

Incidence of Soilborne Wheat Mosaic Virus and Its Reported Vector, *Polymyxa graminis*, in Field-Grown Soft Red Winter Wheat

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ABSTRACT

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The incidence of soilborne wheat mosaic virus (SBWMV) and *Polymyxa graminis* in four resistant (Hart, Purdue, IL 87-7394, and IL 85-2655) and four susceptible (Cardinal, Rosette, Michigan Amber, and Maryland 75-266-46) soft red winter wheat cultivars was investigated in field experiments during 1989-1990. Soilborne wheat mosaic virus antigen in roots and shoots was detected by enzyme-linked immunosorbent assay (ELISA). *P. graminis* root infections were assessed with bright field microscopy. Both resistant and susceptible cultivars became infected with the virus shortly after planting in the fall. Virus antigen was detected by ELISA in shoots of all resistant and susceptible plants until dormancy. When growth resumed in the spring, detectable viral antigen was significantly ($P \leq 0.05$) lower in the shoots of resistant cultivars. In contrast, the detectable antigen in roots of resistant cultivars was significantly ($P \leq 0.05$) lower than in susceptible cultivars for almost the entire 1989-1990 field season. The lower incidence of SBWMV in roots was associated with fewer shoot infections after dormancy. Although not statistically different, the number of resting spores of *P. graminis* per centimeter of root was generally lower in resistant than in susceptible cultivars. The results of this study suggest that mechanisms of resistance to soilborne wheat mosaic may involve a reduction in rates of virus particle assembly, movement, and/or replication.

Polymyxa graminis Ledingham, the reported vector of soilborne wheat mosaic virus (SBWMV) (6,18), is an obligate root parasite of winter wheat

(*Triticum aestivum* L.) and other grasses (2,13). Motile zoospores released from resting spores and subsequently from intracellular sporangia initiate root infections shortly after fall planting of winter wheat (13,17,23). SBWMV was first recognized in Illinois and Indiana in 1919 (14) and is now known to occur in other winter wheat growing areas of the United States, southern Europe, China, and Japan (3,7). Cardinal wheat is a soft red winter wheat cultivar widely grown in Illinois. It exhibits excellent

yields, good winterhardiness, and disease resistance to powdery mildew, wheat yellow mosaic (wheat spindle streak mosaic) virus, leaf rust, loose smut, and Septoria leaf blotch (10). It is, however, susceptible to SBWMV (8), and the potential for disease outbreaks in Illinois is greatly increased with the repeated cultivation of this and other susceptible cultivars.

At present, the only practical means to manage soilborne wheat mosaic is with resistant cultivars (5,11,15,19). The nature of resistance to soilborne wheat mosaic remains unclear. Direct observations by Larsen et al (12) argued against resistance to the fungal vector alone. However, Lapierre et al (11) found differing levels of resistance to *P. graminis* in cultivars of autumn-sown wheat exhibiting resistance to SBWMV. Others have suggested that resistance is more likely to the virus itself. Tsuchizaki (22) noted that resistant cultivars did not become infected with SBWMV when planted into infested soil, but *P. graminis* infected roots of both resistant and susceptible cultivars. Virus resistance may be expressed as a reduction in virus replication, virion assembly, or virus movement (1,9,12,20).

Although winter wheat undergoes profound physiological changes before, during, and after dormancy, little is known about how these physiological

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Table 1. Percentage of plants in which ELISA readings indicated the presence of soilborne wheat mosaic virus (SBWMV) antigen^x in resistant and susceptible cultivars of soft red winter wheat collected during the 1989–1990 growing season^y

Cultivar	11/30/89	1/11/90	1/29/90	2/12/90	2/28/90	4/6/90	5/1/90
Resistant roots							
Hart	33.33 c ^z	30.00 cd	56.67 c	6.67 b–d	23.33 b	23.33 b	44.00 cd
Purdue	27.78 c	35.00 b–d	56.67 c	3.33 cd	26.67 b	16.67 b	40.00 cd
IL85-2655	27.78 c	20.00 d	63.33 bc	0.00 d	23.33 b	16.67 b	20.00 d
IL87-7394	38.89 c	15.00 d	53.33 c	10.00 b–d	26.67 b	13.33 b	60.00 bc
Susceptible roots							
Cardinal	72.22 b	83.33 ab	96.67 a	26.67 a–c	100.00 a	74.17 a	96.00 a
Rosette	94.44 ab	90.00 a	100.00 a	43.33 a	93.33 a	56.67 a	72.00 ab
Maryland	100.00 a	80.00 a–c	96.67 a	16.67 b–d	76.67 a	66.67 a	84.00 ab
Michigan Amber	77.78 ab	70.00 a–d	86.67 ab	30.00 ab	76.67 a	73.33 a	88.00 a
LSD	22.60	50.85	28.78	26.65	26.95	28.44	26.87
Resistant shoots							
Hart	100.00 a	65.00 ab	96.67 a	33.33 b	40.83 b	23.33 c	20.00 de
Purdue	100.00 a	75.00 ab	96.67 a	23.33 b	60.00 b	30.00 c	16.00 e
IL85-2655	100.00 a	65.00 ab	83.33 b	23.33 b	57.50 b	23.33 c	12.00 e
IL87-7394	100.00 a	50.00 b	100.00 a	26.67 b	56.67 b	16.67 c	44.00 cd
Susceptible shoots							
Cardinal	100.00 a	100.00 a	100.00 a	70.00 a	100.00 a	96.67 a	96.00 a
Rosette	100.00 a	100.00 a	100.00 a	90.00 a	100.00 a	100.00 a	68.00 bc
Maryland	100.00 a	80.00 ab	100.00 a	80.00 a	93.33 a	90.00 ab	92.00 ab
Michigan Amber	100.00 a	100.00 a	93.33 ab	83.33 a	93.33 a	73.33 b	88.00 ab
LSD	0.00	47.84	12.69	37.30	32.80	22.65	25.44

^xPositive readings were designated as those above the mean absorbance plus four standard deviations of at least six wells containing extracts from uninfected controls.

^yOn each collection date, five samples of each cultivar were collected per replication, and the mean percent positive ELISA readings across six replications was calculated.

^zFor each collection date and tissue type, means followed by the same letter are not significantly different from each other ($P = 0.05$).

changes might affect resistance to the virus. Therefore, the objectives of this study were to 1) determine the incidence of SBWMV in resistant and susceptible soft red winter wheat before and after dormancy, 2) determine the distribution of *P. graminis* in the roots of cultivars resistant and susceptible to SBWMV, and 3) determine whether the incidence of *P. graminis* is correlated with that of SBWMV.

MATERIALS AND METHODS

Field experiment. During 1989–1990, a field trial was set up at the Agronomy-Plant Pathology South Farm at Urbana, IL. In early October, four resistant (Hart, Purdue, IL87-7394, and IL85-2655) and four susceptible (Cardinal, Rosette, Maryland, and Michigan Amber) soft red winter wheat cultivars were planted into individual plots six rows wide and 4 m long in a field known to be infested with *P. graminis* and SBWMV. Treatments (cultivars) were replicated six times in a randomized complete block design. Sampling began in November after tillers formed. On each date, five plants were collected from each plot (a total of 30 plants per cultivar). Plant roots were collected to a depth of 15 cm. Sampling was halted in December because of freezing of the field soil but continued in January until heading began in May (Table 1).

The incidence of SBWMV antigen in shoots and roots of individual plants was examined instead of pooling leaf samples from several plants and then analyzing selected subsamples. For each collection date, levels of SBWMV antigen in roots

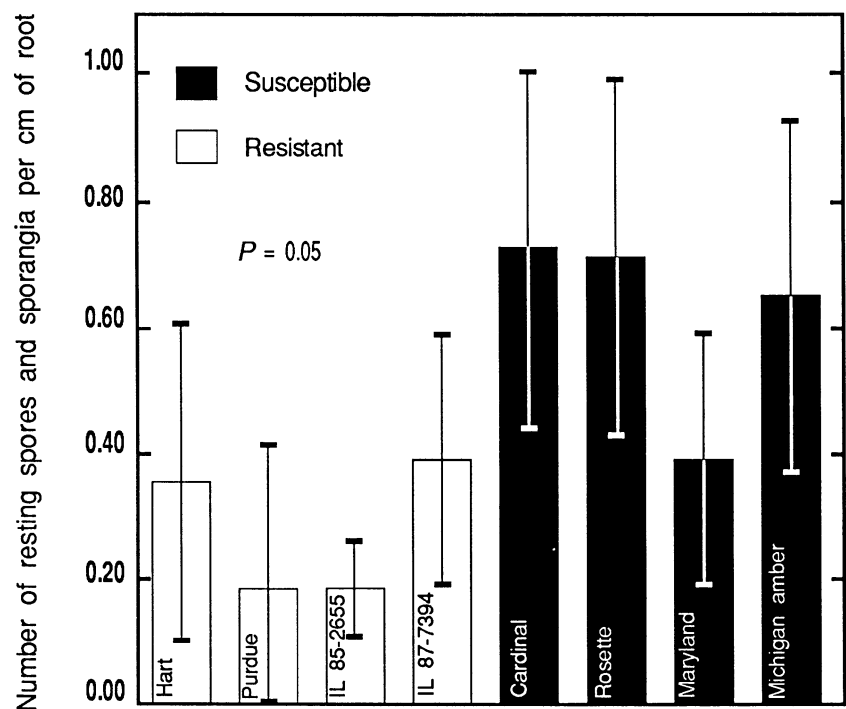


Fig. 1. Number ($\log_{10} [Y + 1]$) of resting spores and sporangia of *Polymyxa graminis* counted in roots of resistant and susceptible cultivars of soft red winter wheat. Samples were collected on 30 November 1989.

and shoots of resistant and susceptible cultivars were also measured and compared. The incidence of SBWMV in roots and shoots of all cultivars before and after dormancy was analyzed as a split plot in time. Data were analyzed using standard analysis of variance for randomized complete block design, and the least significant difference was used for mean separation. In addition, the

incidence of SBWMV was compared with that of *P. graminis*.

Roots were washed in tap water to remove field soil and plants were stored at -20 C until processing. A 4-cm-long root segment was removed from each plant and saved for microscopy. The remaining roots and shoots of each plant were separated and passed through a sap extractor (MEKU, Erik Pollahne and

Co., Am Weingarten 14, Germany). Each sample of extract was diluted (1:10, w/v) in 0.01 M phosphate-buffered saline (PBS) at a pH of 7.4 and stored at -80°C . SBWMV antigen levels in roots and shoots were determined by enzyme-linked immunosorbent assay (ELISA).

Bright field microscopy. *P. graminis* was observed in 4-cm-long root segments using the techniques of Phillips and Hayman (16) and compared with a known isolate provided by D. J. S. Barr, Biosystematics Research Center, Ottawa, Canada. The number of observed sporangia and characteristic clusters of resting spores (13) per centimeter of root was recorded for all cultivars for the 30 November 1989 collection date.

Antiserum preparation. Polyclonal antiserum to SBWMV antigen was provided by J. L. Sherwood, Oklahoma State University. Before using the antiserum, it was cross-adsorbed with uninfected wheat plant extracts prepared in the following manner. Uninfected 2-mo-old wheat leaves were passed through the sap extractor, and the extract was diluted in 0.01 M PBS, pH 7.4 (1:10 w/v). Antiserum was mixed with the extract (1:1 v/v) and stirred for 1 hr at room temperature and then held overnight at 4°C . The serum-plant extract mixture was centrifuged in a DuPont-Sorvall SS-34 rotor at 10,000 rpm (12,000 g) for 10 min. The supernatant was centrifuged again through a Sephadex G-25 spin column at 300 rpm for 5 min. Last, traces of serum were removed from the column by adding 0.5 ml of 20 mM sodium phosphate, pH 7.0, and centrifugation of the column at 1,000 rpm for 5 min. Serum was then filtered through a $0.22\text{-}\mu\text{m}$ filter and applied to a protein G superose column with fast protein liquid affinity chromatography. The fraction containing purified immunoglobulin (Ig) was collected. Type VII-T alkaline phosphatase (Sigma Chemical Company, St. Louis, MO) was conjugated to the purified Ig as follows. Two milligrams of alkaline phosphatase, 1 mg of purified Ig, and glutaraldehyde were mixed to a final concentration of 0.2%, allowed to stand at room temperature for 2 hr, and dialyzed sequentially in three 1-L volumes of PBS. One of the volume changes was overnight, the other two were at least 4 hr.

ELISA procedure. A double-antibody sandwich ELISA system was used to detect SBWMV antigen in the roots and shoots of collected plants. Round-bottom Immulon I microtiter plates (Dynatech, Chantilly, VA) were coated with $50\ \mu\text{l}$ of $1\ \mu\text{g}/\text{ml}$ anti-SBWMV Ig in 0.05 M sodium carbonate coating buffer, pH 9.6, for 2 hr at room temperature. Processed field samples were added to each well in $50\text{-}\mu\text{l}$ aliquots and allowed to stand at 4°C overnight. Conjugated Ig diluted 1:500 in PBS was

added and incubated overnight at 4°C . Freshly prepared substrate, 1 mg/ml of p-nitrophenyl phosphate, disodium, in 10% diethanolamine buffer, pH 9.8, was added, the plates were allowed to develop, and absorbance ($A_{405\text{nm}}$) of each well was determined with a Dynatech MR 700 programmable microplate reader. A positive threshold was selected as the mean absorbance ($A_{405\text{nm}}$) plus four standard deviations of at least six wells containing extracts from uninfected controls. This threshold provided an acceptable ratio of false positives to false negatives (21). ELISA values and the number of positive or negative samples

were recorded for all cultivars on each collection date.

RESULTS AND DISCUSSION

Antigen of SBWMV was detected in all plants collected on the sampling date in November 1989 (Table 1), and *P. graminis* was detected in all roots. The number of observed resting spore clusters and sporangia per centimeter of root varied considerably with no significant differences in the number of *P. graminis* infection sites between resistant and susceptible cultivars (Fig. 1). Although not statistically different, the number of resting spores and sporangia of *P.*

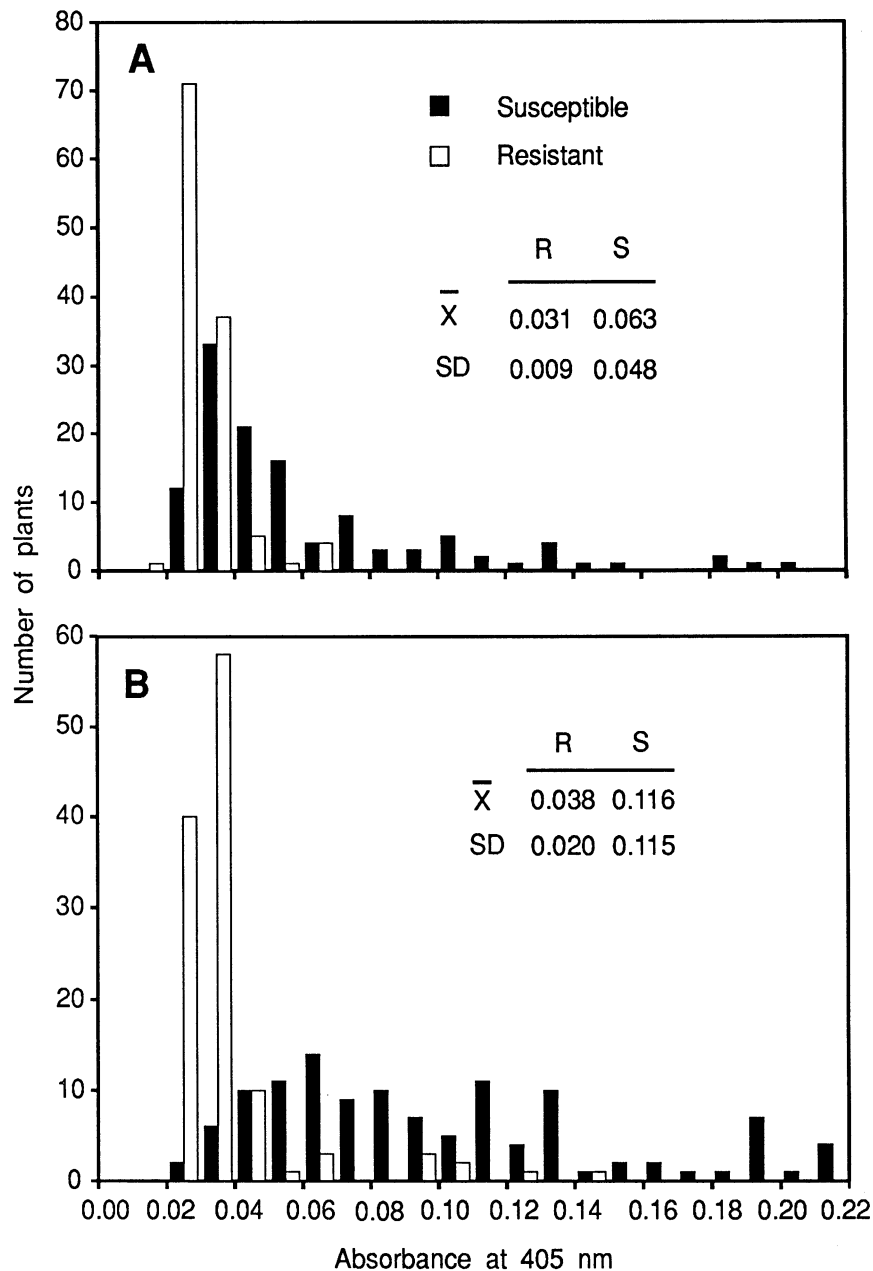


Fig. 2. Frequency distribution of absorbance values measured for (A) individual susceptible and resistant shoots and (B) roots of soft red winter wheat. ELISA readings for each sample were taken from a single well. A total of 30 samples per cultivar were read for this field collection (29 January 1990). Means and standard deviations are given for combined readings of susceptible (S) and resistant (R) shoots and roots. The mean plus four standard deviations of at least six wells containing extracts from uninfected controls was selected as a positive threshold. The calculated threshold for shoots was 0.025 and for roots was 0.028.

graminis per centimeter of root was generally lower in resistant than in susceptible cultivars. The distribution of *P. graminis* in roots was so irregular that there was no clear relationship between the incidence of *P. graminis* and the incidence of SBWMV. However, the lower number of resting spores of *P. graminis* found in roots of resistant cultivars suggests a differential susceptibility to the fungus under these field conditions (Fig. 1). Larsen et al (12) proposed that resistance to viruliferous zoospores played a role in resistance to SBWMV. However, the differing levels of *P. graminis* infections in SBWMV-resistant winter wheat cultivars found by Lapierre et al (11) and *P. graminis* infections of both susceptible and resistant winter wheat cultivars found by Tsuchizaki (22) indicate that further work needs to be done to distinguish between viruliferous and nonviruliferous *P. graminis* root infections before one can determine the role of resistance to zoospores in this disease.

On each collection date from November until the end of January, the detectable incidence of SBWMV in roots of susceptible cultivars was significantly ($P = 0.05$) higher than in resistant cultivars (Table 1). Incidence was similar in roots of some of the susceptible and resistant cultivars at the beginning of February during the onset of renewed growth, but by the end of February, the incidence was again significantly higher in susceptible than in resistant cultivars and remained so through the last collection in May.

Conversely, there was no difference in the incidence of SBWMV detected in the shoots of susceptible and resistant cultivars until the end of January when dormancy ended and active growth resumed. Although the incidence of positive ELISA values during this time was not statistically different, absorbance tended to be higher in shoots and roots of susceptible than in resistant cultivars (Fig. 2). From the time active growth resumed in February until heading in May, the incidence of SBWMV was significantly higher in susceptible than in resistant shoots (Table 1).

On 4 April 1990, seeds of the same resistant and susceptible cultivars were sown adjacent to the fall 1989 planting. Plots were replicated four times. Samples were collected three times beginning 1 mo after planting and analyzed for SBWMV antigen as described previously. The incidence of SBWMV antigen was never greater than 2.5 and 15.0% in roots and shoots of susceptible cultivars and 2.5 and 6.25% in resistant cultivars. Because of these findings, spring infections of soft red winter wheat by *P. graminis* and SBWMV were found to be insignificant.

ELISA values that were higher in susceptible than in resistant cultivars

have been reported previously. Hunger et al (9) suggested the existence of mechanisms that inhibited or slowed capsid production and virion accumulation or production in cultivars resistant to SBWMV. From the higher virion concentrations and ELISA values found in susceptible cultivars than in resistant cultivars, Armitage et al (1) suggested that capsid production and particle

assembly occurred earlier in the season in susceptible cultivars than in resistant cultivars. They agreed with the mechanism proposed by Larsen et al (12) that resistance to SBWMV involves reduced movement of SBWMV in resistant cultivars as well as resistance to infection by zoospores of *P. graminis*. The lower levels of viral antigen found in this study agree with the hypothesis that a reduc-

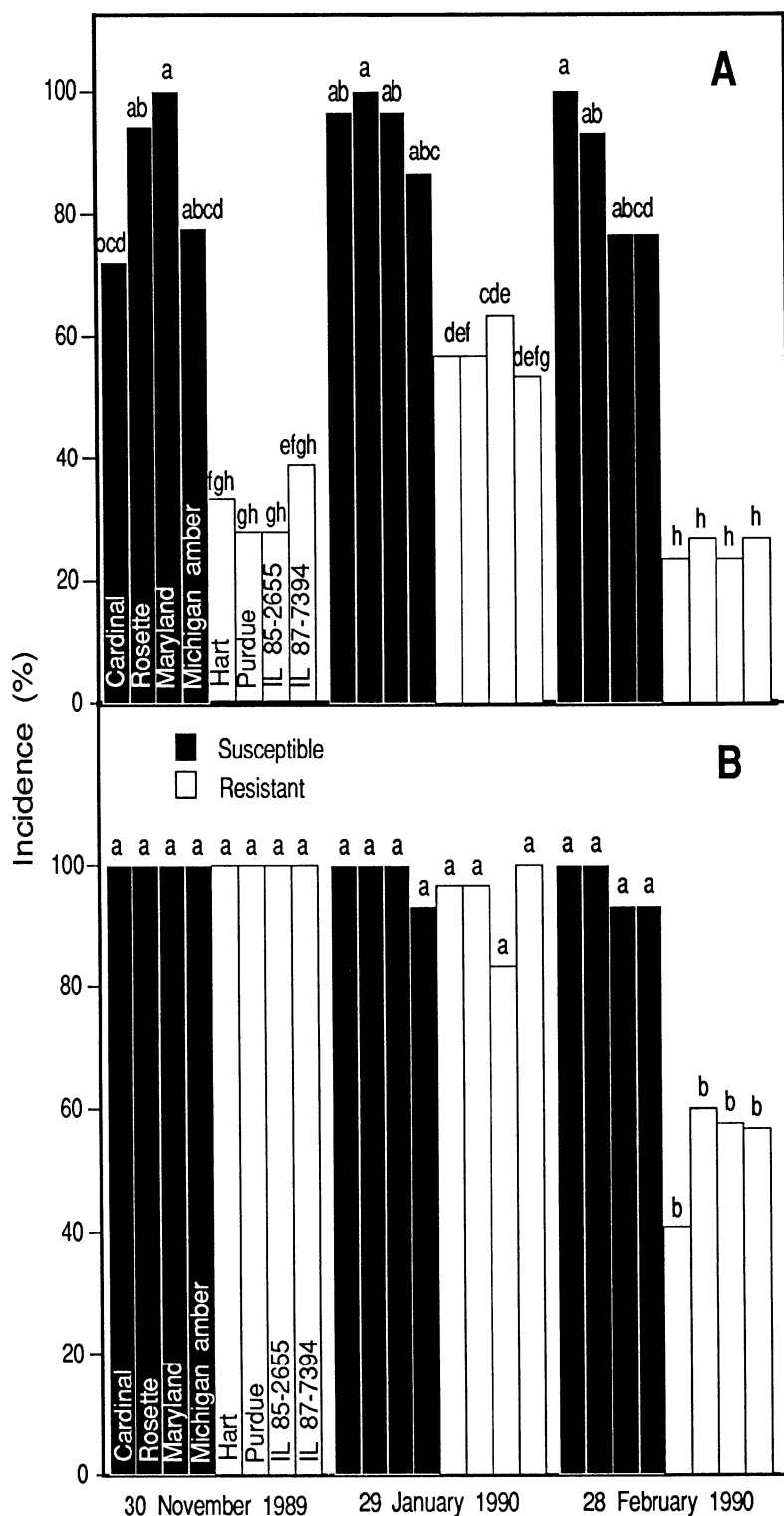


Fig. 3. The percent incidence of SBWMV in (A) roots and (B) shoots of susceptible and resistant winter wheat cultivars over time. Incidence means labeled with the same letter are not significantly different from each other (LSD = 0.05).

tion in replication, particle assembly, and/or movement of virus particles are mechanisms of resistance in soft red winter wheat cultivars.

This study shows that SBWMV moved into roots and shoots of resistant and susceptible cultivars in the fall. However, the virus appeared to accumulate to a greater extent in the roots of susceptible cultivars than in the roots of resistant cultivars. Thus, once growth resumed in the spring, there seemed to be a greater reservoir of virus in the roots of susceptible cultivars than in resistant cultivars (Fig. 3) that moved into actively growing shoots. In field studies, Brakke et al (4) also found SBWMV particles in roots and shoots of symptomatic wheat and concluded that the virus was well distributed throughout symptomatic susceptible hosts, probably accumulating in the fall. Winter wheat cultivars, whether resistant or susceptible to SBWMV, provide a reservoir of vector and virus propagules and contribute to the potential for disease the following season. Even with a multiyear crop rotation, viruliferous *P. graminis* resting spores remain viable in soil debris (13,23).

The observations from this study show that the rates of virus replication and particle assembly in resistant cultivars appear lower after dormancy and that rates of particle movement seem lower, a resistance mechanism suggested by Sherwood et al (20). Given the significant difference in virus incidence in roots of resistant cultivars before and after dormancy, it seems certain that further studies to elucidate the mechanisms responsible for these differences will shed

much-needed light on the nature of resistance to this important wheat pathogen.

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