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Incidence of *Alfalfa mosaic virus*, *Bean pod mottle virus*, and *Soybean mosaic virus* in Nebraska Soybean Fields

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Abstract

The incidence of soybean viruses is increasing across the North Central Region of the United States as indicated by survey efforts and grower reports from several states. To determine the level of virus infection in Nebraska, we surveyed soybean fields for two consecutive years. *Alfalfa mosaic virus* (AMV) was detected in 52% of the fields in 2001 and in 56% of fields in 2002. The incidence of *Bean pod mottle virus* (BPMV) varied more, with 54% of fields testing positive in 2001 and 91% testing positive in 2002. *Soybean mosaic virus* (SMV) was not detected in 2001, but it was detected in 31% of fields in 2002. The widespread distribution of detected SMV in 2002 is suggestive of introduction with seed. The incidence of BPMV was significantly higher in fields planted earlier than the recommended optimum planting date in one of the two years studied. The widespread incidence of AMV and BPMV and the irregular occurrence of SMV indicate that further studies of soybean viral diseases in Nebraska are warranted.

Introduction

Viral diseases of soybean (*Glycine max* L.) can significantly reduce yield, alter seed composition, and cause seed coat mottling (6,8,14). Changes in insect vector populations over the last several years (6,16) have increased the incidence of common soybean viruses throughout the North Central Region of the United States. In this report, we present results of a survey for three soybean viruses in Nebraska: *Bean pod mottle virus* (BPMV) (Genus *Comovirus*), *Soybean mosaic virus* (SMV) (Genus *Potyvirus*), and *Alfalfa mosaic virus* (AMV) (Genus *Alfamovirus*).

Bean pod mottle virus was first identified in Nebraska in 1981 (12). Since that time, BPMV infection has spread tremendously due to increased populations of its vector, the bean leaf beetle (*Cerotoma trifurcata*) (BLB). *Soybean mosaic virus*, which is seed transmitted, occurs in Nebraska. The recent establishment of the soybean aphid (*Aphis glycines*), a vector of SMV, throughout the region increases the potential for this virus to become a production problem (16). The soybean aphid is also a vector for AMV (3). Because alfalfa, a common AMV source plant, is produced in every county in Nebraska (7), soybean aphids could spread the virus throughout the state's soybean crops. Unlike SMV, AMV and BPMV have not been shown to be highly seed transmitted.

Planting date has been found to affect viral incidence (6). Early plantings have higher populations of bean leaf beetles (17), leading to higher BPMV incidence. However, one of the management strategies recommended for SMV is to avoid planting soybeans late (8). Soybean plants co-infected with BPMV and SMV have a greater yield reduction due to the synergism between these viruses (1,2,14). Thus, soybean producers select their planting date based on the level of risk for each virus. Because planting date can be easily manipulated, further understanding its affect on viral incidence would be valuable. This relationship could be ascertained using randomly surveyed fields.

To determine the occurrence of viruses in Nebraska soybean fields, a survey was conducted of commercial fields in 2001 and 2002. The objectives of this research were to determine: (i) the incidence of AMV, BPMV, and SMV within individual fields and within Nebraska; and (ii) if planting date affected viral incidence.

Survey Methodology and Virus Incidence Estimates

During the 2001 and 2002 production season, soybean fields in Nebraska were sampled in cooperation with personnel from the National Agricultural Statistics Service (NASS). Soybean fields were selected using an area-frame sampling method (4). In each of the selected fields, NASS established two assessment plots (1 m²) at randomly selected sites in the field to perform yield estimates. Foliage samples to determine viral incidence in the selected fields were collected outside these randomly identified plots. For each survey year, 120 fields were identified randomly for sampling, but only 94 fields in 2001 and 87 fields in 2002 were sampled based on producer consent.

In each selected field, a surveyor collected foliage samples five paces following the rows toward the center of the field and outside the area randomly identified by NASS. The surveyor removed the upper-most expanded trifoliolate, with the leaflet margins not touching, of the soybean plant closest to their stepping foot and placed it in an empty compartment of a plastic bag. The bag contained ten separate compartments, deep enough to avoid sample contamination between leaflets, made by a heat sealer. This process was repeated until a total of ten trifoliolates were collected from the two identified areas in the field, for a total of twenty trifoliolates from each identified field. Fields were not rated for symptom development and plants were not selected based on symptoms. One sample collection occurred for all fields over the last weeks of both July and August each year.

Sap from each trifoliolate was tested individually for the presence of AMV, BPMV, and SMV. The presence of BPMV and SMV was determined by double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) (5) using methodology following published protocol (11,15) with antibodies for BPMV and SMV provided by Dr. John Hill, Iowa State University. The presence of AMV was determined by indirect ELISA with an antibody provided by Dr. Craig Grau, University of Wisconsin-Madison. Anti-AMV antibody (1:2,000) was coated onto Immulon IB polystyrene microtiter plates (Dynex Technologies Inc., Chantilly, VA) and incubated with plant extract. Next, anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (1:10,000; Sigma-Aldrich, St. Louis, MO) was added, followed by *p*-nitrophenyl phosphate (1 mg/ml). All samples were plated in duplicate wells and inconsistencies (duplicate wells have differing results) with the same sample in duplicate were rerun to ensure accuracy. Absorbance values were read with an Optimax Micro Plate Reader, (Molecular Devices, Sunnyvale, CA) and considered positive when the absorbance values (405 nm) exceeded twice the mean plus 10% of the negative control (buffer and sap from noninoculated healthy soybean leaves). Each ELISA plate was included in the results only if the positive controls on the plate (4 wells) were greater than the negative control standard, as described above.

To estimate incidence within a field, the total number of positive samples within the twenty trifoliolate samples collected at the two locations in the field was converted to a percent incidence. Estimating the incidence among fields was based on the number of positive fields within the total number of fields sampled that year. For each virus, fields testing positive were plotted by county to determine distribution.

Effect of planting date. To determine if planting date affected viral incidence, the number of days the planting date of each field deviated from the optimal date was compared to the viral incidence found using correlation analysis. Optimum planting dates were based on a 10-year average of when soil temperature reached 16°C (13). In the southern half of Nebraska, May 14 was the optimum; in the north, May 17 was the optimum (13). Identified fields were placed into two groups (early and conventional) based on these optimum

planting dates. In fields where virus was detected, the data was analyzed using a mixed model with SAS v.8.2 (SAS Institute Inc, Cary, NC).

Virus Incidence

Of the three viruses, AMV had the most consistent incidence and distribution over the two years studied (Fig. 1). In both years, AMV was detected in approximately 50% of the fields (Table 1), and the majority of the fields had less than 30% field incidence. In 2001, AMV incidence in fields ranged from 0 to 60%, with only two fields having > 50% incidence. In 2002, only two fields had > 50% incidence; however, these had incidences of 80% and 95%. These fields were in the northeastern portion of the state where soybean aphids became established in 2002. The soybean aphid is a vector of AMV (3). This could explain the isolated cases of higher field incidence the second year.

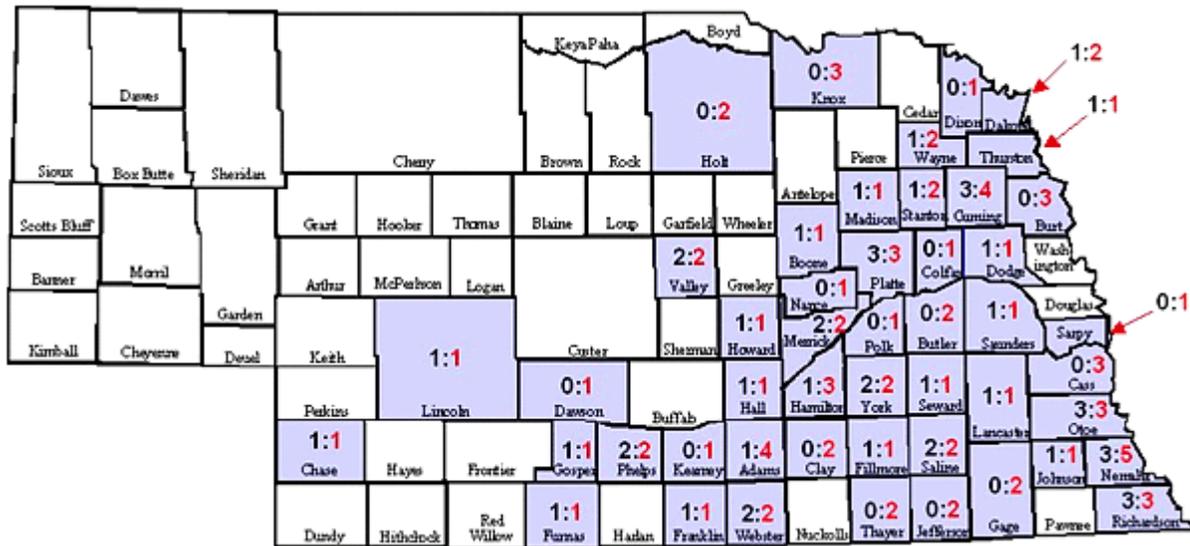


Fig. 1. Distribution of soybean fields with *Alfalfa mosaic virus*-infected plants in 2002. Blue shaded counties denote sampled counties with numbers in each county representing [No. positive fields : No. fields tested].

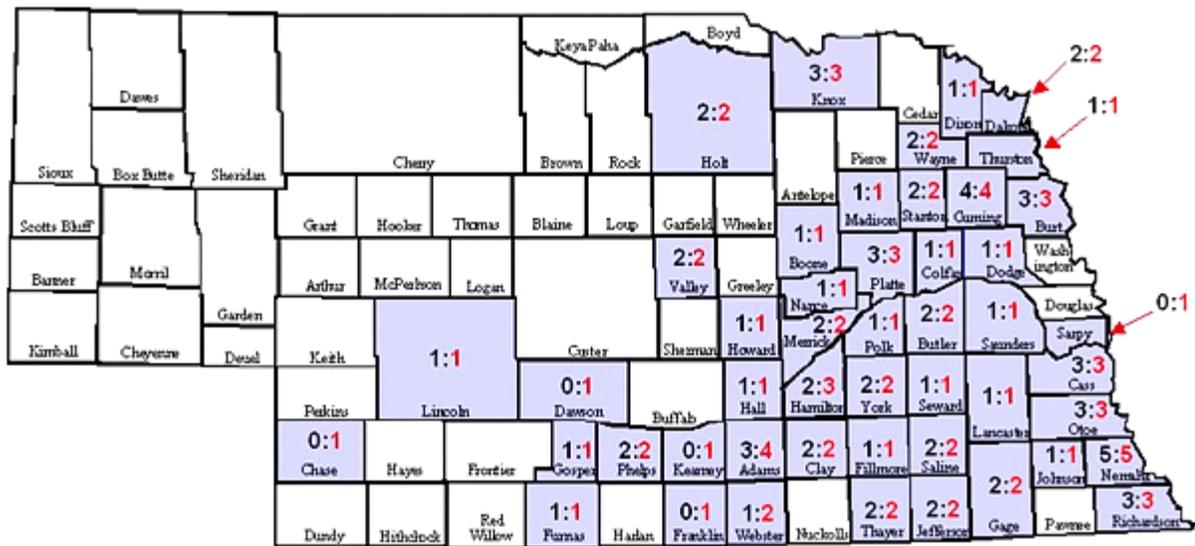


Fig. 3. Distribution of soybean fields with *Bean pod mottle virus*-infected plants in 2002. Blue shaded counties denote sampled counties with numbers in each county representing [No. positive fields : No. fields tested].

Soybean mosaic virus was not detected in 2001, but was detected in 31% of the fields in 2002 (Table 1). The change in SMV incidence coincides with the occurrence of soybean aphids in the region. Soybean aphids were found localized in the northeastern portion of Nebraska in 2002 (T. Hunt, *personal communication*), yet SMV was widely distributed in the Nebraska soybean crop (Fig. 4).

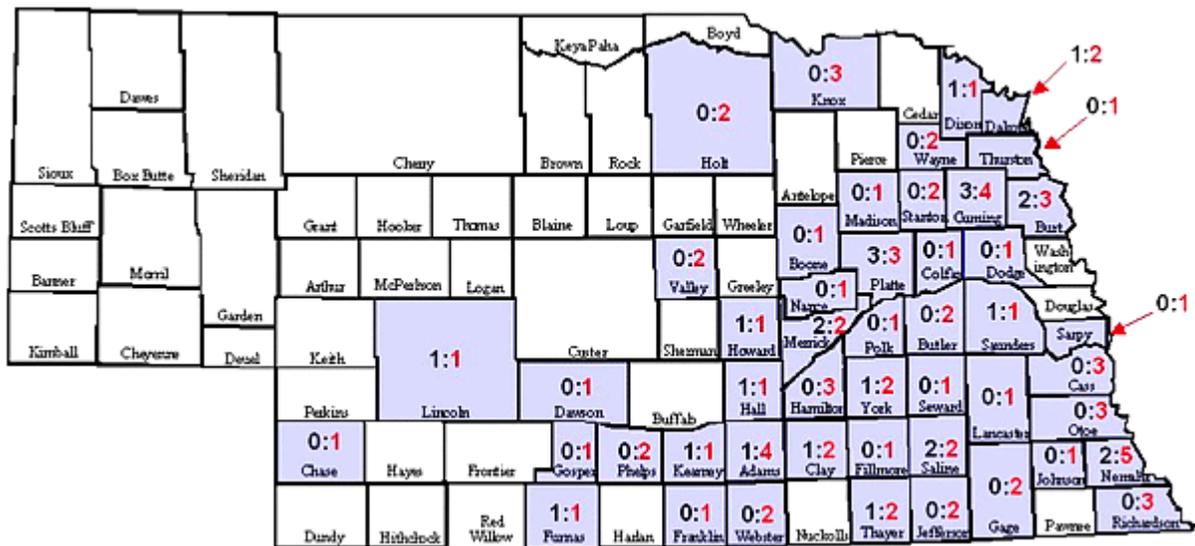


Fig. 4. Distribution of soybean fields with *Soybean mosaic virus*-infected plants in 2002. Blue shaded counties denote sampled counties with numbers in each county representing [No. positive fields : No. fields tested].

Effect of Planting Date on Virus Incidence

When planting date was analyzed, only incidence of BPMV was influenced. Higher BPMV incidence was observed in fields planted before the optimum planting date in 2001 ($P < 0.01$). That year, the average BPMV incidence in fields planted prior to the optimum planting date was 39% compared with 17% in fields planted after the optimum planting date. In 2002, there was no significant effect of planting date on BPMV incidence, with average BPMV field incidence of 43% in fields planted before the optimum planting date and 33% in fields planted afterward. The effect of planting date on the BLB is well documented (17), and we would predict more BPMV in early planted fields, which have more beetles.

Summary

This is the first published soybean virus survey in the state of Nebraska. As insect vector populations change, so too will the incidence of soybean viruses. Changes in BLB populations have been documented in the region over the last several years (10). In 2004, BLB populations dropped in Nebraska (9). If BLB populations remain low, significant amounts of BPMV are not expected; however, when BLB populations grow in the future, BPMV incidence will increase. Therefore, research and continued education is needed to determine the impact of BPMV on soybean production and the best management practices to manage the disease.

Planting date influenced BPMV incidence in 2001, a relationship that has been observed previously (6). The lack of a statistically significant effect of planting date on BPMV incidence in 2002 and a high incidence rate (91%) may indicate a very high BLB population. The extreme pressure and abundance of bean leaf beetles may have negated any effect of planting date. When BLB populations are lower, the influence of planting date may be more pronounced.

The occurrence of AMV raises concern for its potential impact on soybean production. At this time, the effect of AMV on yield is unknown. The soybean aphid can transmit AMV (3), but its transmission efficiency and the virus source are unknown. Further research and surveys are needed to determine how AMV is being transmitted to soybeans.

SMV incidence varied during the two years of this study. *Soybean mosaic virus* is efficiently seed-transmitted (8), and may have been introduced via seed transmission. Given the efficient transmission of SMV by the soybean aphid, demonstrated by Wang and Ghabrial (16), it is conceivable that aphids contaminated the seed production fields in 2001. The seed transmission hypothesis is supported by the low individual field incidence, less than 30% in 24 of the 27 SMV positive fields. Commercial seed producing companies are putting great effort into aphid management. However, because SMV is nonpersistently transmitted, brief probing by the aphid is sufficient for successful transmission (16), making SMV difficult to manage in seed production fields.

Nineteen of the 27 fields with SMV were also positive for BPMV in 2002. All fields were below 40% incidence, and most were less than 10% (Table 1). The synergistic interaction of the viruses infecting the plant could impact yield as well as seed transmission (1,2). The best approach to reduce the impact of SMV appears to be genetic resistance, for which there are known resistance genes (8), and managing seed transmission in seed production fields.

Acknowledgments

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