2016

Soybean vein necrosis virus: impacts of infection on yield loss and seed quality and expansion of plant host range

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Soybean vein necrosis virus: impacts of infection on yield loss and seed quality and expansion of plant host range

by

Melissa Irizarry

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

MASTER OF SCIENCE

Major: Plant Pathology

Program of Study Committee:
Daren Mueller, Co-Major Professor
Steven Whitham, Co-Major Professor
Bryony Bonning

Iowa State University
Ames, Iowa
2016

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DEDICATION

I would like to dedicate this thesis to my mother, Susan Manuel, for teaching me to always reach for the moon. Her unfailing love and many sacrifices have allowed me the opportunity to grow and find my path in life. She is my inspiration.

I would also like to dedicate this thesis to Tomy Norman. He has been willing to join me on this adventure and been so patient. He has been my rock and my champion.

They have been my support without fail through all the ups and downs. I truly feel this thesis would not have happened without their love.
TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS ..................................................................................................................... v

ABSTRACT ........................................................................................................................................ vii

CHAPTER 1  INTRODUCTION AND JUSTIFICATION ................................................................. 1

  Thesis organization ...................................................................................................................... 1
  Literature Review ....................................................................................................................... 2
    Soybean ................................................................................................................................... 2
    Virus and soybean ........................................................................................................ 2
    Tospoviruses ................................................................................................................ 3
    Soybean vein necrosis virus .................................................................................................... 5
    Plant host range ...................................................................................................................... 9
    Thrips ....................................................................................................................................... 11
    The lifecycle of thrips ............................................................................................................ 13
    Thrips and tospoviruses ....................................................................................................... 14
    Soybean thrips plant hosts .................................................................................................... 16
    Cover crops and vegetables in Iowa ..................................................................................... 16
    Management of SVN ............................................................................................................ 17
  References .................................................................................................................................. 20
  Tables ......................................................................................................................................... 27
  Figures ....................................................................................................................................... 29
  Justification .............................................................................................................................. 30

CHAPTER 2  EFFECT OF SOYBEAN VEIN NECROSIS VIRUS ON YIELD AND
SEED QUALITY OF SOYBEAN ........................................................................................................ 31

  Abstract ..................................................................................................................................... 31
  Introduction ............................................................................................................................... 32
  Collection of SVN data in the field ......................................................................................... 34
  Yield parameters ...................................................................................................................... 36
  Seed quality parameters ......................................................................................................... 36
  Discussion ................................................................................................................................ 37
  References ................................................................................................................................. 42
  Tables ....................................................................................................................................... 47
  Figures ...................................................................................................................................... 52
CHAPTER 3  HOST RANGE OF SOYBEAN VEIN NECROSIS VIRUS IN
SPECIALTY AND COVER CROPS AND FEEDING PREFERENCE
OF SOYBEAN THRIPS ON COVER CROPS ........................................... 56

Abstract ........................................................................................................... 56
Introduction ......................................................................................................... 56
Materials and methods .................................................................................... 62
  Plants grown under controlled environmental conditions ......................... 62
  Soybean thrips colonies and SVNV cultures ................................................. 62
  Inoculation techniques .................................................................................. 63
  Thrips feeding preferences in growth chamber ............................................. 65
Results .............................................................................................................. 65
  ELISA positive for buckwheat, melon, and winter pea .................................. 65
  Preferential feeding of soybean thrips on cover crop species ...................... 66
Discussion ......................................................................................................... 67
References ......................................................................................................... 70
Tables ............................................................................................................... 75
Figures .............................................................................................................. 76

CHAPTER 4  GENERAL CONCLUSIONS ................................................................. 79

Summary of yield loss and seed quality changes .......................................... 79
Summary of horticultural and cover crops susceptible to SVNV .................. 80

APPENDIX  SEASONAL PATTERNS OF ADULT NEOHYDATOTHIRPS VARIABILIS
POPULATIONS AND IMPLICATIONS FOR MANAGEMENT IN IOWA SOYBEAN .... 82

Abstract ........................................................................................................... 82
Introduction ........................................................................................................ 83
Materials and Methods ................................................................................... 87
Results .............................................................................................................. 89
  Total thrips numbers vary through season .................................................... 89
  Soybean thrips numbers .............................................................................. 90
  Proportion of total thrips to soybean thrips ................................................ 92
  Weather modeling to predict seasonal adult soybean thrips numbers ......... 92
Discussion ........................................................................................................ 93
  Arrival of adult soybean thrips in Iowa ....................................................... 93
  Weather modeling as a tool for soybean thrips management .................... 94
References ....................................................................................................... 96
Tables .............................................................................................................. 101
Figures .......................................................................................................... 103
I would like to thank my co-advisors Dr. Daren Mueller and Dr. Steven Whitham and my committee members Dr. Bryony Bonning and Dr. Laura Jesse for their guidance and support throughout the course of this research.

I cannot thank Daren Mueller enough. His eternal patience and positive attitude were invaluable. He was always willing to take the time to answer my questions and perk up my spirits. I learned far more than just how to do research from him. He has set a high example of professional achievement and hard work while treating people with fairness and kindness. Steve Whitham was always willing to slowly and repeatedly explain molecular concepts without judgement. Between Steve and Daren, I was never short of a good laugh when I needed it. I am thankful for Erika Saalau-Rojas and Laura Jesse who introduced me to the joys of plant pathology and gave me the opportunity to work in the ISU Plant and Insect Diagnostic Clinic and especially for their friendship. I would also like to thank Jean Batzer for her insightful edits. In addition, I would like to thank my co-authors on the yield, TSV, and thrips papers; my lab mates for collecting field data during hot summer days; and Anna Whitfield, Ismael E. Badillo-Vargas, and Punya Nachappa for their help with establishing a thrips colony. We gratefully acknowledge the assistance of Jason Burkett, Kassie Conner, Mary Delaney (Auburn University), Keith Ames (University of Illinois), Phil Devillez, Dennis Nowaskie, Jeffrey Ravellette (Purdue University), Nathan Bestor, Darison Etienne, Gang Han, Brandon Kleinke, Warren Pierson, Adam Sisson, Stith Wiggs, Ed Zaworski (Iowa State University),
Chase Fritz, Carol Groves, Russell Groves, and Jaime Willbur (University of Wisconsin).

We would also like to thank the Iowa and Wisconsin soybean checkoff for partially funding this project.
Soybean vein necrosis virus (SVNV) rapidly became a widespread soybean (Glycine max) virus within a few years of its initial confirmation in 2008. The economic impact of soybean vein necrosis (SVN) disease remains unknown. Soybean is a crop of global importance with nearly 4 billion bushels of soybeans produced in the United States in 2014. This study was designed to pursue two main questions; is there any yield loss or change in seed quality associated with SVN and are there horticultural or cover crop species that could be serving as sources of SVNV inoculum?

In order to determine if there was any yield loss or change in seed quality associated with SVN, field studies were conducted in six states in 2013, 2014, and 2015. Quantitative parameters, including seeds per pod, pods per plant, yield, and 100-count seed weight, as well as qualitative parameters, including protein and oil concentration, were assessed from plants or seeds collected from research and commercial production fields. Results of this study did not find any impact on yield by SVN. However, seed quality was affected. In Iowa, oil concentration decreased by 0.11% as disease incidence increased by 1% ($P=0.04$). Changes in fatty acid profiles of seed were also observed; linolenic, linoleic and stearic acids decreased between 0.5 and 0.15% in 2 of 6 locations that were tested. These results suggest that infection by SVNV negatively affects soybean seed quality, which may affect the marketability of soybeans for premium markets.
Some tospoviruses, such as *Tomato spotted wilt virus*, are globally important pathogens with extensive host ranges. Because SVNV is a Tospovirus and questions have been raised about soybean being the primary plant host, this study investigated the ability of specialty and cover crops that were commonly present in Iowa to serve as alternative hosts for SVNV. Additionally, insect vector feeding preference was examined on the cover crop species. Eighteen cover crop and seven specialty crops were tested using mechanical and direct thrips inoculations. Presence of SVNV was determined with ELISA. Systemic infection of buckwheat and local infections of melon and winter pea were found. Symptoms were observed on buckwheat and melon. Soybean thrips were found to prefer alfalfa, buckwheat, and crimson clover, and red clover; although they were able to feed on all plant species tested if no other food was presented.

The results from this study indicate changes in fatty acids, oil content, and seed size in soybean seed from SVN symptomatic plants, but no yield loss was seen. Timing of infection may play a role in the extent of damage from SVNV infection. Management of SVNV, as with many viral diseases, may include reduction of inoculum sources and monitoring of insect vectors. This study tested 25 plant species to determine if they could serve as sources of primary inoculum each growing season. Buckwheat, melon, and pea were all found to be susceptible to SVNV infection when direct thrips inoculated. None of these plants are perennials or found in large areas near soybean in the Midwest and thus are not considered to be of concern to soybean farmers.
CHAPTER 1

INTRODUCTION AND JUSTIFICATION

Thesis Organization

This thesis is organized into four chapters. The first chapter contains the literature review and research justification. The second chapter will detail results of a collaborative study on yield and seed quality research conducted with Chris Bloomingdale and Damon L. Smith at the University of Wisconsin-Madison, Nolan R. Anderson and Kiersten A. Wise at Purdue University, Carl A. Bradley at the University of Kentucky, Dennis P. Delaney and Edward J. Sikora at Auburn University, and Nathan M. Kleczewski at the University of Delaware. The third chapter is a summary of results from greenhouse experiments on plant host range of SVNV and soybean thrips feeding preferences. The fourth chapter is a summary and general conclusion of this thesis. The appendix summarizes data about thrips captured in Iowa, which will be combined with thrips capture data from Wisconsin. The references are listed at the end of each chapter.
Literature Review

Soybeans

Soybean (*Glycine max*) is a crop of global importance. Nearly 4 billion bushels of soybeans were produced in the United States in 2014 with more than 500 million bushels coming from Iowa (USDA 2015). Approximately 282 million metric tons of soybeans were produced globally in the 2013/2014 season (McFerron 2014). Soybean protein and oil are of particular importance. Soybeans contain the eight essential amino acids required for human health and are therefore a quality source of complete protein. Soybean is the main crop grown in the United States for use as oil, making up 90% of the oilseed production (USDA 2015). Processed soybeans are used as livestock feed, oil, human food, aquaculture feed, plastics, textiles, and biofuel. The uses and demand for soybean are continually expanding. Despite its widespread production and use in today’s society, soybean existed somewhat on the fringes until World War II. First domesticated in the eleventh century B.C. China, it did not catch the attention of the West until the 20th century. As people experimented and discovered the malleability of soybean resulting in a multitude of uses, acreage began to increase until it became one of the most important crops grown around the world (Hill and Whitham 2014).

Virus and soybean

Currently there are 46 viruses that are known to infect soybeans in the field with eight of these considered to have enough impact to be managed. These are *Alfalfa*
mosaic virus (AMV), Bean pod mottle virus (BPMV), Peanut mottle virus (PeMoV), Peanut stunt virus (PSV), Soybean dwarf virus (SbDV), Soybean mosaic virus (SMV), Soybean vein necrosis virus (SVNV), and Tobacco ringspot virus (TRSV) (Hill and Whitham 2014). SVNV is included on this list because of its widespread prevalence and unknown effect on yield and seed quality.

The deleterious effects of viral infection in soybean are widely documented. For example, Cowpea chlorotic mottle virus (CCMV) and TRSV infections significantly change fatty acid profiles in soybean with a decrease in palmitic, linoleic, and linolenic and an increase in stearic and oleic acids (Harris et al. 1970, Demski et al. 1971, Demski and Jellum 1975). If oil levels drop below accepted standards they may be rejected in the marketplace. Interestingly, premium prices are paid for seed that is high in oleic acid and low in linolenic acids. A similar decrease in oil concentration occurs as a result of infection with CCMV, PeMoV, SMV, and TRSV (Demski and Jellum 1975). Demski et al. (1971) found infection by TRSV in soybean results in larger, but fewer, seeds and this combination produces lower yield overall. Seed size may decrease or increase as a result of viral infection time. For example, SMV was correlated with a reduction in soybean seed size occurring in earlier infections (Ren et al. 1997); in contrast earlier TRSV infection resulted in larger seed size (Demski et al. 1971).

Tospoviruses

The genus Tospovirus takes its name from the type species Tomato spotted wilt virus (TSWV). TSWV has a host range of over 1,000 plant species and a global
distribution making it one of the most destructive and economically important plant viruses, ranking among the top ten globally (Ogada et al. 2013). Over the course of ten years it is estimated that TSWV caused approximately $1.4 billion in losses (Riley et al. 2011). Tomato spotted wilt virus and other tospoviruses are arboviruses transmitted by thrips (Thysanoptera spp.). There are 29 known tospoviruses, including SVNV (Riley et al. 2011, Tennant and Fermin 2015, Zhou and Tzanetakis 2013) (Table 1). Members of the Tospovirus genus can infect at least 1,090 plant species in 80 plant families, including both monocots and dicots (Parrella et al. 2003). The majority of susceptible plants are in the Asteraceae and Solanaceae families.

Tospoviruses belong to the Bunyaviridae family that is uniquely able to infect vertebrates and insects. Tospoviruses are the only genus in the Bunyaviridae that can infect plants, with the other members of the family being animal viruses infecting arthropods, rodents, and humans. As most plant viruses are a product of coevolution between vector and virus, this suggests that the tospoviruses may have originally been a thrips virus that evolved to also infect plants, allowing this genus to become evolutionarily distinct from the rest of the family. Circulative propagative plant viruses are very similar to mosquito-borne viruses in animals (Medeiros et al. 2004). Tospoviruses are not known to exist in nature without the association with thrips.

Tospoviruses are typically placed into two clades, Eurasian (Old World) or American (New World) (Oliveira 2012). Tospoviruses are single strand negative and ambisense RNA viruses that form enveloped, spherical particles with a three part
segmented genome. These segments are termed S (small), M (medium), and L (large) with L being negative sense while S and M are ambisense.

Tospoviruses are transmitted in a persistent and propagative manner (Ullman et al. 1997). Persistent viruses are taken up by the host but then must move into the insect’s digestive system where they pass through the midgut and make their way to the salivary glands. Only at this point can the virus be transmitted to a new host plant. This type of transmission is characterized by a latent period and long-term ability to transmit the virus. In contrast, non-persistent viruses can be transmitted shortly after the insect acquires them. The virus particles stay in the salivary duct and are retransmitted quickly. This type of virus transmission is characterized by no latent period before being able to infect new plants and a short period of infectiousness. A propagative virus replicates within the insect vector in addition to replication within the plant host.

Typical symptoms of tospovirus infection in plant hosts include chlorosis, mottling, necrotic lesions, stunting, sunken and ring spots, as well as wilting (Riley et al. 2011). These symptoms vary depending on the various factors of the plant host and virus strain.

**Soybean vein necrosis virus**

*Soybean vein necrosis virus* was first confirmed in Arkansas and Tennessee in 2008 (Tzanetakis et al. 2009). It was then confirmed in Iowa in 2012 (Smith et al. 2013). It is currently in 16 other states and Canada making it one of the most widespread
soybean viruses in the United States and Canada (Zhou and Tzanetakis 2013). It has not yet been found outside of North America.

Soybean vein necrosis symptoms can be easily mistaken for those of other soybean diseases as well as herbicide injury. Infection leads to local lesions (Zhou and Tzanetakis 2013). Common symptoms include interveinal chlorosis along the leaf vein that can become red-brown lesions leading to necrosis (Figure 1). The veins can be clear, yellow, or dark brown. Symptoms are often worse on the underside of the leaf, possibly due to the preference of thrips to feed in these areas (Bloomingdale et al. 2015). These symptoms can resemble soybean sudden death syndrome (SDS), soybean brown stem rot, and downy mildew of soybean (Bloomingdale et al. 2015). Herbicide injury can also be confused with SVN symptoms as well. As a result of the similarity of symptoms of SVN to other common soybean diseases, the disease may have been present and misdiagnosed prior to its confirmation in 2008.

It is unclear if the virus is moving up each year from the southern United States with insects blown north in the spring or if it may be overwintering in a plant species in the Midwest. Symptoms on soybean in Iowa first appeared around R6 growth stage during 2013 and 2014. The R6 growth stage is when the seed pods are filled but still green and usually occurs in late July/August in Iowa. Symptoms in Arkansas and Tennessee were appearing in June (Zhou et al. 2011). This difference in timing may be an indication that the virus is traveling northward with the thrips annually instead of overwintering in an alternative plant host in the Midwest or it may be overwintering and unable to spread until the annual arrival of the thrips.
Besides visual examination of symptoms, serological tests and PCR primers have been created for use in detection and diagnosis of SVNV (Khatabi et al. 2012 and Zhou and Tzanetakis 2013). Primers for RT-qPCR and nested PCR have been developed with nested PCR being able to detect the virus with increased specificity (Groves et al. 2016). Agdia, Inc. (Elkhart, Indiana) has produced an ELISA serological test available commercially. SVNV has a 16.5-kb tripartite genome typical of a tospovirus. The S and M segments are similar in size to other tospoviruses but the L segment is the largest in the genus (Groves et al. 2016). Five open reading frames and six proteins are encoded between the three genome segments (Zhou and et al. 2011). SVNV has been placed in a new clade along with Bean necrotic mosaic virus (BeNMV) due to their phylogenetic differences from other tospoviruses (Oliveira 2012, Zhou et al. 2011). The discoveries of these two viruses that diverge from the other tospoviruses suggest that there are likely more closely related viruses that will be discovered in the future (Oliveira 2012).

Soybean vein necrosis virus is one of only a handful of tospoviruses that has been found naturally infecting soybean. Tomato spotted wilt virus (TSWV) has only been found in soybean a few times, once in Georgia (Nischwitz et al. 2006), and also with the tospoviruses Groundnut bud necrosis virus (GBNV), Groundnut ringspot virus (GRSV), and Tomato yellow ring virus (TYRV) in the Eastern hemisphere (Khatabi et al. 2012). The origins of SVNV are unclear. It may have evolved from a closely related virus that infects a plant host where soybean thrips also feed. Future work on alternate plant hosts and viruses found in soybean thrips may shed light on the origins of SVNV.
There is some indication that there may be resistance in some soybean cultivars based on symptom variations (Zhou et al. 2011). The high sequence identity of SVNV isolates obtained across cultivars and geographical regions indicates that the symptom variation is unlikely to be caused by differing viral strains and also an indication that soybean may have only recently become a host and may not be the primary host for SVNV (Khatabi et al. 2012, Zhou and Tzanetakis 2013).

Zhou and Tzanetakis (2013) showed a close correlation between symptoms and SVNV infection in soybean by testing 576 symptomatic samples and 127 asymptomatic samples. All symptomatic tissue tested positive for the presence of SVNV and all asymptomatic tissue tested negative. The same results were found with 150 symptomatic and 75 asymptomatic samples (Zhou et al. 2011). Another study showed 94% of symptomatic plants had detectable levels of SVNV while none of the asymptomatic did when assayed by ELISA (Hajimorad et al. 2015).

Despite previous studies pointing to SVNV not moving systemically in soybean, having an absolute association between symptoms and presence of the virus, and only being naturally transmitted via thrips, seed transmission of SVNV in soybean was recently discovered (Groves et al. 2016). This makes SVNV the only tospovirus that is known to be seed transmissible. The plants grown from SVNV infected seed were asymptomatic and showed detectable levels of SVNV throughout the plant. The virus was seed transmitted at a rate of 6% (Groves et al. 2016). It is unknown how the thrips
transmitted non-systemic and seed transmitted systemic infections are related and if they interact within the disease cycle.

**Plant host range**

Because of the local lesions that result from thrips transmission of SVNV, it is suspected that soybean and other legumes may not be the primary plant host of the virus (Khatabi et al. 2012, Zhou and Tzanetakis 2013). A study done by Zhou and Tzanetakis (2013) looked at possible host plant species for the virus. They tested 25 plant species and found that nine were susceptible to infection with SVNV through mechanical inoculation. Susceptible species were hardy mum (*Dendranthema grandiflorum*), ivyleaf morningglory (*Ipomoea hederacea Jacq*), summer squash (*Cucurbita pepo*), soybean, cowpea (*Vigna unguiculata*), mung bean (*Vigna radiate*), *Nicotiana benthamiana*, tobacco (*Nicotiana tabacum*), and *Nicotiana glutinosa*. These plants represented a wide range of families that include Asteraceae, Curcurbitaceae, Leguminosae, and Solanaceae. Hardy mum, ivyleaf morningglory, and summer squash were asymptomatic (Zhou and Tzanetakis 2013).

Legumes are a rarity among tospovirus hosts (Oliveria et al. 2012), which may relate to the divergent lineage of the SVNV and BeNMV. Soybean, cowpea, mung bean, *N. glutinosa*, and tobacco exhibited local lesions. The closely related BeNMV was discovered on common bean (*Phaseolus vulgaris*) (Oliveira et al. 2012), but SVNV did not infect this species. Only *N. benthamiana* had a systemic infection with symptoms. *N. benthamiana* is often used as a model plant for viral studies due to its susceptibility to a
wide variety of viral pathogens (Goodin et al. 2008). Cowpea (75%) and all three Nicotiana (70-81%) species had a substantially greater success rate with mechanical inoculation than the other positive species (10 to 40%). They tested an average of ten plants from each species except Vigna unguiculata and N. benthamiana of which they tested 80 from each species (Zhou and Tzanetakis 2013). Mechanical inoculation techniques for SVNV have a low success rate while direct inoculation using thrips has almost a 100% success rate, indicating the importance of thrips as vectors (Khatabi et al. 2012, Zhou and Tzanetakis 2013). Since the success rate of mechanical inoculation is very low in many species, it is of some concern that not enough plants were tested of some species. In the Zhou and Tzanetakis study only ten plants tested for many species, which may have resulted in false negative conclusions, possibly causing plants capable of hosting SVNV to remain undetected. Species that were found to be negative in the Zhou and Tzanetakis (2013) study were globe amaranth (Gomphrena globosa), palmer amaranth (Amaranthus palmeri), spinach (Spinacia oleracea), dahlia (Dahlia hortensis), broccoli (Brassica oleracea), quinoa (Chenopodium quinoa), common lambsquarters (Chenopodium album), cucumber (Cucumis sativus), peanut (Arachis hypogaea), common bean, cotton (Gossypium), rice (Oryza sativa), common wheat (Triticum aestivum), corn (Zea mays), tomato (Solanum lycopersicum), and pepper (Capsicum annuum) (Zhou and Tzanetakis 2013). They additionally collected ivyleaf morningglory plants from a SVN soybean field and found five of eight plants positive. They noticed that this weed’s range overlaps that of SVNV and soybean thrips perhaps indicating a close tie or important role of this weed in the pathosystems, because weeds are often
considered an important component of plant virus pathosystems (Nutter 1993). However, ivyleaf morningglory is an annual plant that would not provide a source of overwintering inoculum. There was no research to determine if soybean thrips can feed and reproduce on any of the plants that were positive for the ability to be infected with SVNV (Zhou and Tzanetakis 2013). Additionally there are many other crops that are grown across North America that have not been tested.

**Thrips**

There are currently 5,000 known species of thrips in nine families found worldwide. Almost all pest species are in Thripidae which is also the second largest family in the order Thysanoptera and the largest family of Terebrantia with over 2,000 species (Mound 2010).

The soybean thrips (*Neohydatothrips variables*) is the only confirmed species to transmit SVNV (Zhou and Tzanetakis 2013). *Neohydatothrips variabilis* was formerly classified in the genus Sericothrips (Beach 1897, Nakahara 1988). Both genus names appear in recent literature independently. Soybean thrips was first described in Iowa on smartweed and cucumbers in 1896 and originally called *Thrips variables* (Beach 1897). Current taxonomic classification follows: Arthropoda> Hexapoda> Insecta> Thysanoptera>Terebrantia> Thripidae> Sericothripinae> Neohydatothrips. The subfamily Sericothripinae includes three genera. Members of this subfamily have a global distribution and most are associated with flowers with a few breeding on leaves (Mound 2010). *Neohydatothrips variabilis* is the only member of the subfamily
Sericothripinae to be known to transmit a tospovirus. There are 103 species of Neohydatothrips (Mound 2010).

Thrips are tiny insects ranging from 0.6 to 15 mm long with most temperate species being between 1 and 2 mm. Adults have slender bodies with two pairs of narrow wings that are fringed with hairs. The adults of some species such as *Frankliniella fusca* also have a reduced wing form (Riley et al. 2013). Antennae in the Thripidae family usually have 7 to 8 segments of approximately equal size (Riley et al. 2013). Thrips can fly and jump between plants and are very active when observed under a microscope. They are also spread by wind and some species are blown into the Midwest from the southern United States each spring. Other species are able to overwinter in warmer areas in the soil as well as in greenhouses. Previously thought to have a unique rasping-sucking mouthpart that scraped the top of the plant cell away to access the cell nutrients (Lewis 1963), a study found that soybean thrips may actually have a piercing-sucking mouthpart (Huckaba and Coble 1991). Thrips feeding leads to a characteristic silvering damage to foliage. Direct feeding injury from thrips can also cause leaves to curl and if the populations are high this can lead to necrosis. Another indication of thrips feeding is their frass that appears as black specks on the leaf. Thrips damage may also occur on flower buds and fruits due to oviposition (Riley et al. 2013). A few species of thrips cause the development of plant galls. Thrips range in their food sources from fungal spores and hyphae, plants (both leaves and flowers), mosses, and even a few predatory species that feed on other insects (Mound 2010).
The lifecycle of thrips

The length of the lifecycle of thrips species is influenced by genetics and environmental conditions such as temperature and relative humidity. The egg is deposited in the plant tissue, usually within an opening in the stem created by the female thrips ovipositor. Larva then emerge and have two instar stages after which it will cease feeding and go through two pupae stages in the soil. The winged adult will emerge days later. Thrips reproduce quickly and are able to have multiple generations per year. Thrips mainly reproduce sexually, however occasionally parthenogenesis occurs. Soybean thrips have only been observed reproducing sexually.

The lifecycle of a soybean thrips lasts about 2-4 weeks depending on the environmental conditions such as temperature. Lifecycle studies done by Vance (1974) provide most of the known information on soybean thrips development. The following durations of each stage of the lifecycle are mean durations at 22°C and with an eight hour daily light photoperiod. First instar larvae stage lasts about 74 hours, with the larvae feeding near the area it hatched. Both larvae stages can move quickly and hide in the crevices created by the leaf veins. Second instar larvae feed on the leaf and mean duration of this stage is 91 hours. At the end of this period, the larvae will drop directly from the leaf to the soil, where the pre-pupae develop within the top inch. In the laboratory setting, pupae were able to remain on the leaf during this stage if no soil was available. The insect does not feed during this period. The pre-pupal stage lasts 22 hours and the pupae stage lasts 74 hours before the winged adult emerges. The ideal temperature for rapid development was 32°C, but mortality was lower at 26.5°C. At
21°C development was the lowest temperature tested and had the slowest insect development. Overall *N. variabilis* stays in the immature stages longer than some thrips species such as *Thrips tabaci* but a similar amount of time as *F. tritici* (Vance 1974).

**Thrips and tospoviruses**

Studies show that TSWV is acquired by the thrips from the plant tissue at larval stages 1 and 2 as well as when in the adult stage (Wijkamp et al. 1996). However, the insect is only able to transmit the virus if it is acquired during the larval stages and transmission is most effective when acquired during the first larval stage. This is thought to be due to a combination of factors including lack of viral multiplication within the midgut and lack of movement across midgut lining into salivary glands (Riley et al. 2013, Wijkamp et al. 1996). The efficiency of acquisition and subsequent transmission of TSWV is increased when the virus is picked up by the thrips during the early larval stage (Ogada et al. 2013). Transmission to the plant host takes place only in the later instars and adults (Wijkamp et al. 1996, Ullman et al. 1997, Whitfield et al. 2005). Most adults will remain viruliferous for the rest of its life. However, Mautino et al. (2012) showed that some *F. occidentalis* adults may recover from infections of TSWV. There is no vertical transmission from parent to offspring and the virus must be acquired anew by each generation of thrips (Wijkamp et al. 1996).

There are 15 species of thrips known to vector tospoviruses (Riley et al. 2011, Tennant and Fermin 2015, Zhou and Tzanetakis 2013) (Table 2). Fourteen species are in the subfamily Thripinae and the remaining is the vector of SVNV, *N. variabilis* (Zhou and
Tzanetakis 2013), which belongs to the Sericothripinae subfamily. Some viruses such as TSWV are vectored by more than one thrips species while others have a more specific interaction (Ogada et al. 2013).

As is seen in some other pathosystems, the presence of TSWV changes the plant to make it more attractive to its insect vector, Western flower thrips. Thrips feeding on infected pepper plants (Capsicum annum) have increased longevity and survival rates. This can change the population of the thrips to include more infected individuals and thus increasing the rate of viral spread in the plant population (Ogada et al. 2013). This may partly be due to improved nutrition and increased levels of amino acids in the phloem and peripheral cells (Awmack and Leather 2002). This was found to be the case with aphid transmitted viruses (Blua et al. 1994).

The evolutionary origin of the thrips and tospovirus symbiotic relationship is unclear. Mound (2010) explored the divergent vs. convergent hypotheses: i) the host insect/virus relationship developed early in the evolutionary history of thrips and most of these species with the symbiotic relationship are extinct and ii) the host insect/virus relationship is a relatively recent development that occurred independently in several unrelated species of thrips. The challenge with these hypotheses is that the thrips species that vector tospoviruses are genetically, physically, and geographically very different; they are not closely related. Only about 2% of thrips species are vectors. Conversely, tospoviruses appear to depend on the thrips for survival, so it is also unlikely that the viruses evolved recently and independently to become associated with
different thrips species in widespread geographic locations on a diverse range of plant hosts (Mound 2010).

**Soybean thrips plant hosts**

Soybean thrips have been found on many plant species across Central and North America. While there is no comprehensive or compilation study on plant host range or geography, the plants that *N. variabilis* feed on are diverse. Originally described on smartweed and cucumber in Iowa (Beach 1897), *N. variabilis* was subsequently reported on willow, crabapple, and viburnum (Hood 1908). They are commonly found on cotton, soybean, and other legumes such as lima bean (Dupree 1970, Zhou and Tzanetakis 2013). In the Midwest at least 16 plant families have had specimens collected (Gerdes 1979). In Virginia (USDA zone 7), *N. variabilis* is found in large numbers on tomato in spring and fall (Nault and Speese 2002) as well as on cotton in Arkansas, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, and Virginia (Akin et al. 2011). Large numbers of female soybean thrips were found on lilac in California (Bailey 1933).

**Cover crops and vegetables in Iowa**

Tomato spotted wilt virus often overwinters in greenhouses as its thrips species have a wide host range. If SVNV and *N. variabilis* are found to have a wide host range then a similar situation may be possible.
Cover crops are of growing importance in many cropping systems including soybean fields. In 2012, 10 million acres were planted in cover crops with over 300,000 of those being in Iowa (USDA 2012). Benefits of cover cropping include reduction of soil erosion and water runoff, improvement of soil texture and composition, retention of nutrients and weed suppression (Fageria et al. 2005). Cover crops can be used as a short season double crop such as wheat and buckwheat or as an overwintering soil cover with the plants incorporated into the soil in the spring (Sarrantonio and Gallandt 2003). A variety of plant species are used as cover crops, depending on the climate, length of growing season, and desired benefits. Research is being done to understand the impact of increased use of cover crops on many pathogens. The presence of alternative plant hosts near a susceptible crop can influence the impact of the disease within a season. Weeds are known to be a component in some pathosystems including viruses (Duffus 1971, Nutter 1993). In an insect transmitted viral pathosystem, plant species that are preferred by the insect species can also play an important role in the epidemiology of the disease. Cover crops are planted directly in the same fields as soybean, providing geographic accessibility of the thrips vector and SVNV between any susceptible cover crop and soybean.

Management of SVNV

Management of viruses in soybeans usually consists of using resistant cultivars, removing alternative plant hosts that may be providing additional inoculum sources, and using clean seed (Hill and Whitham 2014). Currently there are no documented
sources of plant resistance to SVNV in soybean. Variations in symptom appearance and severity suggest that further studies may find genetically resistant cultivars. Without resistant cultivars, managing thrips populations is the next management strategy although management of virus vectors in soybean has not been economically successful in the past (Hill and Whitham 2014). Thrips are not often managed within soybean fields as they do not usually cause enough damage themselves to be worth the cost of pesticides. Additionally it can be challenging to get enough coverage as thrips spend part of their lifecycle hidden in the soil, flower buds, and stem of the plant protected from the effects of insecticides. Due to their short generation intervals they are also able to develop resistance to insecticides relatively quickly. Thus management of thrips in order to mitigate the effects of SVNV should only be attempted if there is a threat of yield loss or damage to the seed quality. Further complications become apparent when we consider that soybean thrips along with other naturally occurring insect populations play a role in the pollination of soybean (Zhou et al. 2009). Zhou et al. (2009) was unable to find a clear relationship between seed set rates and thrips population numbers and thus it is unknown to what extent the thrips play a role in pollination. Timing of sprays is again important to ensure high enough insect populations at flowering while still controlling pest pressure. Thresholds and disease forecasting models should be developed based on a deeper understanding of the movements, population dynamics, and factors influencing thrips lifecycles and how those factors relate to SVNV spread. Reduction of the presence of alternative plant hosts for the virus and the insect vector is another management strategy that has managed some viral pathogens (Cock 1986,
Cohen et al. (1988, Hilje et al. 2001, Wisler and Norris 2005). Many cultural practices are effective against whitefly-transmitted viruses when implemented on a regional scale, including removal of weeds and crop residue (Hilje et al. 2001). This region-wide implementation of alternate plant hosts is important as many insects including thrips can travel long distances after acquiring the virus depending on weather conditions. Cohen et al. (1988) found whiteflies infected with *Tomato yellow leaf curl virus* seven kilometers from the source of inoculum.

It is also not known if SVNV interacts with other viruses. If further studies indicate synergistic or cross protection interactions, these would also need to be taken into consideration for the development of management plans. Seed transmission must also be considered. If studies show that infection with the seed transmissible types of SVNV results in yield or seed quality losses, then at a 6% transmission rate it has potential to have a large impact.

Consideration should also be given to the question of how the two transmission types of SVNV are connected. It is currently unknown if thrips transmitted and seed transmitted SVNV are acting within separate disease cycles. If so, this scenario contains the possibility of genetic recombination and the development of a symptomatic systemic infection that could be both thrips and seed transmitted thus resulting in greater impact on the crop (Groves et al. 2016). The other possibility is both types are already part of one complex disease cycle. Further research into these questions will guide development of management plans.
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<table>
<thead>
<tr>
<th>Virus name</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Alstroemeria necrotic streak virus</td>
<td>ANSV</td>
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<tr>
<td>Bean necrotic mosaic virus</td>
<td>BeNMV</td>
</tr>
<tr>
<td>Calla lily chlorotic spot virus</td>
<td>CCSV</td>
</tr>
<tr>
<td>Capsicum chlorosis virus</td>
<td>CaCV</td>
</tr>
<tr>
<td>Chrysanthemum stem necrosis virus</td>
<td>CSNV</td>
</tr>
<tr>
<td>Groundnut bud necrosis virus/ Peanut bud necrosis virus</td>
<td>GBNV/PBNV</td>
</tr>
<tr>
<td>Groundnut ringspot virus</td>
<td>GRSV</td>
</tr>
<tr>
<td>Groundnut chlorotic fan-spot virus/ Peanut chlorotic fan-spot virus</td>
<td>GCFV/PCFV</td>
</tr>
<tr>
<td>Groundnut yellow spot virus/ Peanut yellow spot virus</td>
<td>GYSV/PYSV</td>
</tr>
<tr>
<td>Hippeastrum chlorotic ringspot virus</td>
<td>HCRV</td>
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<tr>
<td>Impatiens necrotic spot virus</td>
<td>INSV</td>
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<tr>
<td>Iris yellow spot virus</td>
<td>IYSV</td>
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<tr>
<td>Lisianthus necrotic ringspot virus</td>
<td>LNRV</td>
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<td>Melon severe mosaic virus</td>
<td>MeSMV</td>
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<tr>
<td>Melon yellow spot virus</td>
<td>MYSV</td>
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<tr>
<td>Mulberry vein banding virus</td>
<td>MuBV</td>
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<tr>
<td>Pepper chlorotic spot virus</td>
<td>PCSV</td>
</tr>
<tr>
<td>Pepper necrotic spot virus</td>
<td>PNSV</td>
</tr>
<tr>
<td>Polygonum ringspot virus</td>
<td>PolRSV</td>
</tr>
<tr>
<td>Soybean vein necrosis virus/ Soybean vein necrosis-associated virus</td>
<td>SVNV/SVNaV</td>
</tr>
<tr>
<td>Tomato chlorotic spot virus</td>
<td>TCSV</td>
</tr>
<tr>
<td>Tomato necrotic ringspot virus</td>
<td>TNRV</td>
</tr>
<tr>
<td>Tomato necrotic spot virus</td>
<td>TNSV</td>
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<td>Tomato spotted wilt virus</td>
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<tr>
<td>Tomato yellow ring virus</td>
<td>TYRV</td>
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<tr>
<td>Tomato zonate spot virus</td>
<td>TZSV</td>
</tr>
<tr>
<td>Watermelon bud necrosis virus</td>
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<tr>
<td>Watermelon silver mottle virus</td>
<td>WSMV</td>
</tr>
<tr>
<td>Zucchini lethal chlorosis virus</td>
<td>ZLCV</td>
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Table 2. Thrips species and associated tospoviruses

<table>
<thead>
<tr>
<th>Thrips species</th>
<th>Thrips common name</th>
<th>Tospovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ceratothripoides claratris</em></td>
<td>Oriental tomato thrips</td>
<td>CaCV, TNRV</td>
</tr>
<tr>
<td><em>Dictyothrips betae</em></td>
<td>-</td>
<td>PoIRSV</td>
</tr>
<tr>
<td><em>Frankliniella bispinosa</em></td>
<td>Florida flower thrips</td>
<td>TSWV</td>
</tr>
<tr>
<td><em>Frankliniella cephalica</em></td>
<td>Florida flower thrips</td>
<td>TSWV</td>
</tr>
<tr>
<td><em>Frankliniella fusca</em></td>
<td>Tobacco thrips</td>
<td>TSWV</td>
</tr>
<tr>
<td><em>Frankliniella gemina</em></td>
<td>-</td>
<td>GRSV, TSWV</td>
</tr>
<tr>
<td><em>Frankliniella intonsa</em></td>
<td>Eurasian flower thrips</td>
<td>GRSV, INSV, TCSV, TSWV</td>
</tr>
<tr>
<td><em>Frankliniella occidentalis</em></td>
<td>Western flower thrips</td>
<td>CSNV, GRSV, INSV, TCSV, TSWV, ANSV</td>
</tr>
<tr>
<td><em>Frankliniella schultzei</em></td>
<td>Tomato thrips</td>
<td>CSNV, GRSV, GBNV, TCSV, TSWV</td>
</tr>
<tr>
<td><em>Frankliniella zucchini</em></td>
<td>-</td>
<td>ZLCV</td>
</tr>
<tr>
<td><em>Neohydatothrips variabilis</em></td>
<td>Soybean thrips</td>
<td>SVNV</td>
</tr>
<tr>
<td><em>Scirtothrips dorsalis</em></td>
<td>Chilli thrips</td>
<td>GBNV, GCFSV, GYSV</td>
</tr>
<tr>
<td><em>Thrips palmi</em></td>
<td>Melon thrips</td>
<td>CCSV, GBNV, MYSV, TNRV, TSWV, WSMoV</td>
</tr>
<tr>
<td><em>Thrips setosus</em></td>
<td>Japanese flower thrips</td>
<td>CaCV, TSWV</td>
</tr>
<tr>
<td><em>Thrips tabaci</em></td>
<td>Onion thrips</td>
<td>IYSV, TSWV, TYRV</td>
</tr>
<tr>
<td><em>Unknown</em></td>
<td>-</td>
<td>LNRV, MeSMV, HCRV, MuVBV, PCSV, PNSV, TNSV</td>
</tr>
</tbody>
</table>
Figure 1. Symptoms of *Soybean vein necrosis virus* infection on soybean grown in a growth chamber. A) chlorotic lesions becoming necrotic on soybean leaflet; B) chlorotic lesions on soybean leaflet; and C) chlorotic and necrotic lesions on soybean leaf also with thrips feeding damage.

Justification

*Soybean* is a crop of economic importance, with premiums being paid for special use beans. Any loss in yield or seed quality will impact farmers’ economic situation. The results of this project will be useful for understanding the potential impacts of this disease on soybean in Iowa and elsewhere in the country. This information may help guide decisions about developing management tools for *Soybean vein necrosis virus* and *Neohydatothrips variabilis*. The objectives of this research were to:

1. Assess the effects of *Soybean vein necrosis virus* infection on soybean yield and seed quality.
2. Evaluate cover crop and vegetable species for potential as alternate host for *Soybean vein necrosis virus*. 
CHAPTER 2
EFFECT OF SOYBEAN VEIN NECROSIS VIRUS ON YIELD AND SEED QUALITY OF SOYBEAN


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Abstract

Soybean vein necrosis virus (SVNV) rapidly became a widespread virus of soybean (Glycine max) within a few years of its initial confirmation in 2008. The economic impact of soybean vein necrosis (SVN) remains unknown. Field studies were conducted in six states in 2013, 2014, and 2015 to determine the effect of SVN on soybean yield and seed quality. Quantitative parameters, including seeds per pod, pods per plant, yield, and 100-count seed weight, as well as qualitative parameters, including
protein and oil concentration, were assessed from plants or seeds collected from research and commercial production fields. Results suggest that yield is not impacted by SVN. However, seed quality was affected. In Iowa, oil concentration decreased by 0.11% as disease incidence increased by 1% ($P=0.04$). Changes in fatty acid profiles of seed were also observed; linolenic, linoleic and stearic acids decreased between 0.5 and 0.15% in 2 of 6 locations that were tested. These results suggest that infection by SVNV negatively affects soybean seed quality, which may affect the marketability of soybeans for premium markets.

**Introduction**

Soybean (*Glycine max* (L.) Merr) is grown worldwide, and soybean seeds are used in livestock feed, oil, human food, and biofuel. In the United States, 108 million metric tons of soybeans were produced in 2014 (USDA 2015). Globally, 281.9 million metric tons of soybeans were produced in 2013-14 (McFerron 2014). Soybeans make up 90% of the oilseed produced in the United States and are the largest source of livestock feed protein globally (USDA 2012).

*Soybean vein necrosis virus* (SVNV) belongs to the genus *Tospovirus* in the family *Bunyaviridae*. Tospoviruses are transmitted by thrips (Thysanoptera), a small winged insect. The only known vector of SVNV is the soybean thrips, *Neohydatothrips variabilis* (Beach) (Zhou and Tzanetakis 2013). It is unknown if other thrips species can transmit SVNV. Although tospoviruses can infect over a thousand host plant species, SVNV is the
only known tospovirus to infect soybean in the United States, with the exception of
*Tomato spotted wilt virus* which has been reported in Alabama, Georgia, and Tennessee
(Sikora et al, 2011).

SVNV was first confirmed in soybean in Arkansas and Tennessee in 2008
(Tzanetakis 2009). It has since become widespread across the soybean production
region of the United States and Canada. Common SVN symptoms include interveinal
chlorosis originating from the leaf vein that progress to reddish-brown lesions that
eventually become necrotic. Leaf veins can be clear, yellow, or dark brown with
symptoms often more severe on the underside of the leaf. These symptoms may
resemble other soybean diseases or herbicide injury (Bloomingdale et al. 2015).

Although SVNV has become widespread, the potential impacts on yield and seed
quality are unknown. Of the 46 viruses known to infect soybean, very few are
economically important. Of these, only *Alfalfa mosaic virus* (AMV), *Bean pod mottle
virus* (BPMV), *Peanut mottle virus* (PeMoV), *Peanut stunt virus* (PSV), *Soybean dwarf
virus* (SbDV), *Soybean mosaic virus* (SMV), *Soybean vein necrosis virus* (SVNV), and
*Tobacco ringspot virus* (TRSV) have an impact on yield and seed quality (Demski et al.
1971, Filho et al. 2001, and Hill and Whitham 2014). As soybean is the top oilseed crop
in the United States any drop in oil content of the seed would have an economic impact.
Additionally, soybean farmers can receive premium prices for seeds that have lower
levels of palmitic and linolenic acid as well as higher levels of oleic acid. Linolenic acid
can shorten the shelf life of oil and processors must go through extra steps to
hydrogenate excess linolenic acid from the soybean seed. Fatty acid levels are so
important that breeders are developing soybean cultivars with naturally occurring fatty acids contents closer to the ideals of the marketplace. The objectives of this study were to i) evaluate the impact of SVNV on yield parameters including seeds per pod, pods per plant, yield, and 100-count seed weight, and ii) determine the effect of SVNV on soybean seed quality parameters including protein, oil, and fatty acid concentration of seed.

**Collection of SVN data in the field**

Data were collected in 2013, 2014, and 2015 from six states: Alabama, Delaware, Illinois, Indiana, Iowa, and Wisconsin. Although protocols differed slightly across states due to variations in disease levels, plants and plots were targeted based on symptoms of SVNV infection and whenever possible symptomatic and asymptomatic plants were compared within fields (Table 1). Fields were managed using standard management practices based on local University recommendations for fertility, fungal diseases, insects and weeds. Plant growth stages were based on the description provided by Fehr et al. (1971).

At growth stage R6 symptomatic and asymptomatic plants were marked for later sampling. Representative subsamples of plants were tested for SVNV using an enzyme-linked immunosorbent assay (ELISA; Agdia, Inc., Elkhart, IN) or sent to Agdia, Inc. to confirm pathogen presence. Plants were hand harvested at maturity. Pods per plant, plant yield, seeds per pod, and 100-seed weight were evaluated for each plant. All pods
from each plant were hand-shelled. Incidence data, calculated as the percentage of leaves exhibiting symptoms, was also collected in Delaware, Illinois, and Iowa.

Because asymptomatic plants were hard to find in some fields in Iowa in 2013, the effect of SVN on yield and seed components was measured from plots with differing levels of SVN. At three locations near the towns of Washington, Ottumwa, and Stockport, plots that were two 76 cm rows wide and 3 m long were selected for sampling to provide a range in incidence and severity levels. Within each plot at the R6 growth stage, the total number of fully expanded leaves with SVN symptoms was recorded on all plants. Incidence of disease was determined using the percentage of SVN symptomatic leaves.

Two states, Indiana and Iowa, did additional testing of seed for oil, protein, and fatty acid content. In Indiana, fifteen seeds from each plant were used to determine levels of oils, proteins, as well as fatty acid profiles using a Perten DA 7250 NIR analyzer (Hagersten, Sweden). In Iowa, seeds were tested at the Iowa State University W.M. Keck Metabolomics Research Laboratory using conventional gas chromatography methods (Jaimeson and Reid 1965).

Mixed-model analysis of variance (ANOVA) was used for all asymptomatic and symptomatic samples. Incidence data were subjected to regression analysis, where SVN incidence was the independent variable and each yield variable was the dependent variable. Slopes and intercepts were calculated and r-squared values determined. All statistical analyses were done using SAS v. 9.3 or JMP v. 11 (SAS Institute, Cary, NC).
Yield parameters

Data were collected for a total of 12 location-years. Of these 12 location-years, 11 location-years collected yield data. There was a significant yield difference ($P = 0.01$) between asymptomatic and symptomatic plants in Indiana in 2014 (Table 2). There was a significant positive relationship ($P < 0.01$) in Stockport, Iowa in 2013 (Table 3, Figure 2). All other location-years had no significant relationship.

Data for 100-seed weight were collected in nine location-years. In Alabama, Delaware, and Indiana, 100-seed weight was significantly greater for plants that were symptomatic for SVN compared to asymptomatic plants (Table 2, Table 3, Figure 1). Pods per plant were measured in nine location-years, with only Alabama having a significant result (Table 2). In Alabama, there were significantly more pods per plant on symptomatic plants than asymptomatic ($P = 0.04$). There were no significant effects from SVN on seeds per pod from the five location-years (Tables 2 and 3).

Seed quality parameters

Data on oil, protein, and fatty acid content of soybean seed were collected from seven location-years. Significant changes in oil were seen in three of six location years, while significant changes in protein were seen in only one location-year (Tables 4 and 5). Samples from Indiana had significantly lower total oil concentration in SVN-symptomatic seed compared to asymptomatic seed in the 2014 seeds and the 2015 double-cropped field. In 2015, double-cropped symptomatic had an increase in protein (Table 5).
Laboratory analysis of soybean seeds collected in Iowa in 2013 indicated that total oil levels decreased by 0.11% as percentage of leaves infected increased by 1 percent \((P=0.04)\) in the Ottumwa field (Table 4, Figure 3). Additionally, protein and oil had a strong negative linear relationship in all three fields with oil decreasing as protein increased \((P <0.01)\).

In addition, the fatty acid profile of the oil was different in several location years. In Indiana, seed of SVN-symptomatic plants had less palmitic acid \((P=0.04)\) than asymptomatic plants in 2014, while there was an increase of palmitic acid in the full season symptomatic seed from 2015 (Table 5). No other significant differences in fatty acid profile of the oil were identified from the 2014 or 2015 double-cropped samples. However, the 2015 full season seed had a significant decrease in linoleic and increase in linolenic acid (Table 5). The Washington, Iowa field had a significant positive correlation between SVN incidence and palmitic acid \((P=0.02)\) (Table 4, Figure 4). In 2014, there were significant differences between symptomatic and asymptomatic plants for levels of palmitic, stearic, and linolenic acids in Iowa. Palmitic acid was higher in symptomatic plants \((P <0.01)\) while stearic \((P =0.01)\) and linolenic acids \((P =0.02)\) were lower (Table 5).

**Discussion**

Our results indicate that SVN can influence the quality and chemical composition of harvested seed but likely has little effect on direct yield of soybeans. However, SVN symptoms appeared during the mid to late reproductive stages for all location-years in this study. Protein and oil concentration are important factors in current soybean production systems, with soybeans accounting for the majority of edible vegetable oil
and animal feed proteins globally. Just a small change in protein and oil levels could warrant further study, as a significant decrease in oil production or changes in fatty acid profiles could directly impact the value of soybeans. Soybeans can be sold for a premium if oil and protein levels are high. Conversely, if oil levels drop below accepted standards, they may be rejected in the marketplace. In addition, premium prices are paid for seed that is high in oleic acid and low in linolenic acids. In this study, seed was tested only from Indiana and Iowa, yet several significant changes were observed to both oil concentration and fatty acid profiles of seed from both states.

In Indiana and Iowa, oil concentration decreased significantly in SVN-symptomatic plants. A similar decrease in oil concentration in relationship to infection with *Cowpea chlorotic mottle virus* (CCMV), *Peanut mottle virus* (PeMoV), *Soybean mosaic virus* (SMV), and *Tobacco ringspot virus* (TRSV) was shown previously (Demski and Jellum 1975). Another previous study showed that a negative relationship between total oil and protein occurs in healthy soybean seed with an increase in protein and decrease in oil (Cartter and Hopper 1942). This phenomenon has continued to be demonstrated in further studies and this same relationship was observed in the seed that was tested from Iowa. This negative correlation between oil and protein concentration in seed from infected plants is possibly due to soybean requiring more energy to produce oil in the seed than is needed for protein production (Harris et al. 1970). This effect may be increased when the plant is under stress from viral infection, only leaving enough energy available to create protein but not enough to generate oil (Harris et al. 1970).
Studies in the early 1970s found significant changes to fatty acid profiles in soybean infected with CCMV and TRSV, with a decrease in palmitic, linoleic, and linolenic and an increase in stearic and oleic acids (Harris et al. 1970, Demski et al. 1971, Demski and Jellum 1975). In our study, seed from Indiana in 2014 had a significant decrease in palmitic acid in SVN symptomatic plants. Although not significant, the stearic and linolenic acids were lower in the SVN-symptomatic plants. In 2015, Indiana SVN-symptomatic seed had an increase in palmitic and linolenic acids while having a decrease in levels of linoleic acid. In Iowa in 2014, seed from symptomatic plants had a significant increase in palmitic acid but a decrease in stearic and linolenic acids. The Washington, Iowa 2013 location had increasing concentrations of palmitic acid in correlation to increasing SVN incidence. A decrease in linolenic acid is considered desirable since linolenic acid imparts poor flavor to foods and decreases storage ability of the oil; thus linolenic acid is hydrogenated during processing or reduced through breeding (Howell and Collins 1957, Harris et al. 1970).

Yield loss was not associated with SVNV infection in soybean in any of the six states in this study. Although the trend of increased yield in relation to SVN symptoms observed in Wisconsin in 2013 was not significant, there was a significant yield increase in SVN affected plants in Indiana and Iowa in 2013. Demski et al. (1971) found infection by TRSV in soybean resulted in larger, but fewer, seeds and this combination produced lower yield overall. Our research also found this trend of increased seed size in four location-years: SVN symptomatic plants in Alabama and Indiana and a positive relationship to SVN incidence in Delaware. There was no change in seed size or number
of seeds per plant for the rest of the fields with no data collected on seed size for Indiana or Wisconsin and no data collected on number of seeds for Alabama, Iowa 2013, or Wisconsin. Limiting and confounding factors, such as other diseases present in the field, cultivar differences, as well as environmental conditions may lead to future work under controlled settings to determine the true effects of SVNV infection on yield and seed size.

It has previously been thought that SVNV was only thrips transmitted and did not move systemically through soybean (Zhou and Tzanetakis 2013). However, recent work has shown that SVNV is also seed transmitted, which indicates that the virus can move systemically through the plant (Groves et al. 2016). This viral movement is not well understood and may account for some of the variations seen between the data in this study.

Some of the impact of SVN infection on soybean may be related to the timing of thrips arrival and SVN infection. The interaction between timing of infection and incidence levels of SMV was correlated with a reduction in soybean seed size occurring in earlier infections (Ren et al 1997). Earlier TRSV infection in soybean resulted in larger seed size (Demski et al 1971). In this study, Alabama, Delaware, and Indiana were the only states to see a change in seed size. SVN symptomatic plants in Alabama produced seeds that were larger than those from asymptomatic plants. Seed size in Delaware had a positive relationship to SVN incidence. These two states are both in USDA plant hardiness zones that are milder than the rest of the states in this study. If soybean thrips are able to overwinter in these warmer states, then they may be able to infect the
soybean plants at an earlier growth stage, thus resulting in more impact on seed size. Survey data from Delaware over the last five years supports overwintering or early appearance of thrips in the Mid-Atlantic region (unpublished). Double cropping soybean after another crop is harvested, typically wheat, results in later soybean planting, which may also compound the effect of SVNV infection as the plants are exposed to higher populations of thrips at a younger growth stage. In the Midwestern United States soybean-producing states, symptoms of SVN typically do not appear until the mid to late reproductive phase of the plant. This may be due to vectors not being able to overwinter in cooler northern climates, and therefore SVNV does not arrive until its thrips vector migrate north from warmer climates (MacIntyre-Allen, et al., 2005). These thrips must therefore move into the northern states each season from milder locales. This delay of thrips movement into soybean fields in northern states may be resulting in decreased disease impact.

Further studies on the effects of SVNV infection timing on yield and seed quality are needed to fully understand the potential economic impact of this disease. Controlled environment studies on yield and seed quality may determine the impact of timing of infection and titer levels. Although this study did not observe yield loss associated with SVNV infection, there were significant impacts on seed quality components provides a foundation for future studies on the impacts of SVN on seed quality.
**Contributions of authors**

Melissa Irizarry was responsible for data collection in Iowa. Each author collected data for their respective states and sent data to Iowa for analysis. Melissa Irizarry wrote the first draft of the manuscript, while co-authors edited the manuscript.

**References**


Table 1. Information about field trials investigating yield loss from *Soybean vein necrosis virus*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Sampling unit</th>
<th>Number of samples</th>
<th>Type of data</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>2014</td>
<td>Plants</td>
<td>170</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
</tr>
<tr>
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<td>2014</td>
<td>Plants</td>
<td>175</td>
<td>Incidence</td>
<td>Regression</td>
</tr>
<tr>
<td>Illinois</td>
<td>2013</td>
<td>Plants</td>
<td>40</td>
<td>Incidence</td>
<td>Regression</td>
</tr>
<tr>
<td>Indiana</td>
<td>2014</td>
<td>Plants</td>
<td>690</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Indiana (full season)</td>
<td>2015</td>
<td>Plants</td>
<td>50</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Indiana (double crop)</td>
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<td>Plants</td>
<td>130</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
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<tr>
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<td>Plots</td>
<td>10</td>
<td>Incidence</td>
<td>Regression</td>
</tr>
<tr>
<td>Iowa (Ottumwa)</td>
<td>2013</td>
<td>Plots</td>
<td>24</td>
<td>Incidence</td>
<td>Regression</td>
</tr>
<tr>
<td>Iowa (Stockport)</td>
<td>2013</td>
<td>Plots</td>
<td>20</td>
<td>Incidence</td>
<td>Regression</td>
</tr>
<tr>
<td>Iowa</td>
<td>2014</td>
<td>Plants</td>
<td>50</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>2013</td>
<td>Plants</td>
<td>200</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>2014</td>
<td>Plants</td>
<td>100</td>
<td>Asymp/symp</td>
<td>ANOVA</td>
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</table>
Table 2. Yield and yield component data for location-years where soybean vein necrosis (SVN) symptomatic or asymptomatic plants were hand-harvested in Alabama, Indiana, Iowa, and Wisconsin in 2013, 2014, and 2015.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>2014</th>
<th>2015</th>
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<td></td>
<td></td>
<td>Asymptomatic</td>
<td>Symptom</td>
<td>P-value</td>
</tr>
<tr>
<td>Yield/plant (g)</td>
<td>Alabama</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td></td>
<td>Indiana</td>
<td>16.49</td>
<td>16.74</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Iowa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wisconsin</td>
<td>20.90</td>
<td>24.40</td>
<td>0.06</td>
</tr>
<tr>
<td>100-seed weight (g)</td>
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<td>-</td>
<td>-</td>
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<td>Indiana</td>
<td>15.83</td>
<td>16.72</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Iowa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wisconsin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seeds per pod</td>
<td>Alabama</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>Indiana</td>
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<td>2.27</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Iowa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wisconsin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pods per plant</td>
<td>Alabama</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td></td>
<td>Wisconsin</td>
<td>49.70</td>
<td>56.70</td>
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</table>
Table 3. Linear regression values for yield and yield components for plants with differing levels of soybean vein necrosis (SVN) in Delaware, Illinois, and Iowa in 2013, 2014, and 2015.

<table>
<thead>
<tr>
<th>Seed Parameter</th>
<th>Year</th>
<th>State</th>
<th>Intercept</th>
<th>Slope</th>
<th>$R^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>2014</td>
<td>Delaware</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Illinois</td>
<td>16.64</td>
<td>-0.05</td>
<td>0.02</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Washington)</td>
<td>1804.62</td>
<td>-71.92</td>
<td>0.25</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Ottumwa)</td>
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<td>-21.55</td>
<td>0.04</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Stockport)</td>
<td>2389.75</td>
<td>+182.00</td>
<td>0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>100-seed weight (g)</td>
<td>2014</td>
<td>Delaware</td>
<td>11.41</td>
<td>+0.09</td>
<td>0.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Illinois</td>
<td>16.47</td>
<td>-0.01</td>
<td>&lt;0.01</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Washington)</td>
<td>15.24</td>
<td>+0.08</td>
<td>0.07</td>
<td>0.47</td>
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<tr>
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<td>Iowa (Ottumwa)</td>
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<td>-0.10</td>
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<td>0.11</td>
</tr>
<tr>
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<td>2013</td>
<td>Iowa (Stockport)</td>
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<td>0.25</td>
</tr>
<tr>
<td>Seeds/pod</td>
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<tr>
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<td>-0.01</td>
<td>&lt;0.01</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Washington)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Ottumwa)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Stockport)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pods/plant</td>
<td>2014</td>
<td>Delaware</td>
<td>44.77</td>
<td>0.13</td>
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<tr>
<td></td>
<td>2014</td>
<td>Illinois</td>
<td>40.57</td>
<td>+0.12</td>
<td>0.03</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Washington)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Ottumwa)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Iowa (Stockport)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1Delaware and Illinois yield is per plant (grams) and Iowa is measured in kg/ha.
Table 4. Linear regression data for protein, oil, and palmitic, linolenic, steric, oleic, and linoleic fatty acids content of soybean seed hand harvested from three Iowa fields in 2013 that had differing levels of soybean vein necrosis (SVN).

<table>
<thead>
<tr>
<th>State</th>
<th>Seed Parameter</th>
<th>Intercept</th>
<th>Slope</th>
<th>$R^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington</td>
<td>Protein %</td>
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<td>0.11</td>
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<tr>
<td></td>
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<td>-0.09</td>
<td>0.26</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Palmitic acid %</td>
<td>8.78</td>
<td>+0.20</td>
<td>0.52</td>
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</tr>
<tr>
<td></td>
<td>Linolenic acid %</td>
<td>5.81</td>
<td>+0.01</td>
<td>&lt;0.01</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Steric acid %</td>
<td>4.47</td>
<td>-0.01</td>
<td>0.02</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Oleic acid %</td>
<td>29.14</td>
<td>-0.40</td>
<td>0.11</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid %</td>
<td>51.69</td>
<td>+0.21</td>
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<td>0.53</td>
</tr>
<tr>
<td>Stockport</td>
<td>Protein %</td>
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<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
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<td>Oil %</td>
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<td>+0.09</td>
<td>0.05</td>
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<tr>
<td></td>
<td>Palmitic acid %</td>
<td>10.49</td>
<td>-0.04</td>
<td>0.04</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Linolenic acid %</td>
<td>5.54</td>
<td>+0.01</td>
<td>&lt;0.01</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Steric acid %</td>
<td>5.03</td>
<td>-0.02</td>
<td>0.01</td>
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</tr>
<tr>
<td></td>
<td>Oleic acid %</td>
<td>26.29</td>
<td>+0.53</td>
<td>0.15</td>
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</tr>
<tr>
<td></td>
<td>Linoleic acid %</td>
<td>52.41</td>
<td>-0.58</td>
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<td>0.08</td>
</tr>
<tr>
<td>Ottumwa</td>
<td>Protein %</td>
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<td>+0.04</td>
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</tr>
<tr>
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<td>0.18</td>
<td>0.04</td>
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<td></td>
<td>Palmitic acid %</td>
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<td></td>
<td>Linolenic acid %</td>
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<tr>
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<td>Linoleic acid %</td>
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</table>
Table 5. Protein, oil, and palmitic, linolenic, steric, oleic, and linoleic fatty acids content of soybean seed hand-harvested from plants either symptomatic or asymptomatic for soybean vein necrosis (SVN) in Iowa 2014, Indiana 2014 and 2015.

<table>
<thead>
<tr>
<th></th>
<th>Iowa 2014</th>
<th></th>
<th></th>
<th>Indiana 2014</th>
<th></th>
<th></th>
<th>Indiana 2015 Full season</th>
<th></th>
<th>Indiana 2015 Double crop</th>
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</thead>
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<tr>
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<td>Symptomatic</td>
<td>P-value</td>
<td>Asymptomatic</td>
<td>Symptomatic</td>
<td>P-value</td>
<td>Asymptomatic</td>
<td>Symptomatic</td>
<td>P-value</td>
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</tr>
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<td>&lt;0.01</td>
<td>Protein</td>
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<td>38.99</td>
<td>0.74</td>
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<td>0.01</td>
<td>Oil</td>
<td>21.32</td>
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<td>Oil</td>
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<td>40.36</td>
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<tr>
<td>Oleic</td>
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<td>19.97</td>
<td>0.36</td>
<td>Palmitic</td>
<td>12.19</td>
<td>11.85</td>
<td>0.04</td>
<td>Palmitic</td>
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<td>13.35</td>
</tr>
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<td>55.20</td>
<td>55.68</td>
<td>0.19</td>
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<td>4.82</td>
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<td>7.49</td>
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<td>Oleic</td>
<td>15.82</td>
<td>16.14</td>
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<td>Oleic</td>
<td>16.34</td>
<td>16.74</td>
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<td></td>
<td></td>
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<td></td>
<td>Linoleic</td>
<td>54.36</td>
<td>54.88</td>
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<td>Linoleic</td>
<td>56.38</td>
<td>53.34</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Linolenic</td>
<td>11.23</td>
<td>10.89</td>
<td>0.08</td>
<td>Linolenic</td>
<td>8.57</td>
<td>10.17</td>
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</table>
Figure 1. Linear regression analysis of soybean vein necrosis (SVN) incidence and 100-seed weight in a field where plants were hand-harvested in Delaware in 2014; $y = 11.41 + 0.09x$, $P<0.01$, $R^2=0.21$. 
Figure 2. Linear regression analysis of soybean vein necrosis (SVN) incidence and yield in a field where plants were hand-harvested in Stockport, Iowa in 2013; $y = 35.54 + 2.71x$, $P < 0.01$, $R^2 = 0.65$. 
Figure 3. Linear regression analysis of soybean vein necrosis (SVN) incidence and total seed oil in a field where plants were hand-harvested in Ottumwa, Iowa in 2013; y = 22.00 - 0.11x, P=0.04, R²=0.18.
Figure 4. Linear regression analysis of soybean vein necrosis (SVN) incidence and palmitic acid in a field where plants were hand-harvested in Washington, Iowa in 2013; $y = 8.78 + 0.20x$, $P=0.02$, $R^2=0.52$. 
HOST RANGE OF SOYBEAN VEIN NECROSIS VIRUS IN SPECIALTY AND COVER CROPS AND FEEDING PREFERENCE OF SOYBEAN THRIPS ON COVER CROPS

Abstract

Soybean vein necrosis virus is considered the most widespread soybean virus in North America. Soybean is a crop of global importance with nearly four billion bushels of soybeans produced in the United States in 2014. Some Tospoviruses, such as Tomato spotted wilt virus, are globally important pathogens with extensive host ranges. SVNV is a Tospovirus and previous research indicates that soybean is not the primary plant host. This study investigated the ability of specialty and cover crops that were commonly present in Iowa to serve as alternative hosts for SVNV. Additionally, as the virus is transmitted by soybean thrips, preferential feeding was observed on different cover crops. Eighteen cover crops and seven specialty crops were tested using mechanical and direct thrips inoculations. Presence of SVNV was determined with ELISA. Systemic infection of buckwheat and local infections of melon and winter pea were found. Symptoms were observed on buckwheat and melon. Soybean thrips were found to prefer alfalfa, buckwheat, crimson clover, and red clover; although they were able to feed on all plant species tested if no other food was presented. These data suggest that other crops may harbor SVNV and be a source of inoculum.

Introduction

Soybean (Glycine max) is a crop of global importance. Nearly 4 billion bushels of soybeans were produced in the United States in 2014 with more than 500 million
bushels coming from Iowa (USDA 2015). Approximately 282 million metric tons of soybeans were produced globally in the 2013/2014 season (McFerron 2014). Processed soybeans are used as livestock feed, oil, human food, aquaculture feed, plastics, textiles, and biofuel. The uses and demand for soybean are continually expanding.

Use of cover crops is increasing in many cropping systems including soybean fields. In 2012, 10 million acres were planted in cover crops with over 300,000 of those being in Iowa (USDA 2012). Benefits of cover cropping include reduction of soil erosion and water runoff, improvement of soil texture and composition, retention of nutrients and weed suppression (Fageria et al. 2005). Cover crops can be used as a short season double cropping such as with wheat and buckwheat, as an overwintering soil cover with the plants incorporated into the soil in the spring, and as intercropped living mulch (Sarrantonio and Gallandt 2003). A variety of plant species are used as cover crops, depending on the climate, length of growing season, and desired benefits. Research is being done to understand the impact of increased use of cover crops on many pathogens. The presence of alternative plant hosts near a susceptible crop can influence the impact of the disease within a season. Weeds are known to be a component in some pathosystems including viruses (Duffus 1971, Nutter 1993). In an insect transmitted viral pathosystem, plant species that are preferred by the insect species can also play an important role in the epidemiology of the disease. Cover crops are planted directly in the same fields as soybean, providing spatial accessibility of the thrips vector and SVN between any susceptible cover crop and soybean.
Currently there are 46 viruses that are known to infect soybeans in the field with eight of these considered to have enough impact to be managed; *Alfalfa mosaic virus* (AMV), *Bean pod mottle virus* (BPMV), *Peanut mottle virus* (PeMoV), *Peanut stunt virus* (PSV), *Soybean dwarf virus* (SbDV), *Soybean mosaic virus* (SMV), *Soybean vein necrosis virus* (SVNV), and *Tobacco ringspot virus* (TRSV) (Hill and Whitham 2014). Common symptoms of soybean vein necrosis (SVN) which was first confirmed in Arkansas and Tennessee in 2008 (Tzanetakis et al. 2009) include interveinal chlorosis along the leaf vein that can become red-brown lesions leading to necrosis (Figure 1). The veins can be clear, yellow, or dark brown. Symptoms are often worse on the underside of the leaf, possibly due to the preference of thrips to feed in these areas (Bloomingdale et al. 2015).

*Soybean vein necrosis virus* is a Tospovirus, a genus of arboviruses vectored by thrips. The only thrips species that has been confirmed to transmit SVNV is the soybean thrips (*Neohydatothrips variables*) (Zhou and Tzanetakis 2013). *Soybean vein necrosis virus*, as are all tospoviruses, is a persistent and propagative virus (Ullman et al. 1997). There are 29 known Tospoviruses (Riley et al. 2011, Tennant and Fermin 2015, Zhou and Tzanetakis 2013), which infect a diverse range of plants across the globe. Members of the tospovirus genus can infect at least 1,090 plant species in 80 plant families, including both monocots and dicots (Parrella et al. 2003). The majority of susceptible plants are in the Asteraceae and Solanaceae families. Typical symptoms of a tospovirus infection in plant hosts include chlorosis, mottling, necrotic lesions, stunting, sunken and ring spots,
as well as wilting (Riley et al. 2011). These symptoms can vary depending on the various factors of the plant host as well as virus strain.

*Soybean vein necrosis virus* is one of only a few tospoviruses that has been found infecting soybean. *Tomato spotted wilt virus* was found in Georgia (Nischwitz et al. 2006), with the tospoviruses *Groundnut bud necrosis virus* (GBNV), *Groundnut ringspot virus* (GRSV), TSWV, and *Tomato yellow ring virus* (TYRV) in the Eastern hemisphere (Khatabi et al. 2012).

Because of the local lesions that result from thrips transmission of SVNV, it is suspected that soybean and other legumes may not be the primary plant host of the virus (Khatabi et al. 2012, Zhou and Tzanetakis 2013). A study done by Zhou and Tzanetakis (2013) looked at possible host plant species for the virus. They tested 25 plant species and found that nine were susceptible to infection with SVNV through mechanical inoculation. Susceptible species were hardy mum (*Dendranthema grandiflorum*), ivyleaf morningglory (*Ipomoea hederacea Jacq*), summer squash (*Cucurbita pepo*), soybean (*Glycine max*), cowpea (*Vigna unguiculata*), mung bean (*Vigna radiate*), *Nicotiana benthamiana*, tobacco (*Nicotiana tabacum*), and *Nicotiana glutinosa*. These plants represented a wide range of families that include Asteraceae, Curcurbitaceae, Leguminosae, and Solanaceae. Hardy mum, ivyleaf morningglory and summer squash were asymptomatic (Zhou and Tzanetakis 2013). They additionally collected ivyleaf morningglory plants from a SVNV soybean field and found five of the eight plants tested were positive. They noticed that this weed’s range overlaps with that of SVNV and soybean thrips perhaps indicating a close tie or important role of the weed.
in the pathosystems. As weeds are often considered an important component of viral pathosystems (Nutter 1993). Legume hosts are a rarity among tospoviruses (Oliveira et al. 2012). Soybean, cowpea, mung bean, *N. glutinosa*, and tobacco exhibited local lesions. The closely related BeNMV was discovered on common bean (*Phaseolus vulgaris*) (Oliveira et al. 2012), but SVNV did not infect common bean. Only *N. benthamiana* had a systemic infection with symptoms.

Mechanical inoculation techniques for SVNV have a low success rate while direct inoculation using thrips has a greatly increased success rate, in some plant species nearing 100%, indicating the importance of thrips as vectors (Khatabi et al. 2012, Zhou and Tzanetakis 2013). Species that were found to be negative in the Zhou and Tzanetakis (2013) study were globe amaranth (*Gomphrena globosa*), palmer amaranth (*Amaranthus palmeri*), spinach (*Spinacia oleracea*), dahlia (*Dahlia hortensis*), broccoli (*Brassica oleracea*), quinoa (*Chenopodium quinoa*), common lambsquarters (*Chenopodium album*), cucumber (*Cucumis sativus*), peanut (*Arachis hypogaea*), common bean, cotton (*Gossypium*), rice (*Oryza sativa*), common wheat (*Triticum aestivum*), corn (*Zea mays*), tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) (Zhou and Tzanetakis 2013). However, there was no research performed to determine if soybean thrips can feed and reproduce on any of the plants that tested positive for the ability to be infected with SVNV. Additionally, there are many other crops and weed species across North America that have not been tested.

Soybean thrips have been found on many plant species across Central and North America (GBIF 2012). While there is no comprehensive or compilation study on plant
host range or geography a feeling for the diversity of plants that *N. variabilis* will feed on can be gained from mentions in various papers. *N. variabilis* was originally described on smartweed and cucumber in Iowa (Beach 1897). They are commonly found on cotton, soybean, and other legumes such as lima bean (Dupree 1970, Zhou and Tzanetakis 2013). In the Midwest, specimens have been collected from at least 16 plant families (Gerdes 1979). In Virginia, they are found in large numbers on tomato in spring and fall (Nault and Speese 2002). They were found on cotton throughout the southern and eastern United States (Akin et al. 2011). In California, large numbers of females were found on California lilac flowers (Bailey 1933).

Management of viruses in soybeans usually consists of using resistant varieties, removing alternative plant hosts that may be providing additional inoculum sources, and using clean seed (Hill and Whitham 2014). Currently there are no documented sources of resistance to SVNV in soybean. Another management strategy is to reduce the presence of alternative plant hosts for the virus and the insect vector, which has had success in management of some viral pathogens (Cock 1986, Cohen et al. 1988, Hilje et al. 2001, Wisler and Norris 2005). The two main objectives of this study were to identify specialty horticultural and cover crop species that may serve as a source of SVNV inoculum and determine if soybean thrips showed preferential feeding on these same plant species.
Materials and Methods

Plants grown under controlled environmental conditions

Seeds of eighteen commonly grown cover crop species and seven vegetable species were grown representing nine plant families were planted in 3.5-inch pots (Table 1). In this study, some vegetable species that had previously been found to be negative by mechanical inoculation (Zhou and Tzanetakis 2013) were tested using the thrips inoculation method. The remaining vegetables were chosen because they are common in Iowa gardens and used in horticultural specialty crops markets.

Plants were grown in the greenhouse for mechanical inoculations and in a growth chamber for thrips inoculations. Growth chamber conditions were maintained at photoperiods of 15 light and 9 dark hours at 26°C day and 21°C night temperatures. The light cycle was chosen to mimic Iowa summer growing conditions. Growth chambers were located on the Iowa State University campus and were lit with a combination of florescent and incandescent bulbs. Plants were inoculated for both methods on the first true leaves. Both the original inoculated leaf and also from the youngest leaves were sampled for all broadleaf species 30 days post inoculation. Grass species samples were collected 14 days post inoculation as they naturally shed the older leaves so early collection was necessary to collect the inoculated leaves before senescence.

Soybean thrips colonies and SVNV cultures

Soybean thrips were raised in the growth chamber with photoperiods of 15 light/9 dark hours at 26°C day and 21°C night temperatures. The original soybean thrips
for the colonies were field collected in fall 2014. They were maintained on live SVNV infected soybean plants. This allowed the thrips to thrive in a more natural setting and also to maintain the integrity of the virus, which showed loss of infectivity after two rounds of mechanical inoculations (data not shown). Soybean plants were periodically tested with ELISA (Agdia, Elkhart, Indiana) to confirm presence of the virus. In all ELISA tests, there was a 100% correlation of symptoms and positive assay results for SVNV. Yellow sticky cards were placed in the growth chamber to monitor for any insects such as other thrips species or insects that are capable of transmitting other diseases to soybean. Three thrips cages were maintained within the growth chamber to protect from losses in the event of another pest or pathogen being accidently introduced to the growth chamber.

**Inoculation techniques**

Both mechanical and direct thrips inoculations were used. Mechanical inoculation has a lower success rate but potentially can identify plants that may harbor the virus even if soybean thrips do not feed on it. If other vectors for SVNV are identified in the future, plants that can harbor the virus without soybean thrips would be prime candidates for testing using the newly identified vector species. Thrips inoculation often has a higher success rate but will only work for plants that soybean thrips will feed on. All inoculated plants were tested using DAS-ELISA (Agdia Inc. Elkhart, IN) following manufacturer’s instructions.
**Mechanical inoculation**

Ten plants of each of the 18 cover crop species were mechanically inoculated. Ten technical replicates were used with all 10 plants of the same species tested at the same time. Plants were kept in the dark overnight prior to mechanical inoculation. Symptomatic tissue was ground using a chilled mortar and pestle in chilled sodium phosphate buffer (0.1 M, pH 7.2) with 0.1% (vol/vol) 2-mercaptoethanol added at a 1:10 (wt/vol) ratio (Zhou and Tzanetakis 2013). The sap was hand rubbed onto the test leaves that were dusted with 600 mesh carborundum. Plants were rinsed with tap water after approximately five minutes. Inoculated leaves were marked for later collection.

**Thrips inoculation**

Five plants of each of the cover crop and vegetable species were direct thrips inoculated. Five biological replicates were done with one plant of each species for each replication. Soybean thrips larvae were placed onto SVNV infected leaves for 48 hours and then moved to clean plants until maturity. As adults, the thrips were placed onto the test plant for 72 hours. Five adult thrips were placed on each plant. Leaves were examined for signs of feeding and marked for later collection. Thrips were contained within a small clear plastic bag sealed around the leaf petiole during the acquisition period. During the inoculation period they were placed onto the test plant with only first true leaves available for feeding. A miniature greenhouse was created by covering the plant with a clear plastic bag or clear plastic cup and sealing the bottom edge to the pot with masking tape. Thrips were transferred using a small paint brush and aspirator.
Thrips feeding preference in growth chamber

A plastic nursery flat containing one 3.5” pot of each of the 18 cover crops species in this study was placed into thrips proof cages. Four repetitions of one flat were done. Each flat had a different arrangement of the plant species, ensuring that every plant was on the outside edge and inside rows for two flats each. An open vial containing five adult soybean thrips was placed into each corner of the cage surrounding the test plants, for a total of 20 adult thrips. Plants were three weeks old when exposed to thrips. After three weeks each plant was examined for the presence of adults and larvae as well as signs of feeding. Level of feeding was determined as none, low (<25% of leaves with at least one area of feeding), medium (25-50% of leaves with at least one area of feeding), or high (>50% of leaves with at least one area of feeding).

Results

ELISA positive for buckwheat, melon, and winter pea

Inoculated and new growth leaves were tested using ELISA for the presence of SVNV on all 25 species. All mechanical inoculated plant tissues were negative for SVNV. Three plant species had positive results with thrips inoculation. These were buckwheat, melon and winter pea (Table 1). Buckwheat exhibited symptoms similar to those seen on SVNV systemically infected *N. benthamiana*. The leaves first developed areas of gray ringed melting necrosis and then dark brown stem lesions appeared (Figure 1). The infection appeared to be lethal to the buckwheat plants. Stem tissue also tested positive for SVNV in buckwheat. Melon and winter pea were local infections with inoculated
tissue testing positive and systemic tissue testing negative. Melon exhibited small sunken gray lesions approximately 2-3mm in diameter. Winter pea did not exhibit any symptoms.

**Preferential feeding of soybean thrips on cover crop species**

The eighteen cover crop species were exposed to adult soybean thrips to determine if the thrips had feeding preferences among the plant species. Soybean was not included, as the interest was to identify plants that may be providing habitats for soybean thrips before and after soybean growing season. In addition to levels of feeding damage, the presence of adults and juvenile thrips was recorded (Figure 2). Alfalfa, buckwheat, crimson clover and red clover had the highest levels of feeding damage and also had adults and juveniles present. These were the only plant species with juvenile thrips on the leaves. Species with at least one plant with medium feeding damage were canola, mustard, turnip, and pearl millet. Of these adult soybean thrips were found on mustard, turnip, and pearl millet. Radish, winter pea, hairy vetch, oat, annual ryegrass, sorghum, triticale, and winter wheat had at least one plant with low feeding damage levels. Radish was the only plant species with low feeding level to have soybean thrips present with one plant having adults. False-flax and rye had no feeding damage and no soybean thrips were present.
Discussion

The examination of alternative plant host species for SVNV is a two-part equation. As a virus that is transmitted by an insect vector, it is necessary to determine both what plant species are susceptible to SVNV infection as well as what plant species are preferred by the soybean thrips for feeding. Part one is to know which plants are preferred by soybean thrips, which provides a direction to look for possible overwintering of soybean thrips. Preferred perennial plants such as alfalfa, crimson clover, and red clover may be providing overwintering or early season population growth habitats for soybean thrips prior to moving to soybean fields. The other important part of the SVNV pathosystem is identifying plant species that are able to harbor the virus. Plants that are susceptible to SVNV infection are potential sources of inoculum. It all comes together when a plant species is able to both be infected with SVNV and is preferred by soybean thrips for feeding. These plants will have the most potential for impacting seasonal epidemics of SVNV. In this study, buckwheat fulfilled these requirements by being systemically infected and highly preferred as a feeding source for both juvenile and adult soybean thrips.

Alfalfa, buckwheat, crimson clover and red clover had the highest levels of feeding damage and also had adults and juveniles present. These were the only plant species with juvenile thrips on the leaves. Alfalfa, crimson clover, and red clover are all in the Fabaceae family, the same family as soybean. However, other plants in this family such as winter pea and false-flax had very low levels of feeding and no thrips present on the plants.
Buckwheat is a quick growing short lived annual dicot. It is usually grown later in the season as the second crop in a double-cropping system since it is a short season crop, after soybean or wheat. In the United States in 2012 over 33,000 acres were planted in buckwheat producing over 750,000 bushels (USDA 2012). This was a 6% increase in yield since 2007. Japan is the main importer of buckwheat from the United States (Myers and Meinke 1994). Buckwheat is used to produce buckwheat flour, often sold in health food markets, and as a cover crop. Buckwheat is not frost hardy. Its main uses as a cover crop are quick soil coverage and improvement of soil aggregate stability (Björkman and Shail 2014). Buckwheat was the only systemically infected plant species found in this study. SVNV moved quickly in the plant with symptoms of leaf chlorosis and necrosis, dark brown stem lesions and finally plant death. Buckwheat was a favorite of soybean thrips in this study. It is also known as an excellent attractor of beneficial insects. As the popularity of buckwheat increase in the United States, soybean farmers will need to be aware of the potential for buckwheat as an alternative host for SVNV. The ability of SVNV to cause severe damage to a buckwheat crop will be equally important for buckwheat producers.

Winter pea was found to be capable of harboring a non-systemic local infection of SVNV. However, as it was one of the least preferred plants by the soybean thrips, any potential influence on the spread of SVNV appears to be very minimal.

Melon had a symptomatic local infection and medium level of thrips feeding preference. Cantaloupe melons are a popular fruit in the US with 53,000 acres planted in 2015 (USDA 2016). The majority of commercially produced melons are in California, a
state where SVNV is not a concern. Other major states with commercial production are Colorado, Georgia, Maryland, Pennsylvania, South Carolina, and Texas (USDA 2016).

Additionally, melons are grown across the country in home gardens and by small commercial growers for local markets. The widespread distribution of melon plants may play a part in spreading SVNV, however, in most states the small amount of acreage planted, most of it distant from soybean fields, does not lead to a belief that melon will have a significant impact on disease incidence in soybean. However, it is possible that the opposite may be true for melon growers, who may want to research the effects of SVNV on yield and plant health.

In summary, of the 25 plants tested in this study, only three were found to be hosts of SVNV: buckwheat, melon, and winter pea. Of these three plants, buckwheat is considered to be of potential concern to soybean farmers due to the following factors, increasing use of buckwheat as a cover crop and food crop, proximity to soybean plants, and high feeding preference by soybean thrips. The presence of SVNV in buckwheat and melon may also be a concern to growers of those respective crops. None of the crops tested showed the potential to be the primary host of SVNV and future research may want to focus on weeds commonly found in and near soybean fields such as in ditches surrounding fields. Perennial species with large geographical ranges such as common dandelion and Canada thistle, that may allow the virus to overwinter, are of particular interest.
References


Table 1. Common and scientific names for horticultural and cover crops species that were direct thrips inoculated with *Soybean vein necrosis virus*. Plant species are grouped by family in alphabetical order. ELISA assay results are shown with symptoms observations.

<table>
<thead>
<tr>
<th>Plant family</th>
<th>ELISA</th>
<th>Symptoms</th>
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<tbody>
<tr>
<td><strong>Species</strong></td>
<td>Common name</td>
<td>Infected/ inoculated</td>
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<tr>
<td><strong>Cover crops</strong></td>
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<td></td>
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<td>False-flax</td>
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<td><em>Raphanus sativus</em></td>
<td>Radish</td>
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Table 1. continued

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<td>-</td>
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<tr>
<td></td>
<td><strong>Solanum melongena</strong></td>
<td>Eggplant</td>
<td>0/5</td>
<td>-</td>
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Figure 1. Symptoms of soybean vein necrosis on soybean (Panel A) and on the leaf of buckwheat that was direct thrips inoculated (Panel B).
Figure 2. Feeding damage levels on 18 cover crop species (panel A) and the presence of adult and juvenile soybean thrips (panel B) after three weeks of exposure. Four plants of each species were tested as biological replicates. Feeding damage level was determined as none, low (<25% of leaves with at least one area of feeding), medium (25-50% of leaves with at least one area of feeding), or high (>50% of leaves with at least one area of feeding).
of feeding). Feeding levels were given the following values None=0, low=1, medium=2, high=3. So if 1 plant had none, 1 plant had low, and 2 plants had high feeding levels then $1(0)+1(1)+2(3)=7$ is the reported weighted total.
CHAPTER 4

GENERAL CONCLUSIONS

Soybean vein necrosis virus in its widespread occurrence and relative newness has attracted the interest of plant pathologists and farmers alike. Upon examination of the limited previous research and possible needs of Midwest farmers, this thesis was designed to pursue two main questions; is there any yield loss or change in seed quality associated with SVNV, and are there horticultural or cover crop species that could be serving as sources of SVNV inoculum?

Summary of yield loss and seed quality changes

Field studies were conducted in six states in 2013, 2014, and 2015 to determine the effect of SVN on soybean yield and seed quality. Quantitative parameters, including seeds per pod, pods per plant, yield, and 100-count seed weight, as well as qualitative parameters, including protein and oil concentration, were assessed from plants or seeds collected from research and commercial production fields. Results of this study did not see an impact on yield by SVN. However, seed quality was affected. In Iowa, oil concentration decreased by 0.11% as disease incidence increased by 1% ($P=0.04$). Changes in fatty acid profiles of seed were also observed; linolenic, linoleic and stearic acids decreased between 0.5 and 0.15% in 2 of 6 locations that were tested. These results suggest that infection by SVNV negatively affects soybean seed quality, which may affect the marketability of soybeans for premium markets. Further studies on the effects of SVNV infection timing on yield and seed quality are needed to fully
understand the potential economic impact of this disease. Although this study did not observe yield loss associated with SVNV infection, there were significant impacts on seed quality components, which provide a foundation for future studies within controlled environments on the impacts of SVN on seed quality.

**Summary of horticultural and cover crops susceptible to SVNV and soybean thrips feeding levels**

Of the 25 plants tested in this study only three were found to be hosts of SVNV: buckwheat, melon, and winter pea. Of these three plants, buckwheat is considered to be of potential concern to soybean farmers due to the following factors, increasing use of buckwheat as a cover crop and food crop, proximity to soybean plants, and high feeding preference by soybean thrips. The presence of SVNV in buckwheat and melon may also be a concern to growers of those respective crops. None of the crops tested showed the potential to be the primary host of SVNV and future research may want to focus on weeds commonly found in soybean fields.

Our understanding of the potential impact of SVNV is enhanced by this research and provides a foundation for further research and development of management guidelines. It appears that farmers do not need to be concerned about yield loss when symptoms begin around R6. However, heavy infestations may impact seed size and seed oil and fatty acid contents. At this time there is no indication that thrips should be controlled through pesticides to reduce incidence of SVNV. This may change if earlier infections are seen and depending on the impact of seed transmitted SVNV. Commonly used cover crop grasses should be safe for farmers to use in field as thrips do not feed
heavily on these species and they do not appear susceptible to SVNV infection. Alfalfa, crimson clover, and red clover are preferred thrips feeding species. Careful use of these species should be considered as they may contribute to higher initial thrips populations in soybean.
APPENDIX

SEASONAL PATTERNS OF ADULT *NEOHYDATOTHRIPS VARIABILIS* POPULATIONS AND IMPLICATIONS FOR MANAGEMENT IN IOWA SOYBEAN

Thrips collection data to be combined with data from University of Wisconsin-Madison for a publication. The following paper is preliminary work to be combined with the University of Wisconsin-Madison data and adjusted for specificity for soybean thrips development temperatures.

Abstract

Soybean vein necrosis virus is a widespread pathogen of soybean in North America that is transmitted by soybean thrips (*Neohydatothrips variabilis*). It is unknown if soybean thrips overwinter in the Midwest. Adult soybean thrips population numbers and geographical locations over time of this vector were collected in Iowa using yellow sticky traps. Effects of weather on adult soybean thrips at five Iowa State University research station soybean fields during May to October in 2014 and 2015 were analyzed using stepwise regression modeling. Weather variables used were cumulative degree days, minimum and maximum daily temperatures, weekly and accumulated precipitation. Degree days was the best explanatory variable in all the models and with the best results using a cubic polynomial model of degree days as the only variable and the log of mean *N. variabilis*. This model explained 51% of the data variation of adult
soybean thrips population, providing a preliminary foundation to build a predictive model for forecasting soybean thrips numbers in Iowa soybean fields.

**Introduction**

Soybean is the second most popular crop in the United States and in Iowa where over 9 million acres are planted annually. With the global economic value of soybean, approximately 282 million metric tons of soybeans were produced globally in the 2013/2014 season (McFerron 2014), the emergence of new soybean pathogens garners interest. This interest has recently turned to *Soybean vein necrosis virus*, a tospovirus transmitted by soybean thrips (*Neohydatothrips variabilis*).

Tospoviruses are rarely seen in soybean. The type species of tospoviruses, *Tomato spotted wilt virus* (TSWV), can infect over 1,000 different plant hosts making it one of the most destructive and economically important plant viruses, ranking among the top ten globally (Ogada et al. 2012).

SVNV was first confirmed in 2008 (Tzanetakis et al. 2009) and has since been found in most of the soybean growing regions of North America. Symptoms of SVN disease include chlorotic lesion associated with leaf veins that become red or brown and eventually lead to necrosis. The leaf veins may also be discolored or exhibit clearing (Bloomingdale et al. 2015).

Tospoviruses are transmitted by thrips in a persistent propagative manner (Whitfield et al. 2005). The virus is acquired during the larval stages of the insect and then transmitted to new plants as an adult. There is no vertical transmission from
parent to offspring and the virus must be acquired anew by each generation of thrips (Wijkamp et al. 1996). There are 15 thrips species that transmit the 29 known species of the Tospovirus genus (Riley et al. 2011, Tennant and Fermin 2015, Zhou and Tzanetakis 2013).

Thrips are a diverse family of insects. Most thrips species are not plant pests or of any economic concern. However some, such as Western flower thrips (*Frankliniella occidentalis*), cause billions of dollars in damage each year. Thrips cause damage through feeding as well as spreading pathogens (Jones 2005, Nault and Speese 2002).

Soybean thrips are a 1-mm long slender insect with fringed wings. They feed primarily on the leaves, causing silvering, leaf curling, and, if the populations are very high, leaf necrosis. Thrips populations in soybean are rarely managed as they usually cause limited damage, well below the economically viable threshold for application of pesticides. Soybean thrips have been found on many plant species across Central and North America (GBIF 2012). While there is no comprehensive or compilation study on plant host range or geography a feeling for the diversity of plants that *N. variabilis* will feed on can be gained from mentions in various papers. *N. variabilis* was originally described on smartweed and cucumber in Iowa (Beach 1897). They are commonly found on cotton, soybean, and other legumes such as lima bean (Dupree 1970, Zhou and Tzanetakis 2013). In the Midwest, specimens have been collected from at least 16 plant families (Gerdes 1979). In Virginia, they are found in large numbers on tomato in spring and fall (Nault and Speese 2002). They were found on cotton throughout the southern
and eastern United States (Akin et al. 2011). In California, large numbers of females were found on California lilac flowers (Bailey 1933).

Thrips can fly and jump between plants. They are also spread by the wind and some species are blown into the Midwest from the southern United States each spring. Other species are able to overwinter in warmer areas in the soil as well as in greenhouses. It is unknown where the northern boundary is for successful overwintering of soybean thrips and it is assumed that they move into the Midwest from southern states each spring (Muller 1994). This study investigated the question of when soybean thrips appear in Iowa to determine if they are overwintering in the state.

Management of viruses in soybeans usually consists of using resistant cultivars, removing alternative plant hosts that may be providing additional inoculum sources, and using clean seed (Hill and Whitham 2014). Currently there are no documented sources of plant resistance to SVNV in soybean. Without resistant cultivars, managing thrips populations is the next management strategy, although management of virus vectors in soybean has not been economically successful in the past (Hill and Whitham 2014). It can be challenging to get enough insecticide coverage as thrips spend part of their lifecycle hidden in the soil, flower buds, and stem of the plant protected from the effects of insecticides. Due to their short generation intervals they are also capable of developing resistance to insecticides relatively quickly. Thus management of thrips in order to mitigate the effects of SVNV should only be attempted if there is a threat of yield loss or damage to the seed quality.
When management of thrips in other crops was deemed necessary, weather-based forecasting models have been developed and implemented to facilitate efficient timing of pesticide applications. Thrips activity, including development, population growth, and dispersal flights are temperature dependent (Morsello et al. 2008). Dry weather is also favorable to thrips populations as precipitation events can increase juvenile mortality and delay flight events (Kirk 1997, Morsello et al. 2008). Weather-based forecasting models developed for tobacco thrips (Frankliniella fusca) and onion thrips (Thrips tabaci), vectors for tospoviruses, found that up to 63% of the variation of dispersal could be accounted for by the combination of temperature described as degree days and amount of precipitation (Groves et al. 2003, Morsello et al. 2008).

Degree days are also used in weather models to predict the chances for fruit scarring by citrus thrips and populations of tobacco and western flower thrips in cotton and peanut (Olatinwo et al. 2011, Schweizer and Morse 1997). Degree days are often used in insect forecasting models to determine the optimum time to apply insect management practices and can be calculated in several ways. The concept behind degree days is that insects require a certain amount of heat to develop during each life stage (Logan et al. 1976). Each insect species has a lower and upper temperature threshold for development. Degree days calculate the accumulation of time when the weather temperatures have been between those two thresholds since a beginning date that is set for the insects’ behavior in that geographical region.

The objective of this study was to gather data on the numbers of adult soybean thrips in Iowa soybean fields and determine if there was a pattern consistent with
soybean thrips moving into the state each spring or possibly overwintering. This data was also compared against weather data to find any relationships that might provide a preliminary basis for future weather prediction models that could be used to enhance soybean thrips and SVNV management in Iowa.

**Methods and Materials**

Thrips collection occurred at five Iowa State University research farms during the 2014 and 2015 seasons from May to September with beginning and ending dates determined by weather, planting, and harvesting times (Table 1 and 2). The five collection locations represent the northwest (NW), northeast (NE), central (CT), southwest (SW), and southeast (SE) geographical regions of Iowa (Figure 1).

Four 7.62 by 12.7 cm yellow sticky cards (Sensor brand, BASF, Florham Park, NJ) were hung vertically at each location at 27 meter intervals in soybean fields at or just above the canopy level. Both sides of the cards contained glue were used to collect insects. These cards were replaced weekly and dated when collected from the field. The cards were placed in clear plastic zip top bag or wrapped in clear plastic wrap and stored at 4°C until assessment.

All adult thrips present on the surfaces of the cards were counted. Cards were counted for total numbers of thrips and also numbers of adult *N. variabilis* under microscopic examination of at least 100X magnification. Dichotomous keys were used for identification of *N. variabilis* (Hoddle et al. 2012). Only adult soybean thrips were
counted in this study as juvenile thrips are less mobile and species differentiation is difficult during the larval and pupal stages.

Cards were grouped by week for ease of comparison (Table 5). Arithmetic means (average) of both total thrips and *N. variabilis* were calculated for each location-date. Means were used instead of the total number counted as not every location on every week had all four cards as some cards were lost or damaged in field. The use of means also allowed for balancing of samples to provide a representative quantity for statistical analysis as some reps at a given location-date had widely varying counts. Ratio was calculated as the mean of *N. variabilis* divided by the mean of total thrips and provided the proportion of thrips that were *N. variabilis* showing how population composition changed over time. The ratio of means is an indication of the changing adult soybean thrips population over the growing season.

Weather data for each location was obtained from the Iowa Environmental Mesonet system (Iowa State University) (Table 1). Each collection location was assigned a Mesonet station based on proximity. Weekly temperature (°C) and precipitation (mm) data used for analysis corresponding to sticky card collections were determined from daily data reports (Table 2). From these data, minimum and maximum weekly temperature, weekly and accumulated precipitation and degree days were calculated. Degrees days, beginning January 1, were determined as follows: Degree Days = Average daily temperature – lower base temperature – (maximum daily temperature – upper base temperature) = (max. + min.) / 2 - lower base – (max. - upper base). Degree day values were pre-calculated by the Mesonet database using a lower base of with
40°Fahrenheit (4.4°C) and 86°Fahrenheit (30°C) as the upper temperature ceiling. These temperature thresholds are for soybean growing days. There is no published data on the minimum temperature that soybean thrips will tolerate during their development. The optimum development temperature is 26.5°C and they have been shown to tolerate a maximum temperature of 32°C under lab conditions (Vance 1974). Some species of thrips have lower temperature thresholds of 10 to 11°C. The weather data, including degree days, from Mesonet were used for this preliminary work to give a general idea if there was a possible relationship that warranted further inquiry. JMP software (Version Pro 11, SAS Institute Inc., Cary, NC) was used for statistical analysis.

**Results**

Numbers of adult *N. variabilis* in Iowa over 2014 and 2015 did not increase until mid-July with southern locations reaching peak numbers earlier than northern locations. There were a few scattered *N. variabilis* in the first few weeks, perhaps overwintering in greenhouses or traveling up early. As seen in previous studies of other thrips species (Groves et al. 2003, Morsello et al. 2008), soybean thrips population increased in relation to accumulated degree days. However, there was no relationship to precipitation.

**Total thrips numbers vary through season**

In 2014, the NW location mean of total thrips counted remained relatively low and constant throughout the season (Figure 2), with the highest average count at this
location being 28.5 in week 15. The location in NE Iowa had the highest one-week average count of all locations for 2014 in week 6 at 272.5 thrips. The only location to see its highest numbers early in the season with numbers dropping off by July and remaining relatively low for the rest of the season was SW Iowa. While all locations had a somewhat bell-shaped curve increase and decrease, the timing of these fluctuations was spread out over the season. The peak occurred first in SW Iowa, then NE, followed by CT and SE in the same week and trailed by NW Iowa at the end of the season.

In 2015, there was less variation in the timing of peaks of total thrips counts, all locations peaked within 6 weeks of each other and the highest three, SW, NW, and CT were within 3 weeks (Figure 2). The location in NW Iowa peaked in week 12 at 7.8 and the location in NE Iowa remained low throughout the season peaking in week 8 at 18.3. The CT location had the highest number in weeks 9 at 90.8 and week 11 at 92.8. The SW location had the highest number of all the locations in 2015 in week 9 at 196.3. The SE location peaked latest of all the 2015 locations in week 14 with a mean of 33 total thrips.

**Soybean thrips numbers**

All locations in 2014 had more *N. variabilis* during the second half of the season (Figure 2). The NW Iowa location peaked in week 11 with 17.25 mean *N. variabilis*. NE Iowa had the highest numbers during the 2014 season, peaking in weeks 13 and 14 with 35.75 and 34.5 respectively. The CT location peaked in week 13 at 15.5 *N. variabilis*. The SW location peaked in week 10 at 25.5 and the SE location also reached its highest numbers
in week 10 with 19.75 *N. variabilis*. All locations showed a steady increase and decrease in population numbers over the season.

In 2015, the NW location reached highest numbers in week 15 at 14.25 mean *N. variabilis*. NE Iowa peaked in week 8 with 8.5 (Figure 2). The CT location had the highest mean *N. variabilis* counts of the year in Iowa on week 11 with 29.25. The SW and SE locations both had their highest numbers during weeks 9 through 11. SW Iowa had 17.75 *N. variabilis* in week 9 and 18.75 in week 10. The SE location had 17.00 in both weeks 10 and 11. Populations remained low for all locations until weeks 7 and 8. The NE, SW and SE Iowa locations all began their increase during weeks 7 and 8 and continued to increase until their peak week. The CT location jumped sharply up between weeks 9 and 11 and then decreased rapidly. The NW location was the last location to begin increasing in *N. variabilis* numbers and did not do so until week 12.

In both years, the NW location was the last location in Iowa to have population numbers start to increase. The SE and SW locations both peaked in weeks 9 through 11. The NE and CT locations both peaked later during 2014 than 2015. Both of these locations also had the most noticeable differences between the years and their peak numbers. The NE location in 2014 was at 35.75 but in 2015 only reached 8.5 *N. variabilis*. The CT location peaked in 2014 at 15.5 but in 2015 it had the highest numbers at 29.3. Soybean thrips in 2014 appeared to have a trend of slightly higher overall numbers throughout the growing season than 2015.
Proportion of total thrips to soybean thrips

In all locations in 2014 and 2015, except for SE Iowa, the ratio of N. variabilis to total thrips increased steadily through the season, where the proportion of N. variabilis in the total thrips populations increased with time (Figures 5 and 6). At the SE location again the ratio increased as the season progressed but then it decreased after week 11 in both 2014 and 2015. This is the only location with a substantial influx of other thrips species towards the end of the growing season.

Weather modeling to predict seasonal adult soybean numbers

Statistical analysis using stepwise regression modeling showed that the log of mean N. variabilis, to normalize the data, and degree days in a cubic polynomial regression was the best model with a R^2 of 0.64, and all variables significant to <0.001. The results were similar with the best model for 2015 being a quadratic polynomial regression of degree days and log of mean N. variabilis. This model resulted in a R^2 of 0.54. The model most relevant for both years together was the cubic polynomial regression model of degree days and log of mean N. variabilis. This model had an R^2 of 0.51, accounting for 51% of the variation of N. variabilis adult numbers with all variables significant with p-values of <0.001 and an ANOVA P>F of <0.001 (Figure 3). This suggests that approximately 51% of the changes in numbers of adult soybean thrips may be predicted by the increase in accumulated degree days.
Discussion

Arrival of adult soybean thrips in Iowa

Our findings suggest that *N. variabilis* may not be overwintering in Iowa and is rather moving into the state from areas with warmer overwintering temperatures. There is evidence of soybean thrips overwintering in Delaware USDA hardiness zone 7a (average annual extreme minimum temperature of 0 to 5°F) (Kleczewski unpublished). However, Delaware is in a warmer climate than Iowa's USDA zone 5A and 5B (Table 1). A temperature threshold has been described that determines when certain thrips species take dispersal flights (Morsello et al. 2008). This temperature threshold for flight was higher than the lower developmental temperature for one thrips species, *Limothrips cerealium* (Lewis 1973). However, population numbers in the warmer areas may also need to reach a population level before the thrips will take dispersal flights. Storm conditions or prevailing winds may also influence when and how far thrips travel. Soybean thrips may be arriving earlier in the season than our data suggest, along with other thrips species, but then may feed on another preferred plant other than soybean. The low incidence of SVNV during the study years make it difficult to determine if there was a correlation of thrips numbers with incidence of SVNV (Irizarry et al. unpublished). Other factors may have affected disease levels, such as the amount of available inoculum. If the soybean thrips populations are relatively constant each year and do not fluctuate in relation to the amount of SVNV incidence, then management of thrips numbers may not be as important as knowledge of potential inoculum levels to mitigate
an outbreak in a given year. Continued collection of thrips is needed to correlate with SVNV incidence and severity in order to verify this hypothesis.

**Weather modeling as a tool for soybean thrips management**

This study suggests that degree days may serve as a basis for predictive modeling for soybean thrips population growth in Iowa. If future studies indicate that soybean thrips populations should be managed in order to control SVNV, then this predictive model of population growth could be a first step in determining the optimal timing for effective insecticide applications. We found that soybean thrips arrived in Iowa soybean fields in mid-July. After soybean thrips arrive many factors may influence their population growth curve. Factors that have been shown to influence thrips growth rates include abiotic factors such as temperature, moisture, and precipitation and biological factors such as predators and competition from other insect species (Funderburk et al. 2016, Manna et al. 2012, Morsello et al. 2008, Stumpf and Kennedy 2005, 2007).

Using a single variable such as degree days simplifies the data collection needed to run the model. However, the inherent complexity of living organisms means that the addition of one or more variables would likely increase the accuracy of a model. One limitation of the analysis was the degree days data used in this study was calculated with 40 degrees Fahrenheit (4.4°C) as a minimum threshold and 86 degrees Fahrenheit (30°C) as the maximum. These levels were not based on the preferences of *N. variabilis*. If the thresholds were adjusted for *N. variabilis* then we may find that degree days would account for more of the variation in the data.
There is no published data on the minimum temperature that soybean thrips will tolerate during their development. The optimum development temperature is 26.5°C and they have been shown to tolerate a maximum temperature of 32°C under lab conditions (Vance 1974). This suggests that the minimum base temperature used in this study for calculating degree days is likely lower than the one that would be used for a N. variabilis specific degree days model. In addition, thrips prefer hot dry weather, so relative humidity or leaf wetness may increase the accuracy of a predictive model. There also may be a correlation with the soybean growth stages. For example, canopy closure stabilizes relative humidity, temperature fluctuations, and provides protection from rain and wind (Mayse 1978). This more protected environment may provide more suitable conditions for soybean thrips to reproduce. Furthermore flowering may provide additional nutrient dense food in the form of pollen resulting in increased reproduction and survival rates as is the case with other thrips species (Hulshof and Vanninen 2003).

Since SVNV is first being observed in Iowa in early August around R6 stage (Irizarry et al. unpublished) while soybean thrips numbers began increasing around R3, there was likely a latent period between the thrips population growth and assumed inoculation of plants and the appearance of disease symptoms a few weeks later. This correlated with lab observations where symptoms develop on soybean 12-14 days post inoculation. This suggests two hypotheses: i) the soybean thrips may have carried the virus with them from the southern United States which would mean inoculum levels in the South may indicate a chance for epidemic, ii) soybean thrips acquired the virus from a local inoculum source once they arrived; such as an alternative plant host or from the
seed transmitted SVNV on soybean. If the thrips were acquiring the virus from seed infected soybean then the virus would likely undergo changes within the thrips body to become the form that creates the local lesions we see on thrips inoculated soybean.

Future research should include N. variabilis sampling during years of high SVNV incidence to determine if there was a correlation of soybean thrips numbers with incidence of SVNV, sampling of soil and preferred alternative plant species in early spring to determine if N. variabilis is overwintering in Iowa, and experiments to determine the lower developmental temperature threshold for N. variabilis to adjust weather model for increased specificity. This study provided a basis for the development of a weather based forecasting model to allow for efficient management strategies of soybean thrips to be recommended to soybean farmers.

References


Queensland.


Table 1. Iowa State University research farm locations where thrips were collected on yellow sticky card during the 2014 and 2015 growing seasons. The name of the farm, closest town, latitude and longitude of the farm, geographical area of Iowa that the farm represents and USDA hardiness zone of the farm are presented. The location of the Iowa Environmental Mesonet station that was used for weather data for each farm is also recorded.

<table>
<thead>
<tr>
<th>Research farm</th>
<th>Town</th>
<th>Latitude, longitude</th>
<th>Area of state and abbreviation</th>
<th>USDA hardiness zone</th>
<th>Mesonet location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armstrong</td>
<td>Lewis</td>
<td>41.31030 5, -95.17400 1</td>
<td>Southwest (SW)</td>
<td>5A</td>
<td>Atlantic</td>
</tr>
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<td>Field Extension Education</td>
<td>Boone</td>
<td>42.00886 3, -93.79097 3</td>
<td>Central (CT)</td>
<td>5A</td>
<td>Boone</td>
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<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>Nashua</td>
<td>42.93677 0, -92.57002 2</td>
<td>Northeast (NE)</td>
<td>5A</td>
<td>Charles City</td>
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<tr>
<td>Northwest</td>
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<td>5A</td>
<td>Spencer</td>
</tr>
<tr>
<td>Southeast</td>
<td>Crawfordville</td>
<td>41.20411 9, -91.48590 1</td>
<td>Southeast (SE)</td>
<td>5B</td>
<td>Washington</td>
</tr>
</tbody>
</table>

1 Zone 5A (average annual extreme minimum temperature of -20 to -15°F); zone 5B (average annual extreme minimum temperature of -15 to -10°F).
Table 2. Week number and corresponding dates of collection periods of thrips on yellow sticky cards at five Iowa State University research farms during 2014 and 2015.

<table>
<thead>
<tr>
<th>2014</th>
<th>Dates</th>
<th>2015</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>2 June - 8 June</td>
<td>Week 1</td>
<td>31 May – 6 June</td>
</tr>
<tr>
<td>Week 2</td>
<td>9 June – 15 June</td>
<td>Week 2</td>
<td>7 June – 13 June</td>
</tr>
<tr>
<td>Week 3</td>
<td>16 June – 22 June</td>
<td>Week 3</td>
<td>14 June – 20 June</td>
</tr>
<tr>
<td>Week 4</td>
<td>23 June – 9 June</td>
<td>Week 4</td>
<td>21 June – 27 June</td>
</tr>
<tr>
<td>Week 5</td>
<td>30 June – 6 July</td>
<td>Week 5</td>
<td>28 June – 4 July</td>
</tr>
<tr>
<td>Week 6</td>
<td>7 July – 13 July</td>
<td>Week 6</td>
<td>5 July – 11 July</td>
</tr>
<tr>
<td>Week 7</td>
<td>14 July – 20 July</td>
<td>Week 7</td>
<td>12 July – 18 July</td>
</tr>
<tr>
<td>Week 8</td>
<td>21 July – 27 July</td>
<td>Week 8</td>
<td>19 July – 25 July</td>
</tr>
<tr>
<td>Week 9</td>
<td>28 July- 3 August</td>
<td>Week 9</td>
<td>26 July – 1 August</td>
</tr>
<tr>
<td>Week 10</td>
<td>4 August – 10 August</td>
<td>Week 10</td>
<td>2 August – 8 August</td>
</tr>
<tr>
<td>Week 11</td>
<td>11 August -17 August</td>
<td>Week 11</td>
<td>9 August – 15 August</td>
</tr>
<tr>
<td>Week 12</td>
<td>18 August – 24 August</td>
<td>Week 12</td>
<td>16 August – 22 August</td>
</tr>
<tr>
<td>Week 13</td>
<td>25 August – 31 August</td>
<td>Week 13</td>
<td>23 August – 29 August</td>
</tr>
<tr>
<td>Week 14</td>
<td>1 Sept. – 7 Sept.</td>
<td>Week 14</td>
<td>30 August – 5 Sept.</td>
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<tr>
<td></td>
<td></td>
<td>Week 17</td>
<td>20 Sept. – 26 Sept.</td>
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<tr>
<td></td>
<td></td>
<td>Week 18</td>
<td>27 Sept. – 3 Oct.</td>
</tr>
</tbody>
</table>
Figure 1. Locations of Iowa State University research farms where thrips were collected on yellow sticky cards in 2014 and 2015. These locations represent the five geographical areas of northwest (NW), northeast (NE), central (CT), southwest (SW), and southeast (SE) Iowa.
Figure 2. A) Average number of thrips identified on yellow sticky cards collected weekly at five research farms in between 2 June and 21 September 2014. B) Average number of *Neohydatothrips variabilis* identified on yellow sticky cards collected weekly at five research farms in between 2 June and 21 September 2014. C) Average number of thrips identified on yellow sticky cards collected weekly at five research farms in between 31 May and 10 October 2015. D) Average number of *N. variabilis* identified on yellow sticky cards collected weekly at five research farms in between 31 May and 10 October 2015.

Figure 3. Cubic polynomial regression model of degree days and weekly means of *Neohydatothrips variabilis* (log for normalization) collected on yellow sticky cards at five Iowa State University research farms during 2014 and 2015; $R^2=0.51$, $P<0.001$. 

\[ \text{N. variabilis (log)} \]

\[ \text{Degree days} \]