Temporal and spatial spread of Soybean mosaic virus in soybeans transformed with the coat protein gene of SMV

Todd Alan Steinlage
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Recommended Citation
Steinlage, Todd Alan, "Temporal and spatial spread of Soybean mosaic virus in soybeans transformed with the coat protein gene of SMV" (2001). Retrospective Theses and Dissertations. 17414.
https://lib.dr.iastate.edu/rtd/17414

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Temporal and spatial spread of *Soybean mosaic virus* in soybeans transformed with the coat protein gene of SMV

by

Todd Alan Steinlage

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Pathology
Major Professors: Forrest W. Nutter, Jr. and John H. Hill

Iowa State University
Ames, Iowa
2001
Graduate College

Iowa State University

This is to certify that the Master's thesis of

Todd Alan Steinlage

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
# TABLE OF CONTENTS

## INTRODUCTION
- Thesis organization 4
- Literature cited 5

## CHAPTER I. LITERATURE REVIEW
- Soybean mosaic virus disease 9
- Virus detection 11
- Host resistance 13
- Pathogen-derived resistance 14
- Epidemiological modeling 16
- Literature cited 17

## CHAPTER II. TEMPORAL AND SPATIAL SPREAD OF SOYBEAN MOSAIC VIRUS IN SOYBEANS TRANSFORMED WITH THE COAT PROTEIN GENE OF SMV
- Abstract 23
- Materials and Methods 27
- Results 32
- Discussion 39
- Acknowledgments 45
- Literature cited 46

## CONCLUSIONS 63

## ACKNOWLEDGMENTS 65
INTRODUCTION

Soybean (*Glycine max*) is among the most important crops for production of edible oils and proteins in the temperate and sub-tropical regions of the world (American Soybean Association). *Soybean mosaic virus* (SMV) is one of the most common viruses that infects soybean, and is found worldwide (Cho *et al.*, 1977; Dhingra and Chenulu 1980). The virus may have first entered the United States in shipments of soybean seeds from China in 1915 (Clinton 1916).

Soybean plants infected with SMV may lack discernible symptoms in some soybean cultivars, while other cultivars display very severe symptoms. The infecting strain, soybean cultivar, time of infection, and environmental conditions can all affect the degree of symptom expression; however, mottling on leaves may be masked at temperatures above 27° C. Many cultivars grown in the Midwest do not express strong symptoms when infected with SMV (Conover 1948; Irwin and Shultz 1981; Walters 1963).

Plant infection by SMV can also reduce grain quality. Seed may become mottled and show alterations in oil content (Dunleavy *et al*., 1970; El-Amrety *et al*., 1985; Ross 1977). Reductions in the number of seeds produced per plant and seed weight may also occur, with early infection causing the greatest losses (Bryant *et al*., 1982; Halbert and Irwin 1981; Ross 1969).

Infected seed is the source of primary inoculum in the North Central states and it is thought to be the primary means of long-distance dissemination (Hill *et al*., 1980). Dissemination within and among fields is accomplished by over thirty
species of aphids (Edwardson and Christie 1991; Halbert et al., 1981; Lucas and Hill 1980) in a non-persistent manner (Irwin and Goodman 1981). This allows severe epidemics of SMV to occur. Aphids can acquire and transmit the virus quickly, making insecticide sprays ineffective for their control (Satapathy 1998). In some cases, insecticide sprays have been shown to agitate aphids and cause them to fly prematurely after only a few probes of a host plant, thus spreading the virus more quickly (Satapathy 1998; and Thresh 1983).

Determination of the effectiveness of management tactics and strategies depends on the ability to model pathogen progress and accurate assessments of pathogen incidence (Nutter 1997; Nutter et al., 1998). Methods of virus detection that are more accurate, reliable, and sensitive than symptom expression are needed to evaluate potential management tactics and strategies. Serological methods like the Enzyme Linked Immunosorbent Assay (ELISA) can provide accurate, reliable, and sensitive pathogen detection for viruses in plant tissue (Diaco et al., 1985; Hill et al., 1981; Hill and Durand 1986). Sensitive techniques, such as ELISA, may allow accurate and reliable quantification of pathogen progress, as well as the ability to quantify relationships between pathogen incidence and soybean yield and quality.

Several single dominant resistance genes have been identified in soybean (Chen et al., 1994; Cho and Goodman 1979; Jayaram et al., 1992); however, most resistance genes are not effective against all SMV strains (Lim 1985), and resistance-breaking strains have emerged due to strong directional selection
An alternative to the single dominant gene resistance strategy is the use of a rate-reducing strategy (Nutter and Kuhn 1993; Padgett et al., 1990). This approach allows some infection in the host population while substantially slowing the rate of plant-to-plant spread to minimize yield and quality reductions (Nutter and Kuhn 1993; Padgett et al., 1990; Parlevliet 1979).

A single (recessive) rate-reducing gene strategy effectively reduced the rate of *Tobacco etch virus* (TEV) epidemics in bell pepper, without generation of resistance-breaking virus strains (due to its effectiveness against all strains, thus reducing directional selection pressure) (Padgett et al., 1990).

In addition to the more traditional approach of breeding programs to introduce single resistance genes into plant cultivars, disease resistance may also be achieved by the insertion of genes, gene sequences, or sequences of nucleic acids from the genetic code of a plant pathogen (Sanford and Johnson 1985). For viruses, this form of resistance, termed "Pathogen-derived resistance," typically involves the insertion of a viral coat protein (CP) gene. The effectiveness of this method was first demonstrated by Powell-Abel et al. (1986) with transgenic tobacco expressing the coat protein of *Tobacco mosaic virus* (TMV). The development and use of pathogen-derived resistance has subsequently been successfully implemented by many researchers in recent years (Beachy 1997; Kallerhoff et al., 1990; Kawchuk et al., 1990; Nelson et al., 1987; Stark and Beachy 1989; Tennant et al., 1994; Van Dun and Bol 1988; Van Dun et al., 1987). A concern exists that pathogen-derived resistance may not be
(or remain) effective against all virus strains, as shown by the work of Herrero et al. (2000) and Tennant et al. (1994). This may influence implementation and adoption in a virus management strategy.

A chimeric coat protein gene of SMV strain N was recently inserted into soybean cultivar '9341', to yield six transformed lines (Wang et al., 2001). Greenhouse and growth chamber tests with mechanically transmitted SMV showed at least two of these lines had reduced SMV incidence and also significantly delayed symptom development. These soybean lines may provide adequate levels of rate-reducing resistance at the host population level but such lines first need to be evaluated under field conditions, where varying numbers and species of viruliferous (virus carrying) aphids may challenge the resistance throughout the season (Quemada et al., 1991). Field experiments need to be conducted to quantify the effect of transgenic soybean lines on SMV pathogen progress in time and space and to quantify the effects of this resistance strategy on the yield and quality of soybean grain.

**Thesis organization**

This thesis consists of a general introduction, a literature review, a manuscript, a general conclusion, and acknowledgements. The general introduction describes the importance of SMV, and the rationale for field-testing transgenic soybean lines. Chapter 1 is a review of pertinent literature concerning the biology and management of SMV. Chapter 2 is a manuscript that will be
submitted to *Phytopathology* for publication. The conclusion section summarizes the results and suggests possible future research.

**Literature cited**


infections by CMV strains transmitted mechanically or by aphids. Phytopathology 81: 794-802.


CHAPTER I. LITERATURE REVIEW

Soybean mosaic virus disease

Soybean (*Glycine max*) is among the most important crops grown in the temperate and sub-tropical regions of the world for production of edible oils and proteins (American Soybean Association). The acreage of soybean in Iowa is second only to that of corn (*Zea mays*). *Soybean mosaic virus* (SMV) is one of the most common virus diseases of soybean, with worldwide distribution (Cho *et al.*, 1977; Dhingra and Chenulu 1980). The virus is thought to have entered the United States in shipments of soybean seeds from China in 1915 (Clinton 1916).

Soybean plants infected with SMV may lack noticeable symptoms in some soybean genotypes while symptoms are quite severe in others (Cho and Goodman 1979; Chen *et al.*, 1991). The infecting strain, soybean cultivar, time of infection, and environmental conditions can all affect the degree of symptom expression (Cho and Goodman 1979; Chen *et al.*, 1991; Irwin and Goodman 1981). Infection may result in leaf mottling, rugosity (blistering), distortion, stunting, and premature chlorosis; however, mottling on leaves may be less apparent at temperatures greater than 27°C (Irwin and Goodman 1981; Irwin and Shultz 1981). Many cultivars grown in the Midwest do not express strong symptoms (Conover 1948; Irwin and Shultz 1981; Nutter *et al.*, 1998; Walters 1963).

Plant infection by SMV can also lead to reductions in grain quality. Seed may become mottled and infection may cause alterations in oil content (Dunleavy...
et al., 1970; El-Amrety et al., 1985; Ross 1977). Reductions in yield components such as the number of seeds per plant and seed weight may also occur, with early infection resulting in greater reductions (Bryant et al., 1982; Halbert and Irwin 1981; Ross 1969).

Long-distance dissemination of the virus occurs primarily through the dissemination of virus-infected seed (Hill et al., 1980), while virus dissemination among and within fields is facilitated by over thirty species of aphids (Edwardson and Christie 1991; Halbert et al., 1981; Lucas and Hill 1980). Many of these aphid species inhabit the southern United States and are believed to be brought into soybean production areas from south to north in low-level jet streams during the summer months (Berger et al., 1987; Hewings and Eastman 1995; Irwin and Thresh 1990). Aphids have been shown to transmit SMV in a non-persistent manner (Irwin and Goodman 1981; Lucas and Hill 1980), meaning they harbor the virus only in the mouthparts and do not circulate the virus through the insect hemolymph. The virus is initially acquired by aphids after probing an infected plant and the virus can be transmitted to other host plants after a brief probe (within seconds). The virus is also easily lost after a single probe which is in contrast to persistently transmitted viruses that must first circulate through the hemolymph to reach the salivary glands in order for the virus to become (and remain) transmissible. Persistently-transmitted viruses may not be available for transmission for several days to several weeks from the time of virus acquisition, due to the required latent period in the insect vector; however, once an insect is
able to transmit the virus, it remains viruliferous (capable of transmitting the virus) for long periods of time. Insecticide sprays to control insect vectors are often not effective for the management of non-persistent viruses due to the rapidity with which the vector can acquire and transmit the virus (Satapathy 1998). Moreover, insecticide sprays have been shown to alter aphid behavior by agitating aphids and inducing them to take flight prematurely after just a few initial probes, thus spreading non-persistent viruses more quickly within and among fields (Satapathy 1998; Thresh 1983).

**Virus detection**

Detection of viruses within plant tissues can be accomplished by numerous methods, including the use of live indicator plant species, electron microscopy, polymerase chain reaction (PCR), and serological methods. A particularly cost-effective and sensitive serological method is the Enzyme Linked Immunosorbent Assay (ELISA) (Hill *et al*., 1981). This assay uses specific antibodies to an antigenic molecule to capture the molecule. Subsequently, the captured molecule is recognized by a specific antibody labeled with an enzyme. This enzyme then reacts with a colorless substrate to produce a colored product, which can then be observed visually or quantified using an ELISA plate reader (a modified spectrophotometer, which measures the amount of light absorbed at a wavelength specific for a given enzyme/substrate complex).

ELISA can utilize both polyclonal and monoclonal antibodies for the detection of virus antigens. Polyclonal antibodies are mixtures of several types
of antibodies specific to many different epitopes (sites on the antigen). Antibodies are usually produced in warm-blooded animals, and these animals produce antibodies to many antigenic substances, including possible contaminants associated with the immunogen or feed provided to the animal. Due to the possibility of nonspecific binding, which could cause false positive results, polyclonal antisera should first be cross-absorbed with healthy plant tissue to remove unwanted antibodies that might otherwise react to healthy plant tissue (McLaughlin et al., 1980).

Alterations to the light-chain regions of antibodies can occur during enzyme conjugation. This can result in diminished immunoreactivity, which may alter or lower the sensitivity of an assay (Koenig 1978). An alternative approach to direct conjugation of an enzyme to the detection antibody is to couple the antibody to a low molecular-weight biotin molecule. Biotin has very high affinity for avidin, which can be conjugated to the enzyme of choice. Adding biotin instead of an enzyme to the antibody reduces the alteration of the immunoreactive sites of the antibody as well as increasing sensitivity of the assay (Diaco et al., 1985). Many types of avidin and enzyme conjugates are commercially available. Concentrations of SMV as low as 1 ng/ml can be detected by ELISA (Hill and Durand 1986).

Sensitivity of an ELISA is maximized by optimizing the antibody concentrations used. Known positives and known negatives are utilized to establish a binding ratio (or positive:negative ratio, also known as the P:N ratio).
The antibody concentration that yields the highest P:N ratio, yet maintains acceptably low negative control values, is then chosen (Hill et al., 1981).

**Host resistance**

SMV is a member of the genus *Potyvirus* within the family *Potyviridae* (Barnett 1991, Hollings and Brunt 1981). Viruses in this group are long flexuous rods consisting of single-stranded positive-sense RNA, surrounded by monomeric coat protein sub-units (El-Afifi 1978; Francki 1985). SMV isolates are often classified into strains (groups) based on symptom phenotype on a set of differential soybean hosts, with strains being designated as G1-G7, G7a or C14 (Buzzell and Tu 1984, Cho and Goodman 1979; Hartwig and Keeling 1982).

Several single dominant resistance genes have been identified in different soybean genotypes (Chen et al., 1994; Cho and Goodman 1979; Hill 1999; Jayaram et al., 1992); however, single gene resistance is often not effective against all SMV strains (Cho and Goodman 1979; Lim 1985). This is because resistance-breaking strains may soon emerge due to strong directional selection (Cho and Goodman 1979). A viable alternative to the use of the single gene resistance strategy may be the use of a rate-reducing strategy (Nutter and Kuhn 1993; Padgett et al., 1990). This approach allows some infection in the host population and relies on slowing the spread of the pathogen from plant-to-plant to minimize yield reductions (Nutter and Kuhn 1993; Padgett et al., 1990; Parlevliet 1979). Rate-reducing resistance may be facilitated by reduced host receptivity to virus infection, slowed viral replication within infected cells, reduced
translocation within the host, and/or by interfering with virus acquisition and transmission by vectors (Nutter 1997; Nutter and Kuhn 1993). This strategy has been shown to be effective in reducing the rate of disease progress of Tobacco etch virus (TEV) epidemics in bell pepper, without exerting directional selection for resistance-breaking strains (presumably due to the effectiveness of this resistance strategy against all strains of the virus, which should reduce directional selection for resistance-breaking strains) (Padgett et al., 1990).

Pathogen-derived resistance

As an alternative to the more traditional breeding approaches that introduce one or more genes into an adapted cultivar to reduce the rate of pathogen spread in crops, rate-reducing resistance may potentially be achieved by the insertion of genes, gene sequences, or sequences of nucleic acids that originate from a pathogen's genetic code (Sanford and Johnson 1985). This form of resistance to a virus, termed "Pathogen-derived resistance," typically involves the insertion of a viral coat protein (CP) gene, followed by the selection of transformed lines that display near-immunity (lack of infection, virus replication, and disease symptoms). Powell-Abel et al. (1986) first pioneered this strategy using the coat protein of Tobacco mosaic virus (TMV) to obtain transgenic tobacco which demonstrated the effectiveness of this approach. The development and use of pathogen derived resistance has accelerated in recent years, with many more examples of successful implementation (Beachy 1997; Kallerhoff et al., 1990; Kawchuk et al., 1990; Nelson et al., 1987; Tennant et al.,
1994; Van Dun and Bol 1988; Van Dun et al., 1987). Stark and Beachy's work with the coat protein of SMV showed that some protection could be gained against infection by other potyviruses in tobacco (Stark and Beachy 1989). Still, some concern exists that pathogen-derived resistance may not be (or remain) effective against all virus strains, as shown by the work of Herrero et al. (2000) and Tennant et al. (1994). This will influence both the implementation and adoption as a virus management strategy.

Recently, a chimeric coat protein gene of SMV strain N (group G2) was inserted into soybean cultivar '9341', to yield six transformed lines (Wang et al., 2001). Four of the transformants were found to have retained the coat protein insertion, while two lines were segregants that had lost the insertion. Three of the four transformants that retained the insertion were found to express SMV coat protein, while one line did not. At least two transformants expressed some level of resistance to SMV infection in greenhouse/growth chamber studies utilizing mechanically-transmitted virus (Wang et al., 2001). As Quemada et al. (1991) point out, mechanical inoculation exposes the plant to a high level of virus for a short time (usually a single virus strain), while vector populations in a field challenge the host plant with varying numbers of viruliferous aphids and strains throughout the growing period. Therefore, field experiments need to be conducted to quantify the effect of transgenic soybean lines on SMV temporal and spatial progress and to quantify the effects of this resistance strategy on the yield and quality of soybean grain.
Epidemiological modeling

To properly employ epidemiological models for quantitative comparisons among virus management strategies, it is first necessary to determine optimum plot sizes, and to develop efficient and reliable sampling methods, sample sizes, and sampling intervals for data collection (Nutter and Parker 1996). Nutter et al., (1998), developed a sampling protocol that divided soybean plots into quadrats in order to track the temporal and spatial spread of SMV in non-transgenic soybeans. By establishing a point source of inoculum in each plot, sampling of quadrats each week, and then testing each quadrat for the presence of SMV by ELISA, the analyses of SMV epidemics were made possible. Furthermore, this study employed the use of strain-specific monoclonal antibodies which permitted the differentiation of an introduced inoculum point source (strain G-5) of SMV from exogenous sources (strains) of SMV. Although exogenous virus strains were detected in quadrats, these occurred infrequently and randomly and did not significantly alter pathogen progress curves. This work showed that SMV in soybean could be quantified temporally and spatially using a quadrat-based sampling scheme (Nutter et al., 1998).

Soybean lines that possess rate-reducing resistance to SMV may be capable of increasing both soybean yield and seed quality, while reducing selection pressure on the virus population for resistance-breaking strains to arise. By sampling field plots at specific time intervals, the rate of pathogen progress over time and space can be quantified (Nutter et al., 1998; Padgett et al., 1990).
The use of sensitive detection tools (such as the ELISA) to accurately assess pathogen incidence allows for the use of epidemiological models which can then be used to quantify and compare the effect of soybean lines on epidemic development.

The objectives of this research were to:

1) Quantify and compare the temporal and spatial spread of SMV strain AL-5 in field plots of transgenic and non-transgenic soybeans, and

2) Determine the effects of transgenic resistance on yield components, presence of SMV seed infection, and the percentage of mottled seed.

**Literature cited**


CHAPTER II. TEMPORAL AND SPATIAL SPREAD OF SOYBEAN MOSAIC VIRUS IN SOYBEANS TRANSFORMED WITH THE COAT PROTEIN GENE OF SMV

A paper to be submitted for publication to Phytopathology

Todd A. Steinlage, John H. Hill, and Forrest W. Nutter, Jr.

Department of Plant Pathology, Iowa State University, Ames 50011

Abstract

Soybean lines, transformed with the coat protein gene of Soybean mosaic virus (SMV), were evaluated for SMV disease resistance by quantifying the temporal and spatial spread of SMV strain AL-5 released from a point source in the field. SMV spread, detected during 1999 and 2000 by ELISA, was most appropriately described by the Gompertz model. Two SMV coat protein transformed lines (genotypes) had significantly lower infection rates and significantly lower final pathogen incidence values ($P \leq 0.05$) as compared to the non-transformed control. Ordinary runs analysis revealed within-plot spread was more clustered in plots with the higher rates of temporal pathogen progress. Soybean lines with the lowest infection rates had significantly less seed-coat mottling as compared to the non-transformed control and significantly higher yields in 2000. This is the first field-study demonstrating the effectiveness of pathogen-derived resistance on the temporal and spatial dynamics of pathogen spread in soybean.
Soybean mosaic potyvirus (SMV) causes one of the most common viral diseases of soybean and is found in most soybean growing areas in the world (Cho et al., 1977; Dhingra and Chenulu 1980; Hill 1999). Infection by SMV can result in a number of symptoms ranging from visually undetectable to quite severe (Cho and Goodman 1979; Chen et al., 1991). Symptoms may be masked at temperatures above 27°C, while many cultivars do not express strong symptoms regardless of temperature (Conover 1948; Irwin and Shultz 1981; Walters 1963).

Infection by SMV may cause severe yield losses due to abortion of flowers and reduced number and weight of seed (Hill 1999; Ross 1969). Early plant infection usually results in greater losses (Bryant et al., 1982; Halbert and Irwin 1981; and Ross 1969). Reduced grain quality, as a result of SMV-induced seed discoloration (often described as hilum bleeding or seed coat mottling), can also occur, and has resulted in the assessment of financial penalties to farmers when mottled grain is brought to the grain elevator (Bryant et al., 1982). In major soybean-production areas of the northern United States, the primary inoculum source of SMV is thought to originate from SMV-infected plants that arise from infected seed (Hill et al., 1980). Secondary spread within and among soybean
fields occurs through the activity of more than thirty different aphid species (Edwardson and Christie 1991; Halbert et al., 1981; Lucas and Hill 1980).

Several single resistance genes to SMV infection have been identified (Buzzell and Tu 1984; Chen et al., 1991; Cho and Goodman 1979; Hill 1999; Jayaram et al., 1992); however single gene resistance is often not effective against all known strains of SMV. Thus, resistance-breaking strains often emerge due to the strong directional selection pressure that is created when single resistance genes are deployed (Cho and Goodman 1979; Lim 1985). An alternative approach to the single gene resistance strategy is a strategy that employs a rate-reducing effect on all SMV strains. This strategy allows some infection within a plant population, yet reduces the rate of plant-to-plant pathogen spread to minimize adverse effects on grain yield and quality (Padgett et al.; Parlevliet 1979). Reductions in pathogen spread may be due to reduced host receptivity, slowed virus replication within cells, reduced translocation within infected host cells and in plants, and/or interference with acquisition and transmission by insect vectors (Nutter and Kuhn 1993). For example, this resistance strategy effectively reduced the rate of Tobacco etch virus (TEV) epidemics in bell pepper, without a breakdown of resistance. Presumably this was due to decreased selection pressure for the emergence of new strains (Padgett et al., 1990).

In addition to the more traditional approaches of plant breeding programs that introduce single resistance genes into adapted host cultivars, disease
resistance may also be achieved by the insertion of genes, gene sequences, or sequences of nucleic acids from the genetic code of a plant pathogen (Sanford and Johnson 1985). This form of resistance, termed "pathogen-derived resistance," typically involves insertion of viral coat protein (CP) genes. The effectiveness of this method for resistance to plant viruses was first demonstrated by Powell-Abel et al. (1986) with transgenic tobacco expressing the coat protein of Tobacco mosaic virus (TMV). The development and use of pathogen-derived resistance has subsequently been successfully implemented by several other researchers in recent years (Beachy 1997; Kallerhoff et al., 1990; Kawchuk et al., 1990; Nelson et al., 1987; Stark and Beachy 1989; Tennant et al., 1994; Van Dun and Bol 1988; Van Dun et al., 1987); however, the final selection product has typically been for plant genotypes that express near-immunity to the pathogen, resulting in potentially strong directional selection for resistance-breaking strains.

A chimeric coat protein gene of SMV strain N was recently inserted into soybean cultivar '9341' to yield six transformed lines (Wang et al., 2001). Four of the transformants were found to have retained the coat protein insertion, while two lines were segregants that lost the insertion (Wang et al., 2001). Three of the four transformants that retained the insertion were found to express coat protein, while one line did not (Wang et al., 2001). Greenhouse and growth chamber tests with mechanically-transmitted SMV showed at least two of the transformed soybean lines had reduced virus incidence and delayed symptom
development when challenged with the four SMV strains that were tested (Wang et al., 2001). Some of these transgenic soybean lines may provide adequate levels of rate-reducing resistance at the host population level but these soybean lines first need to be evaluated under field conditions using epidemiological models to quantify and compare virus epidemics (Nutter and Parker 1996; Nutter et al., 1998), where varying numbers of viruliferous aphids and SMV strains may challenge the pathogen-derived resistance throughout the season (Quemada et al., 1991). Therefore, the objectives of this study were to conduct field experiments to quantify and compare the effect of transgenic and non-transgenic soybean lines on the temporal and spatial dynamics of SMV epidemics and to quantify the effects of transgenic resistance and non-transgenic soybean lines on components of soybean yield and quality.

**Materials and Methods**

**Field plots.** Field plots were planted on 26 May, 1999 and 24 May, 2000 at the Iowa State University Curtiss Farm, in Ames, Iowa. Virus-free seed of five transformed lines (3-24, 3-3, 7B-11, 6-13-1, 7B-10-9), as well as the non-transgenic isolate (9341), were planted each year (total of six treatments). One line (7B-10-9) was transformed, but was selected as a segregant that did not retain the SMV CP transcript (Wang et al., 2001).

The six treatments (soybean genotypes) were planted in a randomized complete block design with three replications. Each plot consisted of 8 rows, 10.5 m long and 6 m wide, with a row spacing of 0.75 m. Seed was planted at a
density of six seeds per 30 cm, and later thinned to four plants per 30 cm. Plant borders consisted of the two outside rows, as well as 1.5 m of row in from both ends of each plot; thus, there were six sampling rows, each 7.5 m long. Sampling rows were divided with wooden stakes into 30 cm quadrats (25 per row). Seed in quadrat position 13 of rows 3 and 4 were removed after planting and replaced with the SMV susceptible cultivar 'Williams 82'. There was a 9 m area of tilled earth between plots in order to reduce interplot interference in 1999, while 2 rows of the soybean variety 'Kenwood 94' were planted within each buffer area in 2000 to serve as trap plants (insecticide-treated) for bean leaf beetles, which were prevalent in Iowa during both years of the study.

After the plants had reached growth stage V-3 (two trifoliate nodes above the unifoliate node with fully developed leaves) (Fehr and Caviness 1977), the 'Williams 82' plants in the 13th quadrats were mechanically inoculated with aphid-transmissible SMV strain AL-5 (GeneBank accession no. AF242844), on 25 and 26 June, in 1999; and on 3, 4 and 22 July, in 2000. These SMV-inoculated plants served as point sources of inoculum for pathogen (SMV) spread within each plot.

**Data collection.** This study utilized a systematic, quadrat-based sampling design with all quadrats being sampled. Each quadrat (30 cm section of row) was considered a sampling unit, and all individual plants were samples within that sampling unit, according to the methods of Nutter et al. (1998). This involved recording the row and position of each quadrat within a plot (150
quadrats/plot). Sampling was done by removing the newest fully-expanded trifoliate (near the plant apex) from each plant within a quadrat and then placing these leaves into pre-labeled plastic bags prior to testing by ELISA. Each quadrat was sampled weekly beginning 7-10 days after inoculation of the 'Williams 82' and there were ten sampling dates in both 1999 and 2000.

**Virus assay.** Quadrat samples were refrigerated until sap was expressed from leaves using a sap extractor (Ravenel Specialties Corp., Seneca, SC), and sap from each quadrat (sampling unit) was diluted in approximately 5 ml of 0.05 M sodium phosphate buffer, pH 7.2. Quadrat samples then were frozen at -20°C until testing in duplicate wells by enzyme linked immunosorbent assay (ELISA) (Nutter et al., 1998).

The biotin-avidin double antibody sandwich (DAS) ELISA (Diaco et al., 1985) used anti-SMV monoclonal S4 capture antibody (Hill et al., 1989), coated onto Immulon 1B polystyrene microtiter plates (Dynex, Chantilly, VA). The S4 monoclonal antibody was used at 1.0 μg/ml, and biotinylated polyclonal anti-SMV antibody was used at 0.5 μg/ml, according to the maximum signal to noise ratio (Hill et al., 1981). Alkaline phosphatase conjugated Extravidin (Sigma Chemical Co., St. Louis) was applied at a 1:40,000 dilution. The substrate consisted of p-nitrophenyl phosphate (pNPP) at 1 mg/ml in 10% diethanolamine, pH 9.6. Absorbance at 405 nm was determined using a Bio-Tek ELx800 Universal Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT). A quadrat sample was considered positive for SMV if the absorbance value of duplicate wells was
equal to or greater than twice the value of the mean of the negative controls (sap from healthy soybean leaves). Pathogen incidence for each soybean plot was determined for each sampling date by dividing the number of quadrats testing positive for SMV by the total number of quadrats in each plot (150), and then multiplying by 100.

**Temporal analysis.** To aid in the determination of which pathogen growth model was most appropriate to quantify and compare all SMV epidemics, the percentages of infected quadrats versus time (as affected by soybean line) and the change in virus incidence (dy/dt) versus time were first graphed for each epidemic (plot), and then examined (Nutter 1997; Nutter and Parker 1996). Based on the shapes of these curves, the mean values for pathogen incidence were transformed (Gomez and Gomez 1984) using three potential models that might best fit all data (Nutter 1997; Nutter and Parker 1996). These models were the linear (non-transformed), the logistic, and the Gompertz population growth models (Statistical Analysis System, SAS Institute, Cary, NC; and the EPIMODEL program (Nutter and Parker 1996)). Simple linear regression of transformed incidence values versus time were used to estimate model parameters and statistics. The $F$-statistic, the coefficient of determination ($R^2$), the standard error of the estimate for transformed pathogen incidence (SEE$_y$), and a visual inspection of residual plots, were used to determine the goodness of fit of each population growth model (Nutter 1997; Nutter and Parker 1996). After selecting the most appropriate model, the slope of each regression line was then
used as a quantitative measure of temporal pathogen progress. Slopes were tested for significance by testing for parallelism (Gomez and Gomez 1984). The day of year of epidemic onset for each epidemic was operationally-defined as the day of year on which pathogen incidence reached the 0.05 level (5% incidence) in order to compare soybean lines.

**Spatial analysis.** Maps of SMV-infected quadrats over time were used to analyze the spatial pattern of infected quadrats in each plot (epidemic). The EPIVIRUS program (F. W. Nutter, Jr. unpublished) was used to perform ordinary runs analyses (Campbell and Madden 1990; Madden et al., 1982). A run was defined as a series of like events, in this case a series of infected or non-infected quadrats (Madden et al., 1982). The number of observed runs was compared to the number of expected runs that could occur by random chance by calculating a z-score for a one-sided t-test, with a z score of less than −1.64 indicating rejection of the null hypothesis of randomness in favor of clustering (at $P \leq 0.05$).

**Soybean yield, yield components, and quality.** Prior to harvest, the number of plants in each row was counted. After plant senescence and drying in the field, plots were hand-harvested on 14-17 October in 1999, and 13 October in 2000 by cutting the plants at the soil line with pruning shears and placing all plants within a row in a burlap bag. The four central rows of each plot were individually harvested, except that 'Williams 82' quadrats were harvested separately. Following harvest, plants in bags were further dried on a greenhouse bench. Soybean seeds were then cleaned using a stationary thresher, weighed,
and 100-seed weights, and incidence of seed-coat mottling (%) were obtained for each soybean row. To determine the effect of pathogen-derived transgenic resistance on grain quality, an additional 100-seed sub-sample from each row was passed through an Infratec 1229 infrared grain analyzer (Tecator, Hoganas, Sweden) for protein and oil analysis, to obtain measurements of soy oil and protein content.

The 100-seed weight sample from each row was combined for each plot and a 100-seed subsample of the 400 seeds from each plot were tested using immunocapture PCR to determine the presence or absence of seed-borne SMV infection (Wang et al., 2001). For detection of potential seed-borne Bean pod mottle comovirus (BPMV), the same soybean sample was tested using a biotin-avidin ELISA as described for SMV, except that anti-BPMV polyclonal antibody was substituted for anti-SMV antibody.

Data analysis. Mean separations for date of epidemic onset, final incidence, yield, yield components, and quality assessments were tested for using the Least Significant Difference test ($P \leq 0.05$) (Statistical Analysis System, SAS Institute, Cary, NC). In addition to ANOVA, linear regression was used to quantify the relationship between final SMV incidence ($x$) with yield, yield components, and quality factors as affected by soybean lines.

Results

Pathogen progress curves for 1999 and 2000 (Fig. 1) demonstrated that there were differences among soybean genotypes with regards to the day of year
that epidemic onset began (Table 1). For both years, epidemic onset was delayed the most by lines 3-24 and 7B-11 and began the earliest in lines 6-13-1 (day of year 141 in 1999 and day of year 200 in 2000) and 3-3 (day of year 167 in 1999 and day of year 198 in 2000). The dates of epidemic onset for the non-transgenic control 9341 and segregant 7B-10-9 were similar (day of year 201 and 204 in 1999, and day of year 202 and 204 in 2000, respectively). The dates of epidemic onset in transgenic lines 3-24 and 7B-11 were significantly ($P \leq 0.05$) delayed beyond the date of epidemic onset in transgenic line 6-13-1 in 1999, while lines 3-24 and 7B-11 significantly delayed epidemic onset ($P \leq 0.05$) beyond the dates of epidemic onset compared with all other lines in 2000 (Table 1).

Although final SMV incidence was somewhat lower for all lines in 1999 compared to the 2000 season, the order of final SMV incidence levels among lines was highly consistent. Final pathogen incidence in 1999 was highest in line 3-3, with 35% of the quadrats infected by SMV at seasons end, while lines 3-24 and 7B-11 had significantly lower ($P \leq 0.05$) (compared with all other genotypes) final incidence values of approximately 8% (Table 2). Non-transgenic cultivar 9341 had a final pathogen incidence of 21%, while final incidence in lines 7B-10-9 and 6-13-1 were 23% and 26%, respectively. In 2000, all lines had higher levels of final incidence relative to 1999, with a final incidence of 68% in the non-transformed control (9341). In 2000, final incidence was significantly lower in lines 3-24 (23%), 7B-11 (26%), and 6-13-1 (37%), compared with all other lines
Pathogen incidence was 53% in line 7B-10-9 and 56% in line 3-3, both of which were not significantly different than the non-transgenic control (9341).

Overall, the rate of temporal pathogen spread was faster in 2000 compared to the 1999 growing season. Based on the evaluation criteria for model selection (F-test, $R^2$, low SEE, examination of residuals, and choosing the simplest model), the 1999 and 2000 SMV epidemics were best described by the Gompertz model (Nutter 1997; Nutter and Parker 1996). F-statistics ranged from 64.37 to 640.59, and all were highly significant at $P \leq 0.0001$, indicating a very strong linear relationship between the change in gompit pathogen incidence and the change in time. The high coefficients of determination ($R^2$) (0.87 to 0.99) indicated that time explained 87 to 99% of the variation in the change in gompit pathogen incidence (Nutter 1997) (Table 3). Standard errors of the estimate for $Y$ were low (0.03 to 0.18 gompits), which showed time could be used to accurately predict pathogen increase with respect to time as affected by soybean genotype and year.

In 1999, infection rates of SMV progress ranged from 0.005 gompits/day (line 3-24) to significantly higher ($P \leq 0.05$) rates of spread in line 7B-10-9 (0.015 gompits/day) and the non-transformed control 9341 (0.014 gompits/day) (Table 3, Fig. 2). Lines 7B-11 and 6-13-1 had rates of 0.007 and 0.010 gompits/day, respectively, and both are significantly slower than the non-transgenic control.
cultivar 9341. Line 3-3 had a rate of 0.012 gompits/day, which was not significantly different from 9341 and line 7B-10-9.

The 2000 epidemics were much faster than the 1999 epidemics, with rates of pathogen progress ranging from 0.015 (line 3-24) to a significantly higher rate of 0.040 gompits/day in the non-transgenic control cultivar 9341 ($P \leq 0.05$) (Table 3, Fig. 2). Lines 6-13-1 and 7B-11 had significantly slower rates of SMV spread (0.022 gompits/day and 0.017 gompits/day, respectively). Lines 3-3 and 7B-10-9 had rates of 0.031 and 0.033 gompits/day, respectively, which was approximately twice as fast as rate-reducing lines 3-24 and 7B-11.

Spatial maps were generated by mapping the X-Y coordinates of infected quadrats with the software program EPIVIRUS (F. W. Nutter, Jr. unpublished). For both years, spatial patterns were somewhat variable, however, within-field spread was generally more clustered in plots with the highest rates of pathogen progress (Tables 3 and 4). In 1999, the rate-reducing line 3-24 showed random spatial patterns in all three replications as did rate-reducing line 6-13-1 (Table 4). Soybean line 3-3, a non-rate-reducing line, also predominately showed random spatial patterns, with clustering occurring only after day of year 239 (27 August) in replicate 2. The virus epidemics in line 7B-11 plots were mostly random except for replication 2, which displayed clustering between day of year 214 and 239 (2 August to 27 August). The susceptible, non-transgenic cultivar 9341 showed clustering as the season progressed for all replicates, beginning on day of year 207 in replication 1. Line 7B-10-9 also showed clustering of virus infected
quadrats as the season progressed, but clustering occurred later in the season than in cultivar 9341, beginning on day of year 221 in replication 3.

As in 1999, the ordinary runs analysis of the 2000 epidemics showed random spatial patterns of infected quadrats in all replications of the rate-reducing line 3-24 (Table 5). Virus infected quadrats in line 6-13-1 were generally randomly distributed throughout time and space. Soybean line 3-3 had clustered spatial patterns beginning on day of year 220 and 227 for replications 1 and 2, respectively, while quadrats in replication 3 were randomly infected for the entire season. Line 7B-11 showed some clustering, but only very late in the season (after day of year 250, 7 September) for all three replications. The non-transformed cultivar 9341 showed little clustering in replications 1 and 2, but significant clustering occurred in replication 3 from day of year 220 until the end of the season. Line 7B-10-9 showed clustering of infected quadrats starting on day of year 220 in replications 1 and 3, while SMV infected quadrats in replication 2 were randomly distributed throughout the season.

In 1999, plot yields among transgenic and control lines showed few significant differences, with line 6-13-1 having the highest yield (746.3 kg/ha). This line was significantly different (P ≤ 0.05) from line 3-3, that had a yield of 656.2 kg/ha (Table 6). None of the remaining lines were significantly different from either of these lines. In 2000, however, two rate-reducing transgenic lines (6-13-1 and 3-24) had significantly higher yields (370.2 kg/ha and 383.4 kg/ha, respectively) than the non-transformed cultivar (9341) (Table 6). Regression of
final SMV incidence on yield revealed that there was not a significant linear relationship between SMV incidence and yield in 1999. In 2000, however, there was a significant linear relationship ($P \leq 0.10$, with $R^2 = 0.54$), with yield decreasing by 1.87 kg/ha for each 1% increase in final SMV incidence (Fig. 3A).

Using ANOVA and mean comparisons, few significant differences occurred for yield components among soybean lines in 1999 for number of seeds per plant, or weight of seeds per plant, although 100-seed weight was significantly greater in transgenic line 7B-10-9 (13.7 g) compared to line 7B-11 (13.3 g) (Table 6). In 2000, the number of seeds/plant was significantly greater ($P \leq 0.05$) in rate-reducing lines 3-24 and 6-13-1 (169.6 seeds/plant and 170.5 seed/plant, respectively), compared to the non-transgenic control, 9341 (105.5 seeds/plant) (Table 6). Seed weight/plant had similar results to the number of seed per plant, with rate-reducing lines 3-24 (at 21.2 g seed/plant) and 6-13-1 (at 20.2 g seed/plant) having significantly higher seed weights than the control 9341 (12.7 g seed/plant). Regression of final SMV incidence on yield components (number of seeds/plant, seed weight/plant, and 100-seed weight) revealed no significant linear relationships in 1999. In 2000, however, regression of final SMV incidence on seed weight/plant was significantly linear ($P \leq 0.09$, $R^2 = 0.56$) with seed weight/plant decreasing by 0.13 g for each 1% increase in final SMV incidence (Fig. 3C).

Using ANOVA and mean comparisons, seed quality, as determined by measuring the percentage of soy protein and soy oil in the harvested grain,
showed no significant differences among soybean lines in 1999. In 2000, soy oil content was significantly higher (\( P \leq 0.05 \)) in all lines relative to the non-transgenic control cultivar (9341), while soy protein was significantly higher (\( P \leq 0.05 \)) in non-transgenic 9341 compared to all other lines (Table 7). As with ANOVA, regression of final SMV incidence on soy oil (%) and soy protein (%) in 1999 revealed no significant linear relationships; however, regression of final SMV incidence on soy oil (%) in 2000 showed a significant negative linear relationship (\( P \leq 0.01, R^2 = 0.82 \)) as oil content decreased 0.02% for each 1% increase in final SMV incidence (Fig. 4A). Regression of final SMV incidence on protein (%) in 2000 also revealed a significant (but positive) linear relationship (\( P \leq 0.03, R^2 = 0.73 \)) with soy protein increasing by 0.03% for every 1% increase in final SMV incidence (Fig. 4B).

Seed coat mottling, an important soybean quality factor whereby low seed coat mottling is desirable, was significantly reduced by the rate-reducing transgenic lines in both 1999 and 2000, although the reductions were more dramatic in 1999 than in 2000. Generally, mottling of seeds from all transgenic lines was less than that observed on the transgenic control line 7B-10-9, and the non-transgenic control cultivar 9341. Based upon regression analysis, the relationship between final SMV incidence and percentage seed coat mottling in 1999 was not significant; however, in 2000 there was a significant linear relationship (\( P \leq 0.06, R^2 = 0.62 \)) showing that the percentage of seed coat
mottling increased by 0.30% for each 1% increase in final SMV incidence (Fig. 4C).

In 1999, seed-borne SMV was detected by immunocapture RT-PCR only in the non-transgenic control cultivar 9341; in 2000 seed-borne SMV was detected in all lines except rate-reducing transgenic lines 3-24 and 7B-11. Harvested seed was also tested by quantitative ELISA for the presence of Bean pod mottle comovirus (BPMV), which was found in all seed lots in 1999 and 2000. There was a significant linear relationship between the relative amount of BPMV detected and the incidence of seed mottling in both 1999 (P ≤ 0.09, R²=0.54) and 2000 (P ≤ 0.06, R²=0.61) (Fig. 5). Relative amounts of seed-borne BPMV were higher in 2000 than in 1999, when final SMV incidence was lower.

**Discussion**

The slope of the Gompertz transformation can be used as an estimate of the rate of pathogen progress with respect to time to allow for the comparison of SMV epidemics (Nutter 1997). Clearly, the highest rates of pathogen progress were measured in 2000 in soybean cultivar 9341 and the segregant 7B-10-9. Although considerably slower in 1999, the highest infection rates occurred in the same lines in both years. Pathogen progress in lines 3-24 and 7B-11 was significantly reduced resulting in yields higher than the controls in 2000. Although the overall rate of SMV spread in line 6-13-1 was also moderately reduced, epidemic onset in this line occurred first in both years. Conversely,
epidemic onset in rate-reducing lines 3-24 and 7B-11 were consistently delayed along with having the lowest rates of spread in the study. Compared to cultivar 9341 in 1999, epidemic onset in line 3-24 occurred 10 days later. In 2000, epidemic onset in line 3-24 occurred 23 days later compared to the non-transgenic control cultivar 9341. Epidemic onset in line 7B-11 was delayed by 29 days compared to cultivar 9341 in 1999, while line 7B-11 delayed onset in 2000 by 19 days (compared to cultivar 9341).

The data are very consistent with regards to final SMV incidence. The highest final SMV incidence (68%) occurred in 2000 on non-transgenic cultivar 9341. In 1999, final incidence attained a maximum of only 35% in line 3-3. These results are comparable to those reported by Nutter et al. (1998) in experiments with the cultivar 'Corsoy 79' performed from 1991 to 1994 using SMV strain G-5. Using the same sampling design and testing procedures, the final SMV incidence on transgenic line 3-3 in 1999 was similar to the approximate 45% final incidence found in 1994, and the final incidence on non-transgenic cultivar 9341 in 2000 was similar to the approximate 65% final incidence reported in 1991. Variation in epidemics from year to year may be related to differing aphid species and populations, relative transmission efficiencies of vectors, the timing of aphid flights, environmental factors affecting plant growth, as well as the aggressiveness of the infecting virus strain (in terms of temporal spread). The average temperature in July was higher in 1999 than 2000. Precipitation was also higher in 1999, which could affect aphid reproduction as well as plant vigor.
Clustering of infected quadrats, as revealed by ordinary runs analyses, may have been influenced by the relative susceptibility of a given line in terms of the rate of disease progress. The amount of clustering compares well with rates of pathogen progress, as higher amounts of clustering appear to be associated with higher rates of virus spread. A susceptible genotype should have a greater probability of infection when challenged with a virus (Wang et al., 2001), resulting in a greater probability of clustering if vectors predominately travel short distances after acquiring the virus. There does not appear to be any pattern as to how the plots with significant patterns of clustering were arranged in the 1999 and 2000 field experiments. In 2000, ordinary runs analysis identified significant clustering only in the non-rate-reducing lines (3-3, 9341, and 7B-10-9). Significantly fewer clustered quadrats were found in rate-reducing lines 7B-11 and 6-13-1, and none were found in transgenic line 3-24.

Few significant yield differences occurred among soybean lines in 1999. However, in 2000, when final SMV incidence and infection rates were higher, regression analysis revealed significant linear relationships between final incidence and yield with 54% of the variation in yield explained by final SMV incidence. The remaining 46% of the variation was presumably due to biotic factors such as insects (e.g. bean leaf beetle feeding) and other pathogens such as BPMV. During the years this study was conducted, populations of the bean leaf beetle, a vector of BPMV, were high and BPMV was epidemic in Iowa. The presence of BPMV in our experimental plots was confirmed by detection of the
seed-borne comovirus in soybean seed harvested from the plots. Previous reports have shown BPMV and SMV act synergistically (Calvert and Ghabrial 1983) and yield of soybeans infected by both viruses may be reduced (Ross 1968; Ross 1969; Quiniones et al., 1971). In 2000, the yield of the non-transgenic 9341 was reduced while yields of the two best rate-reducing lines were significantly higher. The absence of any relationship between final virus incidence and 100-seed weight, and the variation in reports of effects of virus infection on this character, are consistent with previous results (Bryant et al., 1982; Ross 1968, Ross 1969; Quinones et al., 1971).

Oil and protein content of seed showed few differences when tested by ANOVA, but regression of final SMV incidence for all soybean lines versus oil and protein in 2000 showed oil and protein showed that reduced oil content and increased soy protein content was significantly associated with final SMV incidence. This is consistent with the previous report of Demski and Jellum (1975) showing similar association between virus infection and oil and protein content.

Quality reductions in 2000 may be related to a synergistic interaction (Calvert and Ghabrial 1983) between BPMV and increased SMV incidence. The increased final incidence of SMV in 2000 could result in increased BPMV replication and presumably higher relative amounts of seed-borne BPMV. Results of this study show that relative amounts of seed-borne BPMV were higher in 2000 than in 1999. Further, increased relative amounts of seed-borne
BPMV detected in harvested seed were associated with detection of seed-borne SMV (1 of 6 lines in 1999, 4 of 6 lines in 2000), except for the rate-reducing lines 3-24 and 7B-11 where, consistent with Wang et al. (2001), seed-borne SMV was never detected. Infection of plants by SMV may allow higher amounts of seed-borne BPMV.

Because SMV and BPMV both induce seed-coat mottling that is indistinguishable, it is probable that BPMV was associated with seed mottling in both 1999 and 2000 while SMV incidence had an effect only in 2000 (Fig. 4C, Fig. 5). In 1999, there was not a linear relationship between percentage of seed-coat mottling and final SMV incidence; however, in 2000 62% of the variation in seed coat mottling could be explained by accumulated SMV incidence (Fig. 4C). Therefore, in 1999 mottling was attributed to factors other than SMV (such as BPMV), while in 2000 only 38% of the mottling was not associated with SMV incidence (Hill et al., 1980). This is consistent with analyses that relate percentage mottling to relative amounts of seed-borne BPMV (Fig. 5).

Comparison of $R^2$ values for 1999 ($R^2 = 0.54$) and 2000 ($R^2 = 0.61$) suggests that over 50% of variation in the amount of mottling was related to relative amounts of seed-borne BPMV in both years. We propose that the higher mottling observed in 2000 is, at least in part, consistent with the higher final incidence of SMV since the probability of coinfection was very high.

Unfortunately, data measuring final incidence of BPMV is not available. Since Wang et al. (2001) reported that no mottling occurred in seeds harvested from
SMV-inoculated plants of lines 3-24 and 7B-11 and no seed-borne SMV was ever detected by immunocapture RT-PCR in these lines, we suggest that most of the virus-induced mottling observed in these lines was caused by BPMV.

Seed transmission of SMV is greatly reduced if infection occurs after the onset of flowering (Bowers and Goodman 1979). We suggest that rate-reducing lines that delay the onset of SMV epidemics until well after flowering (as occurred in our study), would significantly reduce seed transmission. In 1999 and 2000, flowering occurred in 50% of the soybean plants on day of year 207 and 205, respectively. Lines 3-24 and 7B-11 reached epidemic onset 4 and 23 days post flowering in 1999, and 20 and 16 days post flowering in 2000, respectively. Thus, an added benefit of rate-reducing resistance should be to also reduce SMV seed transmission. Results of this study, in which no seed-borne SMV was detected by immunocapture RT-PCR in lines 3-24 and 7B-11, are consistent with this hypothesis. The delay in epidemic onset for both 3-24 and 7B-11, relative to time of flowering, should significantly reduce (or eliminate) virus transmission to seed from season to season; this would be expected to substantially affect the amount of initial inoculum available for future epidemics. Conversely, seed-borne SMV was detected in all other lines when epidemic onset of these lines occurred prior to flowering during both 1999 and 2000.

Durability, defined as the length of time during which a resistance remains usable, may also be increased by using a rate-reducing strategy of pathogen resistance (Padgett et al., 1990; Parlevliet 1979). The previous report of Wang et
al. (2001) did not reveal potential for rate-reducing resistance in line 6-13-1. However, data from this study suggested this line may have some utility, although it was not as apparent as that of lines 3-24 and 7B-11. The high sequence identity of the SMV coat protein gene suggests, as discussed by Wang et al. (2001), that the rate-reducing resistance of lines 3-24, 7b-11 and 6-13-1 should remain durable. Evidence for slowed pathogen progress in lines 3-24, 7B-11, and 6-13-1 suggests potential use of these lines for such a resistance strategy. In contrast to immunity, some infection of the plant population with SMV may occur, but most SMV infections will occur late (after flowering) and should have little or no effect on yield and quality due to plant compensation from neighboring healthy plants.

This report is also, to our knowledge, the first demonstration of pathogen-derived resistance to any pathogen of soybeans in the field.

Acknowledgments

I would like to thank Alan Eggenberger, Mohammad Reza Hajimorad, and Dave Volkers for their expert advice and constant friendship. This research was supported by Hatch Act and State of Iowa funds allocated to the Iowa Agriculture and Home Economics Experiment Station and, in part, by the Iowa Soybean Promotion Board, Pioneer Hi-Bred International, Inc., the Iowa Center for Advanced Technology Development, and the USDA North Central Biotechnology Initiative. Project Nos. 2428 and 3394 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa.
Literature Cited


TABLE 1. Time to 5% *Soybean mosaic virus* incidence (day of year) for transgenic soybean lines and non-transformed cultivar 9341 in the 1999 and 2000 epidemics in Ames, IA.

<table>
<thead>
<tr>
<th>Line</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-24</td>
<td>211 a&lt;sup&gt;x&lt;/sup&gt;</td>
<td>225 a</td>
</tr>
<tr>
<td>3-3</td>
<td>167 ab</td>
<td>198 b</td>
</tr>
<tr>
<td>7B-11</td>
<td>230 a</td>
<td>221 a</td>
</tr>
<tr>
<td>6-13-1</td>
<td>141 b</td>
<td>200 b</td>
</tr>
<tr>
<td>9341&lt;sup&gt;y&lt;/sup&gt;</td>
<td>201 ab</td>
<td>202 b</td>
</tr>
<tr>
<td>7B-10-9&lt;sup&gt;z&lt;/sup&gt;</td>
<td>204 ab</td>
<td>204 b</td>
</tr>
</tbody>
</table>

<sup>x</sup>Numbers followed by the same letter within the same column are not significantly different from each other at *P* ≤ 0.05 using LSD.

<sup>y</sup>Non-transgenic soybean cultivar used as a control.

<sup>z</sup>Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
TABLE 2. Final *Soybean mosaic virus* incidence for transgenic soybean lines and non-transformed cultivar 9341 in the 1999 and 2000 epidemics in Ames, IA.

<table>
<thead>
<tr>
<th>Line</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-24</td>
<td>8.4 c</td>
<td>23.1 d</td>
</tr>
<tr>
<td>3-3</td>
<td>35.1 a</td>
<td>56.0 ab</td>
</tr>
<tr>
<td>7B-11</td>
<td>8.2 c</td>
<td>26.0 cd</td>
</tr>
<tr>
<td>6-13-1</td>
<td>26.0 ab</td>
<td>37.1 bcd</td>
</tr>
<tr>
<td>9341(^y)</td>
<td>20.7 b</td>
<td>68.0 a</td>
</tr>
<tr>
<td>7B-10-9(^z)</td>
<td>22.7 b</td>
<td>53.3 abc</td>
</tr>
</tbody>
</table>

\(^x\)Numbers followed by the same letter within the same column are not significantly different from each other at \(P \leq 0.05\) using LSD.

\(^y\)Non-transgenic soybean cultivar used as a control.

\(^z\)Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
TABLE 3. Gompertz model parameters and statistics describing the progress of Soybean mosaic virus strain AL-5 in transgenic soybean lines and non-transformed cultivar 9341 during 1999 and 2000 in Ames, IA.

<table>
<thead>
<tr>
<th>Year and soybean line</th>
<th>Intercept</th>
<th>Slope</th>
<th>$R^2$</th>
<th>SEE&lt;sub&gt;y&lt;/sub&gt;&lt;sup&gt;w&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-24</td>
<td>-2.29</td>
<td>0.005 a</td>
<td>0.94</td>
<td>0.04</td>
</tr>
<tr>
<td>3-3</td>
<td>-3.24</td>
<td>0.012 b</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>7B-11</td>
<td>-2.66</td>
<td>0.007 a</td>
<td>0.96</td>
<td>0.04</td>
</tr>
<tr>
<td>6-13-1</td>
<td>-2.71</td>
<td>0.010 c</td>
<td>0.97</td>
<td>0.05</td>
</tr>
<tr>
<td>9341&lt;sup&gt;y&lt;/sup&gt;</td>
<td>-3.80</td>
<td>0.014 bcd</td>
<td>0.89</td>
<td>0.12</td>
</tr>
<tr>
<td>7B-10-9&lt;sup&gt;z&lt;/sup&gt;</td>
<td>-4.07</td>
<td>0.015 bd</td>
<td>0.95</td>
<td>0.08</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-24</td>
<td>-4.45</td>
<td>0.015 a</td>
<td>0.87</td>
<td>0.13</td>
</tr>
<tr>
<td>3-3</td>
<td>-7.29</td>
<td>0.031 b</td>
<td>0.95</td>
<td>0.18</td>
</tr>
<tr>
<td>7B-11</td>
<td>-4.91</td>
<td>0.017 ac</td>
<td>0.95</td>
<td>0.09</td>
</tr>
<tr>
<td>6-13-1</td>
<td>-5.49</td>
<td>0.022 ac</td>
<td>0.92</td>
<td>0.16</td>
</tr>
<tr>
<td>9341&lt;sup&gt;y&lt;/sup&gt;</td>
<td>-9.19</td>
<td>0.040 d</td>
<td>0.97</td>
<td>0.15</td>
</tr>
<tr>
<td>7B-10-9&lt;sup&gt;z&lt;/sup&gt;</td>
<td>-7.81</td>
<td>0.033 bd</td>
<td>0.95</td>
<td>0.18</td>
</tr>
</tbody>
</table>

<sup>w</sup> Standard error of the estimate for y.
<sup>x</sup> Numbers followed by the same letter within the same column are not significantly different from each other at $P \leq 0.05$ using LSD.
<sup>y</sup> Non-transgenic soybean cultivar used as a control.
<sup>z</sup> Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
<table>
<thead>
<tr>
<th>Soybean line</th>
<th>Replication</th>
<th>Day of Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>187</td>
<td>194</td>
</tr>
<tr>
<td>3-24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.149</td>
<td>18.149</td>
</tr>
<tr>
<td>2</td>
<td>2.759</td>
<td>-0.959</td>
</tr>
<tr>
<td>3</td>
<td>7.133</td>
<td>2.759</td>
</tr>
<tr>
<td>3-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.973</td>
<td>0.973</td>
</tr>
<tr>
<td>2</td>
<td>1.714</td>
<td>0.794</td>
</tr>
<tr>
<td>3</td>
<td>3.872</td>
<td>3.872</td>
</tr>
<tr>
<td>7B-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.149</td>
<td>18.149</td>
</tr>
<tr>
<td>2</td>
<td>2.044</td>
<td>-0.892</td>
</tr>
<tr>
<td>3</td>
<td>18.149</td>
<td>18.149</td>
</tr>
<tr>
<td>6-13-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.680</td>
<td>-0.496</td>
</tr>
<tr>
<td>2</td>
<td>5.798</td>
<td>4.440</td>
</tr>
<tr>
<td>3</td>
<td>1.610</td>
<td>1.658</td>
</tr>
<tr>
<td>9341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.044</td>
<td>-0.892</td>
</tr>
<tr>
<td>2</td>
<td>18.149</td>
<td>7.099</td>
</tr>
<tr>
<td>7B-10-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.149</td>
<td>18.149</td>
</tr>
<tr>
<td>2</td>
<td>1.784</td>
<td>1.784</td>
</tr>
<tr>
<td>3</td>
<td>7.133</td>
<td>5.414</td>
</tr>
</tbody>
</table>

*A z-score followed by * indicates a value less than -1.64, indicating nonrandomness (i.e. clustering).

Non-transgenic soybean cultivar used as a control.

Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
TABLE 5. Ordinary runs analyses (z-scores\textsuperscript{x}) for Soybean mosaic virus epidemics as affected by transgenic soybean lines and non-transgenic cultivar 9341 in Ames, IA, during 2000 growing season.

<table>
<thead>
<tr>
<th>Soybean line</th>
<th>Replication</th>
<th>Day of Year</th>
<th>192</th>
<th>199</th>
<th>206</th>
<th>213</th>
<th>220</th>
<th>227</th>
<th>234</th>
<th>241</th>
<th>250</th>
<th>259</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-24</td>
<td>1</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>4.061</td>
<td>0.000</td>
<td>-1.257</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.149</td>
<td>6.097</td>
<td>6.097</td>
<td>5.394</td>
<td>4.897</td>
<td>4.897</td>
<td>2.768</td>
<td>2.876</td>
<td>2.150</td>
<td>3.135</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>43.856</td>
<td>11.706</td>
<td>11.706</td>
<td>8.783</td>
<td>8.783</td>
<td>8.783</td>
<td>2.178</td>
<td>1.678</td>
<td>1.678</td>
<td>-0.389</td>
<td></td>
</tr>
<tr>
<td>3-3</td>
<td>1</td>
<td>43.856</td>
<td>1.577</td>
<td>0.994</td>
<td>-0.393</td>
<td>-3.480*</td>
<td>-4.499*</td>
<td>-4.300*</td>
<td>-4.003*</td>
<td>-3.853*</td>
<td>-3.829*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.856</td>
<td>8.783</td>
<td>8.783</td>
<td>0.378</td>
<td>-0.743</td>
<td>-2.420*</td>
<td>-2.587*</td>
<td>-2.353*</td>
<td>-1.892*</td>
<td>-1.634</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.798</td>
<td>-0.445</td>
<td>-0.239</td>
<td>0.273</td>
<td>-0.320</td>
<td>-0.236</td>
<td>0.171</td>
<td>0.319</td>
<td>0.514</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>7B-11</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>7.133</td>
<td>7.133</td>
<td>7.133</td>
<td>7.133</td>
<td>0.060</td>
<td>-3.068*</td>
<td>-3.480*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.000</td>
<td>43.856</td>
<td>18.149</td>
<td>4.984</td>
<td>3.458</td>
<td>3.458</td>
<td>3.458</td>
<td>3.255</td>
<td>-1.970*</td>
<td>-1.717*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.000</td>
<td>7.133</td>
<td>1.647</td>
<td>1.647</td>
<td>1.647</td>
<td>0.714</td>
<td>-2.876*</td>
<td>-2.873*</td>
<td>-2.873*</td>
<td>-2.106*</td>
<td></td>
</tr>
<tr>
<td>6-13-1</td>
<td>1</td>
<td>0.000</td>
<td>2.387</td>
<td>-0.045</td>
<td>-2.083*</td>
<td>-1.824*</td>
<td>-1.313</td>
<td>-1.313</td>
<td>-1.313</td>
<td>-0.933</td>
<td>-0.792</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.856</td>
<td>8.783</td>
<td>8.783</td>
<td>8.783</td>
<td>7.136</td>
<td>1.552</td>
<td>-1.329</td>
<td>-1.267</td>
<td>-1.267</td>
<td>-0.356</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.149</td>
<td>4.984</td>
<td>-2.126*</td>
<td>-0.678</td>
<td>-0.185</td>
<td>-0.098</td>
<td>0.495</td>
<td>-0.474</td>
<td>-0.417</td>
<td>-0.690</td>
<td></td>
</tr>
<tr>
<td>9341\textsuperscript{y}</td>
<td>1</td>
<td>8.783</td>
<td>7.136</td>
<td>3.224</td>
<td>-1.531</td>
<td>-3.738*</td>
<td>-1.940*</td>
<td>1.468</td>
<td>1.162</td>
<td>0.708</td>
<td>0.517</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.783</td>
<td>8.783</td>
<td>2.923</td>
<td>1.304</td>
<td>0.365</td>
<td>-2.475*</td>
<td>-1.133</td>
<td>-0.446</td>
<td>-0.118</td>
<td>-0.172</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.149</td>
<td>18.149</td>
<td>7.133</td>
<td>0.000</td>
<td>-3.719*</td>
<td>-3.177*</td>
<td>-3.823*</td>
<td>-3.117*</td>
<td>-3.117*</td>
<td>-3.209*</td>
<td></td>
</tr>
<tr>
<td>7B-10-9\textsuperscript{z}</td>
<td>1</td>
<td>43.856</td>
<td>7.133</td>
<td>4.460</td>
<td>1.489</td>
<td>-1.766*</td>
<td>-3.829*</td>
<td>-3.660*</td>
<td>-4.325*</td>
<td>-3.922*</td>
<td>-3.768*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.149</td>
<td>5.414</td>
<td>3.872</td>
<td>1.179</td>
<td>-1.634</td>
<td>0.440</td>
<td>-0.357</td>
<td>-0.805</td>
<td>-0.892</td>
<td>-0.458</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.149</td>
<td>18.149</td>
<td>18.149</td>
<td>0.225</td>
<td>-2.448*</td>
<td>-2.998*</td>
<td>-3.442*</td>
<td>-2.169*</td>
<td>-1.790*</td>
<td>-1.116</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{x}A z-score followed by * indicates a value less than -1.64, indicating nonrandomness (i.e. clustering).

\textsuperscript{y}Non-transgenic soybean cultivar used as a control.

\textsuperscript{z}Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
<table>
<thead>
<tr>
<th>Year</th>
<th>Soybean Line</th>
<th>Yield/plot (kg/ha)</th>
<th>Number of seeds/plant</th>
<th>Seed weight/plant (g)</th>
<th>100 seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>3-24</td>
<td>696.6 ab</td>
<td>168.0 a</td>
<td>22.6 a</td>
<td>13.5 ab</td>
</tr>
<tr>
<td></td>
<td>3-3</td>
<td>656.2 b</td>
<td>158.6 a</td>
<td>21.3 a</td>
<td>13.5 ab</td>
</tr>
<tr>
<td></td>
<td>7B-11</td>
<td>679.6 ab</td>
<td>170.3 a</td>
<td>22.5 a</td>
<td>13.3 b</td>
</tr>
<tr>
<td></td>
<td>6-13-1</td>
<td>746.3 a</td>
<td>174.0 a</td>
<td>23.1 a</td>
<td>13.3 ab</td>
</tr>
<tr>
<td></td>
<td>9341</td>
<td>698.5 ab</td>
<td>164.1 a</td>
<td>21.9 a</td>
<td>13.3 ab</td>
</tr>
<tr>
<td></td>
<td>7B-10-9[z]</td>
<td>715.3 ab</td>
<td>159.6 a</td>
<td>21.9 a</td>
<td>13.7 a</td>
</tr>
<tr>
<td>2000</td>
<td>3-24</td>
<td>383.4 a</td>
<td>169.6 a</td>
<td>21.2 a</td>
<td>12.5 a</td>
</tr>
<tr>
<td></td>
<td>3-3</td>
<td>329.1 ab</td>
<td>145.3 ab</td>
<td>17.0 ab</td>
<td>11.7 a</td>
</tr>
<tr>
<td></td>
<td>7B-11</td>
<td>315.3 ab</td>
<td>140.4 ab</td>
<td>16.7 ab</td>
<td>11.9 a</td>
</tr>
<tr>
<td></td>
<td>6-13-1</td>
<td>370.2 a</td>
<td>170.5 a</td>
<td>20.2 a</td>
<td>11.9 a</td>
</tr>
<tr>
<td></td>
<td>9341[y]</td>
<td>254.4 b</td>
<td>105.5 b</td>
<td>12.7 b</td>
<td>12.1 a</td>
</tr>
<tr>
<td></td>
<td>7B-10-9[z]</td>
<td>323.7 ab</td>
<td>148.4 ab</td>
<td>17.6 ab</td>
<td>12.0 a</td>
</tr>
</tbody>
</table>

*Numbers followed by the same letter within the same column are not significantly different from each other at $P \leq 0.05$ using LSD.
*Non-transgenic soybean cultivar used as a control.
*Transformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.

Table 6. Yield and yield components for transgenic soybean lines and non-transgenic cultivar 9341 as affected by *Soybean mosaic virus* during 1999 and 2000 in Ames, IA.
TABLE 7. Quality factors for transgenic soybean lines and non-transgenic cultivar 9341 as affected by Soybean mosaic virus during 1999 and 2000 in Ames, IA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Soybean Line</th>
<th>Oil (%)</th>
<th>Protein (%)</th>
<th>Percentage mottled seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>3-24</td>
<td>17.0 a</td>
<td>35.2 a</td>
<td>9.8 c</td>
</tr>
<tr>
<td></td>
<td>3-3</td>
<td>17.2 a</td>
<td>35.3 a</td>
<td>8.3 c</td>
</tr>
<tr>
<td></td>
<td>7B-11</td>
<td>17.1 a</td>
<td>35.4 a</td>
<td>11.2 c</td>
</tr>
<tr>
<td></td>
<td>6-13-1</td>
<td>17.2 a</td>
<td>35.0 a</td>
<td>24.0 b</td>
</tr>
<tr>
<td></td>
<td>9341γ</td>
<td>17.2 a</td>
<td>35.2 a</td>
<td>33.0 a</td>
</tr>
<tr>
<td></td>
<td>7B-10-9z</td>
<td>17.0 a</td>
<td>35.3 a</td>
<td>21.3 b</td>
</tr>
<tr>
<td>2000</td>
<td>3-24</td>
<td>18.0 a</td>
<td>34.0 c</td>
<td>16.1 bc</td>
</tr>
<tr>
<td></td>
<td>3-3</td>
<td>17.5 b</td>
<td>34.9 b</td>
<td>21.8 b</td>
</tr>
<tr>
<td></td>
<td>7B-11</td>
<td>17.9 ab</td>
<td>34.6 bc</td>
<td>21.3 b</td>
</tr>
<tr>
<td></td>
<td>6-13-1</td>
<td>17.8 ab</td>
<td>34.4 bc</td>
<td>15.3 c</td>
</tr>
<tr>
<td></td>
<td>9341γ</td>
<td>16.9 c</td>
<td>35.8 a</td>
<td>31.5 a</td>
</tr>
<tr>
<td></td>
<td>7B-10-9z</td>
<td>17.7 ab</td>
<td>34.6 bc</td>
<td>30.2 a</td>
</tr>
</tbody>
</table>

*Numbers followed by the same letter within the same column are not significantly different from each other at P ≤ 0.05 using LSD.
γNon-transgenic soybean cultivar used as a control.
zTransformed soybean line, selected as a segregant that did not retain the SMV coat protein transcript, used as a control.
Fig. 1. *Soybean mosaic virus* incidence in transgenic soybean lines and non-transgenic cultivar 9341 during A, 1999 and B, 2000 in Ames, IA. Dashed line indicates the 5% incidence level, operationally defined as epidemic onset.
Fig. 2. Gompertz transformed *Soybean mosaic virus* incidence in transgenic soybean lines and non-transgenic cultivar 9341 during A, 1999 and B, 2000 in Ames, IA.
Fig. 3. Relationship between final *Soybean mosaic virus* incidence in transgenic and non-transgenic soybeans during 1999 and 2000 in Ames, IA and A, yield (kg/ha); B, number of seeds/plant; C, seed weight (g)/plant; and D, 100-seed weight (g). Points without a regression line indicate non-linear relationships.
Fig. 4. Relationship between final Soybean mosaic virus incidence in transgenic and non-transgenic soybeans during 1999 and 2000 in Ames, IA and A, percentage oil; B, percentage protein; and C, mottling incidence (%). Points without a regression line indicate non-linear relationships.
Fig. 5. Relationship between the relative quantities of seed-borne Bean pod mottle virus (BPMV) on incidence of seed mottling in soybean lines during 1999 and 2000 in Ames, IA. A ratio indicating the relative amounts of seed-borne BPMV was calculated by dividing the average absorbance value from ELISA for a specific soybean line (4 wells/plot) by the average absorbance value of the negative controls.
CONCLUSIONS

*Soybean mosaic virus* (SMV) is a serious worldwide pathogen of soybeans. Infection with SMV can reduce soybean yield as well as seed quality. Many cultivars currently grown in the Midwest do not express strong symptoms when infected with SMV, making visual disease determination difficult. Tools such as the Enzyme Linked Immunosorbent Assay (ELISA) are needed for accurate assessments of pathogen incidence, which can be used in modeling epidemics. Modeling allows for accurate comparisons to be made between management strategies.

Management of SMV typically involves deployment of single dominant resistance genes that confer complete resistance to infection. These genes are not effective against all virus strains, and may cause selection pressure for resistance-breaking strains. A rate-reducing resistance strategy allows infection to occur, while reducing the rate of pathogen progress. This strategy should be non-strain-specific, which would reduce selection pressure in the virus population.

Rate-reducing resistance may be achieved by insertion of genetic sequences from the pathogen. This form of resistance is termed "Pathogen-derived resistance" and can be acquired by transforming plants with the coat protein (CP) gene of the virus. This was accomplished in soybean with the CP gene of SMV strain N, yielding six transformed lines. Four lines possess the CP transcript, and three of these lines express coat protein. Two lines did not
maintain the CP transcript. Greenhouse tests showed the transformed lines to possess resistance to SMV infection. Evaluation under field-conditions is necessary to confirm greenhouse tests, as well as for modeling epidemics for comparison of soybean lines.

Field-tests in 1999 and 2000 demonstrated rate-reductions in viral progress for two soybean lines, relative to the non-transformed control. One of these lines expresses CP, while the other possesses the transcript but does not express CP. These two lines also performed well in reducing seed coat mottling, relative to the non-transformed control.

This work shows that pathogen-derived resistance can reduce the rate of SMV epidemics, as well as reducing quality losses. This study also demonstrates the benefits of accurately modeling epidemics for comparison of management strategies.

Future work will be needed with other virus strains to determine if there is strain-specificity in the resistant soybean lines. These inoculations should include both mechanical and aphid inoculations. Before these soybeans can be released to the public, further testing may be required to further determine the potential for environmental risks.
ACKNOWLEDGMENTS

I would like to thank Dr. John Hill and Dr. Forrest Nutter, Jr., my co-major professors for the tremendous learning experience, as well as their patient guidance. Thanks go to Dr. Xiao-Bing Yang, Dr. Larry Pedigo, and Dr. Gary Munkvold for serving on my Program of Study Committee and for making this research better by their helpful suggestions. I would also like to thank Dr. Alan Eggenberger, Dr. Mohammad Reza Hajimorad, Dave Volkers, and all the members of the Nutter lab for their expert advice and constant friendship.

This research was supported by Hatch Act and State of Iowa funds allocated to the Iowa Agriculture and Home Economics Experiment Station and, in part, by the Iowa Soybean Promotion Board, Pioneer Hi-Bred International, Inc., the Iowa Center for Advanced Technology Development, and the USDA North Central Biotechnology Initiative. Project Nos. 2428 and 3394 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. This work was performed at Ames Laboratory under Contract No. W-7405-Eng-82 with the U.S. Department of Energy. The United States government has assigned the DOE Report number IS-T 2229 to this thesis.