; Wiese and Hooper, 1971).

Previous studies on wheat spindle streak mosaic have relied primarily on symptom expression as a measure of plant infection. We produced an antiserum to WSSMV that is able to detect the presence of virus antigen in both root and leaf tissue (Carroll et al., 1995). The antiserum was used to follow natural infection within a population of winter wheat plants during two crop cycles in order to elucidate the epidemiology of the disease. The objectives of this study were to determine the dynamics of virus antigen occurrence in roots and leaves of susceptible winter wheat cultivars and the timing of symptom expression from crop emergence through senescence.

## Materials and methods

### *Field site*

The field site, used for assessing resistance of winter wheat cultivars to WSSMV, was located in Ithaca, NY and had been continuously planted with winter wheat for over 10 years (Bergstrom et al., 1994). Consistently high WSSMV incidence has been observed on the site. Soilborne wheat mosaic furovirus, also vectored by *P. graminis* (Adams, 1990), was absent from the site. The soil is a somewhat poorly drained Niagara silt loam. In both years, the field was fertilized with 225 kg/ha of 10-20-20 NPK incorporated prior to planting; plants were topdressed with 84 kg/ha ammonium nitrate at crop greenup in April. Each year, the previous wheat crop was harvested in July and the field was plowed and harrowed prior to planting the next crop.

### *Field plot design, sample collection and preparation*

In 1992/93, two WSSMV-susceptible cultivars of winter wheat, Frankenmuth and Augusta, were grown separately in two border plots each, surrounding a rectangular winter wheat breeding trial. Plots consisted of six rows, spaced 18 cm apart. Seed were sown on September 16, 1992. Samples were collected weekly or every other week beginning 3 wk after sowing and continuing throughout the crop cycle until plant senescence. Samples were not obtained when the ground was frozen from mid-January through early April. Samples were obtained from two plots at the northeast corner of the field (the north border containing Frankenmuth and the east border containing Augusta). At each sampling date, five aggregate samples from each cultivar were collected at regular distances from randomly selected rows in each border plot. Each aggregate sample included at least five adjacent plants and the surrounding soil from an area of approximately 100 $cm^2$ to a depth of 10 to 15 cm. Samples were bulked by cultivar and a subsample of 22 plants of each cultivar was prepared for enzyme-linked immunosorbent assay (ELISA). Roots were separated from aerial portions of plants and tested for the presence of WSSMV coat protein antigen by ELISA. Roots of samples collected from October to early May were ground in 0.1 M ammonium citrate buffer, pH 6.5 (1:10 w/v). From late May to July, sap was expressed from entire root systems using a rolling leaf press and diluted 1:10 v/v in ammonium citrate buffer, pH 6.5. From October to early April, aerial portions of plants were tested only if the roots tested positive for WSSMV; after early April, leaves of all plants were assayed for WSSMV. Leaf segments were removed with a surface disinfested cork borer from the most recently, fully expanded leaves. These segments were ground in ammonium citrate buffer, pH 6.5 (1:10 w/v). Homogenized samples were kept on ice, clarified with chloroform (1:1 v/v) and diluted 1:4 (v/v) in ammonium citrate buffer, pH 6.5, to yield a final dilution of 1:40. Prior to destructive analysis, each plant was inspected for symptoms (yellow streaks, yellow to light green mosaic) on the uppermost leaves and given a plus (symptomatic) or a minus (asymptomatic) rating. Root and leaf samples from uninfected and WSSMV-infected plants main-

tained in a growth chamber at 8 to 13 °C were used as controls.

In 1993/94 the two susceptible cultivars were grown in four complete blocks, each block was 18.3 m long and consisted of 12 rows spaced 18 cm apart (strips of six rows of each cultivar). The two cultivar strips in each block were divided into thirty 61-cm-long segments. At each sampling interval, one plant of each cultivar was removed from five randomly chosen segments within each block. Within each segment, the single plant of each cultivar was removed from the second row in from the adjacent cultivar strip. In this way, plants of each cultivar were removed from soil locations in close proximity that might contain comparable levels of inoculum and a more direct comparison of virus incidence between the two cultivars could be obtained. The randomized complete block design allowed for a test of nonrandom distribution of the virus in the vector population in the soil and its possible effect on disease incidence. Seed was sown on September 23, 1993. Samples were collected every 2 wks beginning 3 wks after sowing and continuing throughout the growing season until plant senescence (except when the ground was frozen from January through early April). The 20 plants of each cultivar were prepared for ELISA by expressing sap from the entire root system and all green leaves using a rolling leaf press. Sap was prepared for ELISA and symptoms were rated as described above. Root and leaf samples from uninfected and WSSMV-infected plants maintained in a growth chamber at 8 to 13 °C were used as controls.

*Serological assays*

Polyclonal antiserum to WSSMV (Carroll et al., 1995) was used in indirect ELISA in 1992/93 and in double antibody sandwich (DAS)-ELISA in 1993/94 as described by Carroll et al. (1995). Each sample was loaded in two wells and each microtiter plate included uninfected and infected controls. Following the addition of substrate, absorbance values at 405 nm were obtained at 15, 45, 75, and 110 min for indirect ELISA and at 20, 120, and 240 min for DAS-ELISA. Samples were considered positive when their mean absorbance values at 405 nm were 3X those of uninfected sap.

Percent incidence of WSSMV was calculated as the number of root or leaf samples testing positive by ELISA, divided by the total number of samples assayed. Following arcsine square root transformation of percentages, differences in WSSMV incidence in roots and leaves between the two cultivars were analyzed by one-way analysis of variance (ANOVA) using Minitab (1987) and 1993/94 data were also analyzed by SAS ANOVA with time as a covariate. In 1993/94, mean incidence in the four blocks was compared similarly using one-way ANOVA to gain an indication of the dispersion of the virus in the field plot. On each sampling date, the mean of the positive absorbance values was calculated to determine when the highest ELISA values occurred in roots and leaves. The presence of antigen in roots, leaves, or both was determined for each plant assayed in each year.

*Temperature data*

Maximum and minimum daily temperatures for air (at 1.5 m above ground) and soil (at 0.1 m below ground) were obtained from the Department of Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY, for the Game Farm Road weather station, located within 0.5 km of the field site. Mean daily air and soil temperatures were calculated and plotted over time from the time seed was sown until plant senescence.

Polymyxa graminis *analysis*

From October to January of 1992/93, root subsamples saved from the plants testing positive for WSSMV antigen were mounted in sterile water on a slide and examined with a compound microscope for *P. graminis* structures. The root pieces were scored for the presence of thalli or plasmodia, zoosporangia, and cystosori. No attempt was made to differentiate amoeboid zoosporangial thalli from cystogenous plasmodia.

## Results

*1992/93, year one*

Soil temperatures below 15 °C, favorable for WSSMV infection, were reached two weeks after sowing (Figure 1A). In autumn, WSSMV incidence remained below 20% in roots (Figure 1B) and below 15% in leaves (Figure 1C). WSSMV antigen was detected in roots and leaves of Frankenmuth earlier than in Augusta. Incidence of WSSMV increased to over 80% in roots and over 90% in leaves during spring. Maximum WSSMV incidence in roots, 82% in Frankenmuth and 77% in Augusta, and in leaves, 91% in Frankenmuth and 73% in Augusta, both occurred in

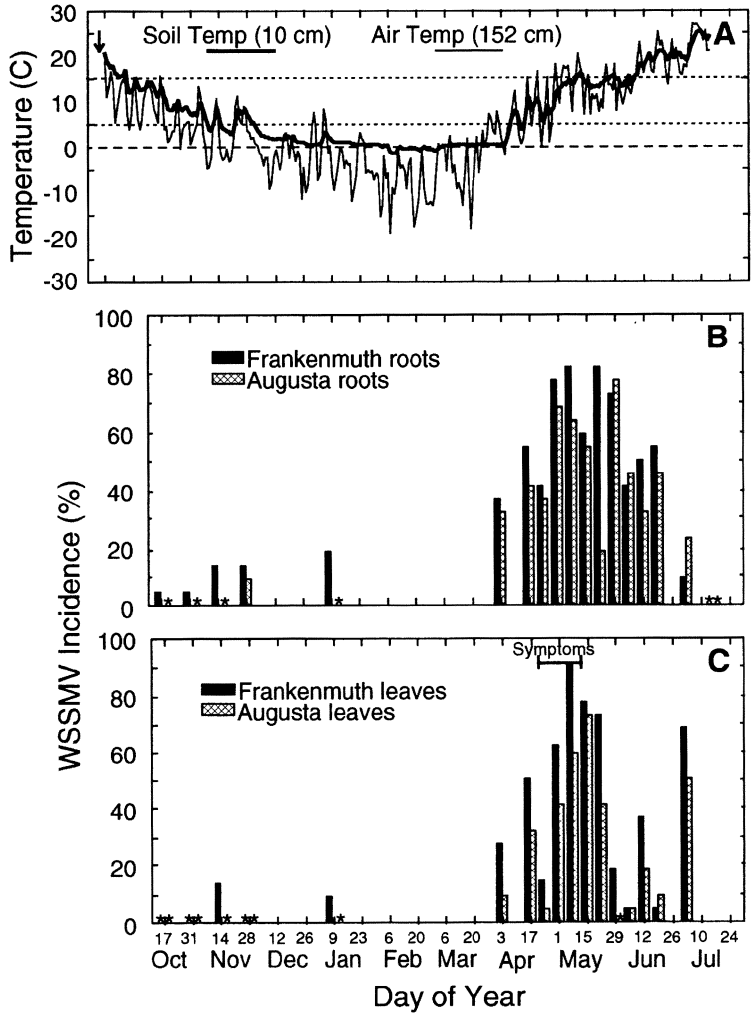


*Figure 1.* (A) Mean daily temperature for 1992/93 from the day after seed was sown (arrow) until plant senescence. Bold line is the soil temperature at a 10 cm depth and the narrow line is the air temperature at a 152 cm height. Dotted line delineates 0 °C and the two stippled lines border the conducive soil temperature range of 5 to 15 °C. (B and C) The percent wheat spindle streak mosaic bymovirus incidence for 1992/93 as determined by indirect ELISA. Zero incidence is indicated by an asterisk. (B) The percent virus incidence in root samples. (C) The percent virus incidence in leaf samples. Leaves were not assayed in the final July sample because tissue was senescent.

May (Figure 1B and 1C). Maximum WSSMV incidence in both tissues coincided with soil temperatures in the 5 to 15 °C range (Figure 1A). When WSSMV incidence was analyzed using one-way ANOVA, no significant differences between the two cultivars were found.

Symptoms developed on overwintered leaves and the first and second leaves that emerged during a three week period, from April 20 to May 4, when soil temperatures were between 5 and 15 °C (Figure 1C). Only a mild mosaic developed, without the characteristic bright yellow streaks and spindles. Symptoms did not develop on new leaves as soil temperatures rose above 15 °C. The average percentage of plants with symptoms was 77% (max 86%) for Frankenmuth and 56% (max 64%) for Augusta. The highest mean positive ELISA values in leaves and roots were found during the period of symptom development.

In autumn, WSSMV antigen was detected in only roots or in both roots and leaves of individual Frankenmuth plants (Figure 3A), whereas only roots of Augusta tested positive (Figure 3B). In early spring, a majority of the plants tested positive in roots and leaves, while in late spring, most plants tested posi-

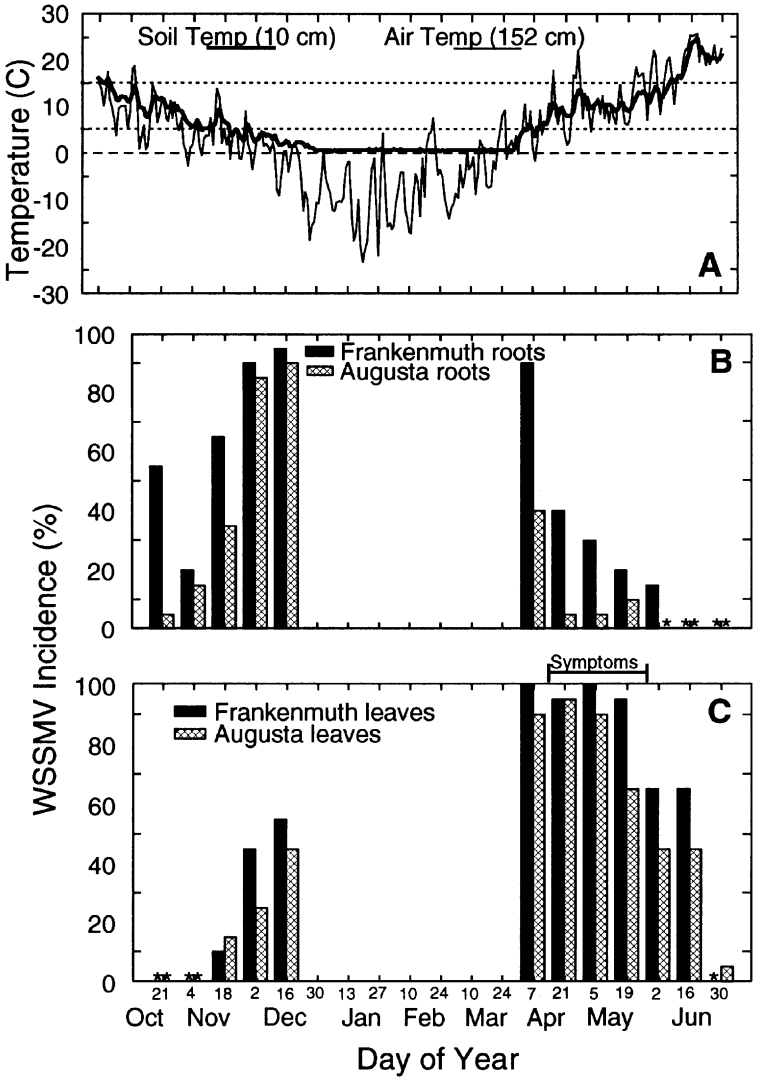


*Figure 2*. (A) Mean daily temperature for 1993/94 from the day after seed was sown until the final sample was taken. Bold line is the soil temperature at a 10 cm depth and the narrow line is the air temperature at a 152 cm height. Dotted line delineates 0 °C and the two stippled lines border the conducive soil temperature range of 5 to 15 °C. (B and C) The percent wheat spindle streak mosaic bymovirus incidence for 1993/94 as determined by double antibody sandwich ELISA. Zero incidence is indicated by an asterisk. No samples were analyzed after plants were senescent. (B) The percent virus incidence in root samples based on the total incidence in each of the four blocks. Standard deviation bars not shown, differences between block means were not significant. (C) The percent virus incidence in leaf samples based on the total incidence in each of the four blocks. Standard deviation bars not shown, differences between block means were not significant.

tive in roots only. Interestingly, the final sample in which flag leaves were tested (other leaves were senescent) showed a substantial increase in the proportion of plants testing positive for WSSMV antigen in leaves only. These flag leaves were asymptomatic.

*1993/94, year two*

Soil temperature was 15 °C the day seed was sown and dropped below 15 °C two days later (Figure 2A). In contrast to the previous autumn, WSSMV incidence in roots rose to maximum levels and in leaves rose to above 50% by December 16 (Figure 2B and 2C). Maximum WSSMV incidence in roots of 95%

in Frankenmuth and 90% in Augusta corresponded to a period when soil temperatures were between 1 and 4 °C. The highest mean positive ELISA values in roots of both cultivars were also found at this time (Dec 16). In root samples assayed during April and May, mean positive ELISA values dropped before soil temperature rose above 15 °C. By the first spring sample, WSSMV incidence in leaves of Frankenmuth had increased to 100% when soil temperatures were at or below 5 °C. Maximum WSSMV incidence of 95% in leaves of Augusta was reached in the second spring sample, coinciding with soil temperatures between 5 and 15 °C. When WSSMV incidence was analyzed using one-way ANOVA for the main effect of cultivar, root incidence was higher in Frankenmuth ($P = 0.01$, $F - 6.69$, $df = 1$) and no significant differences in leaf incidence were found. Analyses using ANOVA with time as a covariate gave similar results. No significant effects on WSSMV incidence by the blocks were found for roots or leaves of either cultivar ($P > 0.9$, $F < 0.15$, $df = 3$), indicating that virus was distributed uniformly in the plot.

In autumn, although WSSMV incidence in leaves was high and soil temperatures were at or below 15 °C, leaves remained symptomless prior to vernalization. Symptoms developed on overwintered leaves and leaves that emerged prior to stem elongation during a five week period, from April 20 to May 19, two weeks longer than the previous year (Figure 2C). A more severe mosaic than the previous year developed with characteristic yellow streaks and spindles. During stem elongation, symptoms failed to develop on young leaves even though soil temperatures remained below 15 °C for two more weeks. The average percentage of plants with symptoms was 98% (max 100%) for Frankenmuth and 93% (max 100%) for Augusta. In assayed leaves of both cultivars the highest mean positive ELISA values coincided with the onset of symptoms.

The distribution of WSSMV antigen in individual plants (Figure 3C and D) followed a similar pattern in autumn for both cultivars; only roots or both roots and leaves tested positive. In spring, only leaves or both roots and leaves tested positive, with the exception of one Frankenmuth plant in early June that tested positive in roots only.

### Polymyxa graminis *analysis*

Data were collected only in year one, 1992/93. Virus-infected root segments that were examined for *P. graminis* contained numerous thalli/plasmodia and a few zoosporangia in autumn (October and November) samples. January samples contained numerous zoosporangia. Cystosori were found in samples collected November 28 and January 7.

## Discussion

Serological assay of individual plants proved to be a sensitive and effective method for estimating the dynamics of WSSMV in winter wheat during two natural crop cycles in New York. Using this approach we corroborated conclusions of Slykhuis (1975b) in Ontario, Canada, on the timing of WSSMV transmission and symptom expression. Our data strongly suggest that WSSMV transmission occurs in autumn and also in spring, and that symptoms occur only in spring, even in plants infected in autumn. Under natural conditions we found evidence that WSSMV moves slowly from roots to leaves and this is in line with the findings of Schenck et al. (1995) under controlled conditions. We discovered that acquisition and spread of virus by the fungal vector may be possible throughout the crop cycle. We showed that the cultivar Frankenmuth is more susceptible to WSSMV than the cultivar Augusta.

If, as Slykhuis (1975b) concluded, transmission of WSSMV from *P. graminis* to wheat plants occurred primarily in autumn, we expected to detect WSSMV antigen in the majority of the crop by the time the ground froze. This was the outcome in year two, 1993/94, but not in year one, 1992/93. In year one, WSSMV incidence in roots was low in autumn and early winter and did not increase to maximal levels until May. This demonstrated that plants can be infected in autumn (as in year two) and strongly suggested that infection continues into spring (as in year one). Throughout the spring of year one, the continued detection of antigen in only roots and not leaves of a proportion of the plants suggested that transmission of virus to these plants occurred less than one month previously (i.e., in late winter or early spring) because virus had not yet moved into leaves (Schenck et al., 1995). This supports the conclusion that low incidence of antigen-positive roots in autumn of year one indeed was due to low incidence of autumn transmission of virus rather than to titers below serological detection (i.e., less than 70 pg of WSSMV per $\mu$l of root sap (Carroll et al., 1995)).

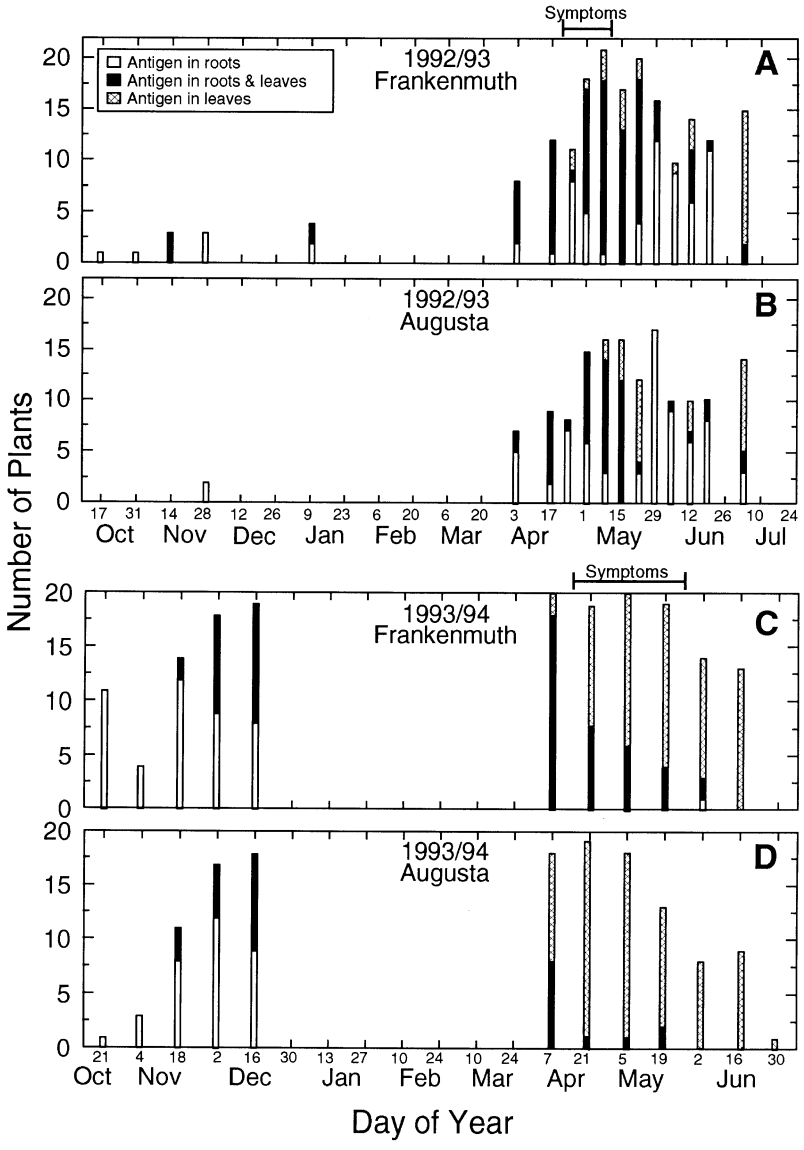


*Figure 3*. (A, B, C, and D) Stacked bar graph showing the number of plants on a sampling date that tested positive for wheat spindle streak mosaic bymovirus in roots only, in both roots and leaves, or in leaves only. A and B data for 1992/93 are based on results from indirect ELISA and C and D data for 1993/94 are based on results from double antibody sandwich ELISA. A. No Frankenmuth plants tested positive for virus in the final spring sample. (B) No Augusta plants tested positive for virus in four out of the five autumn and winter samples and in the final spring sample. (C) No Frankenmuth plants tested positive for virus in the final spring sample.

Both autumn periods were conducive for development of the vector and transmission of WSSMV according to temperature criteria established by Slykhuis and Barr (1978), so the reason for the large differential in root incidence in the two autumns is not apparent. Rainfall at this site was two to three inches above normal in September and October of year one, and at normal levels in September and October of year two. Therefore, soil moisture was not a limiting factor for infection by the vector in either year. Warmer soil temperatures at the time seed was sown in year one (1992/93) may have promoted infection by the vector (Slykhuis, 1975b), but inhibited replication of the virus. Pre-experiment soil inoculum (i.e., viruliferous *P. graminis* population) level may have been lower in year one than in year two. Epidemics of wheat spin-

dle streak mosaic may not be strictly monocyclic; soil inoculum levels may increase during autumn through secondary cycles of root infection.

The presence of zoosporangia of *P. graminis* in virus-infected root samples examined in October 1992 indicated that secondary spread of WSSMV could be occurring from this time on. Rao (1968), working with soilborne wheat mosaic furovirus, provided evidence that the zoosporangial stage of *P. graminis* is repetitive. We attempted to transmit WSSMV to Frankenmuth seedlings under controlled conditions in a growth chamber using root pieces collected in November 1992 and in December 1993 that contained *P. graminis* structures and virus, but these attempts were unsuccessful. Cystosori become most abundant in roots by late spring (Ledingham, 1939), yet we found cystosori in virus-infected roots as early as November. Since WSSMV was found in roots from 3 wk after sowing until crop senescence, this demonstrated that virus could be acquired continuously by the vector for primary inoculum during the majority of the growing season. If acquisition of bymoviruses by *P. graminis* occurs at low frequency, as reported by Adams et al. (1988), then the presence of WSSMV in roots from autumn through spring would maximize opportunities for virus acquisition and spread.

Symptoms developed only in spring when soil temperatures were between 5 and 15 °C following periods with highest mean positive ELISA values in leaves. We observed typical symptoms in year two, which had a high incidence of autumn infection, whereas we observed only mild mosaic in year one, which had a low incidence of autumn infection. In year one, in Frankenmuth and Augusta, respectively, 14% and 17% more plants contained WSSMV in leaves than developed symptoms. In year two, only 2% of plants with virus in leaves failed to develop symptoms. This suggested that a proportion of the plants were infected too late in the crop cycle to allow symptoms to develop in year one. Infection of roots during spring, rather than autumn, may lead to reduced accumulation of virus in leaves, possibly due to slow movement of the virus from roots to leaves (Schenck et al., 1995). In both cultivars and in both years, we detected WSSMV antigen in roots one month prior to its detection in leaves, providing field evidence that virus moves slowly from roots into leaves. In year one, 1992/93, the incidence of plants testing positive for antigen in asymptomatic flag leaves increased when soil and air temperatures were approximately 20 °C, a temperature that in one hour inactivates the virus *in vitro* (Slykhuis, 1975a). It is possible that antigenicity of virus was retained in these leaves, but not infectivity. We detected WSSMV antigen in asymptomatic leaf tissue both prior to and after the appearance of symptoms, regardless of soil or air temperature. In electron microscopic observations of leaf sections, Hooper and Wiese (1972) found that membranous inclusions formed in the cytoplasm of infected cells prior to the development of macroscopic symptoms in wheat leaves. Possible deleterious effects of virus in symptomless plants would help explain large yield reductions that have been associated with the disease even though symptoms appear only briefly in spring.

The two cultivars used in this study have both been rated as susceptible based on the incidence of symptomatic plants at the time of peak symptom development in field trials (Miller et al., 1990). Our data suggest that Frankenmuth is slightly more susceptible to WSSMV than Augusta. Frankenmuth sustained higher WSSMV incidence in roots and leaves than Augusta and, in year one, its roots were infected earlier. These findings support those of Miller et al. (1992) who found in their temperature-driven yield loss model that WSSMV induced slightly less yield reduction in Augusta than in Frankenmuth for each additional day in the conducive temperature range. Little is known about the mechanisms of WSSMV resistance in winter wheat, although inhibition of the movement of virus from roots to leaves has been implicated in some resistant cultivars based on the ability to infect them by mechanical inoculation of leaves (Jackson et al., 1976). Serological assay offers an additional tool for determining, more precisely, levels of wheat resistance to WSSMV and also possible mechanisms of resistance.

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