Identity, Prevalence, and Distribution of Viral Diseases of Winter Wheat in New York in 1988 and 1989

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ABSTRACT

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In 1988 and 1989, 112 winter wheat (*Triticum aestivum*) fields from the major wheat-producing counties of New York were surveyed systematically for the presence of viral pathogens. Forty plants were sampled from each field at each of two growth stages and assayed by enzyme-linked immunosorbent assay (ELISA) for wheat spindle streak mosaic virus (WSSMV), barley yellow dwarf virus (BYDV), soilborne wheat mosaic virus (SBWMV), and wheat streak mosaic virus (WSMV). WSSMV and BYDV were widespread in winter wheat in New York in 1988 and 1989. WSSMV was detected at incidence levels ranging from 0 to 100% of plants infected and was determined to be the principal cause of early spring yellowing of winter wheat in New York in these years. The cultivar Geneva consistently incurred a low incidence of WSSMV infection. BYDV was detected at incidence levels ranging from 0 to 18% of plants infected and, in 1988, a significant negative correlation was found between BYDV incidence and date of planting. WSMV, a virus not previously reported in the state, was detected in several fields at less than 0.5% incidence in both years. SBWMV was not detected in any fields surveyed despite environmental conditions conducive to this disease.

Winter wheat (*Triticum aestivum* L.) has been grown for pastry flour and for feed and straw on New York farms for many years (4). From 1979 to 1988, an average of 61,000 ha per year of wheat was planted in the state, producing grain valued at $20.6 million per year (3). For many years, farmers, extension personnel, and researchers have observed virus-like symptoms in New York winter wheat stands. During surveys in 1986 and 1987, streaking, yellowing, and stunting attributed to infection with various phytopathogenic viruses were observed in many fields (24), suggesting that further investigation into the nature and prevalence of viral diseases of this crop was warranted.

In 1946, McKinney (11,12) observed a disorder near Ithaca, NY, similar to that later described as wheat spindle streak mosaic (WSSM) by Slykhuis (25) in Ontario. This disease is now known to be caused by wheat spindle streak mosaic virus (WSSMV), a member of the barley yellow mosaic virus group. Flexuous rods resembling WSSMV particles have been observed in electron microscope leaf dip preparations from symptomatic plants collected during the past decade (G. C. Bergstrom, T. A. Zitter, and H. W. Israel, *unpublished*), but "early spring yellowing" was often attributed to nutrient deficiencies, "wet feet," and root rots. Although WSSMV is now considered to be a strain of wheat yellow mosaic virus (WYMV), the present study includes only this strain of WYMV and, thus, it will be referred to as WSSMV (28,30).

Since 1957, five distinct isolates of barley yellow dwarf virus (BYDV) have been identified from small grain cereals in New York, mainly spring oats collected near Ithaca (17). These isolates were distinguished by their specificity of aphid transmission and serological properties. Since 1969, PAV-like isolates have been the most frequent BYDV isolates identified in the state (17,24).

In addition to WSSMV and BYDV, two other viruses were considered to be of potential importance for winter wheat in New York. Soilborne wheat mosaic virus (SBWMV) is vectored by the same organism (*Polymyxa graminis* Ledingham) that transmits WSSMV, and the symptoms it causes closely resemble the early spring yellowing of WSSM. SBWMV has been reported at damaging levels in the western, central, and eastern United States (6,12,28) and Canada (L. Seaman and Y. C. Paliwal, *unpublished*). Wheat streak mosaic virus (WSMV) is also widespread and damaging in North America (5,12,28) and its vector, the wheat curl mite (*Aceria tulipae* Keifer), is present in New York.

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The current study was undertaken to assess the identity, prevalence, and distribution of viral diseases on winter wheat in New York. The study combined systematic sampling of New York's winter wheat hectarage with serological detection of the four viruses mentioned earlier.

MATERIALS AND METHODS

Field selection. In 1988, 65 farms were selected randomly from Agricultural Stabilization and Conservation Service (U.S. Department of Agriculture) lists of wheat growers in the major wheat-producing counties of the state. The number of farms selected was proportional to the reported hectarage of wheat harvested in each county in 1986 (1). Two additional farms were selected without randomization in Steuben County to represent the Southern Tier region where conditions are more marginal for wheat production. Fields were located by Cornell Cooperative Extension field staff, and on those farms with more than one wheat field, a single field was chosen either at random or on the basis of convenient access. In 1989, a subset composed of 47 of these 67 farms was selected randomly (with the exception of two farms in Steuben County, which differed from those chosen in 1988) and single fields were selected as described earlier.

Sampling. In 1988, plants were sampled at early stem elongation, growth stage (GS) 30–31 on the scale of Zadoks et al (29) and at spike emergence (GS 47–59). In 1989, plants were sampled at late tillering (GS 25–30) and at booting to spike emergence (GS 45–53). At each growth stage, 40 tillers were selected along a systematic "M" pattern which covered approximately a 2-ha area. Border effects were avoided by beginning sampling at least 10 m from the edge of each field. In addition to the systematic sample, individual plants showing viruslike symptoms were selected and assayed independently, but these results were not included in incidence ratings or statistical analyses.

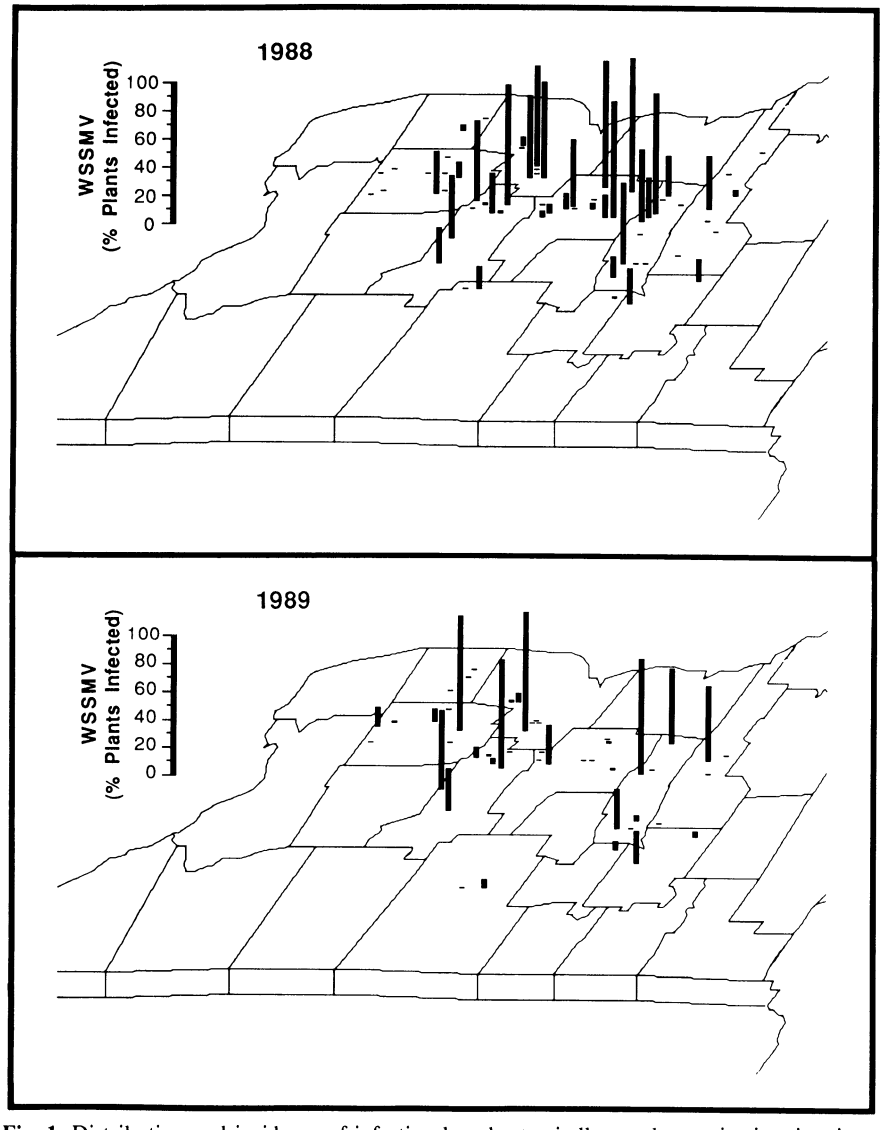

Fig. 1. Distribution and incidence of infection by wheat spindle streak mosaic virus in winter wheat fields surveyed in New York in 1988 and 1989.

Disease rating and virus identification. Incidence ratings (percentage of plants infected with each virus) were determined as follows. In 1988, at GS 30–31, each 40-tiller sample was divided into groups of plants with streaking, general chlorosis, or no viruslike symptoms. Groups of 12 or more plants were further subdivided into smaller batches, and each of these batches was assayed by enzyme-linked immunosorbent assay (ELISA) (7) for the various viruses. At GS 47–59 in 1988 and at both sampling dates in 1989, each 40-tiller sample was rated for viruslike symptoms, then divided randomly into eight five-tiller batches for ELISA.

WSSMV ratings were determined using a combination of visual symptoms and ELISA results. If a batch with streaking symptoms did not have a positive WSSMV ELISA, it was considered to be uninfected. In 1988, asymptomatic batches with positive WSSMV ELISA (three out of 43 asymptomatic batches) were considered to contain one infected plant plus an adjustment factor calculated from the overall proportion of batches of this type (16). In 1989, plants with and without WSSM symptoms were assayed together in random five-tiller batches. Thus, asymptomatic WSSMV infections were detectable only in samples where the total number of batches with positive WSSMV ELISA exceeded the number of symptomatic plants in the sample.

WSSMV ELISA followed the PAS-ELISA procedures of Zagula et al (30) with the following modifications. Antibody and protein A-alkaline phosphatase conjugate were diluted in phosphate-buffered saline (PBS) solution containing 2% nonfat dry milk. Leaf tissue samples were homogenized in 1:5:1.5 (w/v/v) PBS containing 0.05% Tween-20 (PBS-T) and chloroform. Samples were centrifuged, and the clarified supernatant was diluted 1:8 (v/v) in PBS-T. This supernatant was incubated in microtiter plates for 2 hr at 20 C while the second WSSMV antibody solution was incubated overnight at 4 C.

BYDV ratings were based on ELISA results alone. At GS 30–31 in 1988, incidence estimates could not be calculated because batch samples differed in size and were not grouped randomly. At this date, therefore, an overall positive or negative BYDV rating was assigned to each field. At GS 47–59 in 1988 and at both sampling dates in 1989, an estimate of the incidence of BYDV infection was calculated from the proportion of five-tiller batches that tested positive for BYDV in each field as follows (16): Incidence = $100[1 - (\text{number of negative batches/total number of batches})^{1/5}]$.

BYDV ELISA followed the procedures of Rochow (19) and was performed at all sampling dates using the same batch samples and tissue homogenates described above for WSSMV ELISA (without the

1:8 dilution of clarified supernatant in PBS-T). Antisera for PAV, MAV, and RPV isolates of BYDV were combined for an initial composite assay, and samples that tested positive were then retested by ELISA to identify individual isolates. RMV ELISA was performed separately. All polyclonal BYDV antisera were prepared by W. F. Rochow, USDA-ARS, Ithaca (19), except for the RMV antiserum used at GS 45–53 in 1989, which was produced at Purdue University, against a New York RMV isolate (27).

SBWMV ELISA (performed at the earlier growth stages in both years) and WSMV ELISA (performed at the later growth stages) followed the procedures of Lommel et al (9), except that the same antigen preparations used for WSSMV ELISA (without dilution of the clarified supernatant in PBS-T) were used to coat microtiter wells. This procedure made it possible to use the same processed sample for all assays. Antisera for SBWMV, WSMV, and WSSMV were a gift from S. A. Lommel (10).

Information on cultivar, planting date, cropping history, pesticide use, and yield was obtained from a questionnaire administered to participating farmers by Cornell Cooperative Extension field staff (13). To test for relationships between these factors and the various virus ratings, analyses of variance were carried out using the SAS general linear models procedure, and when significant differences were detected, means were compared using least significant differences ($P = 0.05$) on arcsine, square root-transformed data (23). Linear regression followed the SAS PROC REG procedure (23).

RESULTS AND DISCUSSION

Wheat spindle streak mosaic virus. In both 1988 and 1989, the early spring yellowing of wheat plants was quite pronounced because of extended periods of cool temperature. The symptoms initially appeared as light, chlorotic streaks on young, emerging leaves and progressed to a severe mottling with a yellow to necrotic scorching of leaf tips and stunting of plants. This yellowing was often most pronounced in low-lying, poorly drained areas of fields. Symptomatic plants were found to be infected with WSSMV, but not with SBWMV, which causes a similar disease syndrome. The presence of streaking on young, emerging leaves was diagnostic in separating WSSM symptoms from nonsporulating powdery mildew lesions. However, by the second week of May, as mean daily temperatures rose, emerging leaves of infected plants no longer showed streaking, thus WSSM symptoms became more difficult to distinguish.

Field samples ranged from 0 to 100% of plants infected by WSSMV, however no geographical trend was identified (Fig. 1). The presence or absence of WSSM symptoms correlated well with ELISA results, suggesting that asymptomatic infections were not common. Wheat cultivars differed significantly ($P < 0.001$) in incidence (Fig. 2), with Geneva fields consistently incurring less than 2% infection in both years. In 1989, one field of the cultivar Harus was sampled and found to be free of detectable levels of WSSMV. Among the remaining cultivars, no statistically significant differences were found in 1988, but in 1989 Fredrick and Houser fields had intermediate incidences, whereas Frankenmuth and Ticonderoga fields had significantly higher incidences ($P < 0.05$) (Fig. 2). No statistically significant relationships were found between incidence of WSSMV infection and planting date, duration of rotation out of wheat, or any other factor reported on the grower questionnaire (13). Although the overall incidence was lower in 1989 than in 1988, this difference could be explained by an increase in the proportion of fields planted to Geneva. The average incidence of WSSMV infection in fields planted to other cultivars showed little difference between years (33% in 1988 and 34% in 1989).

Field plot experiments conducted concurrently in New York demonstrated that WSSMV had significant effects on yields of susceptible cultivars during these same years (13). The apparent resistance of Geneva was also confirmed by field experiments conducted at the Aurora Research Farm in 1988 and 1989 (13–15). These findings, along with the lack of associations with planting date, duration of rotation out of wheat or other cultural practices reported on the questionnaire (13), support the conclusions of other researchers that varietal resistance is the most effective control measure for this disease (8,26).

Barley yellow dwarf virus. Symptoms of barley yellow dwarf (BYD) were absent at the earlier sampling date in each year, however, BYDV was detected by ELISA at these dates in 33% of the fields in 1988 and 11% of the fields in 1989 (Fig. 3). After flag leaf emergence, symptoms were visible as a yellowing of the upper one to two leaves in scattered clusters of five to 100 plants. Even at this later growth stage, however, symptoms were not a reliable indicator of infection, because BYDV was detected by ELISA in many asymptomatic plants.

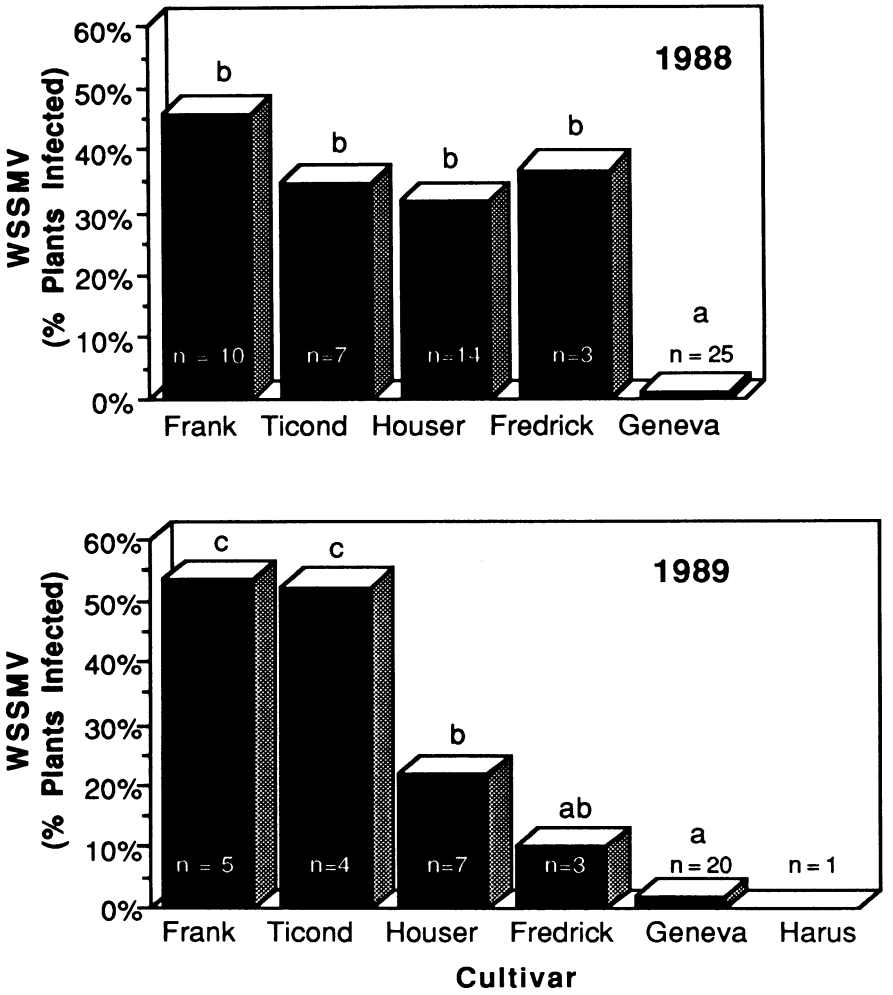

Fig. 2. Incidence of infection by wheat spindle streak mosaic virus among winter wheat cultivars surveyed in 1988 and 1989. Bars with different letters are significantly different ($P = 0.05$). n = Number of fields of each cultivar sampled. Frank = Frankenmuth, Ticond = Ticonderoga.

The percentage of plants infected with BYDV ranged from 0 to 18% with a statewide mean of 1.4% at GS 47–59 in 1988 and a statewide mean less than 1% (range = 0–13%) at both sampling dates in 1989. The reasons for the low incidence of BYDV infection in winter wheat during these years are not clear. In the fall of 1987, hard frosts (below −3 C) did not occur in the region until 6–12 November, and in 1988 a single hard frost occurred on 31 October followed by mild temperatures until 23 November. Thus, weather conditions following winter wheat emergence in both years appeared to be conducive to local aphid movement.

The identity of individual isolates of BYDV is summarized in Table 1. The majority of isolates appeared to be serologically similar to PAV (18) although, in 1989, the RMV isolate accounted for 33 and 56% of the BYDV-positive samples tested at GS 25–30 and GS 45–53, respectively. In addition to the systematically sampled tillers, additional symptomatic plants were collected and assayed separately. Every one of these isolates (not included in Table 1) resembled PAV. These observations concur with the findings of others (20–22) that PAV-like isolates cause more severe symptoms than other BYDV isolates.

In a study using symptomatic winter wheat plants collected near Ithaca from 1967 to 1976, Rochow (17) reported that 63% of BYDV isolates resembled PAV. Although the percentages of isolates collected in this earlier study closely resemble those of the current study, this resemblance is deceptive because the earlier study, using only symptomatic plants, may have selected in favor of PAV-like isolates. Nevertheless, the 1988 and 1989 samples also reflect a predominance of PAV-like isolates, even when the sampling preference for PAV-infected (symptomatic) plants was removed. These data provide continuing support to earlier observations of a long-term (1957–1976) increase in the relative occurrence of PAV-like isolates and a decrease in MAV-like isolates in New York (17).

The geographic distribution of BYDV across the area surveyed was relatively uniform in both years (Fig. 3). No significant differences were found between wheat cultivars, although a significant association ($P < 0.05$) was found between planting date and incidence of BYDV infection in 1988 (Fig. 4). In 1989, only one field tested had greater than a 6% BYDV incidence. Furthermore, the range of planting dates was much narrower in 1989 (37 days) than in 1988 (54 days). Thus, confirmation of the association with planting date was difficult in 1989.

Current recommendations for wheat production in New York include delaying planting until after the Hessian fly-free date to avoid BYDV infection (2). The results from 1988 implied that delayed planting reduced the incidence of BYDV infection. Very late planting can, however, reduce winter hardiness and conflict with other farm activities such as corn harvest. No simple procedure exists to relate the low BYDV incidence observed in 1988 and 1989 to the epiphytotic potential of this virus in past or future years. Thus, continued BYDV monitoring will be necessary in order to ascertain the emphasis that should be placed on this control recommendation.

Other wheat viruses. Wheat streak mosaic virus was detected by ELISA in one field in 1988 and four fields in 1989. Streaking on young foliage resembled WSSM symptoms. However, by the booting to spike emergence stages, symptoms of the two diseases were easily distinguished because WSMV-infected plants continued to produce streaking on young foliage even after the temperatures rose above the optimal range for WSSM symptom development. In 1988 and 1989, the virus occurred only at trace levels (less than 0.5% plants infected) and, thus, could not have had a significant effect on yield. Although this is the first published report of WSMV in New York, the virus was isolated from wheat near Aurora, NY, in 1968 (W. F. Rochow, *unpublished*). Given this earlier observation, it appears likely that WSMV has been present at low levels in New York winter wheat for many years.

SBWMV was not detected in any of the randomly selected fields in 1988 or 1989. The virus was detected, however, in plants with severe rosetting symptoms

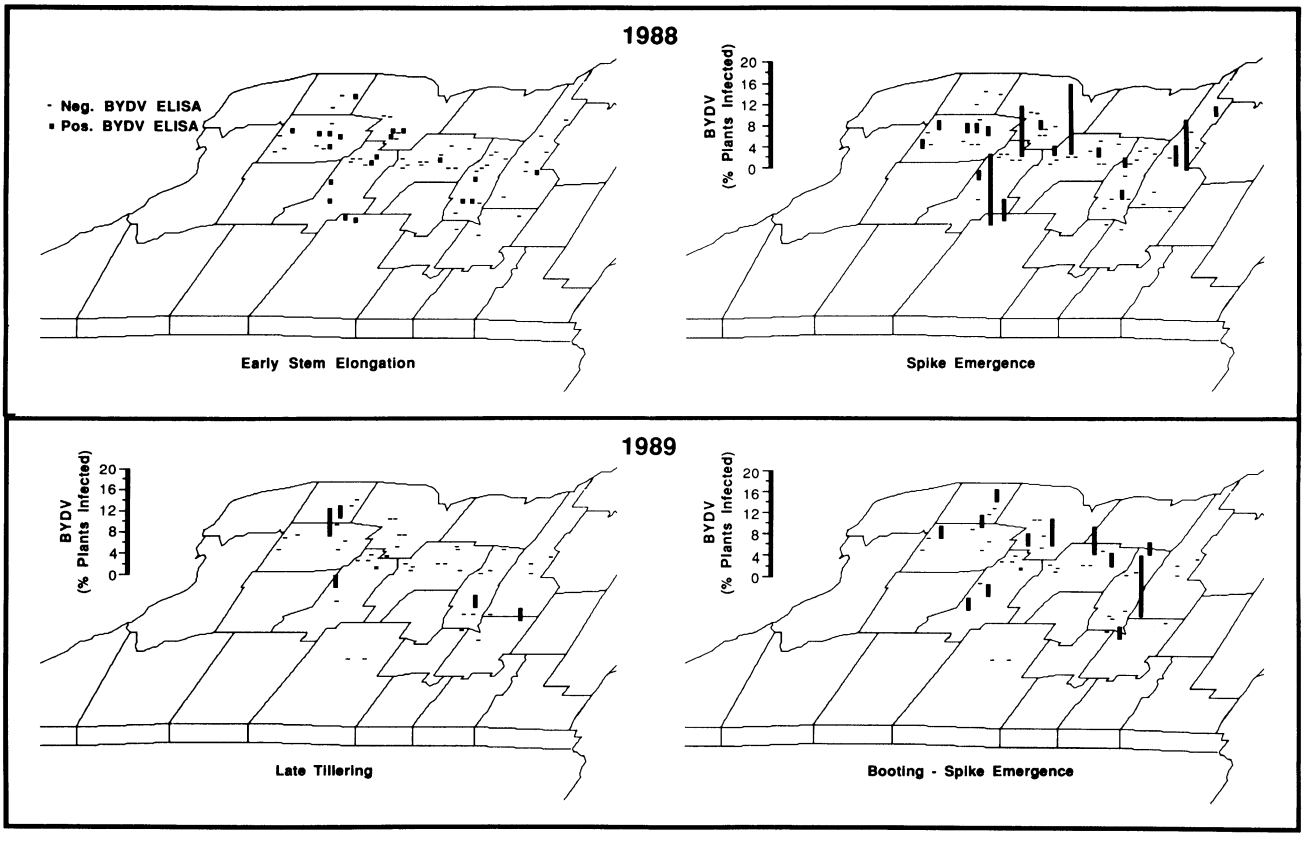

Fig. 3. Distribution and incidence of infection by barley yellow dwarf virus in winter wheat fields sampled at two growth stages in 1988 and 1989.

Table 1. Identity and relative occurrence of individual isolates[a] of barley yellow dwarf virus detected in New York winter wheat in 1988 and 1989

Year	Growth stage[b]	No. samples with positive ELISA				
		PAV	MAV	RPV	RMV	Total
1988	30–31	18	1	2	1	22
1988	47–59	14	0	2	3	19
1989	25–30	3	0	1	2	6
1989	45–53	7	0	0	9	16
Total 1988 & 1989		42 (67%)	1 (2%)	5 (8%)	15 (24%)	63 (100%)
1967–1976[c]		72 (63%)	5 (4%)	13 (11%)	24 (21%)	114 (100%)

[a]Isolates were distinguished serologically according to Rochow (19).
[b]Growth stage according to Zadoks et al (29).
[c]From Rochow (18).

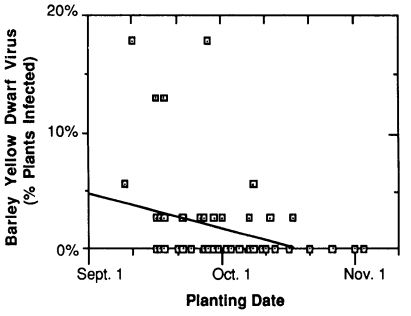

Fig. 4. Relationship of winter wheat planting date and incidence of infection by barley yellow dwarf virus in winter wheat fields surveyed at spike emergence in New York in 1988.

sampled in a plant breeding nursery near Ithaca in 1987 and assayed together with survey samples in 1988. Furthermore, SBWMV-infected plants from Kansas, Kentucky, and Indiana were included in these tests and confirmed that the ELISA was effective in detecting SBWMV. SBWMV has been reported throughout the western, southern, and eastern U.S. (12) and recently at several sites in Ontario (L. Seaman and Y. C. Paliwal, *unpublished*). Optimal conditions for transmission and replication of this virus are similar to those for WSSMV, and *P. graminis* is able to transmit either virus. Thus, SBWMV should be able to develop and persist under the environmental conditions of New York. The most likely explanation for its absence is a lack of SBWMV inoculum. Although WSMV and SBWMV were not found at damaging levels during the years studied, their possible spread is of concern and justifies continued field monitoring in order to assess the potential impact of these viruses on wheat in New York.

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