Development of tools for the management of *Soybean vein necrosis orthotospovirus* and *Tobacco streak ilarvirus* in soybean (*Glycine max* (L.) Merr.)

by

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DEDICATION

I dedicate this work to my parents, Rafael Zambrana Negrón and Nilda Echevarría Figueroa, whose sacrifices, love, and encouragement helped me achieve my goals. To my grandmother, Luz Consuelo Figueroa, who always encouraged me to continue studying - I am proud to be your granddaughter and second grandchild to obtain a Ph.D. in STEM. I also dedicate this to Dr. Carlos Marti Figueroa, whose love and support has sustained me throughout these years.

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ABSTRACT

Glycine max (L.) Merr. (soybean) is an important oilseed and cash crop grown for animal feed, vegetable oil, and protein for human consumption. However, viral pathogens have historically contributed to yield and economic losses and affect soybean seed health and quality. *Soybean vein necrosis virus* (Tospoviridae; SVNV) and *Tobacco streak virus* (Bromoviridae; TSV) represent an emerging and a re-emerging pathogen, respectively, of soybean that are transmitted by seed and change the seed's quality. Other transmission pathways for both viruses include thrips (Thysanoptera: Thripidae), where SVNV is transmitted in a persistent, propagative manner by three species of thrips, and TSV is transmitted in a non-specific manner via the physical movement of infected pollen by several species of thrips. Several management strategies exist for controlling plant viruses, such as cultural practices, vector management (e.g., chemical control, epidemiological modelling), and host resistance. The work presented here is aimed at developing tools for the management of SVNV and TSV in soybean.

Management of TSV is challenging due to the multiple modes of transmission, widespread susceptibility of commercial soybean, and lack of reliable diagnostic tests for the virus. Furthermore, symptomatic plants remain unnoticed until the end of the season due to delayed maturity caused by TSV-infection. Two sensitive assays were developed for the detection of TSV: a fluorescent dye-based quantitative RT-PCR assay and a nested RT-PCR. These assays are molecular diagnostic tools for the rapid and accurate detection of TSV that can aid in assessing or establishing management strategies such as monitoring outbreaks, screening soybean cultivars/accessions for resistance to the virus, or cultural practices aimed at reducing spread or removing inoculum in soybean fields.

Another tool to manage plant viruses is host resistance to prevent disease and reduce the initial inoculum in the field. Currently, there are no soybean genotypes known to be resistant to SVNV. Selected soybean genotypes with favorable agronomical characteristics and known resistance or susceptibility to other pathogens were evaluated for their response to SVNV infection (disease) in a controlled growth environment. Evaluations included measuring soybean vein necrosis severity and viral titer after inoculations with SVNV-infected soybean thrips. The genotypes 51-23, 91-39, and SSR 51-70 were categorized resistant and 52-82B, Williams 82, LG94-1906, and Dwight as susceptible to SVNV. The resistant and susceptible genotypes to SVNV identified in this study can be used in future soybean breeding efforts.

Modelling the patterns of insect movement and crop colonization that result in virus transmission can help time management strategies that will mitigate the impact of the pathogen in crop production. Thrips transmit 2% of insect-transmitted plant viruses and have an intimate association with species in the Tospoviridae. Their developmental cycle and feeding behavior are tightly linked to their ability to transmit tospoviruses in a persistent manner. The appearance of symptoms of SVNV in the field has been associated with an increase in thrips flight. A degree-day phenology model and a weather-based logistic regression model were developed using multi-year capture data from Wisconsin fields. The phenology model was used to describe the seasonal trends of thrips that transmit SVNV. The weather-based model was developed to evaluate environmental weather variables to evaluate the effects of such variables on insect populations and flight risks. These models are tools to understand the epidemiologically important vectors of SVNV in the field and can be used to further develop disease and insect management strategies to limit transmission.

CHAPTER 1. Literature Review

Soybean (*Glycine max* (L.) Merr.)

History

Soybean (*Glycine max* (L.) Merr.) is an important oilseed crop worldwide belonging to the *Fabaceae* family. The cultivated soybean was domesticated during the Zhou Dynasty in Northern China from the perennial wild plant *G. soja* (Sieb. & Zucc.), about 3000 years ago around the 11th century B.C. (Anderson *et al.* 2019; Burton 1997; de Oliveira and Arias 2017; Hartman *et al.* 2011). The center of diversity of the crop is located between Southwest and Northeast China near the Yellow River Valley and the Yangtze River Valley (de Oliveira and Arias 2017). After domestication, soybean cultivation spread from the 1st century to the 15th century A.D. into other regions in East Asia including Korea, Japan, Thailand, Northern India, Philippines, and others (Burton 1997; de Oliveira and Arias 2017). In the 18th century, soybean was introduced into the Western Hemisphere first in Europe, where it was used as an ornamental, and then in the United States of America (de Oliveira and Arias 2017; Hartman *et al.* 2011). The first report of soybean grown in the United States of America (USA) was in the state of Georgia in 1765 (Anderson *et al.* 2019; de Oliveira and Arias 2017). The cultivation of soybean in the 20th century in the USA was as a forage crop for grazing animals (Anderson *et al.* 2019; de Oliveira and Arias 2017; Hart 2017).

Production and uses

The production of soybean shifted from forage to an oilseed crop after World War II when the agricultural trade was disrupted and there was pressure for a domestic source of fat and oils (Hart 2017). Soybeans were used for making processed foods, such as margarine, but became of

greater importance after the discovery of soybean meal for animal feed obtained after crushing the seed for oil (Hart 2017; Hartman *et al.* 2011). Introduction of cultivars from other locations led to the establishment of production and breeding research programs. Efforts of these initial programs focused on improving traits derived from its wild ancestor from a viney plant to a bushy and upright row crop, increasing seed size and quality, and flowering time and type (indeterminate or determinate). Maturity groups were developed by breeders for growing soybeans in different zones in the USA, based on photoperiod and seasonal temperatures (Anderson *et al.* 2019).

The majority of soybean production occurred mainly in the southeastern states in the early 20th century (Hart 2017). In 1924 soybean production, planting, and yields were low with 179,200 hectares (ha) planted and 122,388 metric tons (MT) of grain produced, which yielded 739.75 kg/ha. Production slowly increased from the 1920s to 1940s and by 1941 had increased to 4 million ha planted, 2.91 million MT produced and 1,499 kg/ha in yields (USDA NASS 2020). Commercial cultivation of soybean had moved from southeastern states to the upper Midwest (Hart 2017). The growth of the crop peaked in 1979 with 28.5 million ha planted, 61.5 million MT of grain produced, and 2,158 kg/ha in yields (USDA NASS 2020). After a slight decrease, production and planting increased again in the late 1990s and has continued on an upwards trajectory to recent times (Anderson *et al.* 2019; USDA NASS 2020). Currently, production is at 112.5 million MT, 33 million ha planted, and average yields of 3,375 kg/ha (USDA NASS 2021). The United States of America is one of the top two soybean-producing countries accounting for 28% of the worldwide production and soybean is the second largest row crop in the country (FAOSTAT 2019; American Soybean Association 2020; Hart 2017). The North-Central region of the USA produces

approximately 80% of the total production in an area known as the corn-soybean belt (Anderson *et al.* 2019; NOAA 2021).

Soybean is an important cash crop in the USA and North-Central region and a major oilseed crop worldwide. The seed is typically composed of approximately 18% oil and 36% protein and is used as a fresh vegetable, or in processed foods, industrial products, or substitutes for animal meats and dairy (Anderson *et al.* 2019; Hartman et al 2011; Thrane *et al.* 2017). Fifty nine percent of the worldwide oilseed production is from soybean, and 28% of the vegetable oil and 69.3% of protein meal consumed worldwide is derived from soybean (American Soybean Association 2020). In the United States, soybean oil is the top vegetable oil consumed representing 56% of the total (American Soybean Association 2020). Soy oil is used for pharmaceuticals, cosmetics, food products such as margarine, salad dressings, and cooking oils (Hartman *et al.* 2011; American Soybean Association 2020). Oleic acid is a particular desirable fatty acid in soybean oil and beneficial for farmers since they can receive premiums for this oil or from using soybean varieties with higher oleic acid (Kleczewski 2018). Linoleic acid is an omega-3 fatty acid in soybean oil, but it gives foods a rancid flavor (Fehr 2007; Howell and Collins, 1957). Soybean varieties that produce seeds with higher oleic acid and lower linoleic acid are favorable for increased shelf life and human health benefits, and have recently been the focus of soybean breeding programs (Fehr 2007).

Following the removal of oil from the soybean seed, the remaining meal is processed into soy foods for animal feed and human consumption (American Soybean Association 2020). Ninety eight percent of soybean meal is used for animal feed, where 55% is used for poultry, 25% for swine, and to a lesser extent for beef, dairy, and pet food (Hartman *et al.* 2011; American Soybean

Association 2020). Soy protein is considered a high-quality, complete protein as it provides all the essential amino acids necessary for human diets and it is of equal quality to animal protein (Anderson *et al.* 2019; Hartman *et al.* 2011; Thrane *et al.* 2017). High-protein flour obtained from soybean enhances baked products, snack bars, noodles, and the hull is used for fiber breads, cereals, and snacks (Hartman *et al.* 2011; American Soybean Association 2020). Food-grade soybean varieties have been developed by breeding programs, for which farmers receive higher premiums, to use for soy food products such as tofu, soymilk, miso, edamame, etc. (Anderson *et al.* 2019).

Viral diseases

Despite soybean having the highest increases in production area in the last 40 years compared to other major field crops (Hartman *et al.* 2011), diseases are one of the limiting factors to its production, yield, and economic gains. Total yield losses between 2010-2016 were estimated at 58.8 million MT with higher losses in the Northern region (49 million MT) compared to the South (Allen *et al.* 2017). Economic losses in the U.S. due to diseases have been estimated to be up to $95.8 billion dollars with the Northern region having 5 times higher losses than the south (Bandara *et al.* 2020). Viral diseases have contributed a total of 5.1 million MT in yield loss and $1.4 billion dollars in economic losses (Allen *et al.* 2017; Bandara *et al.* 2020; Crop Protection Network, 2019).

Alfalfa mosaic virus (AMV), *Bean pod mottle virus* (BPMV), *Soybean mosaic virus* (SMV), *Tobacco streak virus* (TSV), *Tobacco ringspot virus* (TRSV), and *Soybean vein necrosis virus* (SVNV) are pathogens of soybean that contribute to the yield and economic losses of soybean

and affect soybean seed health and quality (Allen *et al.* 2017; Kopisch-Obuch *et al.* 2008; Wang *et al.* 2005; Wrather *et al.* 2010). Viruses have an effect on seed health when they are transmitted to the plant's progeny (seed-transmission) or are carried within the seed (seed-borne). *Alfalfa mosaic virus*, SMV, TSV, and TRSV (Bowers and Goodman, 1979; Ghanekar and Schwenk, 1974; He *et al.* 2010; Yang and Hamilton, 1974;) are viruses that are seed-transmitted in soybean. BPMV is a seed-borne virus of soybean (Giesler *et al.* 2002) and causes mottling in the seed coat, especially if in a mixed infection with SMV (Hobbs *et al.* 2003). Seed quality refers to the performance potential of a seed lot, and can include germination, vigor, and chemical composition (i.e., protein, oil) for oilseed crops (Ferguson *et al.* 1991). Viruses have been shown to affect seed quality of soybean by changing the chemical composition resulting in higher protein and lower oil (Demski and Jellum, 1975).

Soybean vein necrosis virus

History and host range

Soybean vein necrosis virus (SVNV) is one of the most recently discovered emerging viruses of soybean, originating in the USA. The virus was initially detected in the state of Tennessee in 2008 (Tzanetakis *et al.* 2009), and subsequently in Arkansas, Kansas, Missouri, Illinois, Mississippi, Tennessee, Kentucky in 2009 (Zhou and Tzanetakis, 2013). Following the reports from mostly southern states, the virus was detected in Ohio and New York in 2011 (Bergstrom 2011; Han et al 2013), in 2012 in Wisconsin, Iowa, Michigan, Alabama, and Oklahoma (Ali *et al.* 2013; Conner *et al.* 2013; Jacobs and Chilvers 2013; Smith *et al.* 2013), and in Indiana

in 2013 (Keough 2015). In more recent years, SVNV has been reported in Louisiana (Escalante *et al.* 2018), Pennsylvania (Hameed *et al.* 2008), and Delaware (Kleczewski 2018). Even though the virus is widespread in major soybean-growing regions of the U. S., there are reports of its presence in Canada (Tenuta 2012) and Egypt (Abd El-Wahab and El-Shazly 2017).

SVNV has a narrow host range and most of the reports of its occurrence are in soybean, an economically important host. However, the virus has been reported to infect other legumes such as cowpea (*Vigna unguiculata; V. radiata)* and kudzu (*Pueraria montana*) (Escalante *et al.* 2018; Zhou and Tzanetakis, 2013; Zhou *et al.* 2018). Other hosts include tobacco (*Nicotiana benthamiana; N. tabacum; N. glutinosa*), buckwheat (*Fagopyrum esculentum*), melon (*Cucumis melo*), pumpkin (*Cucurbita pepo*), ivy leaf morning glory (*Ipomoea hedaracea*), and chrysanthemum (*Dendranthema grandiflorum*) (Irizarry *et al.* 2018; Zhou and Tzanetakis, 2013), and others. Ivy leaf morning glory is a weed host that has been found nearby soybean fields and could potentially be a virus reservoir (Zhou and Tzanetakis, 2013; Sikora *et al.* 2018).

Taxonomy and genomics

Soybean vein necrosis virus is a member of the Tospoviridae, in the genus *Orthotospovirus* (Adams *et al.* 2017). The family was formerly the genus tospovirus in the Bunyaviridae, that has recently been designated as the Bunyavirales Order (Adams *et al.* 2017; Rotenberg and Whitfield, 2018). The genome of SVNV, like other orthotospoviruses, is tripartite single-stranded negative sense ribonucleic acid (RNA) (Zhou and Tzanetakis, 2013). Each genomic segment is known as small (S), medium (M), and large (L) according to their nucleotide lengths. The S segment is ambisense and encodes for the nucleoprotein (NP), which binds the genomic RNA segments, and

the non-structural s protein (NSs) (de Haan *et al.* 1990). The NSs is predicted to be suppressor of host defenses (Takeda *et al.* 2002). The M segment encodes for the non-structural m protein (NSm) involved in cell-to-cell movement and interacts with the NP (Leastro *et al.* 2015). The glycoproteins (Gc/Gn) are embedded in the virion's lipid envelope from host-derived membrane (Kikkert *et al.* 1999) and are encoded by the M segment. The Gc/Gn are important for transmission (Nagata *et al.* 2000) and are involved in virus attachment and entry to insect cells (Bandla *et al.* 1997; Han *et al.* 2019; Rotenberg *et al.* 2015;). The larger RNA, the L segment, encodes for the RNA-dependent RNA polymerase that is involved in genome transcription and replication (de Haan *et al.* 1991). The L RNA of SVNV is one of the longest of the genus and has been used to place SVNV in a distinct phylogenetic clade within the family (Zhou *et al*, 2011; Zhou and Tzanetakis, 2019).

Transmission by thrips

Thrips (Thysanoptera: Thripidae) are small arthropods that transmit tospoviruses in a persistent propagative manner (Jones 2005; Rotenberg *et al.* 2015; Rotenberg and Whitfield 2018). To date, three species of thrips have been associated with the horizontal transmission of SVNV: *Neohydatothrips variabilis* (Beach), *Frankliniella tritici* (Fitch), and *Frankliniella fusca* (Hinds) (Keough *et al.* 2016; Zhou and Tzanetakis, 2013). The transmission cycle of tospoviruses is tightly linked with the developmental stage of the thrips that transmit them. In order to be transmitted, it is key for the virus to be acquired by the thrips larva. Once it is acquired, it replicates inside the thrips body and is transstadially passed from the larva to the non-feeding pupa, to the adult. The infected adult then spreads the virus to other hosts when feeding on plant cells (Han *et al.* 2019;

Rotenberg *et al.* 2015; Rotenberg *et al.* 2018). SVNV infection in thrips has been shown to be higher in *Neohydatothrips variabilis*, compared to *F. fusca* and *F. tritici*, in tissues that are important for acquisition and transmission of the virus (Han *et al.* 2019). Transmission rates of SVNV to soybean are also higher in *N. variabilis* than *F. fusca* and *F. tritici* (Keough *et al.* 2016). Therefore, *N. variabilis* is the primary and most efficient vector of SVNV.

Effect on seed health and quality

Another transmission pathway of SVNV occurs vertically through the soybean seed. The virus is the first reported orthotospovirus to be transmitted to the plant's progeny (Groves *et al.* 2016). Affecting seed health, however, is not uncommon for soybean-infecting viruses. *Alfalfa mosaic virus*, SMV, TSV, and TRSV are viruses that also spread through the seed in soybean (Bowers and Goodman 1979; He *et al.* 2010; Ghanekar and Schwenk, 1974; Yang and Hamilton, 1974). In the cases that the virus does not transmit via the seed it can also infect the seed coat as it is the case of BPMV, a seed-borne virus that causes mottling in the soybean seed coat, especially if in a mixed infection with SMV (Hobbs *et al.* 2003; Giesler *et al.* 2002).

The vertical spread of SVNV not only affects seed health but also its quality. Seed quality refers to the performance potential of a seed lot, and can include germination, vigor, and chemical composition (i.e., protein, oil) for oilseed crops (Ferguson *et al.* 1991). Seeds from SVNV-infected plants were reported to have significantly lower oil content, which is negatively correlated with protein content (Anderson *et al.* 2017; Groves *et al.* 2016). Furthermore, significant changes to the fatty acid composition profile were found in seeds from SVNV-infected plants where the oil content had lower oleic acid and higher linoleic acid (Anderson *et al.* 2017). SVNV infection in

soybean threatens the production of a cash crop that is 59% of the worldwide oilseed production and the top vegetable oil consumed in the U.S. (American Soybean Association 2020).

Disease

Soybean vein necrosis symptoms start as vein-associated chlorosis that later become reddish-brown necrotic lesions as they expand through the veins and can continue to progress throughout the season. Symptom appearance varies in the soybean-growing regions, where SVN symptoms typically are observed in the summer months in southern states (Ali and Abdalla 2013; Tzanetakis *et al.* 2019; Zhou and Tzanetakis 2013, 2019) and late summer-early fall months in the northern states (Jacon and Chilvers, 2013; Smith *et al.* 2013). The symptoms are typically observed during the reproductive stages and coincide with an increase in captures of thrips vector populations (Bloomingdale *et al.* 2016; Keough *et al.* 2018; Chitturi *et al.* 2018). In a multi-state field study, no clear effect on yield was observed for SVNV-infected plants (Anderson *et al.* 2017). The virus has been documented to contribute to soybean yield and economic losses along with other soybean-infecting viruses (Allen *et al.* 2017; Bandara *et al.* 2020), but exact percentage of yield reduction and economic losses due to SVNV are unknown.

Tobacco streak virus

History and host range

Tobacco streak virus (TSV) is a pathogen with a worldwide distribution and wide host range. The virus has been reported in North America (Berkeley and Phillips 1943; Fagbenle and Ford 1970), South America (Costa *et al.* 1955; Truol *et al.* 1987), Oceania (Pappu *et al.* 2008;

Sharman *et al.* 2008, 2015), South Africa (Cook *et al.* 1999), Asia (Abtahi and Motlagh 2009; Golnaraghi *et al.* 2007; Prasada Rao *et al.* 2003; Tomaru *et al.* 1985; Vinodkumar *et al.* 2017), and Europe (Brunt 1968; Dijikstra 1983; Moktra *et al.* 2008). In the United States, TSV is widely distributed and has been detected in the Pacific West (Cupertino *et al.* 1984; Kaiser *et al.* 1982; Kong *et al.* 2018), Plains (Sherwood and Jackson 1985; Dutta *et al.* 2015), Midwest (Johnson 1936, 1943; Fagbenle and Ford 1970; Irizarry *et al.* 2016; Melhus 1942; Rabedeaux *et al.* 2005; Wells-Hansen and McManus 2016; Wells-Hansen *et al.* 2016), and Southeast (Bag *et al.* 2019; Padmanabhan *et al.* 2014) regions in a wide variety of hosts. TSV is an understudied and underreported virus of soybean in the USA (Rabedeux *et al.* 2005). Initially reported in soybean fields in Iowa and Ohio (Johnson 1936, 1943; Melhus 1942; Fagbenle and Ford 1970), followed by Oklahoma (Sherwood and Jackson 1985), Illinois (Hobbs *et al.* 2010; Wang *et al.* 2005), and Wisconsin (Rabedeaux *et al.* 2005).

As a generalist pathogen, TSV has a wide host range and infects species of plants in 30 plant families (Padmanabhan *et al.* 2014). Hosts include tobacco (Finlay, 1974), onion (Sivaprasad *et al.* 2010), tomato (Cupertino, *et al.* 1984), quinoa (Kaiser *et al.* 1982), pepper (Gracia & Feldman 1974), cowpea (Vemana & Jain, 2010), groundnut (Cook *et al.* 1999), peas (Vemana *et al.* 2014), chickpea (Sharman *et al.* 2008), asparagus (Brunt and Paludan 1970), dahlia (Brunt 1968; Moktra *et al.* 2008; Pappu *et al.* 2008), cotton (Sharman *et al.* 2008; Vinodkumar *et al.* 2017), sunflower (Dijkstra 1983; Sharman *et al.* 2008; Sharman *et al.* 2015), cranberries (Wells-Hansen and McManus 2016; Wells-Hansen *et al.* 2016), potato (Salazar *et al.* 1981), soybean (Costa *et al.* 1955; Dutta *et al.* 2015; Fagbenle and Ford 1970; Irizarry *et al.* 2018; Kaiser *et al.* 1982), and many others.

Taxonomy and genomics

Tobacco streak virus is the type member of the *Ilarvirus* genus in the Bromoviridae (Bujarski *et al.* 2019; Pallas *et al.* 2013). Ilarviruses are divided into four subgroups based on serological relationships, host range, and sequence similarity and TSV is grouped into subgroup 1 (King *et al.* 2012; Pallas *et al.* 2013). The genome of TSV is composed of three segments of single-stranded, positive sense RNA contained in a non-enveloped quasi-spherical viral particle (Burjarski *et al.* 2019). RNA 1 and RNA 2 encode for the P1 and P2 protein, respectively, which are subunits of the viral RNA polymerase. Both proteins have methyltransferase and helicase domains and need to be associated for the replication activity of the polymerase (Ge *et al.* 1997; Jaspars 1999; Pallas *et al.* 2013; Scott *et al.* 1998; Xin *et al.* 1998). A sub-genomic RNA 4A produces the protein 2b, from RNA 2, and is inferred to be involved in long-distance viral movement and host gene silencing based on similarities and functional homology to the 2b protein of cucumoviruses (Jaspars 1999; King *et al.* 2012; Pallas *et al.* 2013; Xi et al 1998). RNA 3 encodes for the movement protein required for cell-to-cell movement and the coat protein (CP), which is expressed from a sub-genomic RNA 4 that is transcribed from an internal promoter. The CP is involved in genome activation needed to maintain infectivity and in replication (Cornelissen *et al.* 1984; Jaspars 1999; Pallas *et al.* 2013). The *Ilarvirus* CP of ilarviruses has biological equivalence with the CP of AMV- a member of the *Alfamovirus* genus in the same family- and both proteins can be substituted with the other one (van Vloten-Doting 1975).

Transmission

The horizontal and vertical transmission of TSV occurs by pollen, seed, and thrips. Thrips (Thysanoptera: Thripidae) have been associated with the transmission of ilarviruses by physical movement of infected pollen without a virus-vector relationship that is typical of insect-transmitted viruses (Jones 2005). Several species of thrips have been demonstrated to transmit TSV via infected pollen to healthy plants in controlled experiments: *Microcephalothrips abdominalis*, *Thrips tabaci*, *Frankliniella occidentalis* (also a vector of *Tomato spotted wilt virus*), *Thrips parvisvinus*, *Megalurothrips usitatus*, *Frankliniella schultzei,* and *Scirtothrips dorsalis* (Kaiser *et al.* 1982; Klose *et al.* 1996; Prasada Rao *et al.* 2003; Sdoodee and Teakle 1987, 1993; Sharman *et al.* 2015). The vertical transmission of TSV occurs via the seed at rates of 0.7% in cowpea, 28% in *Verbesina encelioides*, 18% in *Ageratum houstonianum*, 48% in *Parthenium hysterophorus*, and up to 90% in soybean depending on the cultivars (Ghanekar and Schwenk 1974; Kaiser *et al.* 1982; Sharma *et al.* 2015). This type of transmission affects seed health and contributes to the initial inoculum in soybean fields (Almeida and Corso 1991). There are no reports of the effect of TSV infection and soybean oil composition, but seed quality is affected. Seeds from infected plants, if the plant produces pods, may appear disheveled and have less seed weight (Rabedeaux *et al.* 2005).

Disease

Infection of TSV in soybean causes bud blight, shepherd's crook, delayed maturity, poor to no pod production, dark green apical leaves with rugosity, necrotic streaks in the stem, stunting, proliferation of leaf and flower buds. Tobacco streak is a mid-season disease, and its highest incidence occurs during the reproductive stages of soybean (Mueller *et al.* 2016; Rabedeaux *et al.* 2005). However, symptomatic plants may be overlooked, and the delayed maturity will cause them to stand out at the end of the growing season in a pattern of random clusters (Ghanekar and

Schwenk 1974; Almeida and Corso 1991). Soybean yield is affected by TSV infection where 10% reductions in grain yield have been reported with high TSV incidence (33%) (Rabedeaux *et al.* 2005) and annual contributions to yield and economic losses have been reported (Allen *et al.* 2017; Bandara *et al.* 2020).

Thrips (Thysanoptera)

Taxonomy and Biology

Thrips are small, elongated insects (0.5-1mm in average length) in the order Thysanoptera ("fringed wings"), with piercing-sucking mouthparts and various feeding habits (Kumar and Onkar, 2021; Lewis 1997b; Stafford *et al.* 2011). The species are further divided taxonomically in two sub-orders, Terebrantia and Tubulifera, that differ in wings structure, ovipositor shape, and larval stages (Mound 1996; Reitz *et al.* 2011). Terebrantia contains 2000 species divided into eight families where Thripidae is one of the largest and most species are considered crop pests. The life cycle of terebrantian thrips takes approximately 20 days to complete, is favored by warm conditions, and consists of an egg, two actively feeding larval stages, two non-feeding pupal stages, and an adult (Mound 1996, 2009; Kumar and Omkar, 2021; Reitz *et al.* 2011). Eggs are inserted into plant tissues after an incision by the adult female's ovipositor (Reitz *et al.* 2011). Following hatching, the larval instars actively feed and then enter the soil for the pupal stages; adult thrips emerge after 1-10 days (Mound 1996, 2009; Kumar and Omkar, 2021; Reitz *et al.* 2011). Adult thrips and larvae exhibit thigmotaxis behavior where they crawl into small spaces in the host plant such as flowers, flower buds, and leaf crevices (Mound 1996; Reitz *et al.* 2011).

Thrips have various feeding strategies where they are polyphagous, or feed on plant tissue (flowers, leaves, pollen), others are mycophagous and feed on hyphae and spores of fungi, or they

can be predatory and feed on other insects (Kirk 1985, 1997b; Kumar and Omkar 2021). The feeding apparatus is composed of a mandibular stylet and two maxillary stylets contained within a mouthcone (Hogenhout *et al.* 2008; Mound 2009; Stafford *et al.* 2012). When feeding, phytophagous thrips use their mandible to pierce the plant tissue. The mandible is subsequently withdrawn, the maxillary stylets are inserted, thrips salivate into the tissue, and the cell contents are emptied (Kirk 1997b; Mound 2009; Stafford *et al.* 2012). Plant tissues appear with silver marks after the rupturing cells and emptying their contents by thrips feeding (Kirk 1997b).

Transmission of tospoviruses

Thrips transmit 2% of insect-transmitted plant viruses and have an intimate association with species in the Tospoviridae. Their developmental cycle and feeding behavior are tightly linked to their ability to transmit tospoviruses in a persistent manner (Jones, 2005; Hogengout *et al.* 2008; Rotenberg *et al.* 2015). The high fecundity, short developmental time, and feeding behavior make the insect an effective viral vector and the ability to reach pest status in some crop systems (Rotenberg and Whitfield 2018; Rotenberg *et al.* 2015; Whitfield *et al.* 2005). Many of thrips that transmit tospoviruses are in the genus *Thrips* and *Frankliniella*, the two largest genera in the Thripidae, and the transmission process has been best described in *Tomato spotted wilt virus* (TSWV), the type member of the family (Jones 2005; Rotenberg and Whitfield, 2018; Rotenberg *et al.* 2015).

The transmission cycle begins after an adult female oviposits in infected plant tissue and the first instar larva, after hatching from the egg, feeds on the infected tissue (Moritz *et al.* 2004; Whitfield and Rotenberg, 2015). The first instar larva has a complete digestive system, and the midgut and salivary glands are fused (Moritz *et al.* 2004). This allows for the tospovirus to be

acquired, infect thrips tissues, replicate and travel from the midgut to the salivary glands. The virus can no longer be acquired after the first instar due to the dissolution and repositioning of tissues that separate the midgut and salivary glands. The thrips body molts, and the virus is transstadially passed through the next developmental stages (Hogenhout *et al.* 2008; Moritz *et al.* 2004; Rotenberg and Whitfield, 2018; Rotenberg *et al.* 2015). The adult stage then inoculates the virus into plant hosts in a process that can last for days to weeks (Han *et al.* 2019; Hogenhout *et al.* 2008; Rotenberg *et al.* 2015).

Feeding on plant cells by the adult thrips is related to tospovirus transmission and occurs when the insect salivates during feeding (Rotenberg and Whitfield 2018; Rotenberg *et al.* 2015; Stafford *et al.* 2011). The behavior is described as 'probing' when the insect inserts the maxillary stylets into the plant tissue and a 'probe' is the time where it inserts and retrieves the stylet (Stafford *et al.* 2011). There are different types of probing during thrips feeding: non-ingestion, short- and long-ingestion. The non-ingestion probes are approximately 1 second long, plant sap is not ingested, and cells are not damaged, but there is salivation during and before the probe (Stafford *et al.* 2011; Stafford *et al.* 2012). Virus delivery into plant cells is thought to occur during salivation of non-ingestion probing and may be important for virus inoculation since it leaves cells intact and available for infection (Rotenberg and Whitfield 2018; Stafford *et al.* 2012). Salivation also occurs in short- and long-ingestion probes where thrips salivate into cells and then extract their contents, but in the latter the feeding is longer and can last approximately an hour (Stafford *et al.* 2011; Stafford *et al.* 2012). Tospovirus-infection in thrips also alters their feeding causing infected females to do more short-ingestion probes and infected males to do more non-ingestion probes (Stafford *et al.* 2011).

Thrips vectors of SVNV

The three species of thrips that transmit SVNV are polyphagous and differ in their transmission efficiencies and life history. Soybean thrips (*Neohydatothrips variabilis* Beach) is a new Thripidae species with the ability to transmit a tospovirus and preferentially feeds on soybean, alfalfa, buckwheat, crimson clover, and red clover (Hesler *et al.* 2018; Irizarry *et al.* 2018). In soybean, they are most likely found feeding on young, upper trifoliates (Irwin *et al.* 1979). Tobacco thrips (*Frankliniella fusca* Hinds) also transmits TSWV, and its hosts include tobacco, tomato, watermelon, cotton, cowpea, maize, crownbeard, wild mustard, and soybean (Jones 2005). Eastern flower thrips (*Frankliniella tritici* Fitch) prefer to feed on the flowers of their plant hosts: cotton, eggplant, tomato, pepper, peach, ornamentals, and weeds such as morning glory, clover, and dandelion (Irwin *et al.* 1979; Sprague *et al.* 2018). Both soybean thrips and eastern flower thrips reproduce in soybeans and are commonly found in soybean fields (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Irwin *et al.* 1979; Keough *et al.* 2018; Reisig *et al.* 2012). The developmental time of eastern flower thrips is four times shorter than soybean thrips and two times shorter than tobacco thrips (Keough *et al.* 2016) and are more abundant in soybean fields (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Keough *et al.* 2018).

Soybean thrips adults have an SVNV transmission efficiency rate of 72%, higher than tobacco (36%) and eastern flower thrips (6%) (Keough *et al.* 2016). The difference in vector competency is due to higher SVNV infection in the midgut and salivary glands of *N. variabilis* and lower infection of *F. fusca* and *F. tritici* (Han *et al.* 2019). SVNV infection on thrips alters their life history and feeding preference traits that may enhance virus transmission. Infected adult soybean thrips prefer to feed on healthy soybean, females produce more offspring, and more first

instars to hatch from leaf tissues (Keough *et al.* 2016). The increase in fecundity and transmission is related to lower SVNV copy number in soybean thrips (Keough *et al.* 2016; Han *et al.* 2019).

Plant virus and vector management

Control approaches

Management of plant viruses and their vectors require a combination of phytosanitary, cultural, biological approaches, genetic/molecular, and chemical means (Bragard *et al.* 2013; Jones 2004, 2006, 2009). Phytosanitary methods against the virus or their vector include certification programs for virus-free propagules (e.g., seeds, vegetative material), roguing of infected plants, sanitation to produce virus-free plants (e.g., thermotherapy, chemotherapy, tissue culture), sterilization of tools, etc. (Jones and Naidu, 2019; Rubio *et al.* 2020). Cultural and physical practices include barrier cropping, light reflective or absorbing plastic to reduce winged or overwintering insects, mulching, plant density, crop rotation, and adjusting planting or harvest dates to avoid high populations of insect vector (Bragard *et al.* 2013; Jones 2009; Jones and Naidu 2019; Hill and Whitham 2014). These measures are low in selectivity and are aimed at reducing the initial inoculum or rate of spread early in the epidemic (Jones 2009; Jones and Naidu 2019; Zitter and Simmons 1980). Detection methods that are quick, sensitive, and reliable are important for the implementation of phytosanitary and cultural methods to avoid introducing virus inoculum and restrict viral spread (Jones 2009; Rubio *et al.* 2020). Biological (e.g., cross protection, biopesticides), chemical, and host resistance approaches decrease virus spread and have higher

selectivity for a particular virus strain or vector depending on the chemistry used (Jones 2009; Jones and Naidu 2019).

Insect-vector control

Chemical control using insecticides are effective measures at reducing insect vector populations, virus transmission and spread depending on the chemistry used (Rubio *et al.* 2020; Zitter and Simmons 1980). The effectiveness depends on the virus-vector pathosystem and transmission type, they are ineffective for non-persistently transmitted viruses and for some persistently transmitted viruses (Jones 2009; Jones and Naidu 2019; Makkouk and Kumari 2000). Many of the insecticides are available as seed treatments and offer protection after emergence (Makkouk and Kumari 2000). Insecticide seed treatments are common in soybean where 51% of USA soybean farmers use them for pest management (Hurley and Mitchell 2016). The drawbacks to the use of insecticides are the development of insecticide-resistant insect vectors if using narrow-spectrum chemistry, off-target effects on beneficial insects when using broad-spectrum chemistries, and altered insect behaviors that may increase transmission (Jones 2009; Jones and Naidu 2019; Zitter and Simmons 1980). The use of insecticides as a control measure has been effective for some soybean-infecting viruses and infective for others (Hill and Whitham 2014). However, insecticides are ineffective at controlling the thrips vectors of tospoviruses and insecticide-resistance has been shown to increase thrips-vector competence and transmission efficiency of TSWV (Bragard *et al.* 2013; Wan *et al.* 2021). Use of chemical control for thrips in soybean is recommended for fields with known disease history and yield loss (Hill and Whitham 2014).

Resistance to viruses

Host resistance is regarded as the most effective and durable strategy for virus disease and vector management and can reduce the spread of the pathogen at any stage in the epidemic (Bragard *et al.* 2013; Jones 2009; Jones and Naidu 2019). Immunization of the host against viral infection can be achieved by breeding, biotechnology, or a combination of both (Rubio *et al.* 2020). There are two types of host resistance against plant viruses based on the source: 1) active or dominant resistance conferred dominant genes, or resistance (R) genes, that encode resistance proteins, 2) passive resistance mediated by recessive genes that encode host proteins that viruses use during the infection and favor viral replication (de Ronde *et al.* 2014; Hashimoto *et al.* 2016; Rubio *et al.* 2020). Within active resistance, there is complete and partial resistance based on the virus ability to establish an infection and move within the host. Complete resistance is when the virus cannot infect the host and in partial resistance the infection can be established but viral accumulation and movement are restricted (Rubio *et al.* 2020). Molecular detection methods, such as quantitative PCR, can be used as tools to evaluate the resistance levels and track the accumulation of the virus in inoculated plants (Gomez *et al.* 2009; Rubio *et al.* 2020)

Genetic resistance can be challenging due to the availability of R genes in wild relatives and resistant cultivars may not yield as highly as their susceptible counterparts (Jones 2009; Jones and Naidu 2019). Furthermore, due to the high selectivity of this approach, new virus strains can arise and break down the resistance (Jones and Naidu 2019; Rubio *et al.* 2020). Using cultivars that are tolerant to a virus (i.e., asymptomatic infection or less severe disease symptoms, no growth or yield reduction) may be an alternative to genetic resistance (Cooper and Jones 1982; Hill and Whitham 2014; Rubio *et al.* 2020). Other alternatives to achieving host resistance to the virus or

vector can be achieved using the RNA-silencing machinery to target either the pathogen or insect, genome-editing, or other biotechnological methods that interrupt viral transmission or immunize the host (Bragard *et al.* 2013; Hill and Whitham 2014; Whitfield and Rotenberg 2015; Rubio *et al.* 2020).

SVNV Management

There are no management strategies or recommendations for SVNV in soybean (Mueller *et al.* 2016; Hill and Whitham 2014). Furthermore, no known sources of resistance are available and there is very little information about cultivars with resistance or tolerance to soybean vein necrosis (Anderson 2017). Management of the vector may be the best strategy to manage the virus until more information about the pathogen and its effect on soybean yield are known (Hill and Whitham 2014). One of the important considerations to take for the control of thrips-transmitted tospoviruses is the timing of arrival of the insect vector, abundance, period of activity and inactivity, and weather conditions during this time (Jones 2004).

Thrips are an early season pest of soybean and the population dynamics and time of activity of the species that transmit SVNV vary throughout the growing season (Hesler *et al.* 2018; Keough *et al.* 2018). Eastern flower thrips, the least efficient at transmitting SVNV, is more abundant than tobacco and soybean thrips (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Irwin *et al.* 1979; Keough *et al.* 2018). The higher abundance of these species may be related to its shorter developmental time (Keough *et al.* 2016). The flight activity of eastern flower thrips shows many dispersal events and peaks in the summer months. Soybean thrips colonize soybeans early in the season but are less abundant than eastern flower thrips. Their flight activity peaks around late

summer early fall (Bloomingdale *et al.* 2016; Keough *et al.* 2018). The flight activity of soybean thrips occurs during the reproductive stages of soybean and has coincided with the observation of SVNV symptoms in the field (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Keough *et al.* 2018). Tobacco thrips is commonly found in the eastern region of the USA and its abundance and flight activity is lower than eastern flower and soybean thrips in soybean fields (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Keough *et al.* 2018; Morsello *et al.* 2008, 2010; Morsello and Kennedy 2009).

Epidemiological information about the virus and its thrips vector, life cycle, hosts, effects on population by environmental factors are important for selection of control measures (Jones 2004). Models that incorporate ambient temperature and the thermal requirements (degree-days) of the insect can be used to describe the phenology of insects that transmit plant pathogens (Jarošík *et al.* 2011; Kirk 1997a). Insect phenology models derive information from degree-day accumulations in the growing season and are utilized to describe seasonal population dynamics, predict insect occurrence and abundance, shifts in phenology, and identify critical times of insect development and risk (Frost *et al.* 2013a; Frost *et al.* 2013b; Kamiyama *et al.* 2020; Hodgson *et al.* 2011; Mueller *et al.* 2010; Olatinwo and Hoogenboom 2014). These models can be used as tools to guide control approaches such as scouting and chemical applications (Damos and Savopoulou-soultani 2010; Nietschke *et al.* 2007).

Other epidemiological information that can be used to guide control measures are environmental weather variables to evaluate the effects of such variables on insect pressure and/or disease risk (De Wolf et al 2003; Olatinwo and Hoogenboom 2014; Willbur et al 2018). Environmental variables such as temperature and rain have been found to affect the population

dynamics of thrips. Temperature can promote the development of thrips and flight take-off, leading to higher populations and earlier peaks in flight activity while decreasing the availability of plant hosts (Keough *et al.* 2018; Kirk 1997a; Morsello *et al.* 2008; Morsello and Kennedy, 2009; Morsello *et al.* 2010; Lewis, 1997; Stacey and Fellowes, 2002). Rain has the opposite effect on thrips individuals and populations where it increases mortality of the instar larvae and hinders adult flights by saturating their fringed wings (Lewis 1997; Kirk, 1997a; Morsello *et al.* 2008; Morsello and Kennedy, 2009). Weather-based models use data such as temperature, rain, humidity, and other variables to inform integrated pest management strategies such as selection of varieties, and planting dates to avoid insect pressure, and chemical applications (Olantiwo and Hoogenboom 2014). These models can also help determine the weather variables influencing insect population dynamics as it relates to the pathogen they transmit (Keough *et al.* 2016; Morsello *et al.* 2008; 2010).

Literature Cited

Abd El-Wahab, A. S., and El-Shazly, M. A. 2017. Identification and characterization of soybean vein necrosis virus (SVNV): a newly isolated thrips-borne tospovirus in Egypt. J. Virol. Sci. 1:76–90.

Abtahi, F. S., and Motlagh, M. K. 2009. Tobacco streak virus Isolated from Lettuce. Pakistan J. Biol. Sci. 12:707–711

Adams, M. J., Lefkowitz, E. J., King, A. M. Q., Harrach, B., Harrison, R. L., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Mushegian, A. R., Nibert, M., Sabanadzovic, S., Sanfaçon, H., Siddell, S. G., Simmonds, P., Varsani, A., Zerbini, F. M., Gorbalenya, A. E., and Davison, A. J. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). Arch. Virol. 162:2505–2538

Ali, A., and Abdalla, O. A. 2013. First Report of Soybean vein necrosis virus in Soybean Fields of Oklahoma. Plant Dis. 97:1664

Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., *et al.* 2017. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Heal. Prog. 18:19–27

Almeida, Ál. M. R., and Corso, I. C. 1991. Effect of Sowing Time on the Incidence of Bud Blight in Soybean (Glycine max L. Merr.). J. Phytopathol. 132:251–257.

American Soybean Association. 2020. Soystats 2020: A Reference Guide to Important Soybean Facts & Figures. Available at: http://soystats.com/wp-content/uploads/SoyStats2020_Web.pdf.

Anderson, E. J., Ali, M. L., Beavis, W. D., Chen, P., Clemente, T. E., Diers, B. W., Graef, G. L., Grassini, P., Hyten, D. L., McHale, L. K., Nelson, R. L., Parrott, W. A., Patil, G. B., Stupar, R. M., and Tilmon, K. J. 2019. Soybean [Glycine max (L.) Merr.] Breeding: History, Improvement, Production and Future Opportunities. In Advances in Plant Breeding Strategies: Legumes, Cham: Springer International Publishing, 7, p. 431–516.

Anderson, N. R. 2017. Effect of Soybean Vein Necrosis on Soybean Yield and Seed Quality, and Symptom Expression on Soybean and Alternative Hosts. [Master's Thesis], Purdue University, ProQuest Dissertations Publishing, 2017. 10604251

Anderson, N. R., Irizarry, M. D., Bloomingdale, C. A., Smith, D. L., Bradley, C. A., Delaney, D. P., Kleczewski, N. M., Sikora, E. J., Mueller, D. S., and Wise, K. A. 2017. Effect of soybean vein necrosis on yield and seed quality of soybean. Can. J. Plant Pathol. 39:334–341.

Bag, S., Tabassum, A., Brock, J., and Dutta, B. 2019. First Report of Tobacco Streak Virus Infecting Summer Squash in Georgia, U.S.A. Plant Dis. 103:1442

Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., and Esker, P. D. 2020. Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS One. 15:e0231141

Bandla, M. D., Campbell, L. R., Ullman, D. E., and Sherwood, J. L. 1998. Interaction of Tomato Spotted Wilt Tospovirus (TSWV) Glycoproteins with a Thrips Midgut Protein, a Potential Cellular Receptor for TSWV. Phytopathology. 88:98–104.

Bergstrom, G. 2011. A New Disease in New York: Soybean Vein Necrosis Virus (SVNV). Cornell F. Crop. Available at: https://blogs.cornell.edu/ccefieldcropnews/2012/03/05/a-new-disease-in-new-york-soybean-vein-necrosis-virus-svnv/.

Berkeley, G. H., and Phillips, J. H. H. 1943. TOBACCO STREAK. Can. J. Res. 21c:181–190

Bloomingdale, C., Irizarry, M. D., Groves, R. L., Mueller, D. S., and Smith, D. L. 2016. Seasonal Population Dynamics of Thrips (Thysanoptera) in Wisconsin and Iowa Soybean Fields. J. Econ. Entomol. 110(1): 133-141

Bowers, Jr., G. R., and Goodman, R. M. 1979. Soybean Mosaic Virus: Infection of Soybean Seed Parts and Seed Transmission. Phytopathology. 69:569–572.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., Macfarlane, S., Peters, D., Susi, P., and Torrance, L. 2013. Status and prospects of plant virus control through interference with vector transmission. Annu. Rev. Phytopathol. 51:177–201.

Brunt, A. A. 1968. Tobacco Streak Virus in Dahlias. Plant Pathol. 17:119–122

Brunt, A. A., and Paludan, N. 1970. The serological relationship between "Asparagus stunt" and Tobacco streak viruses. Phytopathol. Zeitschrift. 69:277–282

Bujarski, J., Gallitelli, D., García-Arenal, F., Pallás, V., Palukaitis, P., Reddy, M. K., and Wang, A. 2019. ICTV Virus Taxonomy Profile: Bromoviridae. J. Gen. Virol. 100:1206–1207.

Burton, J. W. 1997. Soyabean (Glycine max (L.) Merr.). F. Crop. Res. 53:171–186.

Chitturi, A., Conner, K., Sikora, E. J., and Jacobson, A. L. 2018. Monitoring Seasonal Distribution of Thrips Vectors of Soybean Vein Necrosis Virus in Alabama Soybeans. J. Econ. Entomol. 111:2562–2569

Conner, K., Sikora, E. J., Zhang, L., and Burmester, C. 2013. First Report of Soybean vein necrosis-associated virus Affecting Soybeans in Alabama. Plant Heal. Prog. 14:49.

Cook, G., De Miranda, J. R., Roossinck, M., and Pietersen, G. 199AD. Tobacco streak ilavirus detected on groundnut in South Africa. African Plant Prot. 5:13–19

Cooper, J. I., and Jones, A. T. 1983. Responses of Plants to Viruses: Proposals for the Use of Terms. Phytopathology. 73:127–128.

Cornelissen, B. J. C., Janssen, H., Zuidema, D., and Bol, J. F. 1984. Complete nucleotide sequence of tobacco streak virus RNA 3. Nucleic Acids Res. 12:2427–2437

Costa, A. S., Miyasaka, S., and Pinto, A. J. D. 1955. Queima dos brotos da soja, uma moléstia causada pelo vírus da necrose branca ou couve. Bragantia. 14: VII–X.

Crop Protection Network. 2019. Soybean Disease Loss Calculator. Available at: https://loss.cropprotectionnetwork.org/crops/soybeans.

Cupertino, F. P., Grogan, R. G., and Petersen, L. J. 1984. Tobacco streak virus of tomato and some natural weed hosts in California. Plant Dis. 68:331–333.

Damos, P. T., and Savopoulou-Soultani, M. 2010. Development and statistical evaluation of models in forecasting moth phenology of major lepidopterous peach pest complex for Integrated Pest Management programs. Crop Prot. 29:1190–1199

Demski, J. W., and Jellum, M. D. 1975. Single and Double Virus Infection of Soybean: Plant Characteristics and Chemical Composition. Phytopathology. 65:1154–1156

Dijkstra, J. 1983. Tobacco streak virus in sunflower (Helianthus annuus). Netherlands J. Plant Pathol. 89:153–169.

Dutta, M., Ali, A., and Melcher, U. 2015. Detection, discrimination and discovery of a new Tobacco streak virus strain. J. Virol. Methods. 221:15–21.

Escalante, C., Bollich, P., and Valverde, R. A. 2018. Soybean Vein Necrosis Virus Naturally Infecting Yard-Long Bean (Vigna unguiculata ssp. sesquipedalis) and Soybean (Glycine max) in Louisiana. Plant Dis. 102:2047–2047

Fagbenle, H. H., and Ford, R. E. 1970. Tobacco Streak Virus Isolated from Soybeans, Glycine max. Phytopathology. 60:814

Fehr, W. R. 2007. Breeding for modified fatty acid composition in soybean. Crop Sci. 47:S-72-S-87

Ferguson, J. M., Keys, R. D., McLaughlin, F. W., and Warren, J. M. 1991. Seed and Seed Quality. NC State Ext. Publ. :1–28 Available at: https://content.ces.ncsu.edu/seed-and-seed-quality.

Finlay, J. R. 1974. Tobacco streak virus in tobacco. Australas. Plant Dis. 3(4), 71-71.

Food and Agriculture Organization of the United Nations. 2019. FAOSTAT statistics database. Available at: http://www.fao.org/faostat/en/#rankings/countries_by_commodity.

Frost, K. E., Esker, P. D., Van Haren, R., Kotolski, L., and Groves, R. L. 2013a. Factors Influencing Aster Leafhopper (Hemiptera: Cicadellidae) Abundance and Aster Yellows Phytoplasma Infectivity in Wisconsin Carrot Fields. Environ. Entomol. 42:477–490

Frost, K. E., Esker, P. D., Van Haren, R., Kotolski, L., and Groves, R. L. 2013b. Seasonal Patterns of Aster Leafhopper (Hemiptera: Cicadellidae) Abundance and Aster Yellows Phytoplasma Infectivity in Wisconsin Carrot Fields. Environ. Entomol. 42:491–502

Ge, X., Scott, S. W., and Zimmerman, M. T. 1997. The complete sequence of the genomic RNAs of spinach latent virus. Arch. Virol. 142:1213–1226.

Ghanekar, A. M., and Schwenk, F. W. 1974. Seed Transmission and Distribution of Tobacco Streak Virus in Six Cultivars of Soybeans. Phytopathology. 64:112

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. Am. Phytopathol. Soc. 86:1280–1289.

Golnaraghi, A. R., Shahraeen, N., Pourrahim, R., Farzadfar, S., and Ghasemi, A. 2004. Occurrence and relative incidence of viruses infecting soybeans in Iran. Plant Dis. 88:1069–1074

Gómez, P., Rodríguez-Hernández, A. M., Moury, B., and Aranda, M. 2009. Genetic resistance for the sustainable control of plant virus diseases: Breeding, mechanisms and durability. Eur. J. Plant Pathol. 125:1–22.

Gracia, O., and Feldman, J. M. 1974. Tobacco Streak Virus in Pepper. J. Phytopathol. 80:313–323

Groves, C., German, T., Dasgupta, R., Mueller, D., and Smith, D. L. 2016. Seed Transmission of Soybean vein necrosis virus: The First Tospovirus Implicated in Seed Transmission. PLoS One. 11:1–14

de Haan, P., Wagemakers, L., Peters, D., and Goldbach, R. 1990. The S RNA Segment of Tomato Spotted Wilt Virus has an Ambisense Character. J. Gen. Virol. 71:1001–1007

Hameed, A., Rosa, C., and Rajotte, E. 2018. Distribution of Soybean vein necrosis virus in Pennsylvania and its impact on plant growth. PA Soybean Board Final Term Report. Available at: https://pasoybean.org/wp-content/uploads/2018/03/Distribution-of-soybean-vein-necrosis-virus-in-Pennsylvania-and-its-impact-on-plant-growth_2018.pdf

Han, J., Domier, L. L., Dorrance, A. E., and Qu, F. 2013. First Report of Soybean vein necrosis-associated virus in Ohio Soybean Fields. Plant Dis. 97:693

Han, J., Nalam, V. J., Yu, I.-C., and Nachappa, P. 2019. Vector Competence of Thrips Species to Transmit Soybean Vein Necrosis Virus. Front. Microbiol. 10:431

Hart, C. 2017. The Economic Evolution of the Soybean Industry. In The Soybean Genome, eds. Henry T Nguyen and Madan Kumar Bhattacharyya. Cham: Springer International Publishing, p. 1–9.

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. Food Secur. 3:5–17

Hashimoto, M., Neriya, Y., Yamaji, Y., and Namba, S. 2016. Recessive Resistance to Plant Viruses: Potential Resistance Genes Beyond Translation Initiation Factors. Front. Microbiol. 7:1–11

He, B., Fajolu, O. L., Wen, R.-H., and Hajimorad, M. R. 2010. Seed Transmissibility of Alfalfa mosaic virus in Soybean. Plant Heal. Prog. 11:41.

Hesler, L. S., Allen, K. C., Luttrell, R. G., Sappington, T. W., and Papiernik, S. K. 2018. Early-season pests of soybean in the United States and factors that affect their risk of infestation. J. Integr. Pest Manag. 9:1.

Hill, J. H., and Whitham, S. A. 2014. Control of Virus Diseases in Soybeans. In Advances in Virus Research, eds. Gad Loebenstein and Nikolaos Katis. Academic Press, 90, p. 355–390.

Hobbs, H. A., Herman, T. K., Slaminko, T. L., Wang, Y., Nguyen, B. T., McCoppin, N. K., Domier, L. L., and Hartman, G. L. 2010. Occurrences of Soybean Viruses, Fungal Diseases, and Pests in Illinois Soybean Rust Sentinel Plots. Plant Heal. Prog. 11:44

Hobbs, H. A., Sciences, C., Hartman, G. L., States, U., Wang, Y., Hill, C. B., Bernard, R. L., Pedersen, W. L., and Domier, L. L. 2003. Occurrence of Seed Coat Mottling in Soybean Plants Inoculated with Bean pod mottle virus and Soybean mosaic virus. Plant Dis. :1333–1336.

Hodgson, J. A., Thomas, C. D., Oliver, T. H., Anderson, B. J., Brereton, T. M., and Crone, E. E. 2011. Predicting insect phenology across space and time. Glob. Chang. Biol. 17:1289–1300.

Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., and Redinbaugh, M. G. 2008. Insect Vector Interactions with Persistently Transmitted Viruses. Annu. Rev. Phytopathol. 46:327–359.

Howell, R. W., and Collins, F. I. 1957. Factors Affecting Linolenic and Linoleic Acid Content of Soybean Oil 1. Agron. J. 49:593–597

Hurley, T., and Mitchell, P. 2017. Value of neonicotinoid seed treatments to US soybean farmers. Pest Manag. Sci. 73:102–112

Irizarry, M. D., Elmore, M. G., Batzer, J. C., Whitham, S. A., and Mueller, D. S. 2018. Alternative Hosts for Soybean vein necrosis virus and Feeding Preferences of Its Vector Soybean Thrips. Plant Heal. Prog. 19:176–181

Irizarry, M. D., Pathology, P., Groves, C. L., and Pathology, P. 2016. Re-emergence of Tobacco streak virus infecting Soybean in the United States and Canada. Plant Heal. Prog. 17:92–94.

Irwin, M. E., Yeargan, K. V., and Marston, N. L. 1979. Spatial and Seasonal Patterns of Phytophagous Thrips 1 in Soybean Fields with Comments on Sampling Techniques 2. Environ. Entomol. 8:131–140 Available at: https://academic.oup.com/ee/article-lookup/doi/10.1093/ee/8.1.131.

Jacobs, J. L., and Chilvers, M. I. 2013. First Report of Soybean vein necrosis virus on Soybeans in Michigan. Plant Dis. 97:1387

Jarošík, V., Honěk, A., Magarey, R. D., and Skuhrovec, J. 2011. Developmental Database for Phenology Models: Related Insect and Mite Species Have Similar Thermal Requirements. J. Econ. Entomol. 104:1870–1876.

Jaspars, E. M. J. 1999. Genome activation in alfamo- and ilarviruses. Arch. Virol. 144:843–863.

Johnson, F. 1943. Soybean streak in Ohio. Plant Dis. Report. 27:86–87.
Johnson, J. 1936. Tobacco streak, a virus disease. Phytopathology. 26:285–292.
Jones, D. R. 2005. Plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113:119–157.
Jones, R. A. C. 2006. Control of Plant Virus Diseases. Adv. Virus Res. 67:205–244.
Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Res. 141:113–130.
Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res. 100:5–30.
Jones, R. A. C., and Naidu, R. A. 2019. Global Dimensions of Plant Virus Diseases: Current Status and Future Perspectives. Annu. Rev. Virol. 6:387–409.
Kaiser, W. J., Wyatt, S. D., and Pesho, G. R. 1982. Natural hosts and vectors of Tobacco streak virus in Eastern Washington. Phytopathology. 72:1508–1512.
Kamiyama, M. T., Bradford, B. Z., Groves, R. L., and Guédot, C. 2020. Degree day models to forecast the seasonal phenology of Drosophila suzukii in tart cherry orchards in the Midwest U.S. ed. Antonio Biondi. PLoS One. 15:e0227726
Keough, S. A. 2015. Surveying Indiana Soybean for Soybean vein necrosis virus (SVNV) and Analysis of SVNV on Life History Traits and Host Preference of Thrips Vectors.
Keough, S., Danielson, J., Marshall, J. M., Lagos-Kutz, D., Voegtlin, D. J., Srinivasan, R., and Nachappa, P. 2018. Factors Affecting Population Dynamics of Thrips Vectors of Soybean vein necrosis virus. Environ. Entomol. 20:1–7.
Keough, S., Han, J., Shuman, T., Wise, K., and Nachappa, P. 2016. Effects of Soybean Vein Necrosis Virus on Life History and Host Preference of Its Vector, Neohydatothrips variabilis, and Evaluation of Vector Status of Frankliniella tritici and Frankliniella fusca. J. Econ. Entomol. 109:1979–1987.
Kikkert, M., Van Lent, J., Storms, M., Bodegom, P., Kormelink, R., and Goldbach, R. 1999. Tomato spotted wilt virus particle morphogenesis in plant cells. J. Virol. 73:2288–97
King, A. M. Q., Adams, M. J., Carstens, E. B., and Lefkowitz, E. J. 2012. Bromoviridae. In Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, eds. Andrew M.Q. King, M. J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz. Elsevier, p. 965–976.
Kirk, W. D. J. 1997a. Distribution, Abundance, and Population Dynamics. In Thrips as Crop Pests, ed. Trevor Lewis. CAB International, p. 217–258.
Kirk, W. D. J. 1997b. Feeding. In Thrips as Crop Pests, ed. Trevor Lewis. CAB International, p. 119–174.
Kirk, W. D. J. 1985. Pollen-feeding and the host specificity and fecundity of flower thrips (Thysanoptera). Ecol. Entomol. 10:281–289.
Kleczewski, N. M. 2018. Prevalence and cropping system impacts on soybean vein necrosis disease in Delaware soybeans. Plant Heal. Prog. 19:11–12.
Klose, M. J., Sdoodee, R., Teakle, D. S., Milne, J. R., Greber, R. S., and Walter, G. H. 1996. Transmission of Three Strains of Tobacco Streak Ilarvirus by Different Thrips Species Using Virus-infected Pollen. J. Phytopathol. 144:281–284
Kong, A., Long, M., Arakaki, A., and Melzer, M. 2018. First Report of Tobacco Streak Virus Infecting Sunn Hemp (Crotalaria juncea) in Hawaii. Plant Dis. 102:2664
Kopisch-Obuch, F. J., Koval, N. C., Mueller, E. M., Paine, C., Grau, C. R., and Diers, B. W. 2008. Inheritance of Resistance to in Soybean PI 153282. Crop Sci. 48:933.

Kumar, B., and Omkar. 2021. Thrips. In Polyphagous Pests of Crops, ed. Omkar. Singapore: Springer Singapore, p. 373–407.

Leastro, M. O., Pallás, V., Resende, R. O., and Sánchez-Navarro, J. A. 2015. The movement proteins (NSm) of distinct tospoviruses peripherally associate with cellular membranes and interact with homologous and heterologous NSm and nucleocapsid proteins. Virology. 478:39–49

Lewis, T. 1997a. Flight and Dispersal. In Thrips as Crop Pests, ed. T. Lewis. CAB international, p. 175–196.

Lewis, T. 1997b. Thrips as Crop Pests. ed. T. Lewis. CAB international.

Makkouk, K. M., and Kumari, S. G. 2001. Reduction of incidence of three persistently transmitted aphid-borne viruses affecting legume crops by seed-treatment with the insecticide imidacloprid (Gaucho®). Crop Prot. 20:433–437.

Melhus, I. E. 1942. Soybean diseases in Iowa in 1942. Plant Dis. Report. 26:431–434.

Mokra, V., Gotzova, B., Bezdekova, V., Dedic, P., and Ptacek, J. 2008. First report of Tobacco streak virus on Dahlia in the Czech Republic. Plant Dis. 92:484

Moritz, G., Kumm, S., and Mound, L. 2004. Tospovirus transmission depends on thrips ontogeny. Virus Res. 100:143–149.

Morsello, S. C., Beaudoin, A. L. P., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2010. The influence of temperature and precipitation on spring dispersal of Frankliniella fusca changes as the season progresses. Entomol. Exp. Appl. 134:260–271.

Morsello, S. C., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2008. Temperature and Precipitation Affect Seasonal Patterns of Dispersing Tobacco Thrips (Frankliniella fusca) and Onion Thrips, (Thrips tabaci) (Thysanoptera: Thripidae) Caught on Sticky Traps. Environ. Entomol. 37:79–86.

Morsello, S. C., and Kennedy, G. G. 2009. Spring temperature and precipitation affect tobacco thrips, Frankliniella fusca, population growth and Tomato spotted wilt virus spread within patches of the winter annual weed Stellaria media. Entomol. Exp. Appl. 130:138–148.

Mound, L. A. 1996. THE THYSANOPTERA VECTOR SPECIES OF TOSPOVIRUSES. Acta Hortic. 431:298–309

Mound, L. A. 2009. Thysanoptera. In Encyclopedia of Insects, eds. V. Resh, R. Cardé. Elsevier Inc., p. 999–1003.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., and Robertson, A. 2016. In A Farmer's Guide to Soybean Diseases. eds. Daren Mueller, Kiersten Wise, Adam Sisson, Damon Smith, Edward Sikora, Carl Bradley, and Alison Robertson. The American Phytopathological Society.

Mueller, E. E., Frost, K. E., Esker, P. D., and Gratton, C. 2010. Seasonal Phenology of Aphis glycines (Hemiptera: Aphididae) and Other Aphid Species in Cultivated Bean and Noncrop Habitats in Wisconsin. J. Econ. Entomol. 103:1670–1681.

Nagata, T., Inoue-Nagata, A. K., Prins, M., Goldbach, R., and Peters, D. 2000. Impeded Thrips Transmission of Defective Tomato spotted wilt virus Isolates. Phytopathology. 90:454–459

National Oceanic and Atmosphere Administration (NOAA) National Centers for Environmental Information (NCEI). 2021. U.S. Agricultural Belts. Geogr. Ref. Maps. Available at: https://www.ncdc.noaa.gov/monitoring-references/maps/us-ag-belts.php#primary-corn-and-soybean.

Nietschke, B. S., Magarey, R. D., Borchert, D. M., Calvin, D. D., and Jones, E. 2007. A developmental database to support insect phenology models. Crop Prot. 26:1444–1448.

Olatinwo, R., and Hoogenboom, G. 2014. Weather-based Pest Forecasting for Efficient Crop Protection. In Integrated Pest Management: Current Concepts and Ecological Perspective, ed. D. Abrol. Elsevier, p. 59–78.

de Oliveira, M. F., and Arias, C. A. A. 2017. Center for Diversity and Genetic Resources. In Soybean Breeding, eds. Felipe Lopes da Silva, Aluízio Borém, Tuneo Sediyama, and Willian Hytalo Ludke. Cham: Springer International Publishing, p. 29–41.

Padmanabhan, C., Gao, S., Li, R., Zhang, S., Fei, Z., and Ling, K. S. 2014. Complete genome sequence of an emerging genotype of tobacco streak virus in the United States. Genome Announc. 2:6–7.

Pallas, V., Aparicio, F., Herranz, M. C., Sanchez-Navarro, J. A., and Scott, S. W. 2013. The Molecular Biology of Ilarviruses. In Advances in Virus Research, eds. K. Maramorosch, F. Murphy. Academic Press, 87, p. 139-181

Pappu, H. R., Hammett, K. R. W., and Druffel, K. L. 2008. Dahlia mosaic virus and Tobacco streak virus in Dahlia (Dahlia variabilis) in New Zealand. Plant Dis. 92:1138

Prasada Rao, R. D. V. J., Reddy, A. S., Reddy, S. V., Thirumala-Devi, K., Chander Rao, S., Manoj Kumar, V., Subramaniam, K., Yellamanda Reddy, T., Nigam, S. N., and Reddy, D. V. R. 2003. The host range of Tobacco streak virus in India and transmission by thrips. Ann. Appl. Biol. 142:365–368.

Rabedeaux, P. F., Gaska, J. M., Kurtzweil, N. C., and Grau, C. R. 2005. Seasonal Progression and Agronomic Impact of Tobacco streak virus on Soybean in Wisconsin. Plant Dis. 89:391–396.

Reisig, D. D., Herbert, D. A., and Malone, S. 2012. Impact of Neonicotinoid Seed Treatments on Thrips (Thysanoptera: Thripidae) and Soybean Yield in Virginia and North Carolina. J. Econ. Entomol. 105:884–889.

de Ronde, D., Butterbach, P., and Kormelink, R. 2014. Dominant resistance against plant viruses. Front. Plant Sci. 5:1–17.

Rotenberg, D., Jacobson, A. L., Schneweis, D. J., and Whitfield, A. E. 2015. Thrips transmission of tospoviruses. Curr. Opin. Virol. 15:80–89

Rotenberg, D., and Whitfield, A. E. 2018. Molecular interactions between tospoviruses and thrips vectors. Curr. Opin. Virol. 33:191–197

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Front. Plant Sci. 11:1–23.

Salazar, L. F., Abad, J. A., and Hooker, W. J. 1981. Host Range and Properties of a Strain of Tobacco Streak Virus from Potatoes. Phytopathology. 72:1550–1554.

Scott, S. W., Zimmerman, M. T., and Ge, X. 1998. The sequence of RNA 1 and RNA 2 of tobacco streak virus: additional evidence for the inclusion of alfalfa mosaic virus in the genus Ilarvirus. Arch. Virol. 143:1187–1198

Sdoodee, R., and Teakle, D. S. 1993. Studies on the mechanism of transmission of pollen-associated tobacco streak ilarvirus virus by Thrips tabaci. Plant Pathol. 42:88–92

Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by Thrips tabaci a new method of plant virus transmission. Plant Pathol. 36:377–380

Sharman, M., Thomas, J. E., and Persley, D. M. 2008. First report of Tobacco streak virus in sunflower (Helianthus annuus), cotton (Gossypium hirsutum), chickpea (Cicer arietinum) and mung bean (Vigna radiata) in Australia. Australas. Plant Dis. Notes. 3:27.

Sharman, M., Thomas, J. E., and Persley, D. M. 2015. Natural host range, thrips and seed transmission of distinct Tobacco streak virus strains in Queensland, Australia. Ann. Appl. Biol. 167:197–207.

Sherwood, J. L., and Jackson, K. E. 1985. Tobacco Streak Virus in Soybean in Oklahoma. Plant Dis. 69:727.

Sikora, E. J., Conner, K. N., and Jacobson, A. L. 2018. Incidence of Soybean vein necrosis virus in Alabama Soybean Fields. Plant Heal. Prog. 19:76–81.

Sivaprasad, Y., Bhaskara Reddy, B. V., Rekha Rani, K., Raja Reddy, K., and Sai Gopal, D. V. R. 2010. First report of Tobacco streak ilarvirus infecting onion (Allium cepa). New Dis. Reports. 22:17

Smith, D. L., Fritz, C., Watson, Q., Willis, D. K., German, T. L., Phibbs, A., Mueller, D., Dittman, J. D., Saalau-Rojas, E., and Whitham, S. A. 2013. First Report of Soybean Vein Necrosis Disease Caused by Soybean vein necrosis-associated virus in Wisconsin and Iowa. Plant Dis. 97:693

Sprague, D., Funderburk, J., and Lucky, A. 2018. Flower Thrips, Frankliniella tritici (Fitch) (Insecta: Thysanoptera: Thripidae). EENY-720. Available at: https://edis.ifas.ufl.edu/in1236.

Stafford, C. A., Walker, G. P., and Ullman, D. E. 2012. Hitching a ride. Vector feeding and virus transmission. Commun. Integr. Biol. 5:1:43–49.

Stafford, C. A., Walker, G. P., and Ullman, D. E. 2011. Infection with a plant virus modifies vector feeding behavior. Proc. Natl. Acad. Sci. U. S. A. 108:9350–9355.

Stuart, R. R., GAO, Y., and LEI, Z. 2011. Thrips: Pests of Concern to China and the United States. Agric. Sci. China. 10:867–892

Takeda, A., Sugiyama, K., Nagano, H., Mori, M., Kaido, M., Mise, K., Tsuda, S., and Okuno, T. 2002. Identification of a novel RNA silencing suppressor, NSs protein of Tomato spotted wilt virus. FEBS Lett. 532:75–79

Tenuta, A. U. 2012. First Confirmation of Soybean Vein Necrosis Virus in Ontario. OMAFRA F. Crop News. Available at: https://fieldcropnews.com/2012/09/first-confirmation-of-soybean-vein-necrosis-virus-in-ontario/.

Thrane, M., Paulsen, P. V., Orcutt, M. W., and Krieger, T. M. 2017. Soy Protein: Impacts, Production, and Applications. In Sustainable Protein Sources, eds. S.R. Nadathur, J. P. D. Wanasundar, L. Scanlin. Academic Press, p. 23-45

TOMARU, K., SAWA, Y., KUBO, S., KUWATA, S., and SUZUKI, I. 1985. Tobacco streak virus isolated from tobacco plants in Japan. Japanese J. Phytopathol. 51:486–489.

Truol, G. A., Laguna, I. G., and Nome, S. F. 1987. Detección del tobacco streak virus (TSV) en cultivos de soja en Argentina. Fitopatología. 22:15–20

Tzanetakis, I. E., We, R., Newman, M., and Hajimorad, R. 2009. Soybean vein necrosis virus: A new threat to soybean production in Southeastern United States? Phytopathology. 99:S131

United States Department of Agriculture National Agricultural Statistics Service. 2020. Crop Production - Historical Track Record. Available at: https://usda.library.cornell.edu/concern/publications/c534fn92g?locale=en.

United States Department of Agriculture National Agricultural Statistics Service. 2021. Crop Production 2020 Summary. Available at: https://usda.library.cornell.edu/concern/publications/tm70mv177.

Vemana, K., Goud, T. E. S., Reddy, D. L., Venkateswarlu, N. C., Naik, K. S. S., Kumar, D. S., Padma latha, Y., and Desai, S. 2014. First Report of Tobacco streak virus Infecting Pigeon Pea (Cajanus cajan) in India. Plant Dis. 98:287–287

Vemana, K., and Jain, R. K. 2010. New Experimental Hosts of Tobacco streak virus and Absence of True Seed Transmission in Leguminous Hosts. Indian J. Virol. 21:117–127.

Vinodkumar, S., Nakkeeran, S., Malathi, V. G., Karthikeyan, G., Amala Balu, P., Mohankumar, S., and Renukadevi, P. 2017. Tobacco streak virus: an emerging threat to cotton cultivation in India. Phytoparasitica. 45:729–743

van Vloten-doting, L. 1975. Coat protein is required for infectivity of tobacco streak virus: Biological equivalence of the coat proteins of tobacco streak and alfalfa mosaic viruses. Virology. 65:215–225

Wan, Y., Zheng, X., Xu, B., Xie, W., Wang, S., Zhang, Y., Zhou, X., and Wu, Q. 2021. Insecticide resistance increases the vector competence: a case study in Frankliniella occidentalis. J. Pest Sci. (2004). 94:83–91

Wang, Y., Hobbs, H. A., Hill, C. B., Domier, L. L., Hartman, G. L., and Nelson, R. L. 2005. Evaluation of ancestral lines of U.S. soybean cultivars for resistance to four soybean viruses. Crop Sci. 45:639–644.

Wells-Hansen, L. D., and McManus, P. S. 2016. Recovery of cranberry plants infected with tobacco streak virus and incidence and distribution of TSV in the field. Plant Dis. 100:2257–2265.

Wells-Hansen, L. D., Polashock, J. J., Vorsa, N., Lockhart, B. E. L., and McManus, P. S. 2016. Identification of Tobacco streak virus in Cranberry and the association of TSV with Berry Scarring. Plant Dis. 100:696–703.

Whitfield, A. E., Ullman, D. E., and German, T. L. 2005. Tospovirus-Thrips Interactions. Annu. Rev. Phytopathol. 43:459–489

Willbur, J. F., Fall, M. L., Bloomingdale, C., Byrne, A. M., Chapman, S. A., Isard, S. A., Magarey, R. D., McCaghey, M. M., Mueller, B. D., Russo, J. M., Schlegel, J., Chilvers, M. I., Mueller, D. S., Kabbage, M., and Smith, D. L. 2018. Weather-based models for assessing the risk of sclerotinia sclerotiorum apothecial presence in soybean (Glycine max) fields. Plant Dis. 102:73–84.

De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology. 93:428–435.

Wrather, A., Shannon, G., Balardin, R., Carregal, L., Escobar, R., Gupta, G. K., Ma, Z., Morel, W., Ploper, D., and Tenuta, A. 2010. Effect of diseases on soybean yield in the top eight producing countries in 2006 plant health progress plant health progress. Plant Manag. Netw. :1–8.

Xin, H. W., Ji, L. H., Scott, S. W., Symons, R. H., and Ding, S. W. 1998. Ilarviruses encode a Cucumovirus-like 2b gene that is absent in other genera within the Bromoviridae. J. Virol. 72:6956–9.

Yang, A. F., and Hamilton, R. I. 1974. The mechanism of seed transmission of tobacco ringspot virus in soybean. Virology. 62:26–37.

Zhou, J., Aboughanem-Sabanadzovic, N., Sabanadzovic, S., and Tzanetakis, I. E. 2018. First Report of Soybean Vein Necrosis Virus Infecting Kudzu (Pueraria montana) in the United States of America. Plant Dis. 102:1674

Zhou, J., Kantartzi, S. K., Wen, R. H., Newman, M., Hajimorad, M. R., Rupe, J. C., and Tzanetakis, I. E. 2011. Molecular characterization of a new tospovirus infecting soybean. Virus Genes. 43:289–295.

Zhou, J., and Tzanetakis, I. E. 2013. Epidemiology of soybean vein necrosis-associated virus. Phytopathology. 103:966–971.

Zhou, J., and Tzanetakis, I. E. 2019. Soybean vein necrosis virus: an emerging virus in North America. Virus Genes. 55:12–21

Zitter, T. A., and Simons, J. N. 1980. Management of Viruses by Alteration of Vector Efficiency and by Cultural Practices. Annu. Rev. Phytopathol. 18:289–310.

CHAPTER 2. Sensitive and specific qPCR and nested RT-PCR assays for the detection of *Tobacco streak virus* in soybean

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Abstract

Tobacco streak virus (TSV) is a re-emerging and understudied pathogen of soybean (*Glycine max*). Management of TSV is challenging due to the multiple modes of transmission, widespread susceptibility of commercial soybean, and lack of reliable diagnostic tests for the virus. Soybean plants with TSV-like, virus-like, or no symptoms were collected from commercial and research fields in seven counties in Wisconsin. Two sensitive assays were developed for the detection of TSV: a fluorescent dye-based quantitative RT-PCR (qPCR) assay and a nested RT-PCR (nRT-PCR). *Tobacco streak virus* was detected in 47% and 91% of symptomatic samples using the qPCR assay and the nRT-PCR assay, respectively, suggesting that the nRT-PCR assay has higher sensitivity for detecting TSV. The qPCR assay's limit of detection was determined at 10 fg and the assay was used to estimate the viral load in TSV-symptomatic samples. The titer of TSV in these samples was determined by absolute quantification and ranged from 15 fg to 0.796 ng. The two assays reported here provide diagnostic tools for the rapid and accurate detection of TSV that can aid in monitoring outbreaks, assessing management strategies, or screening soybean cultivars/accessions for resistance to the virus.

Keywords: *Tobacco streak virus*, ilarvirus, soybean, pathogen detection, qPCR, nested RT-PCR

Introduction

Tobacco streak virus (TSV) is the type member of the genus *Ilarvirus* (Bromoviridae) and the causal agent of bud blight in soybean (*Glycine max* L. Merrill) in the United States and Brazil (Costa *et al.* 1955; Fagbenle and Ford 1970). In the United States, TSV was first reported in Iowa and Ohio in soybean plants showing symptoms of stunting, shepherd's crook, delayed maturity, poor pod production, dark green apical leaves with rugosity, necrotic streaks in the stem, and bud blight (Johnson 1936, 1943; Melhus 1942; Fagbenle and Ford 1970). It was subsequently reported in Oklahoma (Sherwood and Jackson 1985), Illinois (Hobbs *et al.* 2010; Wang *et al.* 2005), and Wisconsin (Rabedeaux *et al.* 2005). Sporadic reports of the virus suggest it has not been a widespread problem in commercial soybean fields, or that it has gone underreported. Recently, a re-emergence of TSV has been reported in Oklahoma (Dutta *et al.* 2015), Iowa, and Wisconsin (Irizarry *et al.* 2016).

Tobacco streak virus is an understudied virus in soybean and the agronomic impacts on the crop are not fully understood (Rabedeaux *et al.* 2005). The United States is one of the top three soybean producing countries in the world (FAOSTAT, 2018). In 2019, soybean yields were estimated to be 3.2 metric tons per hectare valued at 31.2 billion US dollars (USDANASS 2019, 2020). However, yield losses due to viral diseases have been estimated to be as high as 891,000 metric tons resulting in 1.7 billion US dollars in economic losses (Allen *et al.* 2017; Bandara *et al.* 2020; Wrather and Koenning 2009). Specifically, TSV has been reported to cause a 10% reduction in yield in artificially inoculated field studies (Rabedeaux *et al.* 2005), and there is widespread susceptibility to TSV in soybean accessions and cultivars (Hobbs *et al.* 2012; Rabedeaux *et al.* 2005; Wang *et al.* 2005). Moreover, infected plants are commonly noticed at the end of the growing season due to delayed maturity caused by TSV infection. The latency in which TSV

symptoms are expressed in soybean is a feature shared with other ilarviruses (Pallas et al 2012, 2013).

The genome of TSV is a tripartite positive sense ssRNA contained in a non-enveloped spherical or quasi-spherical particle (Bujarski *et al.* 2019). RNA 1 and 2 encode the components of the viral replicase, while RNA 3 encodes the movement protein (Ge *et al.* 1997; Pallas *et al.* 2013; Scott *et al.* 1998; Xin *et al.* 1998). A sub-genomic RNA 4 is expressed from RNA 3 to produce the coat protein (CP) (Cornelissen *et al.* 1984), a structural protein that is involved in viral pathogenesis and other biological processes (Bol 1999; van Vloten-doting 1975). *Tobacco streak virus* has a worldwide distribution and a broad host range with species in over 30 families that include weeds, ornamentals, vegetables, fruits, and field crops (Abtahi and Motlagh 2009; Dijkstra 1983; Gracia and Feldman 1974; Kaiser *et al.* 1982; Padmanabhan *et al.* 2014; Vemana and Jain 2010; Vemana *et al.* 2013; Vinodkumar *et al.* 2017; Sharman *et al.* 2008, 2015; Sivaprasad *et al.* 2010; Wells-Hansen *et al.* 2016).

The spread of ilarviruses occurs vertically, via the seed, or horizontally through infected pollen transmitted by insect vectors (Card *et al.* 2007; Mink 1992, 1993). Seed transmission of TSV has been reported in weeds and leguminous hosts (Fagbenle and Ford 1970; Ghanekar and Schwenk 1974; Kaiser *et al.* 1982, 1991). Rates of TSV seed transmission of up to 90% have been reported in soybean and varies depending on the cultivar (Fagbenle and Ford 1970; Ghanekar and Schwenk 1974; Kaiser *et al.* 1982, 1991). Infected seeds contribute to the initial inoculum of the virus in the field and results in random clusters of infected plants (Ghanekar and Schwenk 1974). Another transmission pathway of TSV involves insect vectors where several species of thrips (Thysanoptera: *Thripidae*) have been reported to assist in the transmission of the virus by moving infected pollen (Kaiser *et al.* 1982; Klose *et al.* 1996; Prasada Rao *et al.* 2003; Sharman *et al.*

2015; Sdoodee and Teakle 1987, 1993). A study by Almeida and Corso (1991) found that thrips feeding on pollen from weed reservoirs spread TSV into soybean fields and the spread within fields occurred after an increase in thrips populations. The within-field spread by thrips resulted in a clustered distribution of TSV-infected plants (Almeida and Corso 1991).

Management of viral-induced diseases depends on reliable, sensitive and rapid diagnostic tools (Rubio et al 2020). The various transmission pathways of TSV and the latency of symptom expression in soybean highlights the importance of developing detection methods as diagnostic tools for management of the virus. Serological detection methods, such as enzyme-linked immunosorbent assay and dot-immunobinding assay have been used for detection of TSV (Ali 2017; Sharman *et al.* 2008). However, ilarviruses have been reported to cause disease at low titers (Osman *et al.* 2014; Untiveros *et al.* 2010) and serological tests have failed to reliably detect TSV in plants (Ali 2017). These methods have lower sensitivity and decreased ability to detect low viral titers compared to nucleic acid amplification (Boonham *et al.* 2008; Schneider *et al.* 2004; Untiveros *et al.* 2010). Polymerase chain reaction (PCR) is a highly specific nucleic acid amplification method commonly used in diagnostic tests for ilarviruses (Osman *et al.* 2014; Untiveros *et al.* 2010), with increased sensitivity compared to serological tests (Rubio et al 2020). The objective of this study was to develop a quantitative reverse transcription PCR (RT-PCR) assay and a nested RT-PCR assay for the sensitive and specific detection of TSV in soybean. Both assays were compared as diagnostic tools for TSV and the quantitative RT-PCR (qPCR) assay developed here was compared with a previously published qPCR assay (Dutta et al 2015). The titer of TSV in the study samples was estimated with the qPCR assay.

Materials and Methods

Nested primers targeting the TSV CP gene were designed using the IDT PrimerQuest® Tool (https://www.idtdna.com/Primerquest) and the sequence of a TSV isolate (GenBank Accession No. DQ864458.1). The primers TSVCP12F1 and TSVCP715R1 (Table 2) were used for the first-round PCR. The reaction consisted of 12.5 µL of 2X GoTaq Master Mix, 2.5 µL (10 µM) of each first round primer, and 7.5 µL of cDNA (5 ng / µL). The second round PCR included the primer pair TSVCP39F2 and TSVCP681R2 (Table 2), which are internal to TSVCP12F1/TSVCP715R1. The reaction mixture consisted of 12.5 µL of 2X GoTaq Master Mix, 2.5 µL (10 µM) of each primer, 2.5 µL of nuclease-free water, and 5 µL of the PCR product from the first-round. The following cycling conditions were used in an Eppendorf MasterCycler Pro S programmable thermal cycler for each PCR round: 1 min denaturation at 95 ºC, followed by 40 cycles of 20 seconds denaturation at 95 ºC , 30 seconds annealing at 54 ºC , and 45 seconds elongation at 72 ºC , followed by a final extension step of 10 min at 72 ºC. The PCR products were visualized by electrophoresis in 1X TAE on a 1.2% agarose gel containing SYBR Safe DNA gel stain using a 1 kb DNA ladder to estimate fragment size. The expected product sizes were 700bp in the first round and of 643bp in the second round. The resulting products were Sanger-sequenced with the nested primers. The nucleotide basic local alignment search tool (BLAST) (blast.ncbi.nlm.nih.gov) was used to confirm sequence identity as belonging to TSV (Table AI.2). The samples previously confirmed to be positive for common soybean viruses, described in the previous section, and the healthy soybean control DwNT1 were tested with the nested RT-PCR primers to assess off-target amplification. Selected study samples and the healthy control were subjected to RNA extraction and cDNA synthesis as described above, then tested with RT-PCR and primers for the *Glycine max* elongation factor 1-β (Table 2) to confirm RNA extraction and

cDNA synthesis of the healthy control. The reaction components were used as described previously for first-round RT-PCR. The PCR was performed in an Eppendorf MasterCycler Pro S thermal cycler with the following cycling conditions: 2 min denaturation at 95 ºC, followed by 35 cycles of 1 min denaturation at 94 ºC, 30 seconds annealing at 50 ºC , and 1 min of elongation at 72 ºC , followed by a final extension step of 5 min at 72 ºC.

Comparative qPCR

The qPCR primers TSV1789Fnd and TSV1982Rnd designed by Dutta *et al.* (2015; Table 2), which target the CP gene of TSV, were used to assess the detection of TSV in soybean samples collected in Wisconsin samples and to compare its performance with the PCR tools developed in this study. The PCR was performed in a CFX96 Real-time PCR detection system (Bio-Rad) using a reaction mixture that consisted of 10 µL 2x SsoFast EvaGreen Supermix, 0.8 µL (10µM) of each primer, 4.4 µL of nuclease-free water, and 4 µL of cDNA (5 ng / µL). The cycling conditions consisted of 30 seconds denaturation at 95 ºC for, followed by 35 cycles of 5 seconds denaturation at 95 ºC, 5 seconds annealing/extension at 56 ºC, and a final cycle of a melt curve in 0.5 ºC increments from 55 to 95 ºC.

Results

qPCR assay sensitivity

Serial dilutions (100 to $1.0x10^9$ copies) of the plasmid pGEM-T-TSV were used to determine the sensitivity of the qPCR assay by generating a standard curve tested on three replicate PCR plates. The cloned fragment of the CP gene of TSV was detected at concentrations as low as 1 fg (Figure 1; Table AI.1). However, the limit of detection of the assay (Bustin *et al.* 2009) was set at 10 fg or at a Ct = 32, since the 1 fg samples were detected with higher variability (Figure 1;

Table AI.1). Therefore, 10 fg is the concentration where a reliable detection (> 95%) of the target can be achieved in this qPCR assay (Bustin *et al.* 2009).

qPCR assay validation

The TSVCP_Fw1 and TSVCP_Rv1 primer set was optimized with an annealing/extension temperature gradient, melt curve peak analysis, and a test of efficiency. The optimal annealing/extension temperature was assessed with a gradient of 2 ºC increments. All temperatures tested in the gradient generated amplification curves with Ct's between 10 and 11 and a uniform melt curve with a peak at 81ºC indicating amplification of the correct target (Figure 2). However, 61 ºC was chosen as the optimal annealing and extension temperature as it did not increase the Ct values of pGEM-T-TSV. Furthermore, the 61°C annealing temperature consistently led to higher Ct values or Ct values equal to zero in the nuclease-free water controls (Figure 2A) and no peak in the melt curve analysis (Figure 2B). The efficiency of the reaction was calculated with the slope of the regression line from the 10-fold serial dilutions of pGEM-T-TSV. The average efficiency of three independent replicate PCR plates containing the serial dilutions was 94%, with an R^2=0.998 (Figure1).

qPCR assay specificity

To ensure that the qPCR assay reported here (WI qPCR) did not cross-react with other common viruses found in soybean, samples previously confirmed for SVNV, AMV, and BPMV via RT-PCR were used to determine the specificity of the qPCR assay for TSV. The SVNV-sample (Ar2), AMV-sample (102-8-1), BPMV sample (B1), and the asymptomatic samples had undetermined Ct values or values > 34 (Table 3) and no melt curve peak (Figure 3A). The nuclease-free water (NTC) and no-reverse transcription controls (NRT) reaction controls had undetermined Ct values and did not produce a peak in the melt curve (Table 3, Figure 3A). A healthy soybean

control (DwNT1) was tested with the qPCR assay and yielded undetermined Ct values and no melt curve peak (Figure 3B). The amplification of TSV by the WI qPCR primers TSV_Fw1/Rv1 was confirmed by DNA sequencing and nucleotide BLAST of selected samples. The sequences had 94-97% similarity to TSV isolates from Wisconsin and Iowa (Table AI.2).

Additionally, 34 soybean plants showing TSV-or viral-like symptoms and three asymptomatic plants were tested with the qPCR for the presence of TSV. Sixteen samples yielded Ct values ≤ 32.5 and a melt curve peak of 81ºC (Table 3). These samples are above the limit of detection and were deemed positive for TSV. The samples 201-1, 201-4, SB4, SB7, and W101-1 resulted in Ct values > 33, did not produce a melt curve peak and were deemed negative for TSV (Table 3). TSV titer was measured in the samples using the serial dilutions of pGEM-T-TSV and it ranged from 15 fg to 0.796 ng (Figure 4). The samples W101-2, SB9, and 427 had the lowest titers of the virus (15, 17, and 19 fg, respectively) and were just above the limit of detection (10 fg) of the assay. No concentration of TSV was detected in the samples confirmed for SVNV, AMV, or BPMV. No asymptomatic TSV-infection was found using the WI qPCR assay in the samples without viral symptoms.

Nested RT-PCR

The amplification of the TSV CP by the nested RT-PCR (nRT-PCR) were confirmed by Sanger-sequencing and performing a nucleotide BLAST of the sequences. The sequences generated had 99-100% identity to TSV (Table AI.2). Following the confirmation that the nested primers amplify the TSV CP, the detection of TSV by the nRT-PCR was assessed in the samples from Wisconsin. Samples previously confirmed to be positive for AMV, SVNV and BPMV, respectively, were negative in the nRT-PCR (Table 3). Similar to the WI qPCR assay, the nRT-PCR assay is specific for detecting TSV and does not cross-react with other viruses of soybean.

The nuclease-free water (NTC) controls, the no-reverse transcription (NRT) control, and the healthy soybean control did not produce bands at the expected size of 643-700bp and were negative in the nRT-PCR (Table 3, Figure 3C-D), despite confirmation of RNA quality and cDNA synthesis in these samples (Figure 3E). This assay detected TSV in 31 out of 34 TSV-like or virus-like symptomatic samples, compared to 16 out of 34 with the WI qPCR assay (Table 2). None of the asymptomatic samples were positive with the nRT-PCR.

Comparative qPCR

A comparison of the detection specificity by the WI qPCR and nested RT-PCR assays reported in this study with the set of qPCR primers (Dutta qPCR) reported previously by Dutta *et al.* (2015) was made. The study samples were tested with the Dutta qPCR primers and only 4 out of 34 samples produced Ct values between 28 and 30 and a melt curve peak at 83.5-84ºC (Table 3) and were determined to be TSV-positive based on these parameters. Three of the positive samples (W101-4, D5, and 413) were TSV or virus-symptomatic while the third positive sample (423) was asymptomatic (Table 1). The samples W101-4, D5, and 413 were also positive in the WI qPCR and nRT-PCR, however, the cycle thresholds were similar or higher in the Dutta qPCR compared to the WI qPCR (Table 3). Eight samples with TSV-like or virus-like symptoms that were positive in the WI qPCR, produced Ct values but no melt curve peak using the Dutta qPCR assay and were determined to be TSV-negative in (Table 3). The rest of the study samples had undetermined Ct values and no melt curve peak using the Dutta qPCR assay. Of the asymptomatic samples (Table1), only 423 was positive in the Dutta qPCR and was negative in the WI qPCR and nRT-PCR. The AMV sample, SVNV sample, and BPMV sample were negative using the Dutta qPCR assay, corroborating the specificity of the WI qPCR and nRT-PCR assays (Table 3).

Discussion

Tobacco streak virus is an ilarvirus that affects pod and seed production and plant maturity in soybean. Spread of the virus is aided by its wide host range, thrips-assisted pollen transmission, and seed transmission (Ghanekar and Schwenk 1974; Kaiser *et al.* 1982, 1991; Sdoodee and Teakle 1987, 1993). TSV infection and transmission strategies can have detrimental effects on soybean production in the United States. Although the agronomic impacts of TSV in soybean are not well studied, yield reduction and contribution to annual yield losses have been documented (Bandara *et al.* 2019; Allen *et al.* 2017; Rabedeaux *et al.* 2005; Wrather and Koenning 2009). The latency of symptom expression with TSV infections make scouting and disease management challenging. Reliable detection is an essential tool for the management of plant viruses (Lévesque 2001; Martin *et al.* 2000; Rubio *et al.* 2020). The use of the qPCR and nested RT-PCR assays can aid in the implementation of management strategies such as monitoring the field spread of the TSV, planting resistant cultivars, and rogueing infected plants to prevent potential yield losses and maintain healthy seed production fields. Here we report two sensitive nucleic acid amplification-based assays that target the CP gene of TSV in an effort to expand the molecular diagnostic tools available for detecting TSV in soybean.

The performance of the qPCR assay developed in this study (WI qPCR) was measured by assessing the amplification efficiency, optimum annealing temperature, and melt curve peak analysis. The robustness of the WI qPCR assay was assessed with the amplification efficiency determined from the linear regression of the standard curve. Optimum PCR efficiency ranges from 90-110%, low PCR efficiency (<90%) can result from contamination and high PCR efficiency (>110%) from nonspecific amplification (Bustin *et al.* 2009; Taylor *et al.* 2010). The average efficiency of the WI qPCR assay was 94% which falls within the optimal range and indicates that

the product doubled in each cycle (Bustin *et al.* 2009; Taylor *et al.* 2010). Therefore, the WI qPCR assay is efficient at amplifying the TSV CP gene for isolates examined in this study, in a reproducible manner.

Primer specificity is key to any amplification-based diagnostic test. Increased annealing temperatures have been shown to improve specificity of primers (Korbie and Mattick 2008). This effect was also observed in the WI qPCR assay, where an increased annealing/extension temperature reduced background signal produced from reactions with no-template. The melt curve peak analysis showed that a single product was formed and further confirmed the specificity of the primers. Therefore, increasing the annealing/extension temperature decreased the likelihood of a false-positive detection using this assay without sacrificing its sensitivity. The specificity of the WI qPCR and nested RT-PCR (nRT-PCR) assays to amplify TSV was assessed by Sanger-sequencing the PCR products where the resulting sequences had high similarity to published sequences of the TSV CP gene. Furthermore, a healthy control sample and samples that were previously confirmed to be infected with other viruses of soybean were negative in the WI qPCR and nRT-PCR assays. These results show that both assays described in this study do not cross-react with other common soybean viruses or native soybean genes and are specific for detecting TSV.

With two specific and robust assays to detect TSV in soybean originating in Wisconsin, TSV-like or virus-like symptomatic and asymptomatic samples from around the state were assessed for the presence of TSV. TSV was confirmed in 47% of the samples using the WI qPCR assay. Ninety one percent of symptomatic samples, however, were positive for TSV in the nRT-PCR. The disparity of detection between the assays suggests that the samples that were TSV-negative in the WI qPCR assay could have had lower titers of TSV, as it has been reported for

other ilarviruses (Osman *et al.* 2014; Untiveros *et al.* 2010) and were below the limit of detection. The nesting of the primers in the nRT-PCR increases sensitivity (Goode *et al.* 2002; Shen 2019) and could explain why this assay detected TSV in more samples than the WI qPCR assay. Furthermore, the high annealing temperature of the WI qPCR assay used to improve its accuracy may have decreased its sensitivity, though this was not apparent in our results.

Previously, Dutta *et al.* (2015) reported qPCR primers (Dutta qPCR) for the detection of TSV isolates from Oklahoma that could not be detected with serological tests. Wisconsin soybean samples were tested with the Dutta qPCR primers to compare the assay with the WI qPCR and nRT-PCR assays described in this study. The majority of the samples confirmed TSV-positive either by the WI qPCR or the nRT-PCR were TSV-negative using the Dutta qPCR assay and only three samples were positive in all of the assays. Based on these results, we determined that the Dutta qPCR assay cannot reliably detect the particular genotype of TSV in the samples from Wisconsin. It is possible that the WI qPCR or nRT-PCR assays may not detect TSV in samples from other states as this was not assessed in our study. Interestingly, the asymptomatic sample 423 from Wisconsin was positive only using the Dutta qPCR assay, but not with the WI qPCR or nRT-PCR assay. This finding suggests that sample 423 was an asymptomatic infection of a putatively different strain of TSV than the rest of the samples from Wisconsin. Furthermore, the lack of detection of the majority of samples from Wisconsin using the Dutta qPCR suggests that the differences of results may be due to the genetic differences in the TSV population (e.g., Wisconsin strain(s) vs Oklahoma strain(s)). Although the results from assays with two different chemistries (qPCR vs. nRT-PCR) were compared, we have demonstrated an improved sensitivity for detecting the TSV genotype from Wisconsin with the WI qPCR and nRT-PCR assays.

Molecular diagnostic tools that provide reliable and accurate pathogen detection can aid in disease management decisions and implementation (Lévesque, 2001; Rubio *et al.* 2020). In this study, we developed a qPCR assay and a nRT-PCR assay that are robust and can be used together as valuable tools to detect or quantify TSV. Current soybean disease management recommendations for TSV are to plant disease-free seeds and rogue infected plants (Mueller *et al.* 2016). The nRT-PCR and WI qPCR assay can be used to monitor TSV infection in soybean fields and rogue infected plants to avoid yield loss and maintain seed production fields free from the virus. Another strategy to control viruses is to use resistant cultivars (Rubio et al 2020), however information about resistance to TSV is scarce in soybean germplasm/cultivars (Hobbs *et al.* 2012; Rabedeaux *et al.* 2005; Wang *et al.* 2005). Viral load quantification can provide information about resistance levels in germplasm and cultivars (Rubio et al 2020). The WI qPCR assay can be applied as a screening tool to quantify the titer of TSV in germplasm collections or in breeding programs during the development of soybean cultivars (Rubio et al 2020; Shirima et al 2017). The WI qPCR and nRT-PCR assays from this study are reliable and sensitive diagnostic tools for the detection or quantification of TSV in soybean. Implementation of the assays can help with disease management decisions, assessing virus spread, screening sources of TSV resistant germplasm/cultivars, maintain healthy seed production fields, and provide valuable information to farmers about their potential risk of soybean yield loss due to TSV.

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Literature Cited

Abtahi, F. S., and Motlagh, M. K. 2009. Tobacco streak virus Isolated from Lettuce. Pakistan J. Biol. Sci. 12:707–711.

Ali, A. 2017. Rapid detection of fifteen known soybean viruses by dot-immunobinding assay. J. Virol. Methods. 249:126–129

Allen, T. W., Bradley, C. A., Sisson, A. J, Byamukama, E., Chilvers, M. I., Coker, C. M, Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R, Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M, Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, K. A., and Wrather, J. A. 2017. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Prog. 18: 19–27.

Almeida, Ál. M. R., and Corso, I. C. 1991. Effect of Sowing Time on the Incidence of Bud Blight in Soybean (Glycine max L. Merr.). J. Phytopathol. 132:251–257.

Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., and Esker, P. D. 2020. Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS One. 15:e0231141.

Bol, J. F. 1999. Alfalfa mosaic virus and ilarviruses: involvement of coat protein in multiple steps of the replication cycle. J. Gen. Virol. 80:1089–1102.

Boonham, N., Glover, R., Tomlinson, J., and Mumford, R. 2008. Exploiting generic platform technologies for the detection and identification of plant pathogens. Eur. J. Plant Pathol. 121:355–363.

Bujarski, J., Gallitelli, D., García-Arenal, F., Pallás, V., Palukaitis, P., Reddy, M. K., and Wang, A. 2019. ICTV Virus Taxonomy Profile: Bromoviridae. J. Gen. Virol. 100:1206–1207.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., *et al.* 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin. Chem. 55:611–622

Card, S. D., Pearson, M. N., and Clover, G. R. G. 2007. Plant pathogens transmitted by pollen. Australas. Plant Pathol. 36:455–461.

Cornelissen, B. J. C., Janssen, H., Zuidema, D., and Bol, J. F. 1984. Complete nudeotide sequence of tobacco streak virus RNA 3. Nucleic Acids Res: 12:2427-2437.

Costa, A. S., Miyasaka, S., and Pinto, A. J. D. 1955. Queima dos brotos da soja, uma moléstia causada pelo vírus da necrose branca ou couve. Bragantia. 14:VII–X.

Demski, J. W., and Jellum, M. D. 1975. Single and Double Virus Infection of Soybean: Plant Characteristics and Chemical Composition. Phytopathology. 65:1154-1156.

Dijkstra, J. 1983. Tobacco streak virus in sunflower (Helianthus annuus). Netherlands J. Plant Pathol. 89:153–169.

Dutta, M., Ali, A., and Melcher, U. 2015. Detection, discrimination and discovery of a new Tobacco streak virus strain. J. Virol. Methods. 221:15–21.

Fagbenle, H. H., and Ford, R. E. 1970. Tobacco Streak Virus Isolated from Soybeans, Glycine max. Phytopathology. 60:814-820.

Food and Agriculture Organization of the United Nations. 2018. FAOSTAT. Production: Countries by commodity. Available at:

http://www.fao.org/faostat/en/#rankings/countries_by_commodity.
Ge, X., Scott, S. W., and Zimmerman, M. T. 1997. The complete sequence of the genomic RNAs of spinach latent virus. Arch. Virol. 142:1213–1226.
Ghanekar, A. M., and Schwenk, F. W. 1974. Seed Transmission and Distribution of Tobacco Streak Virus in Six Cultivars of Soybeans. Phytopathology. 64:112
Goode, T., Ho, W.-Z., O'Connor, T., Busteed, S., Douglas, S. D., Shanahan, F., and O'Connell, J. 2002. Nested RT-PCR: Sensitivity Controls are Essential to Determine the Biological Significance of Detected mRNA. In RT-PCR Protocols, ed. Joe O'Connell. New Jersey: Humana Press, p. 065–079.
Gracia, O., and Feldman, J. M. 1974. Tobacco Streak Virus in Pepper. J. Phytopathol. 80:313–323.
Hobbs, H. A., Herman, T. K., Slaminko, T. L., Wang, Y., Nguyen, B. T., McCoppin, N. K., *et al.* 2010. Occurrences of Soybean Viruses, Fungal Diseases, and Pests in Illinois Soybean Rust Sentinel Plots. Plant Heal. Prog. 11:44-47.
Hobbs, H. A., Jossey, S., Wang, Y., Hartman, G. L., and Domier, L. L. 2012. Diverse soybean accessions identified with temperature-sensitive resistance to tobacco streak virus. Crop Sci. 52:738–744.
Irizarry, M. D., Pathology, P., Groves, C. L., and Pathology, P. 2016. Re-emergence of Tobacco streak virus infecting Soybean in the United States and Canada. Plant Heal. Prog. 17:92–94.
Johnson, F. 1943. Soybean streak in Ohio. Plant Dis. Report. 27:86–87.
Johnson, J. 1936. Tobacco streak, a virus disease. Phytopathology. 26:285–292.
Kaiser, W. J., Wyatt, S. D., and Klein, R. E. 1991. Epidemiology and seed transmission of two tobacco streak virus pathotypes associated with seed increases of legume germ plasm in eastern Washington. Plant Dis. 75:258–264.
Kaiser, W. J., Wyatt, S. D., and Pesho, G. R. 1982. Natural hosts and vectors of Tobacco streak virus in Eastern Washington. Phytopathology. 72:1508–1512.
Klose, M. J., Sdoodee, R., Teakle, D. S., Milne, J. R., Greber, R. S., and Walter, G. H. 1996. Transmission of Three Strains of Tobacco Streak Ilarvirus by Different Thrips Species Using Virus-infected Pollen. J. Phytopathol. 144:281–284.
Korbie, D. J., and Mattick, J. S. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nat. Protoc. 3:1452–1456.
Lévesque, C. A. 2001. Molecular methods for detection of plant pathogens-What is the future? Can. J. Plant Pathol. 23:333–336.
Martin, R. R., James, D., and Lévesque, C. A. 2000. Impacts Of Molecular Diagnostic Technologies On Plant Disease Management. Annu. Rev. Phytopathol. 38:207–239.
Melhus, I. E. 1942. Soybean diseases in Iowa in 1942. Plant Dis. Report. 26:431–434.
Mink, G. I. 1992. Ilarvirus Vectors. In Advances in Disease Vector Research, ed. Kerry F Harris. New York, NY: Springer, p. 261–281.
Mink, G. I. 1993. Pollen- and seed-transmitted viruses and viroids. Ann. Appl. Biol. 31:375–402.
Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., Robertson, A. 2016. CHAPTER 4: Foliar Diseases. In A Farmer's Guide to Soybean Diseases, eds. Daren Mueller, Kiersten Wise, Adam Sisson, Damon Smith, Edward Sikora, Carl Bradley, Alison Robertson. St. Paul, MN: APS Press, p. 37–74
Osman, F., Al Rwahnih, M., and Rowhani, A. 2014. IMPROVED DETECTION OF ILARVIRUSES AND NEPOVIRUSES AFFECTING FRUIT TREES USING QUANTITATIVE RT-qPCR. J. Plant Pathol. 96:577-583.

Padmanabhan, C., Gao, S., Li, R., Zhang, S., Fei, Z., and Ling, K. S. 2014. Complete Genome Sequence of an Emerging Genotype Of Tobacco Streak Virus in the United States. Genome Announc. 2:6–7.

Pallas, V., Aparicio, F., Herranz, M. C., Amari, K., Sanchez-Pina, M. A., Myrta, A., Sanchez-Navarro, J. A. 2012. Ilarviruses of Prunus spp.: A continued concern for fruit trees. Phytopathology. 102:1108–1120.

Pallas, V., Aparicio, F., Herranz, M. C., Sanchez-Navarro, J. A., and Scott, S. W. 2013. The Molecular Biology of Ilarviruses. In Advances for Virus Research. eds. Karl Maramorosch, Frederick A. Murphy. Cambridge, MA: Academic Press, p.139-181.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45.

Prasada Rao, R. D. V. J., Reddy, A. S., Reddy, S. V., Thirumala-Devi, K., Chander Rao, S., Manoj Kumar, V., *et al.* 2003. The host range of Tobacco streak virus in India and transmission by thrips. Ann. Appl. Biol. 142:365–368.

Rabedeaux, P. F., Gaska, J. M., Kurtzweil, N. C., and Grau, C. R. 2005. Seasonal Progression and Agronomic Impact of Tobacco streak virus on Soybean in Wisconsin. Plant Dis. 89:391–396.

Rozen, S., and Skaletsky, H. 2000. Primer3 on the WWW for general users and for biologist programmers. In Bioinformatics Methods and Protocols. Methods in Molecular Biology. eds. Misener S., Krawetz S.A. Totowa, NJ: Humana Press, p. 365-386.

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Front. Plant Sci. 11:1–23.

Schneider, W. L., Sherman, D. J., Stone, A. L., Damsteegt, V. D., and Frederick, R. D. 2004. Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription-PCR. J. Virol. Methods. 120:97–105.

Scott, S. W., Zimmerman, M. T., and Ge, X. 1998. The sequence of RNA 1 and RNA 2 of tobacco streak virus: additional evidence for the inclusion of alfalfa mosaic virus in the genus Ilarvirus. Arch. Virol. 143:1187–1198

Sdoodee, R., and Teakle, D. S. 1993. Studies on the mechanism of transmission of pollen-associated tobacco streak ilarvirus virus by Thrips tabaci. Plant Pathol. 42:88–92

Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by Thrips tabach a new method of plant virus transmission. Plant Pathol. 36:377–380

Sharman, M., Thomas, J. E., and Persley, D. M. 2008. First report of Tobacco streak virus in sunflower (Helianthus annuus), cotton (Gossypium hirsutum), chickpea (Cicer arietinum) and mung bean (Vigna radiata) in Australia. Australas. Plant Dis. Notes. 3:27-29.

Sharman, M., Thomas, J. E., and Persley, D. M. 2015. Natural host range, thrips and seed transmission of distinct Tobacco streak virus strains in Queensland, Australia. Ann. Appl. Biol. 167:197–207.

Shen, C.-H. 2019. Amplification of Nucleic Acids. In Diagnostic Molecular Biology, ed. Shen, C.-H. Cambridge, MA: Academic Press, p. 215–247.

Sherwood, J. L., and Jackson, K. E. 1985. Tobacco Streak Virus in Soybean in Oklahoma. Plant Dis. 69:727.

Shirima, R. R., Maeda, D. G., Kanju, E., Ceasar, G., Tibazarwa, F. I., and Legg, J. P. 2017. Absolute quantification of cassava brown streak virus mRNA by real-time qPCR. J. Virol. Methods. 245:5–13.

Sivaprasad, Y., Bhaskara Reddy, B. V., Rekha Rani, K., Raja Reddy, K., and Sai Gopal, D. V. R.

2010. First report of Tobacco streak ilarvirus infecting onion (Allium cepa) . New Dis. Reports. 22:17.

Staroscik, A. 2004. Calculator for determining the number of copies of a template. University of Rhode Island Genomics Sequencing Center. Available at: https://cels.uri.edu/gsc/cndna.html.

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M., and Nguyen, M. 2010. A practical approach to RT-qPCR - Publishing data that conform to the MIQE guidelines. Methods. 50:S1–S5.

United States Department of Agriculture National Agricultural Statistics Service. 2020. Crop Values 2019 Summary. Washington, D. C. ISSN: 1949-0372

United States Department of Agriculture National Agricultural Statistics Service. 2019. Statistics by Subject. Available at: https://www.nass.usda.gov/Statistics_by_Subject/.

Untiveros, M., Perez-Egusquiza, Z., and Clover, G. 2010. PCR assays for the detection of members of the genus Ilarvirus and family Bromoviridae. J. Virol. Methods. 165:97–104

Vemana, K., Goud, T. E. S., Reddy, D. L., Venkateswarlu, N. C., Naik, K. S. S., Kumar, D. S., Padma latha, Y., and Desai, S. 2013. First Report of Tobacco streak virus Infecting Pigeon Pea (Cajanus cajan) in India. Plant Dis. 98:287

Vemana, K., and Jain, R. K. 2010. New Experimental Hosts of Tobacco streak virus and Absence of True Seed Transmission In Leguminous Hosts. Indian J. Virol. 21:117–127.

Vinodkumar, S., Nakkeeran, S., Malathi, V. G., Karthikeyan, G., Amala Balu, P., Mohankumar, S., and Renukadevi, P. 2017. Tobacco streak virus: an emerging threat to cotton cultivation in India. Phytoparasitica. 45:729–743.

van Vloten-Doting, L. 1975. Coat Protein Is Required For Infectivity Of Tobacco Streak Virus: Biological Equivalence Of The Coat Proteins Of Tobacco Streak And Alfalfa Mosaic Viruses. Virology. 65:215–225.

Wang, Y., Hobbs, H. A., Hill, C. B., Domier, L. L., Hartman, G. L., and Nelson, R. L. 2005. Evaluation of Ancestral Lines of U.S. Soybean Cultivars for Resistance to Four Soybean Viruses. Crop Sci. 45:639–644.

Wells-Hansen, L. D., Polashock, J. J., Vorsa, N., Lockhart, B. E. L., and McManus, P. S. 2016. Identification of Tobacco streak virus in Cranberry and the Association of TSV with Berry Scarring. Plant Dis. 100:696–703.

Wrather, A., and Koenning, S. 2009. Effects of Diseases on Soybean Yields in the United States 1996 to 2007. Plant Heal. Prog. 10:1-7.

Xin, H. W., Ji, L. H., Scott, S. W., Symons, R. H., and Ding, S. W. 1998. Ilarviruses Encode a Cucumovirus-Like 2b Gene That Is Absent in Other Genera Within the Bromoviridae. J. Virol. 72:6956–6959.

Xu, H., and Nie, J. 2006. Identification, Characterization, and Molecular Detection of Alfalfa mosaic virus in Potato. Phytopathology. 96:1237–1242

Tables and Figures

Table 2.1 Sample designation, symptom type, location of collection, and year of collection for soybean samples used in the study for detecting TSV with qPCR and nested RT-PCR and comparing the assays.

Sample	Symptoms/Virus	Location[b]	Year
201-1	Virus-like	Columbia	2016
201-2	Virus-like	Columbia	2016
201-3	Virus-like	Columbia	2016
201-4	Virus-like	Columbia	2016
W101-1	Virus-like	Columbia	2016
W101-2	Virus-like	Columbia	2016
W101-3	Virus-like	Columbia	2016
W101-4	Virus-like	Columbia	2016
D1	TSV-like	Columbia	2018
D4	TSV-like	Columbia	2018
D5	TSV-like	Columbia	2018
D6	TSV-like	Columbia	2018
LS1	TSV-like	Rusk	2018
LS2	TSV-like	Rusk	2018
107	Asymptomatic	Chippewa	2019
112	TSV-like	Grant	2019
122	Asymptomatic	Chippewa	2019
215	TSV-like	Chippewa	2019
310	TSV-like	Chippewa	2019
413	TSV-like	Columbia	2019
423	Asymptomatic	Columbia	2019
427	TSV-like	Columbia	2019
319-1	TSV-like	Columbia	2019
SB1	TSV-like	Dodge	2019
SB2	TSV-like	Dodge	2019
SB3	TSV-like	Dodge	2019
SB4	TSV-like	Dodge	2019
SB5	TSV-like	Dodge	2019
SB6	TSV-like	Dodge	2019
SB7	TSV-like	Dodge	2019
SB8	TSV-like	Iowa	2019
SB9	TSV-like	Iowa	2019
W04-1	TSV-like	Dane	2019
W04-2	TSV-like	Dane	2019
W04-3	TSV-like	Dane	2019
W04-4	TSV-like	Dane	2019

[a]County in the state of Wisconsin.

Table 2.2 DNA sequence and thermodynamics of primers used in this study

Primer name	Sequence (5'-3')	Tm[a]	Length[b]	Reference
TSVCP_Fw1	CCAACGACGCAATCCCTTTC	59.8	20	This study
TSVCP_Rv1	GCCCGTTACTCCATCAACCA	60	20	This study
TSVCP39F2	GATCCAAAGTCCAGACCATCCA	56.6	22	This study
TSVCP681R2	AGTCTTGATTCACCAGGAAATCTTC	54.9	25	This study
TSVCP12F1	CGCCATGTCTTCTCGTACTAAC	55.2	22	This study
TSVCP715R1	AAGGGAGCTGGTTTGGATATG	54.8	21	This study
TSV1789Fnd	GCTATCGTCTGCAGCCTCGA	59.3	20	Dutta *et al.* 2015
TSV1982Rnd	CCACATCGCACACAGGAATT	55.8	20	Dutta *et al.* 2015
GmELF1BF	CAACTTGCTCCAAGCTTTCC	54.5	20	A. Ranjan, *unpublished*
GmELF1BR	AGGGTGTCCACGGATACAAG	56.6	20	A. Ranjan, *unpublished*

[a] Tm: Melting temperature (ºC) calculated by Primer3 and IDT's OligoAnalyzer tool.

[b]Base pairs

Table 2.3 Detection of *Tobacco streak virus* in soybean samples by the WI qPCR and nested RT-PCR described in this study and comparison with the previously published qPCR assay.

Sample[a]	WI qPCR Mean Ct[b]	Dutta qPCR Mean Ct[b]	WI qPCR Assay result[c]	Dutta qPCR Assay result[d]	nRT-PCR Assay result[e]
201-1	34.91 ± 0.00*	6.58 ± 3.79*	-	-	+
201-2	19.52 ± 0.02	32.91 ± 0.08	+	-	+
201-3	20.14 ± 0.02	32.66 ± 0.20	+	-	+
201-4	34.91 ± 0.00*	udt.	-	-	+
W101-1	35.14 ± 0.27*	udt.	-	-	+
W101-2	32.56 ± 0.16	udt.	+	-	+
W101-3	29.65 ± 0.10	udt.	+	-	+
W101-4	16.78 ± 0.50	30.48 ± 0.05	+	+	+
D1	udt.	udt.	-	-	+
D4	udt.	9.65 ± 3.41*	-	-	+
D5	23.11 ± 0.04	30.98 ± 0.24	+	+	+
D6	udt.	udt.	-	-	+
LS1	udt.	udt.	-	-	+
LS2	udt.	udt.	-	-	+
107	udt.	udt.	-	-	-
112	28.37 ± 0.07	udt.	+	-	+
122	udt.	udt.	-	-	-
215	udt.	udt.	-	-	-
310	udt.	udt.	-	-	+
413	31.02 ± 0.31	29.77 ± 0.13	+	+	+
423	udt.	30.19 ± 0.03	-	+	-
427	32.25 ± 0.00	udt.	+	-	+
319-1	udt.	udt.	-	-	+
SB1	25.84 ± 0.04	34.68 ± 0.26	+	-	+
SB2	27.4 ± 0.13	33.78 ± 0.24	+	-	+
SB3	25.7 ± 0.04	33.69 ± 0.21	+	-	+
SB4	33.6 ± 0.15*	udt.	-	-	+
SB5	25.58 ± 0.21	33.41 ± 0.12	+	-	+
SB6	28.00 ± 0.04	udt.	+	-	+
SB7	34.15 ± 0.04*	udt.	-	-	+
SB8	30.37 ± 0.01	udt.	+	-	+
SB9	32.41 ± 0.03	udt.	+	-	+
W04-1	udt.	udt.	-	-	+
W04-2	udt.	udt.	-	-	+
W04-3	udt.	udt.	-	-	-
W04-4	udt.	udt.	-	-	-
102-8-1	34.41 ± 0.22*	udt.	-	-	-
Ar2	udt.	udt.	-	-	-
B1	34.59 ± 0.07*	33.69 ± 0.05	-	-	-
NRT	udt.	udt.	-	-	-
NTC	udt.	udt.	-	-	-

[a]NRT: No-reverse transcription control; NTC: No-template control (Nuclease-free water); 102-8-1: *Alfalfa mosaic virus* sample; Ar2: *Soybean vein necrosis virus* sample; B1: *Bean pod mottle virus* sample

[b]Mean Ct: average threshold cycle of two technical replicates; "± ": standard error; udt.: undetermined Ct, no template detected

[c,d,e]A denotation with the plus sign (+) or minus sign (-) indicates the sample was positive for TSV or negative for TSV, respectively.

[c]The following criteria were used to determine WI qPCR assay results: sample was positive if the Ct value $\leq$ 32 and the melt curve peak is at 81ºC. If a Ct value was produced but there was no melt curve peak (*), then the sample was considered negative.

[d]The following criteria were used to determine Dutta qPCR assay results: sample was positive if the Ct value $\leq$ 31 and the melt curve peak is at 84ºC. If a Ct value was produced but there was no melt curve peak (*), then the sample was considered negative.

[e]The assay result of the nested RT-PCR (nRT-PCR) assay was determined after visual inspection of products separated on an agarose gel and size estimated using a 1kb molecular ladder. A sample was determined positive if the product size of the first-round PCR was at 700 bp or at 643 bp in the second round. If no product was obtained at the expected size of each round, then the sample was determined negative.

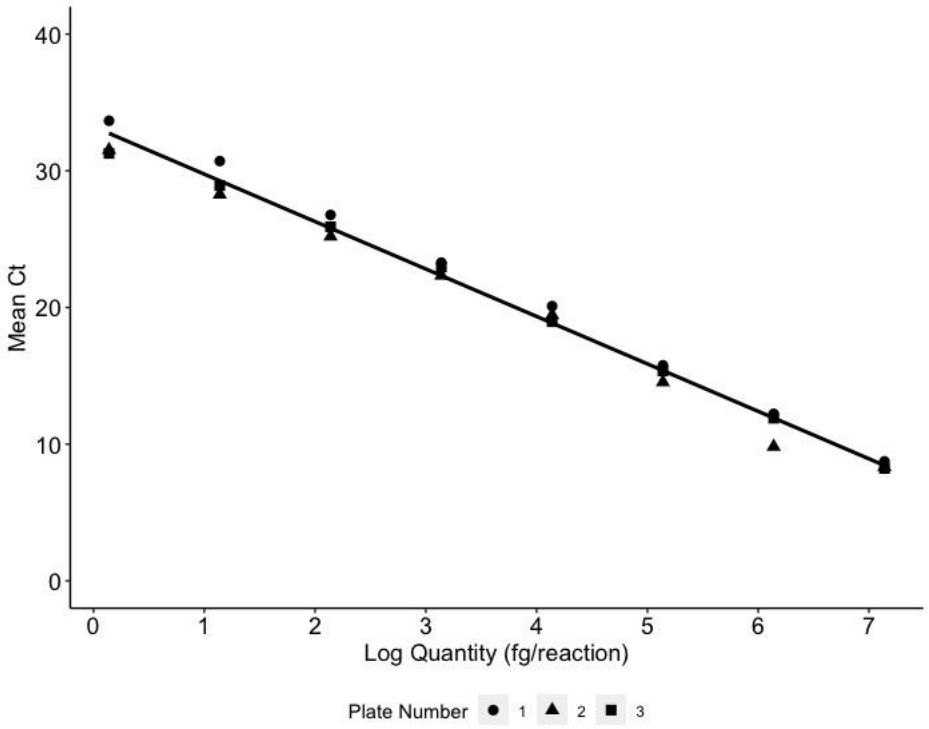

Figure 2.1 Concentration and threshold cycle of serial dilutions of pGEM-T-TSV. Three independent replicate plates were used to generate the equation: $y=-3.47x+33.22$, $R^2=0.998$. Mean threshold cycle (Ct) was calculated based on two technical replicates per serial dilution per plate.

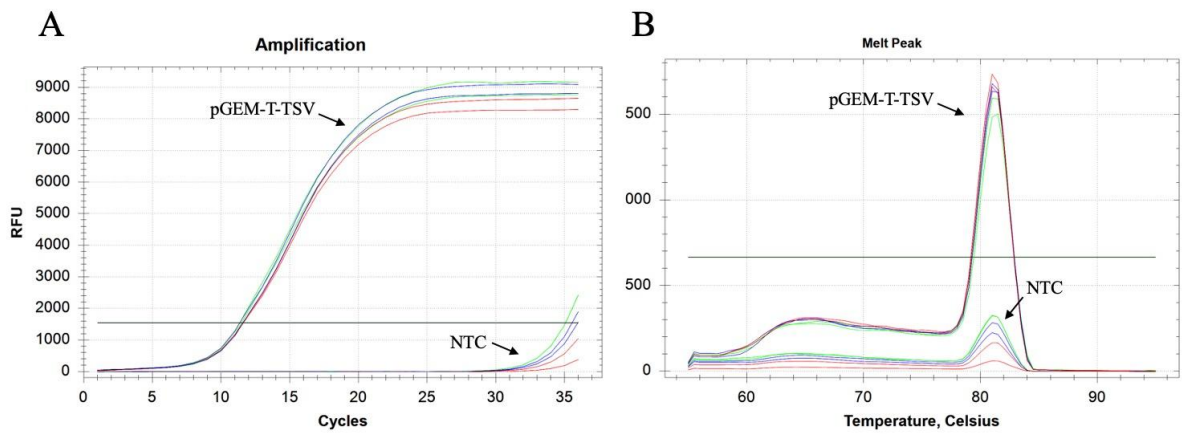

Figure 2.2 Amplification curve (A) and melt curve analysis (B) of annealing temperature gradient of TSVCP_Fw1 and TSVCP_Rv1 primers with pGEM-T-TSV used as the DNA template. A) Amplification curves at 57ºC (blue line), 59ºC (green line) and 61ºC (red line). No-template control (NTC: Nuclease-free water) was used at each of the temperatures and yielded Ct values greater than 35. B) A single peak at 81ºC on the melt curve indicates a single PCR product. NTC showed a melt curve peak below threshold.

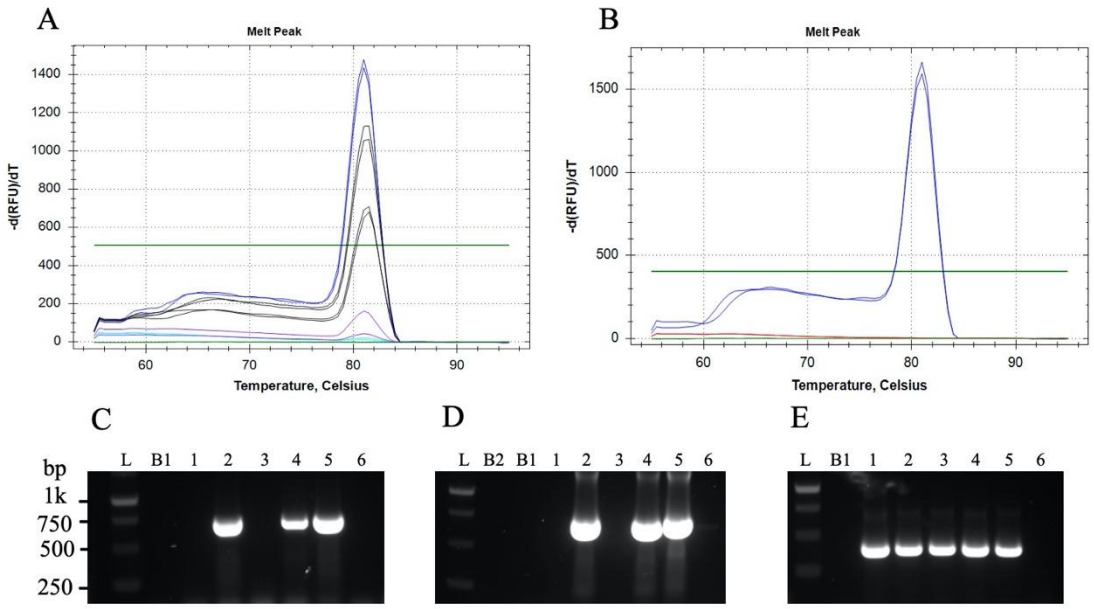

Figure 2.3 Specificity of the qPCR and nested RT-PCR (nRT-PCR) assays. (A) Melt curve peak analysis of the qPCR assay with a TSV-infected soybean sample (W101-4, blue line), pGEM-T-TSV standards (Std-7, Std-8, black line), SVNV-positive sample (Ar2, light blue line), no-template control (green line), no-reverse transcriptase control (purple line). (B) Melt curve peak analysis of the qPCR assay with TSV-infected soybean sample (W101-4, blue line) a healthy soybean sample (DwNT1, red line) and a no-template control (green line). (C) Specificity of the nRT-PCR assay via gel electrophoresis, with first-round nRT-PCR, (D) second-round nRT-PCR, and (E) soybean elongation factor 1-β amplification as a control for RNA quality and cDNA synthesis. Lane L: 1kb molecular ladder; lanes B2, B1: nuclease-free water controls; lane 1: DwNT1; lane 2: 413; lane 3: 423; lane 4: SB5; lane 5: W101-4; lane 6: no-reverse transcription control.

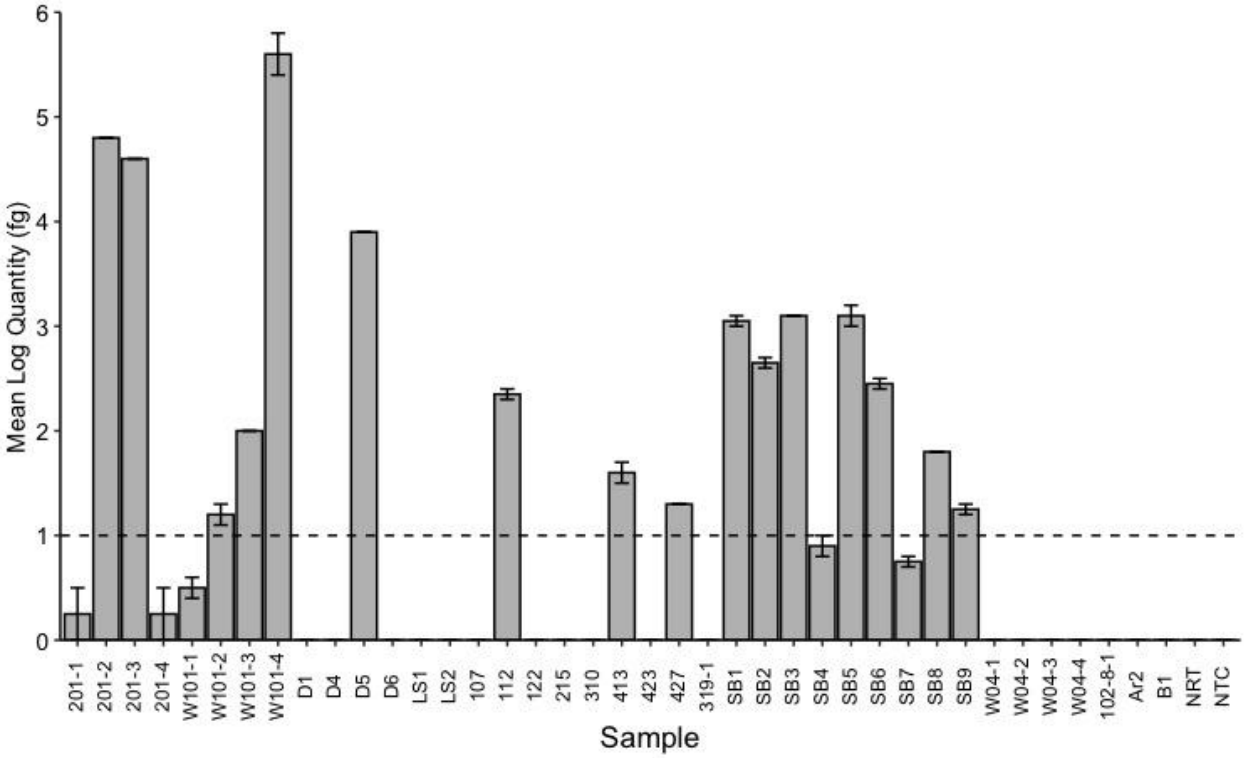

Figure 2.4 Titer of Tobacco streak virus in symptomatic, non-symptomatic or virus-confirmed soybean samples. Mean log quantity determined by calculating the log of the target concentration (fg) compared with pGEM-T-TSV standard dilutions. Dashed line indicates limit of detection at 10 fg. 102-8-1: AMV-positive control, Ar2: SVNV-positive control, B1: BPMV-positive control, NRT: no-reverse transcriptase (no-template) control, NTC: nuclease free water (no-template) control.

CHAPTER 3. Evaluation of selected soybean (*Glycine max*) cultivars and breeding lines for their response to *Soybean vein necrosis virus* infection

Abstract

Soybean vein necrosis virus (SVNV) is an emerging virus of soybean efficiently transmitted by soybean thrips (*Neohydatothrips variabilis*). Host resistance is a durable strategy for controlling viral diseases. However, there is a lack of information about soybean cultivar response to SVNV and whether there is resistance to the virus. Soybean cultivars and breeding lines (genotypes) were selected to be screened in a controlled growth environment for their response to thrips-mediated inoculation with SVNV. Evaluations included measuring soybean vein necrosis severity and viral titer after inoculations with SVNV-infected soybean thrips. Williams 82 and 52-82B were the genotypes with the highest disease severity values and SVNV titer and, thus, were the most susceptible to the virus. The genotypes 51-23 and 91-38 showed little to no disease severity and low SVNV titer. Soybean genotype SSR51-70 was asymptomatic, but the virus was detected and quantified. Our results suggest that 51-23, 91-38, and SSR51-70 are resistant to SVNV, while 51-23 is tolerant. The genotypes that were susceptible in this study can be used to study SVNV biology and epidemiology, while resistant and tolerant lines can be used in future soybean breeding efforts.

Keywords: *Soybean vein necrosis virus*, tospovirus, soybean, breeding lines, varieties, disease severity, virus titer

Introduction

Soybean vein necrosis virus (SVNV) is an orthotospovirus (Tospoviridae) with a ssRNA tripartite genome and a narrow host range where soybean (*Glycine max L. Merril*) is its most economically important host (Irizarry *et al.* 2018; Zhou and Tzanetakis 2013). The virus was reported initially in Tennessee and Arkansas and is now present in the soybean-growing regions of the United States (Zhou and Tzanetakis 2019). Symptoms induced by the virus in soybean include vein-associated yellowing that becomes red-brown irregular lesions, as soybean vein necrosis (SVN) progresses, distributed heterogeneously throughout the leaf. Soybean thrips (*Neohydatothrips variabilis*) are the primary vectors of SVNV and efficiently transmit the virus in a persistent propagative manner (Keough *et al.* 2016; Zhou and Tzanetakis 2013). The virus is reported to affect seed health and quality by being transmitted via the seed and changing the seed oil and protein content (Groves *et al.* 2016; Anderson *et al.* 2017).

Soybean is an important oilseed crop and source of protein with uses in food products, pharmaceuticals, industrial products (e.g., cosmetics, paints, plastics, biodiesel, etc.), and animal feed (Hartman *et al.* 2011; American Soybean Association 2020). The United States is one of the top two producers of soybeans with 122 million metric tons, accounting for approximately 30% of the world soybean production, valued at 31.2 billion dollars and with 3.38 metric tons of average yield per hectare (American Soybean Association 2020; USDA NASS 2021, 2020). Fifty six percent of the vegetable oil consumed in the United States comes from soybean (American Soybean Association 2020). However, diseases limit soybean production and yield. Economic losses associated to soybean diseases are estimated at $96.48 billion US dollars, for an annual average of $4.55 billion dollars, and yield losses of up to 58.8 thousand metric tons. Viral diseases

have contributed to economic and yield losses of up to $1.7 billion US dollars and 910 thousand metric tons, respectively (Allen *et al.* 2017; Bandara *et al.* 2020).

Management practices to control diseases caused by plant viruses are aimed at removing inoculum, limiting dispersal, interrupting or interfering with transmission, and reducing viral accumulation or activation of the plant's defense system. Strategies include rogueing, adjusting planting dates to avoid high populations of insect vectors, planting virus-free seed, chemical control of insect vectors with insecticides, and host resistance (Hill and Whitham 2014; Rubio *et al.* 2021). Chemical control is not a durable strategy for insect-transmitted plant viruses as insecticide resistance can develop (Horowitz *et al.* 2020), the insect's ability to transmit the virus can increase (Wan *et al.* 2021), and chemical control has not been a successful strategy for soybean-infecting viruses in general (Hill and Whitham 2014). The impact of a disease on soybean depends on factors such as weather, production practices, disease susceptibility, and cultivar selection (Mueller *et al.* 2016). Cultivars resistant to a plant virus can prevent or delay their spread and reduce inoculum (Kaweesi *et al.* 2014; Jones 2004; Rubio *et al.* 2020). However, information about soybean varieties with resistance to SVNV is lacking (Hill and Whitham 2014).

Screening soybean genotypes (public or commercial cultivars, or breeding lines) can provide insight about sources of resistance that can be incorporated into breeding programs. For diseases caused by plant viruses, genotype screenings incorporate virus detection, quantification, and symptom severity measurements (Gómez *et al.* 2009). Enzyme-linked immunosorbent assay (ELISA) and quantitative PCR are molecular tools that have been used to estimate virus titer in different pathosystems (Gil-Salas *et al.* 2009; Kaweesi *et al.* 2014; Maruthi *et al.* 2014; Rubio *et al.* 2020). Quantitative PCR is a more sensitive and accurate tool for estimating virus titer

compared to ELISA and can be used to determine resistance levels based on pathogen concentration or accumulation (Balaji *et al.* 2003 Rubio *et al.* 2020; Shirima *et al.* 2017).

Plant genotypes can be classified based on the virus's ability to infect and the plant's response to that viral infection that results in disease symptoms. Resistance is used to describe if the virus cannot infect the plant (complete resistance) or if its movement and accumulation are reduced (partial resistance), compared to a more susceptible standard (Cooper and Jones 1983; Gómez *et al.* 2009; Rubio *et al.* 2020). A combination of viral titer measurements and symptom severity can be used to classify resistance levels to viruses in plants. If viral infection in a plant host produces severe disease symptoms, growth and yield are affected compared to an asymptomatic or non-infected plant of the same genotype, and the virus can multiply and move within the host, then that plant genotype is susceptible. A plant is considered 'tolerant' when the virus can infect and replicate, but the infection causes mild disease symptoms, or results in an asymptomatic reaction, and no significant reductions in growth or yield compared to a susceptible genotype (Cooper and Jones 1983; Gómez *et al.* 2009; Paudel and Sanfaçon 2018; Rubio *et al.* 2020). In a recent study by Anderson (2017), public and commercial soybean cultivars were screened to determine their response to SVNV infection. Of the varieties tested, the SoyNam variety LG94-1906 had the highest SVN severity compared to the other varieties. However, this is the only study that has evaluated soybean varieties for their response to SVNV infection. Thus, our objective was to evaluate selected soybean breeding lines and public cultivars for their response to virus infection and symptom development by measuring SVN severity and quantifying the SVNV nucleoprotein gene to estimate the amount of virus in the plant. Knowing more about the response to infection and possible types of resistance of soybean varieties can influence

management of SVNV such as cultivar selection for growers and identification of sources of resistance for soybean breeding programs.

Materials and Methods

Genotypes and experimental design

Seven soybean genotypes were chosen for this study based on their agronomic qualities, susceptibility or resistance to pathogens of economic importance, availability as public cultivars, and prior knowledge on their genomics (Table 3.1). The genotypes were grown and tested in a controlled environment room at the University of Wisconsin-Madison's Biotron Laboratory Controlled Environment Research Center (biotron.wisc.edu). The room conditions consisted of a cycle of 14 hours of 100% incandescent light at 24 ºC and 24% relative humidity (RH) and 10 hours of darkness at 18 ºC and 18% RH. Seeds were pre-germinated and transplanted, after 4 days in a moist and dark chamber, to 2.54 cm pots containing SunGro propagation mix (Sun Gro Horticulture, Agawam, MA). The pots were placed in cages measuring 81.28 cm in length by 45.72 cm in width by 60.96 cm in height with "No Thrips Insect Screen" (75-mesh) from Greenhouse Megastore (Danville, IL) covering the top and sides. A small front-opening was incorporated into the cage and a 30.48 cm long sleeve made of Bioquip "No-Thrips Insect Screen" (75-mesh; Rancho Dominguez, CA) for access to the inside. Cages containing plants were placed inside the controlled environment room.

Each experimental plant was infested with SVNV-infected adult *N. variabilis* (soybean thrips) when the first trifoliate was fully expanded (V1 stage), with an expanding second trifoliate (Broeske *et al.* 2017; Fehr and Caviness 1977), in order to inoculate plants with SVNV. Thrips were reared in a colony maintained with SVNV-infected soybeans. The uppermost trifoliate (1-2

nodes below apical meristem) were selected to collect adult soybean thrips since that is the location where they are most likely found (Irwin *et al.* 1979). The selected trifoliate was placed in a Petri dish, sealed with parafilm, and placed briefly at 4 ºC to slow down the movement of the thrips. Subsequently, a lightly moistened fine paintbrush, size 05 (Princeton Artist Brush Company, Princeton, NJ) was used to collect one thrips at a time with a delicate sweeping motion. The insects were carefully placed in a 1.5 mL microcentrifuge tube and kept on ice until transported to the controlled environment room. Ten adult soybean thrips were collected per experimental plant for the first infestation. The microcentrifuge tube containing thrips were placed in the soil, next to the plant, and opened. Three days later, five adult soybean thrips were collected in a microcentrifuge tube and added to the plants for a second infestation. The soybean thrips were given an inoculation access period (IAP) of seven days for the first 10 thrips and three days for the 5 thrips. After placing the microcentrifuge tubes with soybean thrips, the plant was quickly covered with a pot-cage made of clear plastic cylindrical tube measuring 12.7cm in diameter, 30.48cm in length, and with a 0.0762 cm wall thickness from Visipak (Arnold, MO). The pot-cage was open on one end and sealed on the other with a flat top poly plug in natural color from Visipak. The flat top was modified with an opening that was covered with 75-mesh from Greenhouse Megastore (Danville, IL), sealed with hot glue, to allow air exchange and maintain a tight seal. To end the IAP, 2.5 mL of the systemic insecticide imidacloprid (1% v/v; formulated as Marathon Granular; OHP Inc., Bluffton, SC) was applied directly to the soil. At 14 days post infestation (dpi), disease severity was measured by counting the number of nodes with SVNV-symptoms (e.g., vein-associated yellowing) and calculated with equation 1. The experiment was a seven (genotype) by two (inoculated or not inoculated) factorial experiment arranged in a randomized complete block

design with three biological replicates. The experiment was repeated twice for a total of three experimental repetitions (runs).

(1)

$$SVN\ severity = \frac{number\ of\ symptomatic\ nodes}{total\ number\ of\ nodes} \times 100$$

RNA isolation and reverse transcription

The center leaflet of the second trifoliate, or V2 growth stage (Broeske *et al.* 2017), was sampled at 14dpi, flash frozen, and stored at -80ºC. Two microcentrifuge tubes of ten thrips and two of five thrips were collected at the time of infestation, as described above, and stored at -80ºC to confirm SVNV-infection in the thrips. The leaflet was ground in a mortar and pestle with liquid Nitrogen and approximately 100mg were used to isolate total RNA with the Maxwell RSC Plant RNA kit with the automated Maxwell RSC Instrument (Promega, Madison, WI) according to the manufacturer's instructions. To extract RNA from the thrips, the Maxwell RSC Plant RNA protocol was performed with the following modifications: 400 µL of homogenization buffer with thioglycerol was added quickly to the frozen microcentrifuge tube and a pellet pestle was used to grind the thrips until small pieces of tissue were observed. Complementary DNA (cDNA) was synthesized from samples by reverse transcribing up to 1 µg of RNA with the 5X iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA). The cDNA was diluted with nuclease-free water to a 1:10 ratio for downstream use.

Quantitative PCR

The estimation of SVNV titer in the assay plants was performed with quantitative PCR (qPCR) and the primers SVNV_NP-Fw/Rv (Table 3.2) targeting the Nucleoprotein (NP) gene. A standard curve was generated with the target sequence from these primers cloned into a TA plasmid. First, the amplicon was generated with reverse transcription polymerase chain reaction

(RT-PCR) and a reaction mixture of 12.5 µL of 5X GoTaq Master Mix (Promega, Madison, WI), 2.5 µL (10 µM) of SVNV-NP_Fw/Rv, and 7.5 µL of 1:10 cDNA dilution (5 ng/µL). The PCR reaction was performed in an Eppendorf MasterCycler Pro S programmable thermal cycler (Eppendorf AG, Hamburg, Germany). The cycling conditions consisted of 2 min denaturation at 95 ºC, followed by 35 cycles of 30 seconds denaturation at 94 ºC, 30 seconds annealing at 57 ºC, and 45 seconds elongation at 72 ºC, and a final extension step of 5 min at 72 ºC. The PCR product was visualized with 1.2% agarose gel electrophoresis, containing SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA), in 1X TAE. The 239bp fragment was cloned into the pGEM-T vector using the pGEM-T Easy Vector System I (Promega, Madison, WI) following the methods described in Zambrana-Echevarría *et al.* (2021). The standard curve was generated by 10-fold dilutions of pGEM-T-SVNV and the copy numbers in each dilution was calculated with a copy number calculator (Staroscik 2004) using the DNA concentration and the size of the plasmid and insert (3254 bp). The number of copies of the plasmid ranged from 1.42 $x10^7$ to 1.42 fg per reaction.

The qPCR reaction consisted of 10 µL of 2X SsoFast EvaGreen (Promega, Madison, WI), 0.8 µL of each (10µM) primer, 4.4 µL of nuclease-free water, and 4 µL of cDNA (5 ng/µL). Two template-free reactions, nuclease-free water and no-Reverse transcription controls, substituting cDNA were included in each plate. Each qPCR reaction was loaded to a 96-well skirted clear-well microplate (Bio-Rad; Hercules CA) and the following cycle conditions were used in a CFX96 Real-time PCR detection system (Bio-Rad; Hercules, CA): 30 seconds denaturation at 95ºC, followed by 35 cycles of 5 seconds denaturation at 95ºC, 5 seconds annealing/extension at 61ºC, and a final cycle of a melt curve in 0.5ºC increments from 55º to 95ºC. Serial dilutions of pGEM-T-SVNV were included in each plate (Figure AI.2) and the absolute quantification of SVNV NP

was performed by the CFX Maestro Software 1.1 (Bio-Rad). Two technical replicates were used for each biological replicate in the qPCR plate per experimental run. The titer of SVNV (fg) was averaged between the technical replicates.

Nested RT-PCR

To confirm the detection of SVNV in the samples, nested primers targeting the Nucleoprotein (NP) gene in the S RNA were used for the reverse transcriptase polymerase chain reaction (RT-PCR). The first-round primers SVNV-F1 and SVNV-R1 (Table 3.2) were added to a reaction mixture that consisted of 12.5 µL of 5X GoTaq Master Mix (Promega, Madison, WI), 2.5 µL (10 µM) of each primer, and 7.5 µL of 1:10 cDNA dilution (5 ng/µL). The PCR reaction was performed in an Eppendorf MasterCycler Pro S programmable thermal cycler (Eppendorf AG, Hamburg, Germany) with the following conditions: 2 min denaturation at 95 ºC, followed by 32 cycles of 30 seconds denaturation at 94 ºC, 30 seconds annealing at 52 ºC, and 1 min elongation at 72 ºC, and a final extension step of 10 min at 72 ºC. The second round of PCR was performed with the primers SVNV-F2 and SVNV-R2 (Table 3.2) that were nested within SVNV-F1/R1. The reaction consisted of 12.5 µL of 5X GoTaq Master Mix (Promega, Madison, WI), 2.5 µL (10 µM) of each primer, 2.5 µL of nuclease-free water, and 5 µL of the first-round PCR reaction. Products were visualized on a 1.2% agarose gel with 1X TAE. Size was estimated with a 1kb molecular ladder (Promega, Madison, WI) for an expected size of 800bp in each round if the sample was positive for SVNV.

Statistical analysis

Data from disease severity measurements and SVNV titer were analyzed separately using generalized linear mixed model analysis of variance in SAS v 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Soybean genotype and treatment were considered fixed effects

and biological replicate (n=9) and experimental run (n=3) were considered random effects in the disease severity and SVNV titer GLMM, respectively. The data were normalized using a log-normal distribution as specified by the 'dist' function. For the SVNV titer model, an extreme outlier corresponding to LG94-1906 was removed from the SVNV titer data set. Mean separations were performed using Fisher's Least Significant Difference (LSD) at P=0.05 with the 'mult' SAS open-source macro (Piepho 2012).

Results

Disease Severity

Overall, SVN severity values ranged from 2-16% across all experiments and genotypes (Table 3.3). The genotypes with the highest average disease severity were 52-82B, LG94-1906, and Williams 82 (Table 3.3). Soybean genotype, treatment, and their interaction were significant (Table 3.4). The thrips-infested genotypes 52-82B, LG94-1906, and Williams 82 had significantly higher (P< 0.01) levels of SVN compared to their non-treated (i.e., non-infested) counterparts. Comparing the infested genotypes, 52-82B had significantly higher SVN severity than 51-23, 91-38, Dwight, and SSR51-70, but was not different from LG94-1906 and Williams 82 (Figure 3.1). Severity of SVN on LG94-1906 and Williams 82 was not significantly different compared to the other infested genotypes evaluated.

SVNV quantification and detection

The titer of SVNV was measured at 14 dpi by quantitative PCR. Treatment had a significant effect on SVNV titer, but there was no significant effect of soybean genotype on the SVNV titer, and no significant interaction was observed between treatment and genotype (Table 3.4). SVNV

was undetectable by qPCR in 51-23 and therefore no SVNV concentration was measured in any of the experiments (Figure 3.2). However, two 51-23 non-treated replicates were positive for SVNV in the nested RT-PCR (Table 3.5). The varieties with the highest SVNV titer on average were 52-82B and Williams 82 followed by Dwight and LG94-1906, although they were not significantly different from each other (Figure 3.2). For 52-82B, the nested RT-PCR detected SVNV in more replicates than the qPCR assay and SVNV was detected and quantified in one of the non-treated replicates of 52-82B (Table 3.5). Only one replicate was positive for SVNV (Table 3.5) in 91-38 for a viral concentration of 0.25 fg, but on average the genotype had low to no detectable SVNV (Figure 3.2). Dwight, LG94-1906, and SSR51-70 were the varieties with the lowest SVNV titers (Figure 3.2). Only one inoculated replicate of Dwight was positive for SVNV (Table 3.5, Figure 3.2). One replicate of LG94-1906 had SVNV titer of 33,878.137 fg in the third experimental run. However, this value was removed from the statistical analysis as it was an outlier in the data set. SSR51-70 had the lowest average titer of all the SVNV-positive genotypes (Figure 3.2).

Discussion

Soybean vein necrosis virus is a wide-spread pathogen in the major soybean growing regions of the United States (Zhou and Tzanetakis 2019). The vegetative spread of the virus via the seed adversely changes the oil and protein content (Groves *et al.* 2016; Anderson *et al.* 2017). For a major oilseed crop like soybean, this negative effect of SVNV on seed health and quality can add to economic impact above yield loss alone (Allen *et al.* 2017; Bandara *et al.* 2020). However, there is scarce information on soybean cultivar response to SVNV infection and there are no reports of resistant varieties (Anderson 2017; Hill and Whitham 2014). Host resistance and cultivar

selection influences the impact a disease will have on a crop, the spread and inoculum of plant viruses, and management practices (Hill and Witham 2014; Mueller *et al.* 2016).

Cultivar screening provides information about the host response to viral infection and quantification of the virus titer can be used to determine resistance levels (Gómez *et al.* 2009; Rubio *et al.* 2020; Shirima *et al.* 2017). The genotypes chosen for this study have different agronomic characteristics ranging from high yields, sequenced genomes, to known resistance to important pathogens of soybean. LG94-1906 and Williams 82 have genome maps publicly available (Grant *et al.* 2010; Schmutz *et al.* 2010) which could be useful for determining the genetic basis of resistance to SVNV in the future. The breeding lines used in this study have also been used as check lines for resistance screenings to *Sclerotinia sclerotiorum*, an important soybean pathogen in the Midwest region of the United States (Webster *et al.* 2020). Therefore, they were chosen due to the information available from these genotypes to evaluate their response to SVNV infection (SVN severity) and to quantify the virus to assess possible sources of resistance or tolerance.

SVN severity values differed among the genotypes, where 52-82B, Williams 82, and LG94-1906 had the highest average values. These results suggest that these genotypes are susceptible to SVNV based on symptoms. LG94-1906 is a publicly available variety (Grant *et al.* 2010) previously reported to be susceptible to SVNV (Anderson 2017). Although LG94-1906 was not the genotype with the highest level of SVN, it is considered susceptible (Anderson 2017) and we agree with this assessment. Quantifying the amount of virus can also be used to evaluate genotypes in breeding programs for resistance to a virus (Rubio *et al.* 2020; Shirima *et al.* 2017). In this study, we estimated the titer of SVNV using qPCR and detected the virus using nested RT-PCR in soybean genotypes subjected to thrips-mediated inoculation of SVNV. Despite the

cultivars not responding to inoculation (no significant differences in SVNV titer), 52-82B and Williams 82 had the highest numerical SVNV titer. This is consistent with these two genotypes having two of the highest SVN severity values. Williams 82 has been reported to be susceptible to many viral pathogens of soybean and has been used as a susceptible check previously (Wang *et al.* 2005). The finding that Williams 82 and 52-82B had the highest SVN severity values and virus titer of all the genotypes suggests that they are susceptible to SVNV. The genotype SSR51-70 had no disease severity values in any of the experiments and one of the lowest titers of SVNV. This suggests there was an asymptomatic infection of SVNV or that the virus was possibly detected early in the infection since disease severity measurements were made at 14 dpi. SSR51-70 may be resistant to SVNV infection and is a good candidate for breeding programs in the future. The genotypes 51-23 and 91-38 had little to no disease severity and SVNV titer was low or undetectable, which suggests that these genotypes are resistant to SVNV. Future research efforts could focus on 51-23, SSR51-70 and 91-38 genotypes to assess SVNV accumulation patterns, SVN severity past 14 dpi, and determine if they are in fact partially resistant or tolerant.

The non-treated controls of the genotypes 51-23, 52-82B, and Williams 82 were asymptomatic but positive in the nested RT-PCR but not in the qPCR. Although the instances were low (i.e., 1-2 biological replicates in the overall experiment), it is possible that this is a detection of SVNV seed-transmission since all of the assay plants came from field-grown seeds (Groves *et al.* 2016). Another alternative explanation for the SVNV-positive, non-treated controls could be related to the assay plants sharing a cage with inoculated treatments as a result of the randomization. SVNV is persistently transmitted and viruliferous thrips prefer to feed on healthy tissue (Keough *et al.* 2016). The SVNV-positive, non-treated plants may have been a result of

thrips from the inoculated treatment, that were not effectively killed by the insecticide application, feeding on the healthy controls and inoculating them with SVNV.

Viral load and severity of symptoms may or may not correlate positively and depends on the variety, virus strain, or which virus is infecting in the disease complex (Kaweesi *et al.* 2014; Satoh *et al.* 2011). SVN severity values did not always correlate with SVNV titer. LG94-1906 had one of the lowest SVNV titers but had the third highest SVN severity values. In contrast, SSR 51-70 had the lowest SVNV titer but no SVN severity. These results suggests that SVNV infection may induce different levels of symptoms in soybean depending on the plant genotype. There are no known SVNV strains (Zhou and Tzanetakis 2019) that could explain differences in SVN symptom severity as with other pathosystems (Satoh *et al.* 2011). Another possible explanation for the low correlation between disease severity and virus load is that the symptoms observed at the time of measurements may have resembled SVNV but in fact were other physiological disorders or that the viral titers were below the detection limit of the qPCR assay. A similar result with the detection limits has been reported for *Tobacco streak virus* (Zambrana-Echevarría *et al.* 2021).

Currently, there is no knowledge on the genetic basis of resistance to SVNV – most likely due the lack of knowledge on resistant genotypes – and SSR51-70, 51-23, and 91-38 are good candidates for future studies on resistance to SVNV. The genome of Williams 82 has been sequenced (Schmutz *et al.* 2010) and public availability of this sequence can lead to the discovery of recessive resistance or host susceptible factors (e.g., translation initiation proteins) that viruses use to propagate. Recessive resistance is an active area of study and can be used as a strategy to control viruses (Hashimoto *et al.* 2016; Hill and Whitham 2014). The genotypes found to be susceptible to SVNV in this study, 52-82B and Williams 82, can be used to identify host factors

used by the virus to establish an infection and target them as potential sources of recessive resistance.

Soybean disease screening in controlled environments have the advantage of not depending on a particular location's inoculum pressure and the ability to control inoculum distribution among assay plants and environmental variables (Gómez *et al.* 2009). A limitation to our study was the dependency on the thrips vector to inoculate SVNV in our assay plants, which may have affected the outcome. The thrips were obtained from a colony reared in the lab and, while adults were selected for the inoculations, the development time was not controlled, and this may affect survival during the IAP. Field evaluation of the genotypes used in this study may be more appropriate in the future, especially since SVN symptom intensity is different when infected plants are grown in a controlled growth environment (Zambrana-Echevarría, *pers. obs.*) or in the greenhouse (Zhou and Tzanetakis 2019).

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Literature Cited

Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, K. A., Wrather, J. A. 2017. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Heal. Prog. 18:19–27

American Soybean Association. 2020. Soystats 2020: A Reference Guide to Important Soybean Facts & Figures. Available at: http://soystats.com/wp-content/uploads/SoyStats2020_Web.pdf.

Anderson, N. R. 2017. Effect of Soybean Vein Necrosis on Soybean Yield and Seed Quality, and Symptom Expression on Soybean and Alternative Hosts. ProQuest Diss. Theses. ISBN: 978-0-355-26259

Anderson, N. R., Irizarry, M. D., Bloomingdale, C. A., Smith, D. L., Bradley, C. A., Delaney, D. P., Kleczewski, N. M., Sikora, E. J., Mueller, D. S., and Wise, K. A. 2017. Effect of soybean vein necrosis on yield and seed quality of soybean. Can. J. Plant Pathol. 39:334–341.

Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., and Esker, P. D. 2020. Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS One. 15:e0231141

Balaji, B., Bucholtz, D. B., and Anderson, J. M. 2003. Barley yellow dwarf virus and Cereal yellow dwarf virus Quantification by Real-Time Polymerase Chain Reaction in Resistant and Susceptible Plants. Phytopathology. 93:1386–1392.

Broeske, M., Gaspar, A., Gaska, J., and Conley, S. 2017. A Visual Guide to Soybean Growth Stages. Integr. Pest Crop Manag. Grow. Guid. Available at: https:// https://ipcm.wisc.edu/downloads/guides/

Chawla, S., Bowen, C. R., Slaminko, T. L., Hobbs, H. A., and Hartman, G. L. 2013. A public program to evaluate commercial soybean cultivars for pathogen and pest resistance. Plant Dis. 97:568–578.

Cooper, J. I., and Jones, A. T. 1983. Responses of Plants to Viruses: Proposals for the Use of Terms. Phytopathology. 73:127–128.

Fehr, W. R., and Caviness, C. E. 1977. Stages of soybean development. *Special Report.* 87

Gil-Salas, F. M., Colyer, A., Boonham, N., Cuadrado, I. M., and Janssen, D. 2009. Resistance screening against Cucumber vein yellowing virus using a real-time (Taqman®) RT-PCR assay in cucumber (Cucumis sativus). Crop Prot. 28:109–112

Gómez, P., Rodríguez-Hernández, A. M., Moury, B., and Aranda, M. 2009. Genetic resistance for the sustainable control of plant virus diseases: Breeding, mechanisms and durability. Eur. J. Plant Pathol. 125:1–22.

Grant, D., Nelson, R. T., Cannon, S. B., and Shoemaker, R. C. 2010. SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res. 38:D843–D846

Groves, C., German, T., Dasgupta, R., Mueller, D., and Smith, D. L. 2016. Seed Transmission of Soybean vein necrosis virus: The First Tospovirus Implicated in Seed Transmission. PLoS One. 11:1–14

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. Food Secur. 3:5–17.

Hashimoto, M., Neriya, Y., Yamaji, Y., and Namba, S. 2016. Recessive Resistance to Plant Viruses: Potential Resistance Genes Beyond Translation Initiation Factors. Front. Microbiol. 7:1–11

Hill, J. H., and Whitham, S. A. 2014. Control of Virus Diseases in Soybeans. In G. Loebenstein, N. Katis (Eds), Advances in Virus Research. 90:355–390. Academic Press.

Horowitz, A. R., Ghanim, M., Roditakis, E., Nauen, R., and Ishaaya, I. 2020. Insecticide resistance and its management in Bemisia tabaci species. J. Pest Sci. (2004). 93:893–910

Irizarry, M. D., Elmore, M. G., Batzer, J. C., Whitham, S. A., and Mueller, D. S. 2018. Alternative Hosts for Soybean vein necrosis virus and Feeding Preferences of Its Vector Soybean Thrips. Plant Heal. Prog. 19:176–181

Irwin, M. E., Yeargan, K. V., and Marston, N. L. 1979. Spatial and Seasonal Patterns of Phytophagous Thrips in Soybean Fields with Comments on Sampling Techniques. Environ. Entomol. 8:131–140.

Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res. 100:5–30.

Kaweesi, T., Kawuki, R., Kyaligonza, V., Baguma, Y., Tusiime, G., and Ferguson, M. E. 2014. Field evaluation of selected cassava genotypes for cassava brown streak disease based on symptom expression and virus load. Virol. J. 11.

Keough, S., Han, J., Shuman, T., Wise, K., and Nachappa, P. 2016. Effects of Soybean Vein Necrosis Virus on Life History and Host Preference of Its Vector, Neohydatothrips variabilis, and Evaluation of Vector Status of Frankliniella tritici and Frankliniella fusca. J. Econ. Entomol. 109:1979–1987.

Maruthi, M. N., Bouvaine, S., Tufan, H. A., Mohammed, I. U., and Hillocks, R. J. 2014. Transcriptional response of virus-infected cassava and identification of putative sources of resistance for cassava brown streak disease. PLoS One. 9.

McCaghey, M., Willbur, J., Ranjan, A., Grau, C. R., Chapman, S., Diers, B., Groves, C., Kabbage, M., and Smith, D. L. 2017. Development and evaluation of *Glycine max* germplasm lines with quantitative resistance to *Sclerotinia sclerotiorum*. Front. Plant Sci. 8:1–13.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., and Robertson, A. 2016. A Farmer's Guide to Soybean Diseases. eds. Daren Mueller, Kiersten Wise, Adam Sisson, Damon Smith, Edward Sikora, Carl Bradley, and Alison Robertson. The American Phytopathological Society.

Nickell, C. D., Noel, G. R., Cary, T. R., and Thomas, D. J. 1998. Registration of 'Dwight' Soybean. Crop Sci. 38:1398–1398.

Piepho, H. P. 2012. A SAS macro for generating letter displays of pairwise mean comparisons. Commun. Biometry Crop Sci. 7:4–13.

Paudel, D. B., and Sanfaçon, H. 2018. Exploring the Diversity of Mechanisms Associated With Plant Tolerance to Virus Infection. Front. Plant Sci. 9:1-20

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Front. Plant Sci. 11:1–23.

Satoh, K., Shimizu, T., Kondoh, H., Hiraguri, A., Sasaya, T., Choi, I.-R., Omura, T., and Kikuchi, S. 2011. Relationship between Symptoms and Gene Expression Induced by the Infection of Three Strains of Rice dwarf virus ed. Jean-Pierre Vartanian. PLoS One. 6:e18094

Schmutz, J., Cannon, S. B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D. L., Song, Q., Thelen, J. J., Cheng, J., Xu, D., Hellsten, U., May, G. D., Yu, Y., Sakurai, T., Umezawa, T., Bhattacharyya, M. K., Sandhu, D., Valliyodan, B., *et al.* 2010. Genome sequence of the palaeopolyploid soybean. Nature. 463:178–183

Shirima, R., Maeda, D., Kanju, E., Ceasar, G., Tibazarwa, F., and Legg, J. 2017. Absolute quantification of cassava brown streak virus mRNA by real-time qPCR. J. Virol. Methods. 245:5–13

United States Department of Agriculture National Agricultural Statistics Service. 2021. Crop Production 2020 Summary. Available at: https://usda.library.cornell.edu/concern/publications/tm70mv177.

United States Department of Agriculture National Agricultural Statistics Service. 2020. Crop Values 2019 Summary. Washington, D. C. Available at: http://usda.library.cornell.edu/concern/publications/k35694332?locale=en.

Wan, Y., Zheng, X., Xu, B., Xie, W., Wang, S., Zhang, Y., Zhou, X., and Wu, Q. 2021. Insecticide resistance increases the vector competence: a case study in Frankliniella occidentalis. J. Pest Sci. (2004). 94:83–91

Wang, Y., Hobbs, H. A., Hill, C. B., Domier, L. L., Hartman, G. L., and Nelson, R. L. 2005. Evaluation of ancestral lines of U.S. soybean cultivars for resistance to four soybean viruses. Crop Sci. 45:639–644.

Webster, R. W., Roth, M., Reed, H., Mueller, B., Groves, C., McCaghey, M., Chilvers, M. I., Mueller, D. S., Kabbage, M., and Smith, D. L. 2020. Identification of Soybean (Glycine max) Check Lines for Evaluating Genetic Resistance to Sclerotinia Stem Rot. Plant Dis.

Zambrana-Echevarria, C., Roth, M., Dasgupta, R., German, T., Groves, C., and Smith, D. L. 2021. Sensitive and specific qPCR and nested RT-PCR assays for the detection of *Tobacco streak virus* in soybean. PhytoFrontiers. PHYTOFR-11-20-0036-R

Zhou, J., and Tzanetakis, I. E. 2013. Epidemiology of soybean vein necrosis-associated virus. Phytopathology. 103:966–971.

Zhou, J., and Tzanetakis, I. E. 2019. Soybean vein necrosis virus: an emerging virus in North America. Virus Genes. 55:12–21.

Tables and Figures

Table 3.1 Soybean cultivars and breeding lines used in this study

Genotype	Characteristics	Reference
PI 518671 'Williams 82'	Susceptible to *Soybean mosaic virus* and *Tobacco streak virus* Mapped genome Publicly available	Chawla *et al.* 2013 Wang *et al.* 2005 Schmutz *et al.* 2010
PI 597386 'Dwight'	High Yield Resistant to *Heterodera glycines* Susceptible to *Sclerotinia sclerotiorum* Publicly available	Nickell *et al.* 1994 McCaghey *et al.* 2017
91-38	High Yield; Food-grade Resistant to *S. sclerotiorum* Germplasm/Breeding line	McCaghey *et al.* 2017
52-82B	High Yield Resistant to *S. sclerotiorum* Germplasm/Breeding line	McCaghey *et al.* 2017
51-23	High Yield Resistant to *S. sclerotiorum* Germplasm/Breeding line	McCaghey *et al.* 2017
SSR51-70	High Yield Resistant to *S. sclerotiorum* Germplasm/Breeding line	McCaghey *et al.* 2017
LG94-1906	Susceptible to *Soybean vein necrosis virus* Mapped genome Publicly available through SoyNam	Anderson, 2017 Grant *et al.* 2010

Table 3.2 Primers used for the detection of *Soybean vein necrosis virus* using nested RT-PCR and quantitative PCR

Primer	Sequence (5'-3')	Tm[a]	Length[b]	Reference
SVNV-NP_Fw	GGAAGCTTACCCCTTCTGGC	58	20	Keough *et al.* 2016
SVNV-NP_Rv	ACTCCTCTCATTTGGGGTGC	57.2	20	Keough *et al.* 2016
SVNV-F1	AGATATAAAGTTGAGACACTATC	47.3	23	This study
SVNV-R1	TGCAACACATCCGGAACTCTG	57.6	21	This study
SVNV-F2	CCTGAATTCATGCCACAAACAGCAGG	60.6	26	This study
SVNV-R2	TTAGCGGCCGCTAAACAGAAAACTCC	61.2	26	This study

[a]Tm: melting temperature (ºC) calculated by IDT's Oligo Analyzer Tool
[b]Base pairs

Table 3.3 Average soybean vein necrosis severity per genotype and experimental run

Genotype	Run 1	Run 2	Run 3	Total Mean
51-23	0%	6%	0%	2%
91-38	0%	0%	0%	0%
52-82B	6%	17%	27%	16%
Dwight	0%	6%	7%	4%
LG94-1906	11%	0%	13%	8%
SSR51-70	0%	0%	0%	0%
Williams 82	28%	0%	7%	11%

Table 3.4 Analysis of variance of soybean vein necrosis severity and virus titer for the fixed effects of genotype, treatment, and their interaction

		Disease Severity		SVNV titer	
Fixed Effects	df	*F* value	*P* value	*F* value	*P* value
Genotype	6	2.52	0.0254	1.13	0.3517
Treatment	1	16.27	0.0001	3.98	0.0487
Genotype*Treatment	6	2.52	0.0254	1.03	0.4103

Table 3.5 *Soybean vein necrosis virus* detection by nested RT-PCR and quantitative PCR for each soybean genotype and treatment.

Genotype	Treatment	nested RT-PCR[a]	qPCR[a]
51-23	Inoculated	0	0
	Non-treated	2	0
52-82B	Inoculated	4	1
	Non-treated	1	1
91-38	Inoculated	1	1
	Non-treated	0	0
Dwight	Inoculated	1	1
	Non-treated	0	0
LG94-1906	Inoculated	5	4
	Non-treated	0	0
SSR51-70	Inoculated	2	1
	Non-treated	0	0
Williams 82	Inoculated	3	3
	Non-treated	1	0

[a]Number of SVNV-positive biological replicates out of nine total replicates across all experiments

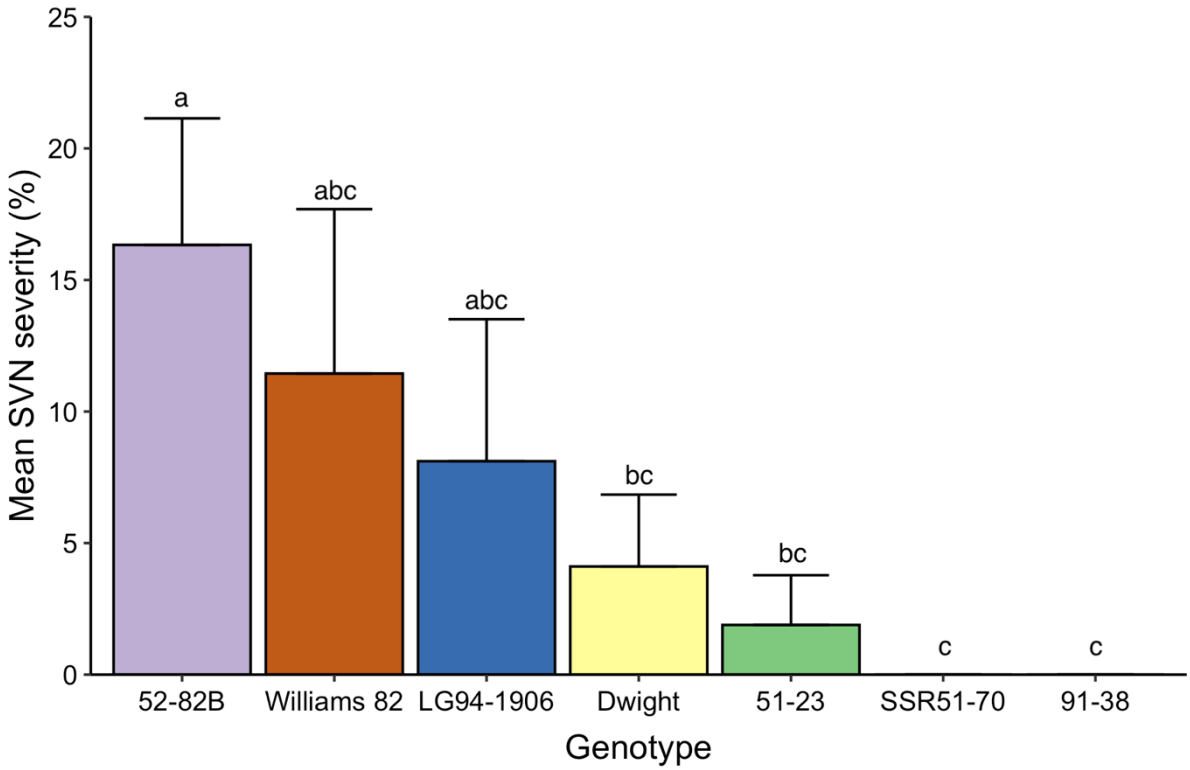

Figure 3.1 Mean soybean vein necrosis (SVN) severity at 14 days post thrips infestation for soybean genotypes tested in a controlled environment over three experimental runs. Whiskers on each bar indicate the standard error of the mean. Bars with the same letter are not statistically different from each other based on Fisher's Least Significant Difference at P=0.05.

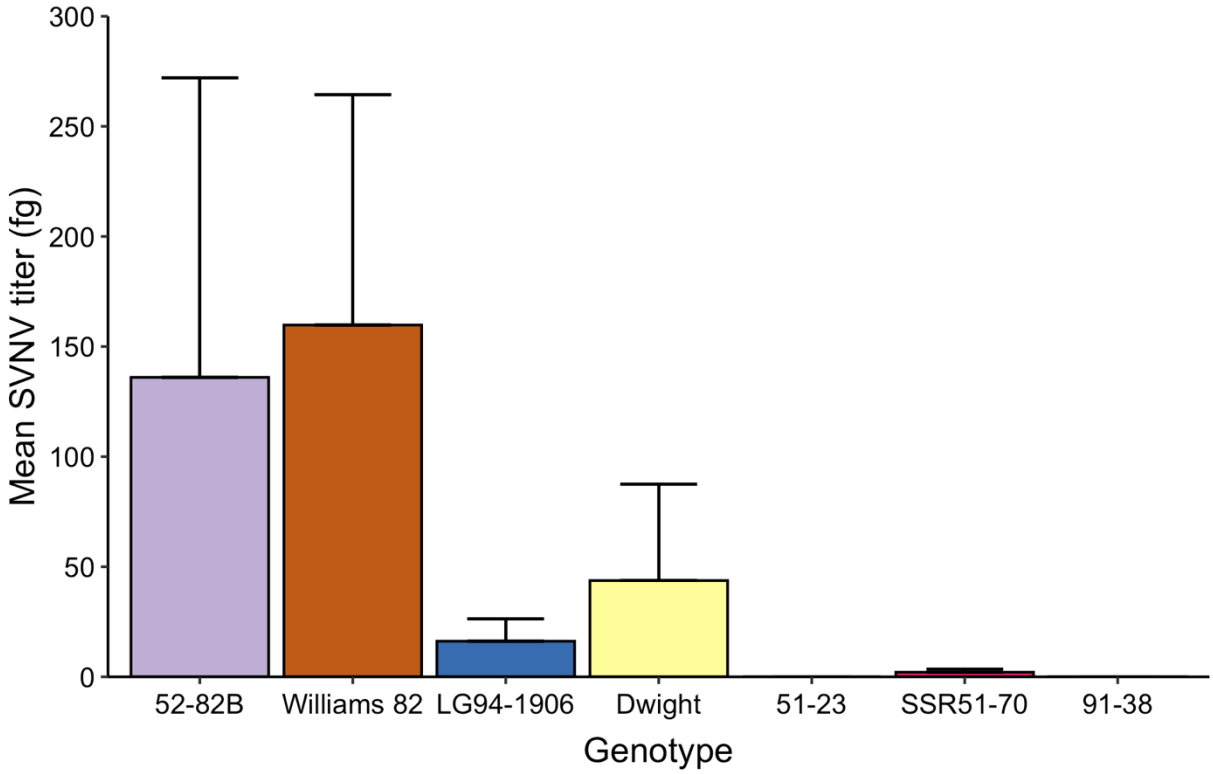

Figure 3.2 Mean titer of *Soybean vein necrosis virus,* measured by quantitative PCR, in soybean genotypes tested in a controlled environment over three experimental runs. Whiskers on each bar indicate the standard error of the mean.

CHAPTER 4. Degree-day and weather-based models to describe the seasonal patterns and factors affecting the dispersal of thrips vectors of *Soybean vein necrosis virus*

Abstract

Thrips (Thysanoptera: Thripidae) are considered a minor, direct pest of soybean, however, their ability to transmit *Soybean vein necrosis virus* (SVNV) may contribute to economic losses due to the effects of the virus on the crop. *Neohydatothrips variabilis* (Beach), *Frankliniella fusca* (Hinds), and *Frankliniella tritici* (Fitch) transmit SVNV in a persistent, propagative manner. Increase in the populations of these species reportedly precede the onset of SVNV symptoms. A generalized additive mixed model (GAMM) was used to describe the seasonal patterns of *N. variabilis*, *F. fusca*, and *F. tritici* using multi-year adult capture data and approximate critical degree days related to their phenology. The GAMM suggested a species-specific pattern, where peaks in flight activity among species varied with *F. fusca* captures culminating at 1687 DD (late June), 2372 DD (late August) for *F. tritici*, and 2625 DD (late August) for *N. variabilis*. SVNV was detected during the periods of high activity of *F. tritici* and *N. variabilis*. Furthermore, a logistic regression modelling approach was used to determine environmental weather variables that correlate with periods of high and low captures for each species of thrips. Significant models describing patterns of *F. fusca* and *N. variabilis* capture included relative humidity and wind speed, respectively, as explanatory variables. The *F. tritici* model was the best fitting and included air temperature and total precipitation as the most explanatory variables. Both GAMM and logistic regression models are useful tools to better understand the epidemiologically important vectors of SVNV in the field, and outcomes of these approaches can be used to further refine disease and insect management strategies to limit transmission.

Introduction

Soybean (*Glycine max* L. Merr.) is an economically important crop used as a source of vegetable oil, human food, and animal feed (Hartman *et al.* 2011). In 2019, the United States (US) production was 112.5 million metric tons with yields of 3.19 metric tons valued at 46 billion US dollars. Soybean is one of the top three commodity crops in Wisconsin. The state produced 2.2 metric tons of soybeans in 2020 which yielded 3,400 kg/ha and was valued at 1 billion US dollars (USDA NASS 2021a, 2021b, 2020c).

Thrips (Thysanoptera: Thripidae) are small, polyphagous insects with piercing-sucking mouthparts and a worldwide distribution. Phytophagous thrips feed on leaves, flowers, and stems where they cause injuries that appear as silver lesions (Kirk 1997b; van de Wetering *et al.* 1998). These insects are regarded as full season pests in soybeans and their feeding habits can cause severe damage if reproducing populations become large under ideal weather conditions (Bloomingdale *et al.* 2016; Hesler *et al.* 2018; Irwing *et al.* 1979). In the Midwest, they often infest only 0-10% of soybean acres at economic levels and for this reason are not actively managed by growers (Hurley and Mitchell 2017; Musser *et al.* 2020). However, their potential to cause losses in soybean result from their ability to transmit plant viruses (Jones 2005). Many of these viruses are in the Tospoviridae and are acquired and transmitted as an indirect outcome of thrips feeding (Rotenberg *et al.* 2015; van de Wetering *et al.* 1998).

Soybean vein necrosis virus (SVNV) is an emerging virus in the *Orthotospovirus* genus in the Tospoviridae. The virus was first reported in 2008 in soybean fields in Tennessee and Arkansas (Zhou *et al.* 2011) and is now widespread in the major soybean growing regions of the USA (Zhou and Tzanetakis, 2019). Symptoms caused by SVNV on soybean start as vein-associated chlorosis that later expands to become irregularly shaped necrotic lesions. There is no clear effect on soybean

yield, however, the oil content and health of the seed is negatively affected (Anderson *et al.* 2017; Groves *et al.* 2016). SVNV is transmitted in a persistent, propagative manner by three species of thrips: *Neohydatothrips variabilis* (Beach), *Frankliniella fusca* (Hinds), and *Frankliniella tritici* (Fitch) (Zhou and Tzanetakis 2013; Keough *et al.* 2016). After the virus is acquired by the early instar larvae, it circulates and replicates in the thrips body and is transtadially transmitted through its developmental stages (Rotenberg *et al.* 2015; Rotenberg and Whitfield 2018; Han *et al.* 2019). Each of the vector species differ in their ability to transmit the virus where *N. variabilis* is considered most efficient at transmitting SVNV and *F. tritici* is regarded as least efficient (Keough *et al.* 2016; Han *et al.* 2019).

The relative abundance of the thrips that transmit SVNV, their population dynamics and flight activity differ during the growing season in soybean fields. *Neohydatothrips variabilis* and *F. tritici* colonize soybean and have been captured within fields. *Frankliniella fusca* has been captured in soybean foliage, but in low numbers (Irwin and Yang 1980; Irwin *et al.* 1979). Studies have reported that *F. tritici* is the most abundant thrips species in soybean fields and its flight activity shows more dispersal events, peaking in early summer in June-July (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Irwin *et al.* 1979; Keough *et al.* 2018). The abundance of *F. fusca* tends to remain low and without notable peaks in flight activity throughout the season (Chitturi *et al.* 2018; Keough *et al.* 2018). Peak flight activity among adult *N. variabilis* occurs later in the season around mid to late August or early September in Iowa, Wisconsin, and Indiana (Bloomingdale *et al.* 2016; Keough *et al.* 2018) and July-August in Alabama (Chitturi *et al.* 2018). The increase in flight activity of thrips that transmit SVNV has been reported to precede the onset of SVNV symptoms (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Keough *et al.* 2018). Assessing the

factors that influence the dynamics of thrips that transmit SVNV will aid in our understanding of virus transmission and the epidemiology of SVNV in soybean fields.

Abiotic factors affect population dynamics, abundance, and phenology of insects (Frost *et al.* 2013; Kamiyama *et al.* 2020). For thrips, rainfall and temperature can have both direct, indirect, as well as both negative and positive effects on population dynamics, including development and flight activity. Rainfall has been observed to have a negative effect on thrips development as it can increase the mortality of immatures (Kirk 1997a; Morsello *et al.* 2008; Morsello and Kennedy, 2009). Flight activity can also be suppressed due to heavy rain when their fringed wings become saturated (Lewis 1997). However, rainfall can indirectly benefit thrips by delaying host senescence (Morsello *et al.* 2008) and increasing availability of food resources. Temperature has a direct positive effect on thrips development (Morsello *et al.* 2008; Morsello and Kennedy 2009; Morsello *et al.* 2010; Keough *et al.* 2018). A rise in temperatures increases thrips populations by hastening generation times (Kirk 1997a) and increasing the conditions conducive for flight (Lewis 1997). Both of these events can occur after a developmental threshold of 5-10ºC and a flight threshold of 17-21ºC is reached (Lewis 1997; Stacey and Fellowes 2002). Higher temperatures may negatively affect thrips populations indirectly by accelerating host senescence and availability as a resource (Kirk 1997a; Keough *et al.* 2018).

Temperature has direct effects on insect phenology influencing transmissibility of insect-transmitted plant viruses, such as SVNV, and the distribution and abundance of the insect vectors (Jones, 2016; Trębicki *et al.* 2017). Increases in temperatures affects insect generation times and frequency, extends flight times, and can shift population peaks in the season (Bergant *et al.* 2005; Canto *et al.* 2009; Jones 2016; Trębicki *et al.* 2017). Phenology models can be used to assess the seasonal patterns of insects (Frost. *et al.* 2013), guiding scouting and pesticide applications (Damos

and Savopoulou-Soultani 2010; Nietschke et al 2007), predicting occurrence and abundance (Frost. *et al.* 2013; Kamiyama *et al.* 2020) together with shifts in phenology due to climate change (Hodgson *et al.* 2011). In Wisconsin, phenology models have been previously developed for important insect pests, including plant-pathogen vectors (Frost *et al.* 2013; Kamiyama *et al.* 2020; Mueller *et al.* 2010). These models incorporate ambient temperature (degree-days) and the thermal requirements of insects needed for development (Jarošík *et al.* 2011; Kirk, 1997). Degree-days represent the accumulation of temperatures above an insect's lower developmental threshold where a developmental stage is completed. The sum of degree-days is used to measure the development of insects related to the accumulation of heat over time (Bergant *et al.* 2005; Jarošík *et al.* 2011). Increases in temperatures can result in a more rapid and/or greater total accumulation of degree-days, which can also result in an extended period conducive for insect development (Bergant *et al.* 2005).

The transmission of SVNV is tightly linked to the development of thrips (Rotenberg *et al.* 2015; Rotenberg and Whitfield, 2018; Han *et al.* 2019) and degree-days have been found to be an important abiotic factor that influences their populations (Morsello *et al.* 2008; Morsello and Kennedy, 2009; Morsello *et al.* 2010; Keough *et al.* 2018). The correlation of SVNV symptoms with vector populations have been based on circumstantial reports or a lack of regular scouting concomitant with thrips monitoring (Bloomingdale *et al.* 2016, Chitturi *et al.* 2018; Keough *et al.* 2018). Modelling the phenology of thrips is important for developing integrated pest management practices (Damos and Savopoulou-Soultani 2010; Kirk, 1997a), understanding the epidemiologically important vectors of SVNV in the field, and influencing disease and insect management strategies to limit transmission.

We report here on the use of a generalized additive mixed model using degree-days to describe the seasonal patterns of *N*. variabilis, *F. fusca*, and *F. tritici* abundance using catch data from 2013-2019 and a generalized linear model to determine the environmental variables influencing thrips flight in Wisconsin. Generalized additive mixed models are a variation of generalized linear models where non-linear relationships between the response variable and predictor variables are described by non-parametric smooth functions (Crawley 2012; Wood 2017). The model describes the data using complex functions as best (linear unbiased) predictors where the underlying trends in the data can be estimated by fitting a smoothing curve that has no specific functional form (Zuur *et al.* 2009; Frost *et al.* 2013; Kamiyama *et al.* 2020). In logistic regression models, on the other hand, the objective is to obtain a best fitting model, that is biologically relevant, of the relationship between the binary, response variable and predictors (De Wolf *et al.* 2002). The response variable can be used to estimate the probability of an event (Wood 2017; De Wolf *et al.* 2002).

Logistic regression models have been utilized to model the relationship of disease with environmental weather variables and make risk assessments (De Wolf *et al.* 2002; Willbur *et al.* 2018). Our goal for this study was based on a two-step modelling approach: 1) use a generalized additive mixed model to describe the seasonal patterns of thrips towards the accurate identification of a risk window(s) for SVNV transmission based on estimated critical degree-days of flight activity, and 2) use a logistic regression model to identify environmental weather variables that influence elevated risk of thrips activity, and 3) validate the logistic regression models using recorded thrips captures.

Materials and Methods

Thrips collection and sampling

The flight activity of *N. variabilis*, *F. fusca*, and *F. tritici* was previously monitored in soybean fields from 2013-2014 in five locations in Wisconsin (Table 1). The trap count data collection and field sites have been described previously in Bloomingdale (2015) and Bloomindale *et al*. (2016).

The activity of *N. variabilis*, *F. fusca*, and *F. tritici* and SVNV were also monitored from 2017 to 2019 at the Arlington Agricultural Experiment Station using sentinel plots (Table 1). The trial consisted of eight replicated plots measuring 36 m^2, each with eight, 0.76m-wide rows. Four of the rows were planted with *Vigna unguiculata* subsp. *unguiculata* (L.) Walp var. 'California Blackeye #5', as the sentinel crop, while four rows (two on each lateral side of the plot) were planted with *G. max* var. 'Dwight'. Yellow panel traps measuring 13.97cm by 22.86cm (Seabright Laboratories, Emeryville, CA) were attached to the top of fiber glass rods (above the canopy) and placed within each plot, between rows of *V. unguiculata*. The first rod with panel trap was placed at 10m from the first row of crops, and the others were subsequently placed ~25m apart. Each year, the panel traps would be placed in the field one month after planting, depending on the weather, or when plants were in vegetative growth stages (V1 or V2). The exposure period was one week with the trapping intervals of 7 days (+/- 2) in all three years. Trapping was initiated on June 20 and ended on September 12 in 2017, May 29 to September 11 in 2018, and June 20 to September 16 in 2019. After the exposure period in the field, clear plastic saran wrap was placed on the adhesive side to preserve the specimens. The traps were then stored at 4ºC or at -20ºC for long-term storage. Each trap consisted of two panels with 72, 6.5 cm^2 cells. Fifty-six of the total cells (28 per panel) were fully covered in adhesive and were considered as the collection region. Twenty

cells (10 per panel) were randomly sampled per trap. Adult thrips of *N. variabilis*, *F. tritici*, and *F. fusca* caught on the panel traps were identified by morphological characteristics and tabulated. The capture weeks were standardized among the three years to match the same calendar week and allow comparisons between years (Table AI1.3). This resulted in 13 weeks of trapping that would allow for the equal downstream analyses.

SVNV detection

Asymptomatic and virus-like symptomatic leaf tissue from cowpea and soybean were sampled each week, concomitant with panel traps. Total RNA was isolated from approximately 100mg of tissue using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA) and the Maxwell RSC Plant RNA kit with the automated Maxwell RSC Instrument (Promega, Madison, WI) according to manufacturer's instructions. Reverse transcription of the RNA template to complementary DNA (cDNA) was performed using the 5X iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA) with random priming and 1 µg of RNA template. The cDNA was subsequently diluted to a 1:10 ratio using nuclease-free water. Detection of SVNV in the samples was achieved using RT-PCR with nested primers, designed by Dr. Ranjit Dasgupta, that amplify the Nucleoprotein (NP) gene. The first-round PCR reaction consisted of 12.5 µL of 5X GoTaq Master Mix (Promega, Madison, WI), 1 µM of SVNV F1 (5'-AGATATAAAGTTGAGACACTATC-3'), 1 µM of SVNV R1 (5'-TGCAACACATCCGGAACTCTG-3'), and 7.5 µL of cDNA in a 1:10 dilution. Two reactions substituting cDNA with nuclease-free water were included as negative controls. The second-round PCR reaction consisted of 12.5 µL of 5X GoTaq Master Mix (Promega, Madison, WI), 1 µM of SVNV F2 (5'-CCTGAATTCATGCCACAAACAGCAGG-3'), 1 µM of SVNV R2 (5'-TTAGCGGCCGCTAAACAGAAAACTCC-3'), 2.5 µL nuclease-free water, and 5.0 µL DNA

from the first-round. Four negative controls were included in the second-round PCR: two reactions substituting DNA with nuclease-free water, and two reactions using the negative controls from the first-round. The PCRs were performed in an Eppendorf MasterCycler Pro S programmable thermal cycler (Eppendorf AG, Hamburg, Germany) using the following conditions: 2 min denaturation at 95 ºC, followed by 32 cycles of 30 seconds denaturation at 94 ºC, 30 seconds annealing at 52 ºC, and 1 min elongation at 72 ºC, followed by a final extension step of 10 min at 72 ºC. Samples with amplicons of 800 bp were determined positive for SVNV with a 1.2% agarose gel stained with SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA).

Phenology model

Daily minimum and maximum air temperatures were obtained from the PRISM Climate Group (2020) for all the years of captures (2013-2019) and locations (Table 4.1). The developmental thresholds have not been pre-determined for the thrips species evaluated in this study. Thus, growing degree-days (DD) were calculated based on the lower (7.2 ºC) and upper (40 ºC) developmental thresholds for *Frankliniella occidentalis* (Pest Prophet 2021; Stacey and Fellows 2002) as a proxy species. Cumulative DD were calculated for each location and year from January 1st to December 31st using the equation (1) with observed air temperatures (Obs Temp), and lower and upper temperatures of developmental threshold (TLower, TUpper, respectively).

(1)

$$\mathrm{DD} = \frac{[\mathrm{Max\ (Obs.\ Temp,\ \ TLower)} + \mathrm{Min\ (Obs.Temp,\ \ TUpper)}]}{2} - \mathrm{TLower}$$

A generalized additive mixed model (GAMM) was generated to describe the seasonal patterns of *N. variabilis*, *F. fusca*, and *F. tritici* abundance (Y_{ij}) as a function of degree-days (x_i). The GAMM was generated from the trap counts from 2013-2104 and 2017-2019, DD, year, and replicate data using a negative Poisson regression. The model was built using the *glmer* function

of the *lme4* package and the critical DD predictions from the GAMM were generated with the *gam* function in the *mgcv* package of R version 3.6.3 (Bates et al. 2015; R Core Team 2020; Wood 2004). The GAMM is described as follows:

(2)

$$Y_{ij} \sim Poisson\left[\mu_{ij(a)}\right]$$

$$g\left[\mu_{ij(a)}\right] = log[\mu_{ij(a)}] = f(x_i) + \beta_j + \varepsilon_a + \delta_{ij}$$

$$\varepsilon_a \sim N(0, \sigma_a^2)$$

In this model, DD (ε_a) was used as a random effect and year, replicate, and location as fixed effects. The intercept (β_j) interpreted as the thrips counts by species explained by DD, $f(x_i)$ is a penalized cubic regression smoothing function of DD, and δ_{ij} represents the residual error of the model estimation. To interpret GAMMs, the smooth functions $f(x_i)$ needs to be graphically represented (Wood 2017). Therefore, the smooth function (i.e., conditional modes) was plotted versus DD (x_i) to represent the expected seasonal trends of capture for each of the thrips' species. The GAMM-predicted critical DD from the conditional modes (CM) were used to approximate thrips phenology (i.e., above average trap catches, peak flight activity, end of flight activity) based on calendar dates corresponding to the DD (Kamiyama et al. 2020). The windows for SVNV transmission were estimated after inspection of the plot for periods with positive CM (above average trap catches) as it related to an increase, peak, and end of the flight activity.

Logistic regression model

Logistic regression model was used to determine biologically relevant weather values influencing thrips flight and identify periods of high or low flight activity. Weather data for each location (Table 4.1) corresponding to 2013-2014 data set were obtained from Dark Sky weather data network (Apple, Inc., Cupertino, CA). Daily mean, maximum, and minimum of air

temperature (ºC), relative humidity (%), wind speed (m/s), dew point (ºC), and total and maximum precipitation (mm) were obtained. Seven-day moving averages for each weather variable were calculated for each date based on average thrips trapping intervals. The GAMM-generated CM were transformed to a binary variable for each trap location and date. If the conditional mode was above average count for a specific date, then it was labeled as '1', and '0' if it was below average. All weather variables and their seven day moving averages, mean counts and CM fits for each thrips species, respectively, and DD (n=125) were included in a Kendall correlation analysis using PROC CORR and the Kendall command in SAS (v.9.4, SAS Institute). Correlations were used to assess biologically relevant weather variables based on their degree of association with the thrips CM fits (Willbur *et al*. 2018). Subsequent logistic regression modelling was performed using the biologically relevant variables and the CM fits for each thrips species using the PROC LOGISTIC procedure with the descending option. Models were evaluated using the Akaike's information criterion, the area under the receiver operating characteristic curve (c statistic). Goodness of fit was evaluated with the Hosmer-Lemeshow test and max-rescaled R^2 statistic used to evaluate model selection.

The resulting logit for each best-fitting model was used to calculate the probability of each thrips species per trapping date and corresponding DD using equation 3. The probability at which sensitivity and specificity values converged for each of the models were used to determine the risk of high flight threshold (high flight potential). Daily mean thrips counts from 2017-2019 were compared with the model probabilities (equation 3) for each thrips species and dates to validate the models.

(3)

$$\text{Probability} = \frac{e^{\text{logit}\,(\mu)}}{(1 + e^{\text{logit}\,(\mu)})}$$

Results

Detection of Soybean vein necrosis virus

Plants from sentinel plots were sampled at the same time as the thrips sticky traps in 2017-2019. SVNV was detected using nested RT-PCR and the results were converted to a binary variable based on positive/negative results (presence/absence) due to unbalanced data and to evaluate presence (SVNV-positive sample) or absence (SVNV-negative sample). Weeks where SVNV was detected in the sentinel plots were compared to DD based on thrips developmental threshold (Figure 4.1). In 2017, SVNV was only detected in late August (2820 DD) to early September (2940 DD). The following year, SVNV was detected in mid-July (1760-1970 DD), at 2940 DD, 2720 DD, and 3060 DD which correspond to several weeks in August. In 2019, SVNV was detected in late August or at 2610 DD and 2720 DD.

Phenology model

A plot of the smooth function versus cumulative DD was generated to represent GAMM-estimated seasonal trends of the *F. fusca*, *F. tritici*, and *N. variabilis* (Figure 4.2). The model suggested that above average flight activity of *F. fusca* begins at 1108 DD (Figure 4.2A). A period of increased activity and above average trap captures occurred between 1108 DD and 1687 DD, which corresponded to late-June and mid-July, respectively. Peak flight occurred at 1697 DD followed by a period of descending activity that ended at 2464 DD (mid-August). For *F. tritici*, the model estimated that above average trap catches start at 1291 DD (late-June) and continue to 2372 DD (Figure 4.2B). Peak flight activity occurred at 2372 DD (late-August), demonstrated by above average trap captures, and ended at 2851 DD (early September) where trap captures decreased thereafter. For *N. variabilis*, the most efficient vector of SVNV, the model estimates that above average flight activity occurred at 1678 DD (mid-July) and continued through 2093 DD

(late July to early August), (Figure 4.2C). Flight activity of *N. variabilis* peaked at 2625 DD (late August) and ended at 3028 DD (mid to late September).

Logistic regression model

The best fitting model for *F. fusca* used maximum, minimum, and mean relative humidity as the most explanatory variables (Table 4.2). This model had a c statistic of 0.779, 57% model accuracy, and 71% sensitivity, 71% specificity at a 64% threshold. Mean relative humidity was negatively correlated to *F. fusca* (equation 4). The *F. tritici* model included mean air temperature and total precipitation as the most explanatory variables, where the former was positively correlated, and the latter was negatively correlated (Table 4.2, equation 5). This model had a c statistic of 0.726, 62% model accuracy, 69.5 % sensitivity and 68.9% specificity at a 76% probability threshold. The *N. variabilis* model included the intercept and maximum wind speed as the most explanatory variables (Table 4.2, equation 6). The model had a c statistic of 0.669, a low maximum rescaled R^2, and 35% accuracy, and 63.2% sensitivity and 62 % specificity at a 42% probability threshold.

(4)

$$F.fusca\ Logit(\mu) = 0.405\ (\text{MaxRH}) + 0.176(\text{MinRH}) - 0.617\ (\text{MeanRH})$$

(5)

$$F.tritici\ Logit(\mu) = 0.090\ (\text{MeanAT}) - 0.045(\text{TotPre})$$

(6)

$$N.variabilis\ Logit(\mu) = 2.227 - 0.705(\text{MaxWS})$$

Model validation was conducted with the 2017-2019 thrips capture data set. The probability risk for each species was calculated by first generating the logit for each species and then using the logits in equation 3 for each trapping date and corresponding DD. The model

predicted high risk (probability >65%) for *F. fusca* at 2240 DD in 2017 (Figure 4.3A), at 1200DD and between 1540 - 2720 DD in 2018 (Figure 3B), and 940-2060 DD in 2019 (Figure 4.3C). The increased counts of *F. fusca* occurred at 2240 DD in 2017 and 1970 DD in 2018 as predicted by the model. However, in 2019, the observed *F. fusca* was below the action threshold and risk prediction. In the *F. tritici* model, periods of high risk (>75% threshold) predicted by the model were at 1890DD and 2240-2530 DD in 2017 (Figure 4.4A), 1760 DD and 2210-2490 DD in 2018 (Figure 4B), and at 1570-1720 DD, 2060 DD, and 2610 DD in 2019 (Figure 4.4C). Mean captures of *F. tritici* did not coincide with similar periods of mean capture in 2017. *F. tritici* captures were above the risk of high flight threshold at 2330 DD in 2018 and 2430 DD in 2019.

The *N. variabilis* model predicted periods of high captures in 2017 above the action threshold (40%) throughout the season. However, *N. variabilis* captures were above the risk of high flight between 2390-2820 DD, which corresponds to August and September (Figure 4.5A). In 2018, high risk of *N. variabilis* was predicted by the model at 2330 DD and 2720 DD (Figure 5B). The observed captures of *N. variabilis* above the threshold occurred between 2210-2490 DD. In 2019, the model predicted high risk at 1280 DD and 2290 DD (Figure 4.5C), but the captures that were above the threshold occurred between 2060 and 2430 DD.

Discussion

Thrips are small, polyphagous arthropods with the ability to transmit tospoviruses (Jones 2005; Rotenberg et al. 2015; Rotenberg and Whitfield 2018). *Soybean vein necrosis virus* (SVNV) is a tospovirus that is widespread in the major soybean-growing regions of the USA (Zhou and Tzanetakis, 2019). Three species of thrips (*Frankliniella fusca*, *Frankliniella tritici*, and *Neohydatothrips variabilis*) are reported to transmit SVNV with different efficiencies (Keough et al 2016). Occurrence of SVNV-symptoms are reported to be follow an increase in thrips flight

activity (Bloomingdale *et al.* 2016; Chitturi *et al.* 2018; Keough *et al.* 2018). Thus, our objective was to 1) describe the seasonal trends of *F. fusca*, *F. tritici*, and *N. variabilis* populations using a generalized additive mixed model (GAMM) and degree-days, 2) use a logistic regression model to identify weather variables influencing thrips activity, and 3) correlate SVNV detection with periods of high and low risk of thrips captures.

The GAMM presented in this study was used as a descriptive tool to assess the seasonal patterns of *F. fusca*, *F. tritici*, and *N. variabilis* in Wisconsin. A smooth curve plot generated from the GAMM represented seasonal trends and critical degree-days related to the insects' phenology. Periods of high or low risk for each of the species and associated flight activity can be determined from interpretation of the plot (Frost et al. 2013; Kamiyama et al. 2020). The trends from the smooth curve plot suggests that there are species-specific patterns associated with the flight dynamics of thrips that transmit SVNV. *Frankliniella fusca* population trends are typically earlier in the season and we observed lower counts in yellow cards. Although it is the second most efficient insect vector in our study, in terms of SVNV transmission (Keough et al. 2016), *F. fusca* may not be an epidemiologically important species for field transmission in our investigations. Patterns of *Frankliniella tritici* capture illustrated increased activity for a longer period, compared to *F. fusca* and *N. variabilis*, that coincided with detection of SVNV in sentinel plots. Specifically, the period between 2000-3000 DD is a time of high risk due to increased, or peak activity of *F. tritici* and *N. variabilis*. These observations suggest a period of elevated risk for SVNV transmission and coincides with a similar period where SVNV was detected in the 2017-2019 trapping years. GAMMs have been used previously to describe seasonal patterns, estimate phenology, and predict insect occurrence (Frost et al. 2013; Kamiyama et al. 2020). The data presented here are limited to certain locations in Wisconsin and observations across multiple

locations and multiple years would be necessary for the development of a more comprehensive, predictive tool.

The weather variables influencing thrips populations and risk probabilities were evaluated with a logistic regression model and the 2013-2014 data set. Both the *F. fusca* and *F. tritici* models had acceptable discrimination as indicated by the c statistic. However, the *F. tritici* model was the most interpretative and biologically relevant model where air temperature was positively correlated, and total precipitation was negatively correlated. Temperature has a positive effect on thrips development and flight (Kirk 1997a; Lewis 1997) and precipitation can increase mortality of immatures and prevent flight (Kirk 1997a; Lewis 1997; Morsello *et al.* 2008; Morsello and Kennedy, 2009). Both of temperature and precipitation have been previously shown to influence absolute thrips population densities and flight activity (Kirk 1997a; Morsello *et al.* 2008; Morsello and Kennedy 2009; Morsello *et al.* 2010; Keough *et al.* 2018). The *F. fusca* model only included mean, maximum, and minimum relative humidity as explanatory variables, which were positively and negatively correlated. Relative humidity may affect thrips initiation of flight and restrict its duration (Lewis 1997). Maximum wind speed was the only explanatory variable in the *N. variabilis* model and was negatively correlated. Thrips are considered weak flyers (Lewis 1997) and captures have been found to decrease as wind speed increases (Smith *et al*. 2016). The risk probabilities predicted by the corresponding model for each of the thrips' species did not accurately predict periods of high capture for many of the trapping years and species in the 2017-2019 validation data set. The logistic regression models are limited by the number of observations in the 2013-2014 and 2017-2019 data sets. Similar to the phenology model, more locations and trapping years are needed to predict risk for thrips flight and develop a comprehensive predictive model.

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Literature Cited

Anderson, N. R., Irizarry, M. D., Bloomingdale, C. A., Smith, D. L., Bradley, C. A., Delaney, D. P., Kleczewski, N. M., Sikora, E. J., Mueller, D. S., and Wise, K. A. 2017. Effect of soybean vein necrosis on yield and seed quality of soybean. Can. J. Plant Pathol. 39:334–341.

Bates, D., Maechler, M., Bolker, B., and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. J. Stat. Softw. 67:1–48.

Bergant, K., Trdan, S., Žnidarčič, D., Črepinšek, Z., and Kajfež-Bogataj, L. 2005. Impact of Climate Change on Developmental Dynamics of Thrips tabaci (Thysanoptera: Thripidae): Can It Be Quantified? *Environ. Entomol*. 34:755–766.

Bloomingdale, C., Irizarry, M. D., Groves, R. L., Mueller, D. S., and Smith, D. L. 2016. Seasonal Population Dynamics of Thrips (Thysanoptera) in Wisconsin and Iowa Soybean Fields. *J. Econ. Entomol*. 110:133–141

Canto, T., Aranda, M. A., and Fereres, A. 2009. Climate change effects on physiology and population processes of hosts and vectors that influence the spread of hemipteran-borne plant viruses. *Glob. Chang. Biol*. 15:1884–1894.

Chitturi, A., Conner, K., Sikora, E. J., and Jacobson, A. L. 2018. Monitoring Seasonal Distribution of Thrips Vectors of Soybean Vein Necrosis Virus in Alabama Soybeans. *J. Econ. Entomol*. 111:2562–2569

Crawley, M. J. 2012. The R Book. 2nd Edition. Chichester, UK: John Wiley & Sons, Ltd.

Damos, P. T., and Savopoulou-Soultani, M. 2010. Development and statistical evaluation of models in forecasting moth phenology of major lepidopterous peach pest complex for Integrated Pest Management programs. Crop Prot. 29:1190–1199

Frost, K. E., Esker, P. D., Van Haren, R., Kotolski, L., and Groves, R. L. 2013. Seasonal Patterns of Aster Leafhopper (Hemiptera: Cicadellidae) Abundance and Aster Yellows Phytoplasma Infectivity in Wisconsin Carrot Fields. *Environ. Entomol*. 42:491–502

Groves, C., German, T., Dasgupta, R., Mueller, D., and Smith, D. L. 2016. Seed Transmission of Soybean vein necrosis virus: The First Tospovirus Implicated in Seed Transmission. *PLoS One*. 11:1–14

Han, J., Nalam, V. J., Yu, I.-C., and Nachappa, P. 2019. Vector Competence of Thrips Species to Transmit Soybean Vein Necrosis Virus. *Front. Microbiol*. 10:431

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. *Food Secur*. 3:5–17.

Hesler, L. S., Allen, K. C., Luttrell, R. G., Sappington, T. W., and Papiernik, S. K. 2018. Early-season pests of soybean in the United States and factors that affect their risk of infestation. *J. Integr. Pest Manag*. 9(1):1-15.

Hodgson, J. A., Thomas, C. D., Oliver, T. H., Anderson, B. J., Brereton, T. M., and Crone, E. E. 2011. Predicting insect phenology across space and time. Glob. *Chang. Biol*. 17:1289–1300.

Hurley, T., and Mitchell, P. 2017. Value of neonicotinoid seed treatments to US soybean farmers. *Pest Manag. Sci*. 73:102–112

Irwin, M. E., Yeargan, K. V., and Marston, N. L. 1979. Spatial and Seasonal Patterns of Phytophagous Thrips in Soybean Fields with Comments on Sampling Techniques. *Environ. Entomol*. 8:131–140.

Irwin, M. E., and Yeargan, K. V. 1980. Sampling Phytophagous Thrips on Soybean BT. In *Sampling Methods in Soybean Entomology*, eds. M. Kogan and D. C. Herzog. New York,

NY: Springer New York, p. 283–304.
Jarošík, V., Honěk, A., Magarey, R. D., and Skuhrovec, J. 2011. Developmental Database for Phenology Models: Related Insect and Mite Species Have Similar Thermal Requirements. *J. Econ. Entomol*. 104:1870–1876.
Jones, D. R. 2005. Plant viruses transmitted by thrips. *Eur. J. Plant Pathol.* 113:119–157.
Jones, R. A. C. 2016. Future Scenarios for Plant Virus Pathogens as Climate Change Progresses. In *Advances in Virus Research*, Academic Press, p. 87–147.
Kamiyama, M. T., Bradford, B. Z., Groves, R. L., and Guédot, C. 2020. Degree day models to forecast the seasonal phenology of Drosophila suzukii in tart cherry orchards in the Midwest U.S. *PLoS One*. 15:1–13.
Keough, S., Danielson, J., Marshall, J. M., Lagos-Kutz, D., Voegtlin, D. J., Srinivasan, R., and Nachappa, P. 2018. Factors Affecting Population Dynamics of Thrips Vectors of Soybean vein necrosis virus. *Environ. Entomol*. 47:734–740.
Kirk, W. D. J. 1997a. Distribution, Abundance, and Population Dynamics. In *Thrips as Crop Pests*, ed. Trevor Lewis. CAB International, p. 217–258.
Kirk, W. D. J. 1997b. Feeding. In *Thrips as Crop Pests*, ed. Trevor Lewis. CAB International, p. 119–174.
Lewis, T. 1997. Flight and Dispersal. In *Thrips as Crop Pests*, ed. T. Lewis. CAB international, p. 175–196.
Morsello, S. C., Beaudoin, A. L. P., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2010. The influence of temperature and precipitation on spring dispersal of *Frankliniella fusca* changes as the season progresses. *Entomol. Exp. Appl*. 134:260–271.
Morsello, S. C., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2008. Temperature and Precipitation Affect Seasonal Patterns of Dispersing Tobacco Thrips, *Frankliniella fusca*, and Onion Thrips, *Thrips tabaci* (Thysanoptera: Thripidae) Caught on Sticky Traps. *Environ. Entomol*. 37:79–86.
Morsello, S. C., and Kennedy, G. G. 2009. Spring temperature and precipitation affect tobacco thrips, *Frankliniella fusca*, population growth and *Tomato spotted wilt virus* spread within patches of the winter annual weed *Stellaria media*. *Entomol. Exp. Appl*. 130:138–148.
Mueller, E. E., Frost, K. E., Esker, P. D., and Gratton, C. 2010. Seasonal Phenology of *Aphis glycines* (Hemiptera: Aphididae) and Other Aphid Species in Cultivated Bean and Noncrop Habitats in Wisconsin. *J. Econ. Entomol*. 103:1670–1681.
Musser, F. R., Catchot, A. L., Conley, S. P., Davis, J. A., DiFonzo, C., Greene, J. K., Lorenz, M. G., Owens, D., Reisig, D. D., Roberts, P., Royer, T., Seiter, N. J., Smith, R., Stewart, S. D., Taylor, S., Tilmon, K., Villanueva, R. T., and Way, M. O. 2019. 2019 Soybean Insect Losses in the United States. *MidSouth Entomologist*. 13(1): 1-23. Available at: www.midsouthentomologist.org.msstate.edu.
Nietschke, B. S., Magarey, R. D., Borchert, D. M., Calvin, D. D., and Jones, E. 2007. A developmental database to support insect phenology models. Crop Prot. 26:1444–1448.
PRISM Climate Group. 2020. Northwest Alliance for Computational Science & Engineering, Corvallis, OR. 2020. Available at: http://prism.oregonstate.edu/
R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
Rotenberg, D., Jacobson, A. L., Schneweis, D. J., and Whitfield, A. E. 2015. Thrips transmission of tospoviruses. *Curr. Opin. Virol*. 15:80–89
Rotenberg, D., and Whitfield, A. E. 2018. Molecular interactions bewteen tospoviruses and thrips

vectors. Curr. Opin. Virol. 33:191–197

Smith, E. A., Shields, E. J., and Nault, B. A. 2016. Impact of Abiotic Factors on Onion Thrips (Thysanoptera: Thripidae) Aerial Dispersal in an Onion Ecosystem. Environ. Entomol. 45:1115–1122

Stacey, D. A., and Fellowes, M. D. E. 2002. Temperature and the development rates of thrips: Evidence for a constraint on local adaptation? *Eur. J. Entomol*. 99:399–404.

Trębicki, P., Dáder, B., Vassiliadis, S., and Fereres, A. 2017. Insect–plant–pathogen interactions as shaped by future climate: effects on biology, distribution, and implications for agriculture. *Insect Sci*. 24:975–989.

United States Department of Agriculture National Agricultural Statistics Service. 2021a. 2020 State Agriculture Overview for Wisconsin. Available at: https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=WISCONSIN.

United States Department of Agriculture National Agricultural Statistics Service. 2021b. Crop Production 2020 Summary. Available at: https://usda.library.cornell.edu/concern/publications/tm70mv177.

United States Department of Agriculture National Agricultural Statistics Service. 2021c. Crop Values 2020 Summary. Washington, D. C. Available at: http://usda.library.cornell.edu/concern/publications/k35694332?locale=en.

van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and Peters, D. 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. *Entomol. Exp*. Appl. :9–15.

Willbur, J. F., Fall, M. L., Bloomingdale, C., Byrne, A. M., Chapman, S. A., Isard, S. A., Magarey, R. D., McCaghey, M. M., Mueller, B. D., Russo, J. M., Schlegel, J., Chilvers, M. I., Mueller, D. S., Kabbage, M., and Smith, D. L. 2018. Weather-Based Models for Assessing the Risk of *Sclerotinia sclerotiorum* Apothecial Presence in Soybean (*Glycine max*) Fields. Plant Dis. 102:73–84

Wood, S. N. 2017. Generalized Additive Models: An Introduction with R. Chapman and Hall/CRC.

Wood, S. N. 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. J. Am. Stat. Assoc. 99:673–686.

Zhou, J., Kantartzi, S. K., Wen, R. H., Newman, M., Hajimorad, M. R., Rupe, J. C., and Tzanetakis, I. E. 2011. Molecular characterization of a new tospovirus infecting soybean. *Virus Genes*. 43:289–295.

Zhou, J., and Tzanetakis, I. E. 2013. Epidemiology of soybean vein necrosis-associated virus. *Phytopathology*. 103:966–971.

Zhou, J., and Tzanetakis, I. E. 2019. *Soybean vein necrosis virus*: an emerging virus in North America. *Virus Genes.* 55:12–21

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. 2009. Mixed effects models and extensions in ecology with R. New York, NY: Springer New York.

Table and Figures

Table 4.1. Trapping locations for 2013-2014 and 2017-2019 trapping periods.

Location	County	Latitude (N)	Longitude (W)	Trapping year (s)
Arlington	Columbia	43.32	-89.33	2013-2014; 2017-2019
Chippewa Falls	Chippewa	44.96	-91.37	2013-2014
Fond du Lac	Fond du Lac	43.73	-88.57	2013-2014
Lancaster	Grant	42.83	-90.78	2013
Galesville	Trempealeau	44.08	-91.28	2013-2014
Platteville	Grant	42.76	-90.43	2014

Table 4.2. Parameters for the *Frankliniella fusca*, *Frankliniella tritici*, and *Neohydatothrips variabilis* models

Model	Variables/Parameters[b]	AIC	c	R^2	Corr.	Sens	Spec.	F. Pos.	F. Neg.
F. fusca	MaxRH, MinRH, Mean RH	139.838	0.779	0.3609	57[c]	71.2[c]	71.3[c]	81.4[c]	58.2[c]
F. tritici	MeanAT, TotPre	130.889	0.726	0.4134	62[d]	69.6[d]	68.9[d]	71.4[d]	86.1[d]
N. variabilis	Intercept, MaxWS	164.249	0.669	0.1103	35[e]	63.2[e]	64.8[e]	62[e]	56.5[e]

[a]Abbreviations: AIC = Aikaike's information criterion, c = c statistic, R^2 = maximum rescaled value, Corr. = percent of model accuracy, Sens. = percent sensitivity, Spec. = percent specificity, F. Pos = percent false positives, F. Neg = percent false positives

[b]Maximum (Max), minimum (Min), mean relative humidity (RH), air temperature (AT), total precipitation (TotPre), widn speed (WS).

[c]Probability threshold of 0.65

[d]Probability threshold of 0.75

[e]Probability threshold of 0.40

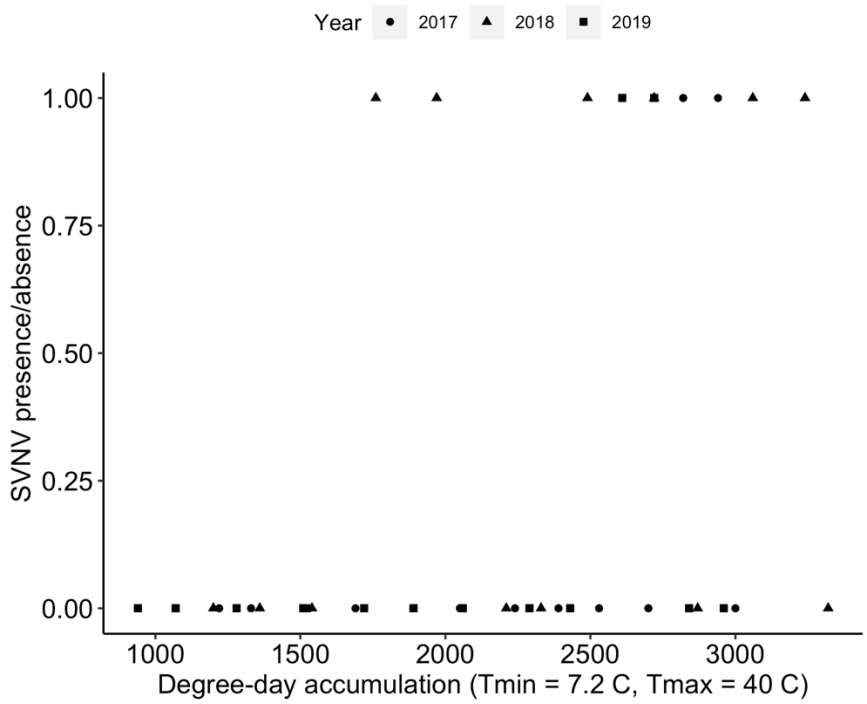

Figure 4.1. Detection of *Soybean vein necrosis virus* (SVNV) in sentinel plots during the 2017-2019 trapping years versus cumulative degree-days. Plants in sentinel plots (soybean, cowpea) were sampled concomitantly with thrips sticky traps in 2017 (circles), 2018 (triangles), and 2019 (squares). SVNV was detected using nested RT-PCR and nucleoprotein gene specific primers. PCR products were evaluated visually with 1.2% agarose. SVNV-positive sample (800 bp PCR product) was labeled as '1' (presence), and SVNV-negative samples (no amplification) labeled as '0' (absent).

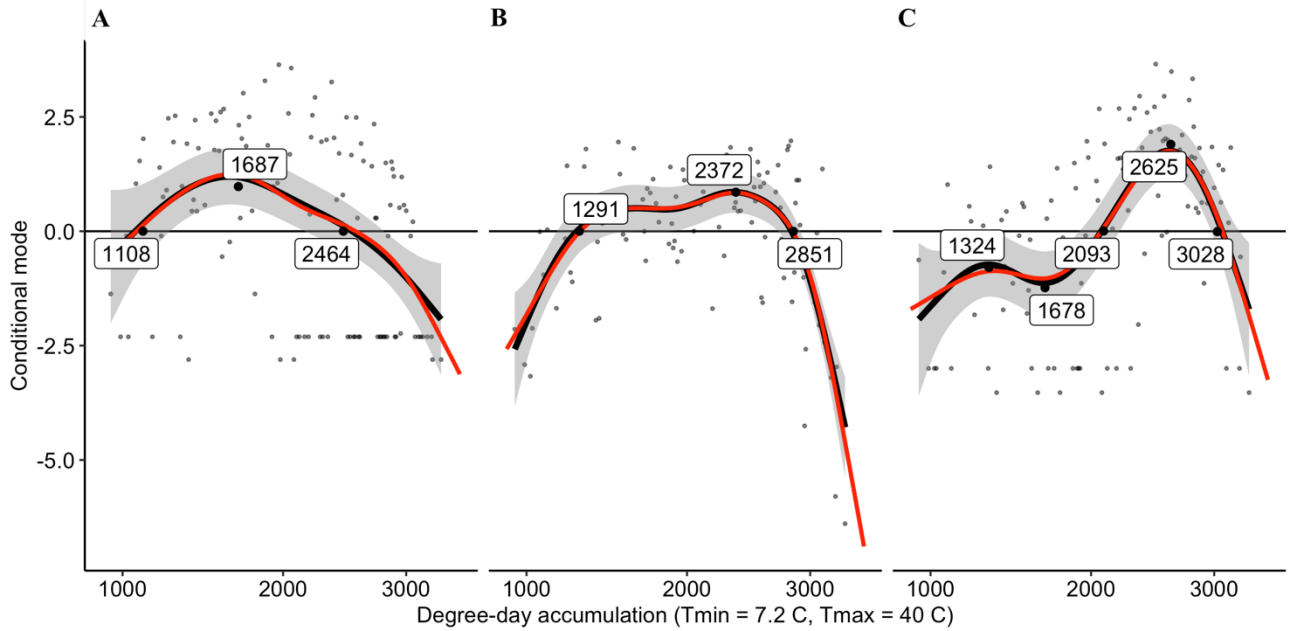

Figure 4.2. Seasonal trends of three species of thrips, that transmit *Soybean vein necrosis virus*, over cumulative degree-days for the 2013-2019 trapping years. Smooth curve plots were produced for *Frankliniella fusca* (A), *Frankliniella tritici* (B), and *Neohydatothrips variabilis* (C) from the GAMM spline fits (conditional modes). Smooth fit lines (black line) are shown with 95% confidence intervals (grey band). Conditional modes (CM, grey circles) are deviations from the mean where '0' indicates average trap catches, positive CM: above average trap catches, negative CM: below average trap catches. GAMM-estimated trends are shown in red. Critical degree-days estimated by the GAMM are shown in white boxes.

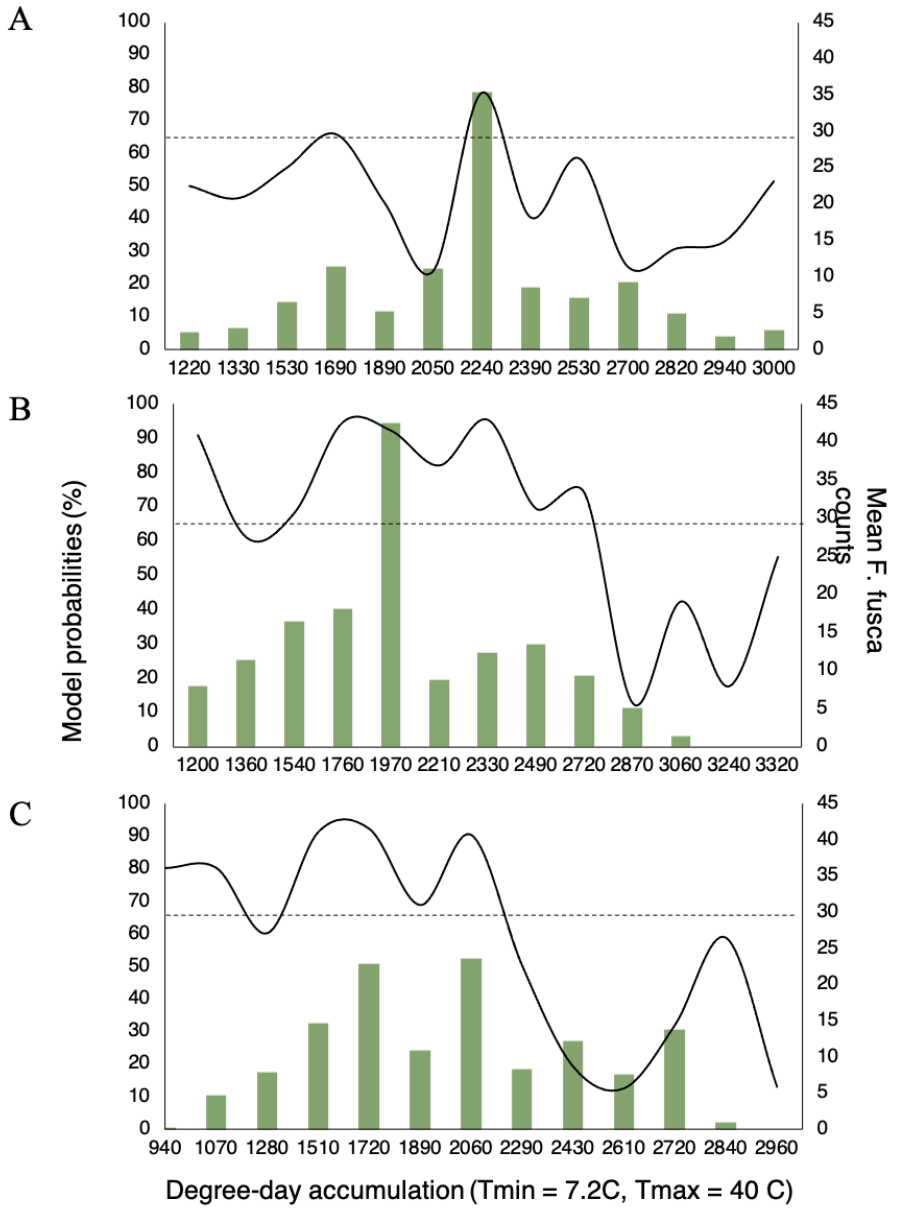

Figure 3. *Frankliniella fusca* logistic regression model validation and the risk of flight threshold. Logistic regression model for *F. fusca* was validated using 2017 (A), 2018 (B), and 2019 (C) data set. Solid line shows the probabilities of *F. fusca* calculated using the logit model parameters (equation 4). Bars represent mean counts of *F. fusca* corresponding to each trapping date represented as cumulative degree-days. Dashed line represents action threshold at a probability of 0.65.

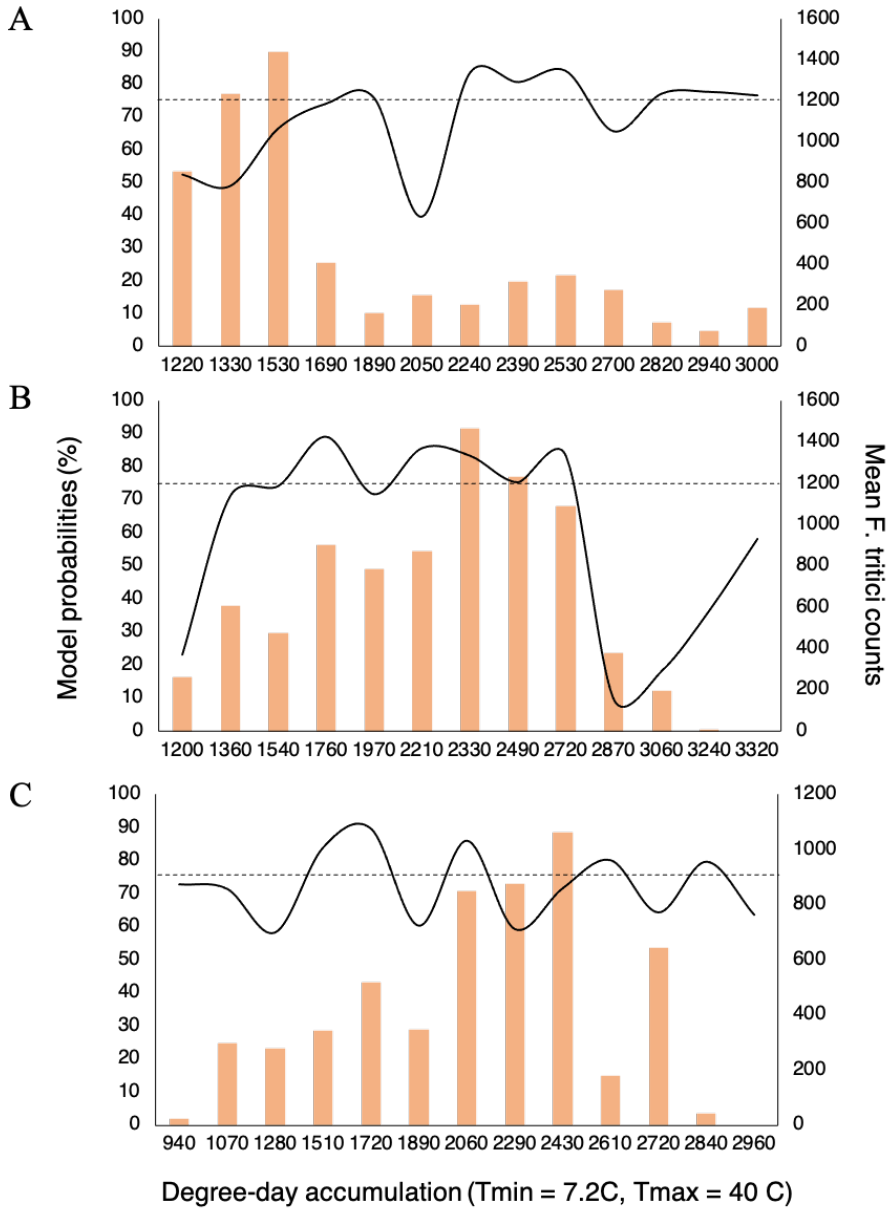

Figure 4.4. *Frankliniella tritici* logistic regression model validation and the risk of flight threshold (75%). Logistic regression model for *F. tritici* was validated using 2017 (A), 2018 (B), and 2019 (C) data set. Black line shows the probabilities of *F. tritici* calculated using the logit model parameters (equation 5). Bars represent mean counts of *F. tritici* corresponding to each trapping date represented as cumulative degree-days. Dashed line represents action threshold at a probability of 0.75.

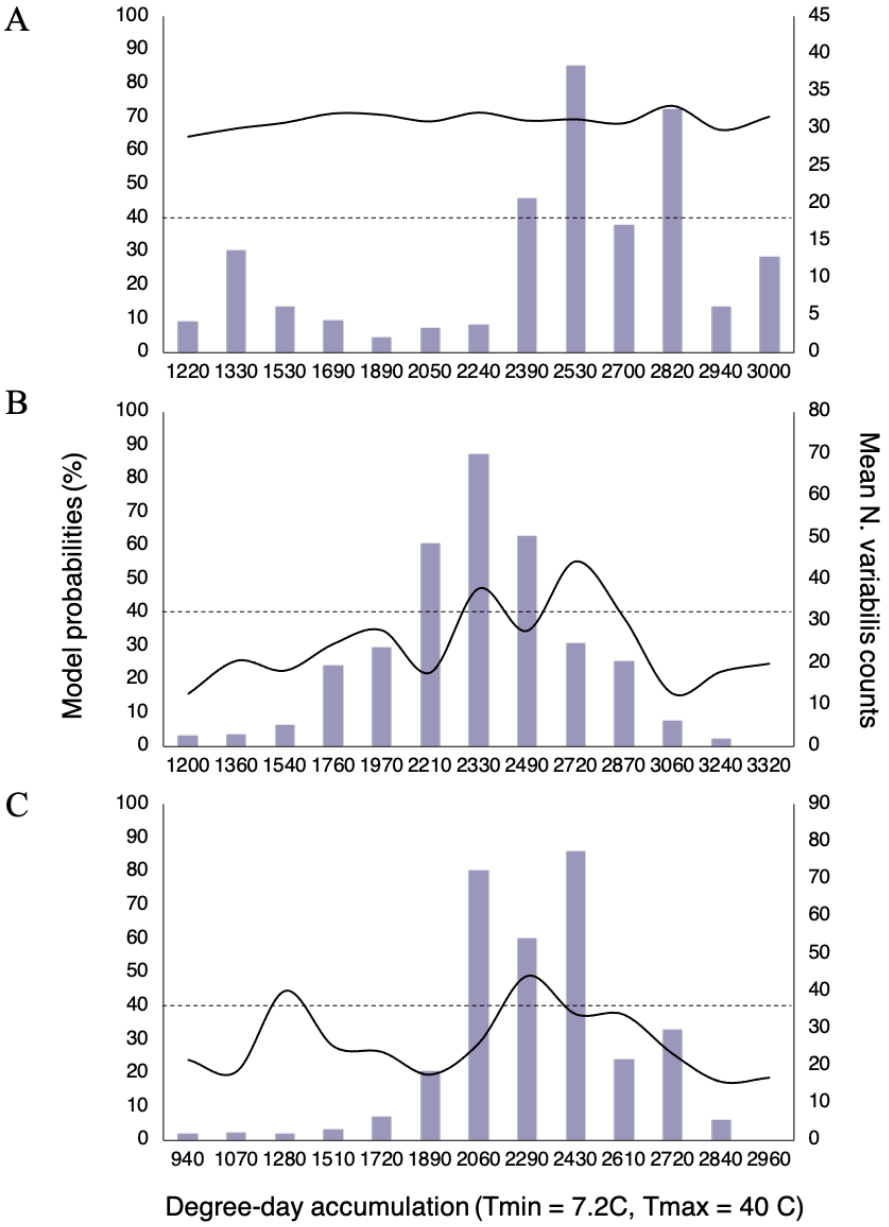

Figure 4.5. *Neohydatothrips variabilis* logistic regression model validation and the risk of flight threshold (40%). Logistic regression model for *N. variabilis* was validated using 2017 (A), 2018 (B), and 2019 (C) data set. Black line shows risk probabilities of *N. variabilis* calculated using the logit model parameters (equation 6). Bars represent mean counts of *N. variabilis* corresponding to each trapping date represented as cumulative degree-days. Dashed line represents action threshold at a probability of 0.40.

CONCLUSION. Future directions for the management of *Soybean vein necrosis virus* and *Tobacco streak virus* in soybean

The assays developed for the detection and quantification of TSV from Wisconsin are tools that can be used to implement strategies manage the virus in soybean such as removing infected plants, testing mother plants in seed production fields, monitoring TSV in fields during the growing season or outbreaks. A previous assay (Dutta *et al.* 2015) failed to reliably detect TSV in soybean samples from Wisconsin. The low and unreliable detection of TSV in Wisconsin samples by the Dutta *et al.* (2015) assay may reflect genetic differences within TSV populations that may be present in different states or regions. Future research can aim at assessing isolate or strain differences in the TSV population from soybean hosts and if the populations cluster based on location (i.e., regions or states). This could be achieved by high-throughput sequencing samples from fields in the major soybean-growing regions of the United States and performing a phylogenetic analysis to determine the existence, if any, of molecularly distinct TSV strains in soybean (Dutta *et al.* 2015).

Quantitative PCR assays are tools to quantify the amount of viral titer and assess resistance in breeding programs (Rubio *et al.*, 2020; Shirima *et al.*, 2017). There is scarce resistance in soybean germplasm to TSV (Hobbs *et al.* 2012; Wang *et al.* 2005). Future work may be focused on screening current commercial varieties, soybean breeding lines (McCaghey *et al.* 2017; Webster *et al.* 2020), and more germplasm accessions for resistance to TSV. The qPCR assay developed (Zambrana-Echevarría *et al.* 2021) can be used as a screening tool to assess resistance levels, type of resistance (i.e., complete or partial), and TSV accumulation patterns related to disease progression and severity (Rubio *et al.* 2020; Shirima *et al.* 2017). The TSV nested RT-

PCR assay is a tool that can be used by diagnostic labs for detection of TSV in samples submitted for virus identification.

Screening of genotypes for their response to SVNV resulted in three resistant (51-23, 91-38, SSR51-70) and five susceptible (52-82B, Williams 82, LG94-1906, Dwight) genotypes to SVNV. Research in the future can aim to evaluate these genotypes in a field setting to represent growth conditions of commercial productions (Gomez *et al.* 2009). Two types of active resistance to plant viruses: complete or partial (Gomez *et al.* 2009; Rubio *et al.* 2020). Further evaluation of soybean vein necrosis and SVNV titer in 51-23, 91-38, and SSR51-70, past 14 days post-infection, would be useful to characterize the resistance level and determine if these genotypes exhibit partial or complete resistance (Maruthi *et al.* 2014; Rubio *et al.* 2020). The genome of Williams 82 has been sequenced (Schmutz *et al.* 2010) and there are genetic maps available for LG94-1906 (Grant *et al.* 2010). These genotypes can be used to assess host susceptibility factors (e.g., host proteins the virus hijacks to propagate in plant cells) to SVNV and target them as potential sources of recessive resistance (Hashimoto *et al.* 2016; de Ronde *et al.* 2014). Moreover, future research can also assess the patterns of accumulation, past 14 days post-inoculation, and systemic movement of SVNV as disease progresses (Kaweesi *et al.* 2014).

Management of the thrips may a good strategy for SVNV due to lack of management recommendations, scarce information about resistant genotypes, and unclear effects of the virus on soybean yield (Anderson 2017; Anderson *et al.* 2017; Mueller *et al.* 2016; Hill and Whitham 2014). Some of the important considerations for control of tospoviruses are thrips abundance, periods of enhanced flight activity, and weather conditions that influence population development together with elevated flight activity (Jones 2004). The phenology model identified critical degree-days for increased and peak activity of thrips that transmit SVNV. These periods are risk windows

for transmission and coincided with detection of the virus in sentinel plots. Furthermore, the logistic regression model identified temperature and precipitation as most explanatory variables for thrips activity. These models were used as descriptive tools in our approach, however future research can be aimed at collecting thrips catch data during more years and in several locations in Wisconsin, but also in other soybean-growing regions. A more robust data set can be incorporated into the thrips phenology model and use it as a prediction tool for thrips (Kamiyama *et al.* 2020). The logistic regression model could also benefit from a robust data set with more observations in order develop risk-assessments and have more data sets to validate the models for each of the thrips' species that transmit SVNV (Willbur *et al.* 2018a, 2018b). The contributions from these models can be used to develop prediction models to evaluate or implement management strategies such as adjusting planting dates to avoid periods of high activity or application of insecticides prior to the risk windows in fields where there is known history and high incidence of SVNV.

Literature Cited

Anderson, N. R. 2017. Effect of Soybean Vein Necrosis on Soybean Yield and Seed Quality, and Symptom Expression on Soybean and Alternative Hosts. ProQuest Diss. Theses. ISBN: 978-0-355-26259

Anderson, N. R., Irizarry, M. D., Bloomingdale, C. A., Smith, D. L., Bradley, C. A., Delaney, D. P., Kleczewski, N. M., Sikora, E. J., Mueller, D. S., and Wise, K. A. 2017. Effect of soybean vein necrosis on yield and seed quality of soybean. Can. J. Plant Pathol. 39:334–341.

Dutta, M., Ali, A., and Melcher, U. 2015. Detection, discrimination and discovery of a new Tobacco streak virus strain. J. Virol. Methods. 221:15–21.

Gómez, P., Rodríguez-Hernández, A. M., Moury, B., and Aranda, M. 2009. Genetic resistance for the sustainable control of plant virus diseases: Breeding, mechanisms and durability. Eur. J. Plant Pathol. 125:1–22.

Grant, D., Nelson, R. T., Cannon, S. B., and Shoemaker, R. C. 2010. SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res. 38:D843–D846

Hashimoto, M., Neriya, Y., Yamaji, Y., and Namba, S. 2016. Recessive Resistance to Plant Viruses: Potential Resistance Genes Beyond Translation Initiation Factors. Front. Microbiol. 7:1–11

Hill, J. H., and Whitham, S. A. 2014. Control of Virus Diseases in Soybeans. In G. Loebenstein, N. Katis (Eds), Advances in Virus Research. 90:355–390. Academic Press.

Hobbs, H. A., Jossey, S., Wang, Y., Hartman, G. L., Domier, L. L., Hobbs, H. A., Jossey, S., Wang, Y., and Sciences, C. 2012. Diverse Soybean Accessions Identified with Temperature-Sensitive Resistance to Tobacco Streak Virus. Crop. Sci. 52(2):738-744

Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res. 100:5–30.

Kamiyama, M. T., Bradford, B. Z., Groves, R. L., and Guédot, C. 2020. Degree day models to forecast the seasonal phenology of Drosophila suzukii in tart cherry orchards in the Midwest U.S. ed. Antonio Biondi. PLoS One. 15:e0227726

Kaweesi, T., Kawuki, R., Kyaligonza, V., Baguma, Y., Tusiime, G., and Ferguson, M. E. 2014. Field evaluation of selected cassava genotypes for cassava brown streak disease based on symptom expression and virus load. Virol. J. 11.

Maruthi, M. N., Bouvaine, S., Tufan, H. A., Mohammed, I. U., and Hillocks, R. J. 2014. Transcriptional response of virus-infected cassava and identification of putative sources of resistance for cassava brown streak disease. PLoS One. 9.

McCaghey, M., Willbur, J., Ranjan, A., Grau, C. R., Chapman, S., Diers, B., Groves, C., Kabbage, M., and Smith, D. L. 2017. Development and evaluation of *Glycine max* germplasm lines with quantitative resistance to *Sclerotinia sclerotiorum*. Front. Plant Sci. 8:1–13.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., and Robertson, A. 2016. A Farmer's Guide to Soybean Diseases. eds. Daren Mueller, Kiersten Wise, Adam Sisson, Damon Smith, Edward Sikora, Carl Bradley, and Alison Robertson. The American Phytopathological Society

de Ronde, D., Butterbach, P., and Kormelink, R. 2014. Dominant resistance against plant viruses. Front. Plant Sci. 5:1–17.

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Front. Plant Sci. 11:1–23.

Schmutz, J., Cannon, S. B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D. L., Song, Q., Thelen, J. J., Cheng, J., Xu, D., Hellsten, U., May, G. D., Yu, Y., Sakurai, T., Umezawa, T., Bhattacharyya, M. K., Sandhu, D., Valliyodan, B., et al. 2010. Genome sequence of the palaeopolyploid soybean. Nature. 463:178–183

Shirima, R. R., Maeda, D. G., Kanju, E., Ceasar, G., Tibazarwa, F. I., and Legg, J. P. 2017. Absolute quantification of cassava brown streak virus mRNA by real-time qPCR. J. Virol. Methods. 245:5–13

Wang, Y., Hobbs, H. A., Hill, C. B., Domier, L. L., Hartman, G. L., and Nelson, R. L. 2005. Evaluation of ancestral lines of U.S. soybean cultivars for resistance to four soybean viruses. Crop Sci. 45:639–644.

Webster, R. W., Roth, M., Reed, H., Mueller, B., Groves, C., McCaghey, M., Chilvers, M. I., Mueller, D. S., Kabbage, M., and Smith, D. L. 2020. Identification of Soybean (Glycine max) Check Lines for Evaluating Genetic Resistance to Sclerotinia Stem Rot. Plant Dis. PDIS-10-20-2193-RE

Willbur, J. F., Fall, M. L., Bloomingdale, C., Byrne, A. M., Chapman, S. A., Isard, S. A., Magarey, R. D., McCaghey, M. M., Mueller, B. D., Russo, J. M., Schlegel, J., Chilvers, M. I., Mueller, D. S., Kabbage, M., and Smith, D. L. 2018a. Weather-based models for assessing the risk of *Sclerotinia sclerotiorum* apothecial presence in soybean (Glycine max) fields. Plant Dis. 102:73–84.

Willbur, J. F., Fall, M. L., Byrne, A. M., Chapman, S. A., McCaghey, M. M., Mueller, B. D., Schmidt, R., Chilvers, M. I., Mueller, D. S., Kabbage, M., Giesler, L. J., Conley, S. P., and Smith, D. L. 2018b. Validating *Sclerotinia sclerotiorum* apothecial models to predict Sclerotinia stem rot in Soybean (*Glycine max*) fields. Plant Dis. 102:2592–2601.

Zambrana-Echevarria, C., Roth, M., Dasgupta, R., German, T., Groves, C., and Smith, D. L. 2021. Sensitive and specific qPCR and nested RT-PCR assays for the detection of Tobacco streak virus in soybean. PhytoFrontiers. PHYTOFR-11-20-0036-R

APPENDIX CHAPTER I. Supplementary Tables and Figures

Table AI.1 Quantitative PCR assay sensitivity determined with serial dilutions of pGEM-T-TSV performed in three replicate plates.

Plate Number	Sample[a]	Mean Ct[c]	Mean Sample Ct[c]	Starting quantity[d]
1	Std-1	8.74 ± 0.05	8.43 ± 0.11	13,800,000
2		8.34 ± 0.15		
3		8.22 ± 0.13		
1	Std-2	12.22 ± 0.12	11.31 ± 0.48	1,380,000
2		9.82 ± 0.10		
3		11.90 ± 0.11		
1	Std-3	15.77 ± 0.16	15.22 ± 0.27	138,000
2		14.54 ± 0.50		
3		15.36 ± 0.09		
1	Std-4	20.10 ± 0.19	19.50 ± 0.22	13,800
2		19.45 ± 0.18		
3		18.96 ± 0.02		
1	Std-5	23.28 ± 0.19	22.86 ± 0.18	1,380
2		22.34 ± 0.18		
3		22.96 ± 0.03		
1	Std-6	26.77 ± 0.19	25.96 ± 0.30	138
2		25.20 ± 0.09		
3		25.90 ± 0.07		
1	Std-7	30.71 ± 0.25	29.30 ± 0.47	13.8
2		28.27 ± 0.01		
3		28.92 ± 0.19		
1	Std-8	33.66 ± 0.53	32.15 ± 0.50	1.38
2		31.53 ± 0.08		
3		31.27 ± 0.22		

[a]Std: standard, serial dilution of pGEM-T-TSV
[c]Mean Ct: average threshold cycle of two technical replicates per plate; "± ": standard error.
[c]Mean Sample Ct: threshold cycle averaged across all plates per standard; "± ": standard error.
[d]Starting Quantity: Concentration in femtograms of pGEM-T-TSV per 4µL in the reaction

Table AI.2 Nucleotide Blast results of selected samples Sanger-sequenced with qPCR and nested RT-PCR primers described in this study.

Sample	Primer [a]	Query cover	E value	Identity (%)[b]	Accession Number [b]	Description
W101-4	TSVCP_Fw1*	98%	$1E^{-35}$	97.85	MT596823.1	TSV RNA 3 WI isolate
SB5	TSVCP_Fw1*	97%	$1E^{-26}$	94.25	MT669385.1	TSV RNA 3 IA isolate
D5	TSVCP_Fw1*	97%	$2E^{-29}$	96.47	MT669385.1	TSV RNA 3 IA isolate
201-1	TSVCP39F2 TSVCP715R2	100%	0.0	100	MT596823.1	TSV RNA 3 WI isolate
201-2	TSVCP39F2 TSVCP715R2	100%	0.0	100	MT596823.1	TSV RNA 3 WI isolate
201-4	TSVCP39F2 TSVCP715R2	100%	0.0	100	MT596823.1	TSV RNA 3 WI isolate
W101-4	TSVCP12F1 TSVCP715R1	100%	0.0	100	MT596823.1	TSV RNA 3 WI isolate
D4	TSVCP12F1 TSVCP715R1	100%	0.0	99.16	X00435.1	TSV RNA 3 complete sequence
LS1	TSVCP39F2 TSVCP715R2	100%	0.0	100	MT596823.1	TSV RNA 3 WI isolate
SB5	TSVCP39F2 TSVCP715R2	96%	0.0	99.62	MT669385.1	TSV RNA 3 IA isolate

[a]TSVCP_Fw1: qPCR primer, TSVCP12F1/TSVCP715R1: nested RT-PCR first-round primers, TSVCP39F2/TSVCP715R2: nested RT-PCR second-round primers.
[*]A consensus sequence was unable to be generated from both qPCR primers TSVCP_Fw1 and TSVCP_Rv1 due to the length of the product (137bp). The TSV Fw1 sequence was used to confirm TSV amplification buy the qPCR primers.
[b]Top result (based on the percentage of identity) of the nucleotide blast alignment search.

Table AI.3. Collection weeks and trapping dates of *Nehydatothrips variabilis*, *Frankliniella fusca,* and *Frankliniella tritici* captures in Wisconsin from 2017-2019.

Week	Standardized Week	2017	2018	2019
1	-	-	May-29	-
2	-	-	June-06	-
3	-	-	June-13	-
4	1	June-20	June-20	June-20
5	2	June-26	June-27	June-26
6	3	July-05	July-03	July-03
7	4	July-11	July-11	July-11
8	5	July-19	July-18	July-18
9	6	July-25	July-26	July-24
10	7	Aug-02	Aug-02	July-30
11	8	Aug-09	Aug-08	Aug-08
12	9	Aug-16	Aug-16	Aug-14
13	10	Aug-23	Aug-22	Aug-22
14	11	Aug-30	Aug-30	Aug-28
15	12	Sept-07	Sept-06	Sept-03
16	13	Sept-12	Sept-11	Sept-10
17	-	Sep-24	-	Sept-16

"-": no sampling date

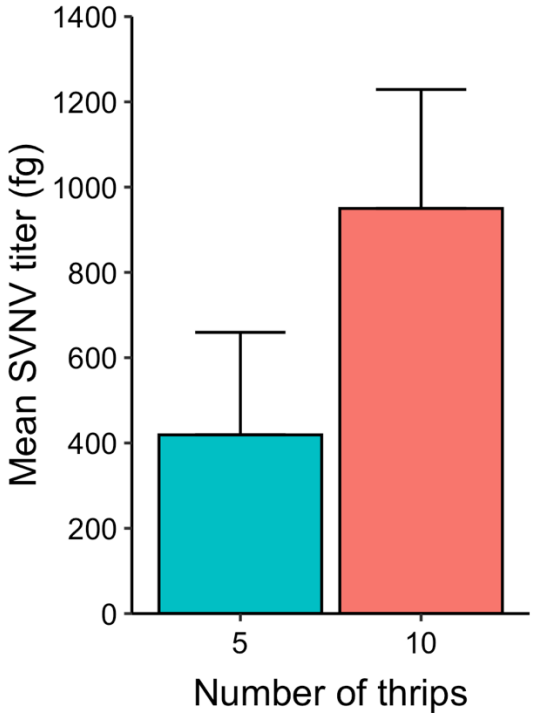

Figure AI.1. Titer of *Soybean vein necrosis virus* (SVNV) in soybean thrips (*Neohydatothrips variabilis*) colony used to infestate soybean plants in a controlled growth environment. Absolute quantification of SVNV was performed nucleoprotein (NP) gene primers by with Keough *et al.* (2016). The concentration of the SVNV NP gene was determined using standard dilutions of pGEM-T-SVNV.

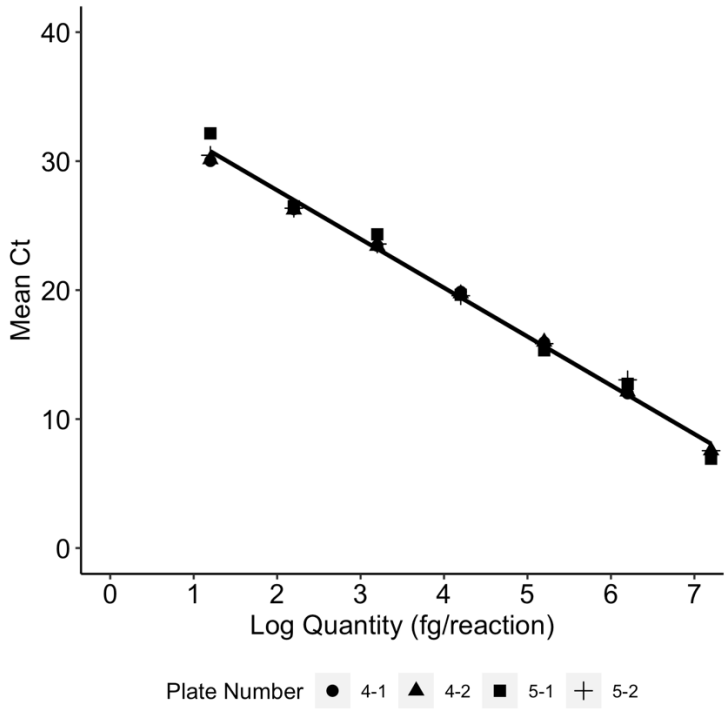

Figure AI.2. Concentration and threshold cycle of serial dilutions of pGEM-T-SVNV. Four independent replicate plates were used to generate the equation: $y=-3.78x+35.29$, $R^2=0.993$. Mean threshold cycle (Ct) was calculated based on two technical replicates per serial dilution per plate.

APPENDIX CHAPTER II. Occurrence of *Soybean vein necrosis virus* and *Tobacco streak virus* in soybean conventional variety trials in Wisconsin

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Materials and Methods

Trial scouting and disease incidence

Experimental research trials of conventional soybean varieties were established by the Dr. Shaw Conley's group in the Department of Agronomy at UW-Madison in the 2018 and 2019 growing seasons. The plots measured 40x600 cm with 0.381m wide rows planted at a 160,000 seeds per acre. In both years, the variety trials were located in Arlington Agricultural Research Station (43.29ºN, -89.33ºW) in Columbia County, Platteville (42.59ºN, -90.42ºW) in Lafayette County, and Chippewa Falls (44.99ºN, -91.41ºW) in Chippewa County. These locations represented the South Eastern, South Central, and North Central soybean growing regions of Wisconsin to assess viral disease incidence and the incidence single and mixed infections of SVNV and TSV. Disease incidence (DI) in the trial was expressed as a percentage and calculated using the equation 1:

(3)

$$DI = \frac{number\ of\ symptomatic\ plants}{(seeding\ rate\ per\ acre) \times \left(\frac{1\ acre}{linear\ ft}\right) \times \left(\frac{plot\ length\ \times\ width}{43{,}560\ ft}\right)} \times 100$$

SVNV and TSV detection

Leaflets were sampled from soybean plants in the conventional variety trials. In 2018, SVNV symptomatic plants were sampled at the end of August. The following year, SVNV-symptomatic, TSV-symptomatic, and asymptomatic plants were sampled in early- to mid-September. Total RNA was isolated using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA) or the Maxwell RSC Plant RNA kit with the automated Maxwell RSC Instrument (Promega, Madison, WI) according to manufacturer's instructions. The RNA was reverse transcribed with the 5X iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA) with random priming. The resulting complementary cDNA (cDNA) was diluted to a 1:10 ratio with nuclease-free water. Nested RT-PCRs for the detection of SVNV and TSV were performed using primer pairs TSVCP12F1/715R1 and TSVCP39F2/681R2 (Table 2.2; Zambrana-Echevarría *et al*. 2021) for the detection of TSV, and primer pair SVNV-F1/Rv1 and SVNV-F2/R2 (Table 3.2) for the detection of SVNV. PCR reactions and cycle conditions are as described previously in Chapter 2 and Chapter 3. Product amplification was confirmed by visual inspection of a 1.2% agarose in 1X TAE stained with SYBR DNA gel stain. Samples with PCR products of 643 bp were determined positive for TSV. Samples with PCR products of 800 bp were determined positive for SVNV.

Results

Trial scouting and disease incidence

The SVN disease incidence was determined for each trial/location in 2018 and 2019. In 2018, the SVN disease incidence was 0% for Chippewa Falls, 0.005% for Columbia, and 0.02% for Grant. In 2019, the SVN disease incidence was 0% for Chippewa Falls, 0.002% for Columbia, and 0.04% in Grant County. The incidence of TSV was only determined in 2019 and it was 0%

for Chippewa Falls, 0.002% for Columbia, and 0.014% for Grant. Overall, Grant county had higher disease incidence (0.02-0.04%) than the other locations.

SVNV and TSV detection

SVNV was detected in Columbia and Grant trials in 2018, and only in the Grant trial in 2019. Although the number of samples collected in each location and each year was unbalanced, the number of SVNV-positive samples was higher in Grant county than in the Chippewa Falls and Columbia (Table AII.1). Mixed infections of SVNV and TSV were detected in Columbia and Grant counties in 2018 and only in Grant county in 2019, although they weren't the majority of the samples our results provide evidence that this phenomenon occurs in nature (Table AII.1). The varieties that were positive for each virus are described in Table 2 for 2018 and Table 3 for 2019. In 2018, nine varieties were among the samples collected from Columbia and Grant County trials. All of the samples collected from the varieties LS24C756N, 266LL, 236LL, DSR-1721/R2Y, and AG24X7 were positive for SVNV (Table AII.2). Half or 60% of samples from the varieties HS23X70, LS2580NHP, Viking 2018N. There were mixed infections of SVNV-TSV detected in samples from all of the aforementioned varieties and in O.2518N.

In 2019, two public varieties (Dane and MN1410) were among the varieties sampled and they were positive for SVNV (Table 3). The majority of samples collected from these varieties (43-50%) were positive for SVNV, and only one was positive for TSV per variety (Table AII.3). For the commercial varieties, all the samples collected from O.2155N, LS2580NHP, and C2300 were positive for SVNV. The variety SVX-4006 was positive for both of SVNV and TSV in the same sample and was the only variety with a SVNV-TSV mixed infection in 2019 (Table AII.3).

Table AII.2.Virus detection summarized by location.

County	Year	SVNV[a]	TSV[b]	SVNV-TSV[c]	Total of samples
Chippewa	2018	nt	nt	nt	0
	2019	0	0	0	9
Columbia	2018	0	0	2	4
	2019	3	2	0	18
Grant	2018	12	0	2	15
	2019	22	1	2	37

"nt": not tested

[a]Number of samples positive *Soybean vein necrosis virus* (SVNV); single infection

[b]Number of samples positive for *Tobacco streak virus* (TSV); single infection

[c]Number of samples positive for SVNV and TSV; mixed infection

Table AII.3. Virus detection per variety in 2018 soybean conventional variety trails.

Variety	SVNV[a]	TSV[b]	SVNV-TSV[c]
HS 23X70	3	0	1
LS 2580NHP	2	0	2
LS 24C756N	3	0	0
Viking 2018N	1	0	1
Viking O.2518N	0	0	1
266LL	2	0	0
236LL	1	0	0
DSR-1721/R2Y	1	0	0
AG24X7	1	0	0

[a]Number of samples positive *Soybean vein necrosis virus* (SVNV); single infection
[b]Number of samples positive for *Tobacco streak virus* (TSV); single infection
[c]Number of samples positive for SVNV and TSV; mixed infection

Table AII.3. Virus detection per variety in 2019 soybean conventional variety trails

Variety	SVNV[a]	TSV[b]	SVNV-TSV[c]
C1838RX	1	0	0
C2300	2	0	0
Dane	3	1	0
HS 28X70	1	0	0
LGS2010	1	0	0
LS 2580NHP	2	0	0
LS 2880NHP	2	0	0
MN1410	2	1	0
O.2155N	3	0	0
O.2518N	1	0	0
Power Plus 25G8	2	0	0
SB90	1	0	0
SVX-4006	1	0	1
SVX-4009	1	0	0
Viking 2018N	2	0	0

[a]Number of samples positive *Soybean vein necrosis virus* (SVNV); single infection
[b]Number of samples positive for *Tobacco streak virus* (TSV); single infection
[c]Number of samples positive for SVNV and TSV; mixed infection