Seasonal Progression, Symptom Development, and Yield Effects of *Alfalfa mosaic virus* Epidemics on Soybean in Wisconsin

Emily E. Mueller and Craig R. Grau, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

ABSTRACT

Mueller, E. E., and Grau, C. R. 2007. Seasonal progression, symptom development, and yield effects of *Alfalfa mosaic virus* epidemics on soybean in Wisconsin. *Plant Dis.* 91:266-272.

The occurrence of *Alfalfa mosaic virus* (AMV) has increased in Wisconsin soybean fields in conjunction with the establishment of the soybean aphid (*Aphis glycines*). Field studies were conducted to determine the seasonal incidence of AMV-infected plants, progression of symptom severity caused by AMV, and the effect of AMV on soybean yield and seed quality. An isolate of AMV, collected from field-grown soybean, was introduced into plots by mechanical inoculation of plants at the V1 growth stage. The goal of the experiment was to achieve an incidence of AMV-infected plants of 0, 50, and 100% in 2002, and 0, 10, 25, 50, 75, and 100% in 2003. Severity of foliar symptoms was rated, and leaf samples were collected for serological assay (enzyme-linked immunosorbent assay [ELISA]) to estimate the incidence of AMV-infected plants from growth stages VC to R5. The maximum incidence of AMV-infected plants was 49% at growth stage R5, yet detection of the virus by ELISA dropped dramatically thereafter in both years. Incidence of AMV-infected plants accounted for 53 to 67% of the variability observed for severity of foliar symptoms in 2002 and 2003, respectively. Maximum yield loss ranged from 32% in 2002 to 48% in 2003 based on the difference in yield between noninoculated plots and plots with the highest incidence of AMV-infected plants. Incidence of AMV-infected plants explained 31% of the variation in yield in 2002 and 26% in 2003. An AMV incidence of 30% or greater was required for yield loss caused by AMV. Results of this study suggest that AMV has the potential to lower soybean yield and warrants further study.

Alfalfa mosaic virus (AMV), the single member of *Alfamovirus* in the family *Bromoviridae*, is distributed worldwide and naturally infects a wide range of plant species (11,14). Although many legume species are reported hosts of AMV, soybean (*Glycine max* (L.) Merr.) has been seldom recognized as a common host of the virus (12). Reports are limited on the detection of AMV in field-grown soybean plants (1,3,18,30). Pierce (21) first reported soybean as a host of AMV in comparative host range studies with bean (*Phaseolus vulgaris* L.). Most subsequent studies involving AMV have used soybean as a diagnostic host (11) or as a model system (6). Symptoms caused by AMV range from mosaic to mottle patterns of contrasting mixes of vivid yellow and dark green leaf tissues (3,5,12). Newly emerged leaves infected by AMV often express bright yellow spots as well as dwarfing and vein browning (5,12). Although symptoms are apparent, soybean plants in the field are frequently asymptomatic based on serological assays (18). Soybean plants

infected by AMV produce only minor amounts of seed with mottled seed coats (12).

Although several species of aphids are reported to transmit AMV (2,12), the rapid dispersal of the soybean aphid (*Aphis glycines* Matsumura) may be a partial explanation for the recurrent detection of AMV in the north-central United States (3,13,18,23,30). Although the green peach aphid (*Myzus persicae* Sulzer) is common to soybean fields (15), Clark and Perry (3) reported that it did not transmit AMV from soybean to soybean, but found the soybean aphid was capable of transmitting AMV from soybean to soybean.

Yield loss estimates specific to AMV are not available, but overall loss attributed to viruses is estimated to have increased almost fivefold in Wisconsin from 1999 to 2002 (28). *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV) are prevalent with symptomatic soybean in Wisconsin (18) and are proven to be yield-limiting pathogens (7,25). In contrast, AMV-infected plants are often asymptomatic, and thus the virus has the perception of minor agronomic importance (1,5,12).

A study was conducted to determine the impact of AMV on soybean health and productivity. The study was warranted because of the frequent occurrence of AMV in soybean fields (18,30), and the common occurrence of a proven vector, the soybean aphid (3,13). Furthermore, AMV-

infected forage legumes such as alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and yellow sweet-clover (*Melilotus officinalis*), putative sources of inoculum (10,16,22,24), are common in the agricultural landscape of Wisconsin. The objectives of this study were to (i) determine the seasonal progress of symptom severity and incidence of AMV-infected plants, (ii) determine the effect of AMV on soybean yield and seed quality in a field environment, and (iii) establish the level of AMV incidence at which soybean yield is reduced by this aphid-transmitted pathogen.

MATERIALS AND METHODS

Field experiments were performed in 2002 and 2003 to monitor the incidence of AMV-infected soybean plants and symptom severity, and evaluate the impact of AMV on soybean yield and seed quality in induced epidemics of AMV. Experiments were conducted at the West Madison Agricultural Research Station in an experimental area with a soil classified as Plano silt loam. Soybean was the previous crop grown at each experimental location.

Plot management. Soybeans were machine-planted at a rate of 30 seeds per meter on 3 May 2002 and 17 May 2003. Plots of each treatment replication were 2.1 m long and were comprised of four rows spaced 0.76 m apart. The two outside rows were planted to cultivar NE 2701, and the two inner experimental rows were planted to the cultivar Colfax. NE 2701 was planted as a border because seed of Colfax was of limited supply in 2002, and repeated in 2003 for continuity between years of the study. NE 2701 (9) and Colfax (8) were selected for this study because of their resistance to SMV, which was needed to minimize the confounding effects of SMV on yield and seed quality (19).

Metribuzin (Sencor, 0.42 kg a.i./ha) and pendimethalin (Prowl, 1.39 kg a.i./ha) were incorporated with a soil groomer for weed control on 3 May 2002. Only pendimethalin (1.39 kg a.i./ha) was applied for weed control on 17 May 2003. Infestations of the bean leaf beetle (*Cerotoma trifurcata* (L.) Forster) in 2002 and high soybean aphid populations in 2003 were controlled with applications of lambda-cyhalothrin (Warrior IEC, 0.02 kg a.i./ha) on 31 May 2002 and 2 July 2003. The insecticide was applied to reduce possible yield loss caused by bean leaf beetles and

Corresponding author: C. R. Grau
E-mail: cg6@plantpath.wisc.edu

Accepted for publication 20 September 2006.

DOI: 10.1094/PDIS-91-3-0266
© 2007 The American Phytopathological Society

BPMV, and the direct effects of feeding by the soybean aphid, which could confound estimates of yield loss and seed quality caused by AMV.

Epidemic induction. The concept of this experiment was to compare yield of soybean grown in plots with differing incidence of AMV-infected plants. In 2002, the controlled introduction of AMV inoculum into designated plots was done to achieve an incidence of 0, 50, and 100% AMV-infected plants. In 2003, inoculum was introduced to achieve incidences of 0, 10, 25, 75, and 100% AMV-infected soybean plants. Treatment plots were arranged in a randomized paired block design with four replications in 2002 and eight replications in 2003.

Isolate AMV-C was recovered from soybean grown in a commercial field in 2001 in Walworth County, WI. The isolate was not purified by local lesion transfer and was regarded as a composite source of inoculum. AMV-C was maintained in susceptible soybean cultivars IA2021 and Colfax in a greenhouse environment. The presence of AMV-C was confirmed by an enzyme-linked immunosorbent assay (ELISA) (Agdia Inc., Elkhart, IN) and the formation of local lesions on inoculated unifoliate leaves of *Phaseolus vulgaris* cv. Bountiful (4). Source plants expressed symptoms that were reported for AMV and were determined to be free of BPMV and SMV by ELISA (Agdia).

Inoculum was prepared from AMV-infected plants of Colfax grown in a greenhouse and infectivity confirmed by bioassay on unifoliate leaves of *P. vulgaris* cv. Bountiful (4) and by ELISA. The last fully expanded trifoliate leaf of each plant was ground in 1 ml of phosphate-buffered saline (PBS, pH 7.2). To introduce AMV-C into the field plots, plants were dusted with Carborundum and inoculated with AMV-C at the V1 growth stage (20) on 6 June 2002 and on 13 June 2003, followed by a second inoculation on 24 June 2003 at the V4 growth stage. The leaf sap-buffer homogenate was applied to both unifoliate leaves with cheesecloth or cotton swabs. Due to the instability and rapid degradation of AMV virus in buffer solution (4), inoculum was frequently replenished during the course of the inoculation.

Assay and sampling procedures. An indirect ELISA was used with a polyclonal AMV antibody to assay field samples and greenhouse-grown plants to detect the presence of AMV-C. Leaves were individually placed into a 7.6 × 7.6 cm grinding bag (Agdia), ground in 1 ml of PBS (pH = 7.2), with 100 µl of the ground homogenate loaded onto a 96-well ELISA plate (Agdia) precoated with carbonate buffer (pH 9.6). Blank wells consisted of PBS and carbonate buffers maintained at the same ratio of sap to carbonate buffer as leaf sample wells. Negative controls consisted of carbonate buffer, PBS, and sap

from virus-free soybean leaflets. Positive controls consisted of carbonate buffer, PBS, and sap from an AMV-C infected leaflet. Absorbance at $A_{405\text{ nm}}$ was assessed using an EL 800 Universal Microplate

Reader (Bio-Tek, Winooski, VT). Samples with absorbance values that exceeded the mean plus two standard deviations of the negative control were regarded as positive. Background, as measured in buffer and

Fig. 1. Incidence of plants infected with *Alfalfa mosaic virus* (AMV) was studied in an induced epidemic in plots of soybean cv. Colfax during the 2002 field season. Treatment variables included a noninoculated control, and plots in which 50 or 100% of plants were inoculated with isolate AMV-C. Plants were mechanically inoculated on 6 June at the V1 growth stage. Leaf samples were collected from 20 individual soybeans in each plot on 3 June, 3, 19, and 31 July, 14 August, and 6 September at the V1, R1, R2, R3, R4.5, and R6 reproductive stages, respectively. Leaf samples were assayed for AMV using enzyme-linked immunosorbent assay (ELISA) to determine the percentage of plants infected by AMV at each sample date. Error bars represent standard error of the mean.

Fig. 2. Incidence of plants infected with *Alfalfa mosaic virus* (AMV) was studied in an induced epidemic in plots of soybean cv. Colfax during the 2003 field season. Treatment variables included a noninoculated control, and plots in which 10, 25, 50, 75, or 100% of plants were inoculated with AMV. Plants were mechanically inoculated on 13 June at the vegetative V1 stage and 24 June at the reproductive V4 stage. Leaf samples were collected from 20 individual soybeans within each research plot on 13 and 24 June, 1, 9, and 18 July, and 8 and 22 August, at the V1, R1, R2, R3, R4, R5, and R6 growth stages, respectively. Leaf samples were assayed for AMV using enzyme-linked immunosorbent assay (ELISA) to determine the percentage of plants infected by AMV at each sample date. Error bars represent standard error of the mean.

negative control wells, was minimal for all ELISAs. All reactions were performed at room temperature.

Within a week after emergence, plots were sampled and plants assayed for seed-transmitted viruses. Twenty soybean plants

were randomly chosen per plot, and one leaflet of the youngest trifoliolate was removed. The leaflets were bulked for each treatment and tested by ELISA for the presence of AMV, SMV, and *Tobacco streak virus* (TSV) using 96-well ELISA

plate kits (Agdia). This sampling took place before treatments were established on 3 June 2002 and 13 June 2003 and was intended to identify seed transmission of the virus. From mid-June until mid-September of both years, plots were sampled by removing a single leaflet from the youngest fully expanded trifoliolate leaf from 20 randomly selected plants within each plot. Leaflets were chilled on ice in the field and were stored at 4°C to minimize inactivation of viral infectivity.

To account for other vector-transmitted viruses, ELISA was used to test the field samples for BPMV and TSV. The BPMV antibody was obtained from John Hill, Iowa State University; and the TSV antibody was purchased from Agdia. Control wells consisted of PBS, negative controls and PBS, and positive controls and PBS. Negative controls were supplied by Agdia and positive controls were from stock greenhouse-grown soybean plants only infected with the virus corresponding to the desired ELISA.

Estimates of symptom severity were made in conjunction with leaf samples for AMV incidence on similar dates and crop growth stages. In 2002, the foliar canopy of individual soybean plots was rated for symptom severity on 3 June, 3, 19, and 31 July, 14 August, and 6 September. These dates correspond to the V1, R1, R2, R3, R4.5, and R6 reproductive stages of soybean (20). Symptom severity was rated in 2003 on 13 and 24 June, 1, 9, and 18 July, and 8 and 22 August at the V1, R1, R2, R3, R4, R5, and R6 growth stages, respectively. Symptoms attributed to AMV were rated in each plot by estimating the percentage of the upper canopy that was symptomatic. Symptoms attributed to virus infection were recorded, but symptomatic and asymptomatic plants were not tested for virus infection status.

Aphid populations. Population density of the soybean aphid was determined for each plot on 19 and 31 July 2002, during the R2 to R3 reproductive stages of soybean plants. Ten randomly selected plants were chosen within each plot, and both alatae and apterous forms of the soybean aphid were counted on the entire plant. Soybean aphid population density was not recorded in 2003 due to an insecticide application to achieve its control.

Soybean yield and seed quality. On 11 October 2002, two inner experimental rows 0.48 m long were harvested from each plot with an Almaco Bundle Thresher (Allen Machine Co., Nevada, IA). Two experimental rows from each plot were harvested 10 October 2003 with an Almaco SP20 Plot Combine. Grain yield was adjusted to 13% moisture content for both years. Seed was assessed to estimate the percentage that expressed mottled seed coats.

Statistical analysis. Using a paired block design in 2002, data for treatment

Fig. 3. Symptom severity attributed to *Alfalfa mosaic virus* (AMV) was assessed for soybean plants cv. Colfax on 3 June, 3, 19, and 31 July, 14 August, and 6 September at the V1, R1, R2, R3, R4.5, and R6 reproductive stages, respectively, throughout the 2002 field season. Symptoms were assessed on a per plot basis by rating the severity of foliar symptoms expressed in the upper entire plot canopy. Treatment variables include a noninoculated control and plots in which 50 or 100% of plants were mechanically inoculated with AMV. Bars represent standard error of the mean.

Fig. 4. Symptom severity attributed to *Alfalfa mosaic virus* (AMV) was assessed for soybean plants cv. Colfax on 13 and 24 June, 1, 9, and 18 July, and 8 and 22 August, at the V1, R1, R2, R3, R4, R5, and R6 growth stages, respectively, throughout the 2003 field season. Symptoms were assessed on a per plot basis by rating the severity of foliar symptoms expressed in the upper entire plot canopy. Treatment variables include a noninoculated control and plots in which 10, 25, 50, 75, or 100% of plants were mechanically inoculated with AMV. Bars represent standard error of the mean.

variables were divided into a treatment portion and a control portion. Data were incorporated into a linear regression model, and treatment means and statistics were generated using PROC GLM (General Linear Model) SAS software for Windows v.8 (SAS Institute Inc., Cary, NC). Each treatment plot was contrasted to the corresponding control plot, using the CONTRAST function. For overall differences, all similar treatments were averaged and analyzed as a randomized complete block design. Without paired control plots, treatment means for 2003, set up as a randomized complete block design, were calculated and compared in PROC GLM, linear regression function in SAS. Utilizing the least-squares difference, treatment differences were considered significant at alpha equal to or less than 0.05. Unbalanced data were analyzed with least-squares linear regression models, under PROC REG SAS procedure. Foliar symptom severity and virus incidence were used as predictors of yield loss in a stepwise multiple linear regression performed in MINITAB for Windows v.12.1 (MINITAB Inc., State College, PA). Yield models were based on sample dates in both 2002 and 2003.

RESULTS

AMV was successfully introduced into field research plots in 2002 and 2003, although the actual incidences of AMV-infected plants, based on ELISA, were lower than expected. Foliar symptoms began to appear within 7 days after mechanical inoculation with AMV. Stunted growth and a yellow-dark green leaf mosaic were the two most common symptoms observed in the field. Symptoms were more evident on older, more mature leaves than on newly emerged trifoliate leaves.

Seasonal progression of AMV and symptom severity. A range in incidence of AMV-infected plants was achieved among the inoculation treatments. In 2002, the incidence of AMV-infected plants for the 50 and 100% treatment plots was not statistically different with the exception of the 14 August sampling date (Fig. 1). In 2002, the percentage of AMV ELISA-positive plants in plots targeted at 50 and 100% peaked at 36 and 49%, respectively (Fig. 1). Although statistical significance was not achieved between the 50 and 100% treatments, the incidence of AMV-infected plants was statistically significant between both inoculation treatments and the non-inoculated control plots (Fig. 1) except at the final sample date on 6 September. In 2003, AMV incidence peaked at 30% in the 50% treatment plots and at 42% in the 75% plots. The incidence of AMV in the 100% treatments increased sharply after 29 July (R5) and peaked at 49% by 22 August, early R6 growth stage (Fig. 2). The incidence of AMV-infected plants was sharply less in early September, R6 growth stage, in 2002 (Fig. 1) and in 2003 (data

not presented). The incidences of AMV-infected plants for the 10 and 25% treatments were less than 15% throughout the season and were not different statistically from the noninoculated control in 2003 (Fig. 2). By the end of each growing season, the incidence of AMV-ELISA positive plants was less than 10% in control plots (Figs. 1 and 2). Plants positive for AMV based on ELISA were not observed prior to the controlled introduction of AMV into the plots.

Significant foliar symptoms were observed in both years and at all sampling dates (R1 to R6) in 2002 or after R2 (2003) when 50% or more of the plants were inoculated (Figs. 3 and 4). Symptom severity in the 50% treatment plots fluctuated around 30%, and symptoms in the 100% treatment plots were greatest in August of both years. Severity of symptoms was greater in the 75 and 100% treatment plots, and they differed from each other and from the control treatment at all sam-

Fig. 5. Relationship between incidence of *Alfalfa mosaic virus* (AMV)-infected plants and foliar symptom severity was compared in manually induced epidemics with cv. Colfax at the West Madison Agricultural Field Research Station in A, 2002 and B, 2003.

Table 1. Effect of *Alfalfa mosaic virus* isolate C (AMV-C) on soybean yield in induced epidemics in 2002 and 2003

2002			2003		
AMV ^v (%)	AMV ^w incidence (%)	Yield (Mg/ha)	AMV ^x (%)	AMV ^y incidence (%)	Yield (Mg/ha)
0	3 a ^z	4.2 a	0	0 a	4.0 a
			10	8 b	3.5 abc
			25	12 b	3.9 ab
50	36 b	3.6 ab	50	30 c	3.8 ab
			75	42 d	3.3 bc
100	49 b	3.2 b	100	49 d	3.0 c

^v AMV-C was introduced mechanically on 3 June 2002 at the V1 growth stage. Percent values indicate proportion of plants inoculated.

^w Incidence of AMV-infected plants on 31 July 2002 at R3 growth stage.

^x AMV-C mechanically introduced on 13 June 2003 at V1 growth stage and 24 June 2003 at V4 growth stage. Percent values indicate proportion of plants inoculated.

^y Incidence of AMV-infected plants on 5 August 2003 at R5 growth stage.

^z Mean values followed by the same letter are not statistically significant at the $P = 0.05$ level of probability.

pling dates after 24 June 2003. However, in 2003, severity of foliar symptoms in the 50% treatment plots did not differ from that of control plots until 1 July to 22 August (Figs. 3 and 4). Also in 2003, the 10 and 25% treatment plots expressed low severity of foliar symptoms during the entire field season and lingered near 10%, reflecting levels of symptom severity observed in noninoculated control plots. Symptom severity increased early in the 2003 season for the 75% treatment, reached a plateau briefly at 30%, and then increased sharply by 22 August to 56% symptomatic canopy. In the 100% treat-

ment plots, the initial foliar symptoms escalated as the season progressed, resulting in over 60% symptomatic canopy by the end of the field season for both years (Figs. 3 and 4). Severity of symptoms attributed to AMV remained low in control plots throughout the season for both years, peaking on 6 September 2002 at 9% and on 22 August 2003 at 12% (Figs. 3 and 4). Foliar symptoms in plots with the 10 and 25% treatments were not significantly different than the noninoculated control plots. The majority of viruslike symptoms were attributed to AMV based on regression analysis. The incidence of AMV-

infected plants accounted for 67% ($P = 0.05$) of the variability in symptom severity in 2002 and 53% ($P = 0.05$) in 2003 (Fig. 5).

Yield and AMV relationships. Yields of plots with introduced AMV were significantly lower than those of control plots when the incidence of AMV-infected plants exceeded 30% (Table 1). An incidence >30% was observed in the 100% inoculation treatment ($P = 0.04$) in 2002 and in the 75 and 100% inoculation treatments ($P = 0.008$) in 2003. An AMV incidence of 30% or greater resulted in yields statistically lower than those of the noninoculated control plots. For both years, the proportion of mottled seed was <1%, and grain moisture, individual seed weight, number of pods per plant, and plant height were not affected by AMV (data not presented).

Severity of foliar symptoms and incidence of AMV-infected plants were both inversely related to soybean yield (Figs. 6 and 7). Symptom severity on 3 July 2002 accounted for 56% of the variation in yield, and incidence of AMV-infected plants on 18 July 2002 accounted for 31% of the variation in yield (Fig. 6). In 2003, symptom severity on 8 August accounted for 26%, and incidence of AMV-infected plants on 31 July accounted for 28% of the variation in soybean yield (Fig. 7).

Other viruses and insects. In 2002, the incidence of BPMV-infected plants ranged from 15 to 20% and was not significantly different among the AMV treatments. BPMV was not detected in 2003. SMV was not detected in either study year.

Soybean aphid colonization in 2002 consisted of a relatively uniform distribution of both winged (alatae) and apterous aphid populations across treatments. Whole plant populations of *A. glycines* were 45 apterous aphids, usually accompanied by one alatae, on 19 July, increasing eightfold by the end of July (data not presented). Population density of the soybean aphid was extremely low in 2003 due to insecticide application.

DISCUSSION

This study presents evidence that AMV is capable of reducing soybean yield in a field environment. In both years of the study, AMV caused yield loss in soybean when introduced at early vegetative growth stages and final incidence of infected plants exceeded 30%. In previous studies, the incidence of AMV-infected plants has exceeded 30% in natural epidemics in Wisconsin (18). In contrast to seed from BPMV- or SMV-infected plants (7,25), mottled seed coats were not observed on grain harvested from plots with a high incidence of AMV-infected plants.

Severity of foliar symptoms and incidence of AMV-infected plants were inversely related to soybean yield. Sampling dates that best predicted yield effects of

Fig. 6. Effect of incidence of plants infected with *Alfalfa mosaic virus* (AMV) and symptom severity on yield of soybean cv. Colfax. Isolate AMV-C was introduced into research plots located at West Madison Agricultural Field Research Station to establish a range of virus incidence in 2002. **A**, Data for incidence of AMV-infected plants is from the 18 July 2002 sampling date. **B**, Data for symptom severity is from the 3 July 2002 sampling date.

AMV were clustered between 3 July and 18 July 2002, and 31 July and 8 August 2003. Severity of foliar symptoms accounted for greater variation in yield than incidence of AMV-infected plants. From the proposed linear relationships, AMV could have a varying effect on soybean yield. The effect of AMV on soybean physiology was not investigated, but the extensive chlorosis of foliage suggests the virus has a significant effect on photosynthesis. AMV is reported to have a significant adverse effect on nodule function (29). Tu et al. (26) reported that SMV disrupted nodulation and leghemoglobin content of nodules for soybean.

While 13 other aphid species are capable of vectoring AMV, *A. glycines* is the only aphid species to successfully reproduce on soybean. The soybean aphid is attracted to soybean and capable of high levels of reproduction (19,23). The incidence of SMV-infected plants has been observed to increase as soybean aphid populations rise in numbers (19). In contrast to expectations, high soybean aphid populations in 2002 did not result in a significant movement of AMV into non-inoculated control plots. The incidence of AMV was <10% in control plots for both years of the study. This differential in incidence allowed an opportunity to contrast soybean yield at high and low incidence of AMV. It is not known whether the origin of AMV in control plots was isolate AMV-C that was moved from inoculated plots or AMV exogenous to the experiment.

The incidence of AMV-infected plants, based on ELISA, increased steadily from the time of inoculation through the later reproductive growth stages. The incidence of ELISA-positive plants decreased by growth stage R6 in both years. Fleysh et al. (6) reported AMV was detectable in leaves, stems, roots, seedpods, and seed coats up to 45 days after inoculation but only in seedpods and seed coats 65 days after inoculation. Virus degradation or lack of virus translocation to new leaves are possible explanations for the decline in AMV detection, as witnessed in AMV-infected tobacco (17). However, the movement of AMV from vegetative to reproductive plant parts may be a more feasible explanation for the decline in detection by ELISA at the later reproductive growth stages. Thus, the timing of leaf collection was crucial for accurate detection of AMV. The late-season decline of AMV-positive plants could lead to an underestimation of AMV incidence in research plots or surveys.

Although an AMV model epidemic was successfully established in the virus-treated plots, the symptom severity and percent ELISA-positive plants assigned to inoculation treatments was not achieved at any growth stage during the field season. Although inoculum was refreshed frequently during inoculations, the instability

of AMV in buffers may have contributed to this experimental outcome (4,14). It was assumed that all plants were nodulated by *Bradyrhizobium japonicum* because of prior soybean cultivation at the site. This point is made because nodulated soybean plants are reported to be more susceptible to AMV compared with non-nodulated plants (27). The lower-than-expected incidence of AMV-infected plants may be the result of AMV distribution in inoculated plants and plant parts sampled at a particular growth stage.

This study demonstrated that AMV reduced yield, but not seed quality, of the

soybean cultivar Colfax. Although the use of more soybean cultivars would have strengthened the study, Colfax was the only soybean cultivar available with resistance to SMV and of a maturity group adapted to Wisconsin. The need for an SMV-resistant cultivar was evident based on previous experiments. The experiment was attempted in 2001 with an SMV-susceptible cultivar, and the incidence of this virus reached 70% in control and AMV treatments. Future research should be directed to the phenotypic responses of other soybean accessions to multiple isolates of AMV. Sources of inoculum have

Fig. 7. Effect of incidence of plants infected with *Alfalfa mosaic virus* (AMV) and symptom severity on yield of soybean cv. Colfax. Isolate AMV-C was introduced into research plots located at West Madison Agricultural Field Research Station to establish a range of virus incidence in 2003. **A**, Data for incidence of AMV-infected plants is from the 31 July sampling date. **B**, Data for symptom severity is from the 8 August sampling date.

not been identified, but forage legumes are suspected sources of AMV inoculum because of their common occurrence in soybean growing regions of Wisconsin. A further unknown, however, is whether aphids such as *A. glycines* commonly probe forage legumes to acquire AMV and transmit to soybean (3). From an epidemiological standpoint, characterizing *A. glycines* and other aphid species with regard to their efficiency at transmitting different, local AMV isolates would also be of academic interest and practical importance.

LITERATURE CITED

1. Allington, W. B., Moorhead, E. L., and Staples, R. 1960. Alfalfa mosaic virus in soybean. *Phytopathology* 50:627.
2. Castillo, M. B., and Orlob, G. G. 1966. Transmission of two strains of cucumber and alfalfa mosaic viruses by single aphids of *Myzus persicae*. *Phytopathology* 56:1028-1030.
3. Clark, A. J., and Perry, K. L. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines*. *Plant Dis.* 86:1219-1222.
4. Crill, P., Hagedorn, D. J., and Hanson, E. W. 1970. Alfalfa mosaic, the disease and its virus incitant: A literature review. University of Wisconsin, Agric. Exp. Stn. Res. Bull. 280.
5. Dorrance, A. E., and Lipps, P. E. 2002. Ohio State University Extension, Onioline. "Soybean Virus Diseases." Profitable Soybean Disease Management in Ohio. Ext. Bull. 895. Ohio State University, Extension. Online publication.
6. Fleysh, N., Deka, D., Drath, M., Koprowski, H., and Yusibov, V. 2001. Pathogenesis of alfalfa mosaic virus in soybean (*Glycine max*) and expression of chimeric rabies peptide in virus-infected soybean plants. *Phytopathology* 91:941-947.
7. Giesler, L. J., Ghabrial, S. A., Hunt, T. E., and Hill, J. H. 2002. *Bean pod mottle virus*. A threat to U.S. soybean production. *Plant Dis.* 86:1280-1289.
8. Graef, G. L., Specht, J. E., Korte, L. L., and White, D. M. 1994. Registration of 'Colfax' soybean. *Crop Sci.* 34:818.
9. Graef, G. L., White, D. M., and Korte, L. L. 2005. Registration of 'NE2701' soybean. *Crop Sci.* 45:410-411.
10. Hagedorn, D. J., and Hanson, E. W. 1963. A strain of alfalfa mosaic virus severe on *Trifolium pretense* and *Melilotus alba*. *Phytopathology* 53:188-192.
11. Hampton, R., Beezner, L., Hagedorn, D., Bos, L., Inouye, T., Barnett, O., Musil, M., and Meiners, J. 1978. Host reactions of mechanically transmissible legume viruses of the northern temperate zone. *Phytopathology* 68:989-997.
12. Hartman, G. L., Sinclair, J. B., and Rupe, J. C. eds. 1999. *Compendium of Soybean Diseases*. 4th ed. American Phytopathological Society, St. Paul, MN.
13. Hill, J. H., Alleman, R., Hogg, D. B., and Grau, C. R. 2001. First report of transmission of *Soybean mosaic virus* and *Alfalfa mosaic virus* by *Aphis glycines* in the New World. *Plant Dis.* 85:561.
14. Hull, R. 1969. Alfalfa mosaic virus. *Adv. Virus Res.* 15:365-433.
15. Irwin, M. E., Ruesink, W. G., Isard, S. A., and Kampmeier, G. E. 2000. Mitigating epidemics caused by non-persistently transmitted aphid-borne viruses: The role of the plant environment. *Virus Res.* 71:185-211.
16. Jones, R. T., and Diachun, S. 1976. Identification and prevalence of viruses in red clover in central Kentucky. *Plant Dis. Rep.* 60:690-694.
17. Kuhn, C. W., and Bancroft, J. B. 1961. Concentration and specific infectivity changes of alfalfa mosaic virus during systemic infection. *Virology* 15:281-288.
18. Lee, M. E., Kurtzweil, N. C., and Grau, C. R. 2002. Prevalence and agronomic effects of viruses in Wisconsin. (Abstr.) *Phytopathology* 92:S139.
19. Lee-Burrows, M. E., Boerboom, C. M., Gaska, J. M., and Grau, C. R. 2005. The relationship between *Aphis glycines* and *Soybean mosaic virus* incidence in different pest management systems. *Plant Dis.* 89:926-934.
20. Pedersen, P. 2004. Soybean growth and development. PM 1945. Iowa State University, University Extension, Ames, IA.
21. Pierce, W. H. 1934. Viroses of the bean. *Phytopathology* 24:87-115.
22. Piñeyro, M. J., Albrecht, K. A., Mondjana, A. M., and Grau, C. R. 2002. First report of *Alfalfa mosaic virus* in kura clover (*Trifolium ambiguum*) in Wisconsin. *Plant Dis.* 86:695.
23. Ragsdale, D. W., Voegtlin, D. J., and O'Neil, R. J. 2004. Soybean aphid biology in North America. *Ann. Entomol. Soc. Am.* 97:204-208.
24. Sherwood, R. T. 1997. Viruses of white clover in pastures of Pennsylvania, New York, and Vermont. *Plant Dis.* 81:817-820.
25. Steinlage, T. A., Hill, J. H., and Nutter, F. W., Jr. 2002. Temporal and spatial spread of soybean mosaic virus (SMV) in soybeans transformed with the coat protein gene of SMV. *Phytopathology* 92:478-486.
26. Tu, J. C., Ford, R. E., and Grau, C. R. 1970. Some factors affecting the nodulation and nodule efficiency in soybeans infected with soybean mosaic virus. *Phytopathology* 60:1653-1656.
27. Tu, J. C., and Tse, S. Y. 1976. Virus infectivity of alfalfa mosaic virus in soybean as influenced by nodulation. *Phytopathol. Z.* 85:170-175.
28. Wrather, J. A., Koenning, S. R., and Anderson, T. R. 2003. Effect of Diseases on Soybean Yields in the United States and Ontario (1999-2002). Online. *Plant Health Progress* doi:10.1094/PHP-2003-0325-01-RV.
29. Wroth, J. M., Dilworth, M. J., and Jones, R. A. C. 1993. Impaired nodule function in *Medicago polymorpha* L. infected with Alfalfa mosaic virus. *New Phytol.* 124:243-250.
30. Ziems, A. D., and Giesler, L. J. 2004. Incidence of *Bean pod mottle virus* and *Alfalfa mosaic virus* in Nebraska soybean fields. (Abstr.) *Phytopathology* 94:S162.