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Alternative Hosts for Soybean vein necrosis virus and Feeding Preferences of Its Vector Soybean Thrips

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Research

Alternative Hosts for *Soybean vein necrosis virus* and Feeding Preferences of Its Vector Soybean Thrips

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Abstract

Soybean vein necrosis virus (SVNV), a tospovirus and one of the most widespread soybean viruses in North America, is primarily transmitted by soybean thrips (*Neohydatothrips variabilis*). Although soybean is not considered the primary plant host for SVNV, there is a dearth of knowledge about alternative host plants for SVNV. We therefore investigated whether commonly present specialty and cover crops in Iowa can serve as alternative hosts for SVNV. Seventeen cover crops and seven specialty crops were tested using mechanical and thrips inoculations. Clear symptoms of SVNV and systemic infection in buckwheat and clear local infection with possible systemic infection on melon were shown. Additionally, we compared soybean thrips feeding on 18 cover crops and determined that they preferred alfalfa, buckwheat, crimson clover, and red clover. Our results suggested that alternative host crops may harbor SVNV and be a possible source of inoculum for soybean.

Soybean (*Glycine max* [L.] Merr.) is highly valued for the high levels of protein and oil in its seed and is processed for livestock feed, oil, human food, aquaculture feed, plastics, textiles, and biofuel. Globally, 351 million metric tons of soybean were produced in 2016 (USDA 2017b). The United States alone produced nearly 109 million metric tons of soybean in 2014 (USDA 2015b).

Management practices to improve soybean crop sustainability such as the use of cover crops are being adopted by some farmers. In 2015, 4% of farmers adopted cover crops on some portion of their fields (6.8 million acres), with the lowest in the Heartland (0.6% of cropland) and the highest in the Southern Seaboard (5.7% of cropland) (USDA 2015a). Reduction of soil erosion and water runoff by cover crops is particularly advantageous with corn and soybean, and benefits also include 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, as an overwintering cover with plants incorporated into the soil in spring, and as an intercropped living mulch (Sarrantonio and Gallandt 2003). The selection of cover crop species depends on climate, length of growing season, and desired benefits. However, plants other than the target crop, such as weeds or possibly cover crops, may be components in pathosystems, including insect-transmitted viruses (Duffus 1971; Nutter 1993). Certain cover crops planted in the same field may serve as reservoir hosts of plant pathogens and provide spatial accessibility of an insect vector from a susceptible cover crop to the target crop.

Soybean vein necrosis virus (SVNV) was initially identified in 2008 in Tennessee and in 2013 in Iowa, and it has been detected across the North Central region of the United States and in Ontario, Canada (Zhou and Tzanetakis 2013). The long-term implications of this disease on yield are not yet known. However, in a 3-year study across six Midwestern states, the virus was shown to decrease seed oil content (Anderson et al. 2017). The tospovirus SVNV is a persistent and propagative virus vectored primarily by soybean thrips (*Neohydatothrips variabilis* Beach) (Ullman et al. 1997; Zhou et al. 2011) and to a lesser extent eastern flower thrips (*Frankliniella tritici* Fitch) and tobacco thrips (*F. fusca* Hinds) (Keough et al. 2016). Typical symptoms of a tospovirus infection, which can vary depending on plant host and virus strain, include chlorosis, mottling, necrotic lesions, sunken spots, ring spots, stunting, and wilting (Riley et al. 2011). In soybean, SVNV causes symptoms including vein clearing, leaf chlorosis, and leaf necrosis. Based on the necrotic response and limited virus spread following thrips transmission, it was proposed that soybean and other legumes may not be the primary plant host of SVNV (Khatabi et al. 2012; Zhou and Tzanetakis 2013). This idea was supported by the observation that SVNV isolates collected from 2008 to 2012 lacked genetic diversity (Zhou and Tzanetakis 2013). Furthermore, natural tospovirus infections appear to be a rarity for leguminous hosts; the major exceptions are SVNV in soybean and *Bean necrotic mosaic virus* in common bean (de Oliveira et al. 2012).

Zhou and Tzanetakis (2013) conducted a host range study using mechanical SVNV inoculation, and they found that nine of 25 species tested could support accumulation of SVNV. The SVNV host plants represented a wide range of dicot families that include Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae. In addition to soybean, cowpea (*Vigna unguiculate* [L.] Walp.), mung bean (*Vigna radiata* [L.] R. Wilczek), *Nicotiana benthamiana* Domin, tobacco (*Nicotiana tabacum* L.), and *Nicotiana glutinosa* L. were susceptible to SVNV. Asymptomatic SVNV hosts include hardy mum (*Dendranthema grandiflorum* [Ramat.] Kitam.), summer squash (*Cucurbita pepo* L.), and ivy leaf morning glory (*Ipomoea hederacea* Jacq.) (Zhou and Tzanetakis 2013). Ivy leaf morning glory is a weed that has an overlapping geographic range with SVNV and soybean thrips, and five of eight ivy leaf morning glory plants from a soybean field tested positive for SVNV, suggesting that this weed species may have a close tie to the SVNV pathosystem (Zhou

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and Tzanetakis 2013). However, there are no reports to our knowledge demonstrating that soybean thrips can feed and reproduce on infected SVNV plant hosts and transmit the virus to soybean.

Soybean thrips were originally reported on smartweed and cucumber in Iowa (Beach 1897) and have been collected from at least 16 plant families in the Midwest (Gerdes 1979). Soybean thrips are commonly found on soybean and other legumes, such as lima bean, and on cotton (Akin et al. 2011; Dupree 1970; Zhou and Tzanetakis 2013), tomato in Virginia (Nault and Speese 2002), and lilac in California (Bailey 1933). The reports of soybean thrips and SVNV host ranges suggest the potential for thrips transmission of SVNV from alternative host plants. Understanding the potential inoculum sources is an important problem, because currently there are no documented sources of resistance to SVNV in soybean. Control of virus diseases in soybean includes planting virus-free soybean seed and removing alternative plant hosts that may provide additional sources of inoculum (Hill and Whitham 2014; Wisler and Norris 2005). The goals of this study were to identify specialty horticultural and cover crop species that may serve as a source of SVNV inoculum and to determine feeding preferences of soybean thrips for these plant species. Because mechanical inoculation techniques for SVNV have a low success rate whereas direct inoculation using thrips has a greater success rate (Khatabi et al. 2012; Zhou and Tzanetakis 2013), both methods were used.

Virus Host Range Study

We evaluated 24 plant species representing nine families including 17 cover crops and seven specialty crops. Specialty crops were selected based on popularity in Iowa vegetable gardens and horticultural specialty crops markets (Table 1) (USDA 2017a). The plants used for SVNV inoculum, rearing thrips colonies, and host range inoculations by thrips were grown in growth chambers under incandescent and fluorescent lights set to 15-h days at 26°C and 9-h nights at 21°C. Soybean thrips (*Neohydatothrips variabilis*) were collected from a field near Ames, Iowa, in the fall of 2014, and colonies were maintained on SVNV-infected soybean plants, which preserved the integrity of the virus for mechanical transmissions. Thrips transmission of our SVNV isolate was necessary, because we observed loss of SVNV infectivity after two passages by mechanical inoculation (data not shown). The presence of SVNV was periodically verified using an enzyme-linked immunosorbent assay (ELISA; Agdia, Elkhart, IN). Three thrips cages were maintained within the growth chamber to protect from losses in the event of another pest or pathogen introduction, which were detected using yellow sticky cards (BASF). Viruliferous adult soybean thrips used for host range inoculations were reared by placing larvae onto leaves of SVNV-infected soybean plants for 48 h, after which the larvae were moved onto noninfected soybean plants until maturity.

Cover and specialty crop plants that were to be mechanically inoculated with SVNV were seeded in 9-cm pots and grown in the greenhouse. Mechanical inoculations were performed on 10 plants of each of the 17 cover crop species. Plants were completely randomized on the greenhouse bench and kept in the dark 14 h prior to mechanical inoculation. SVNV symptomatic soybean leaves were ground using a chilled mortar and pestle in ice-cold sodium phosphate buffer (0.1 M, pH 7.2) containing 0.1% (vol/vol) 2-mercaptoethanol added at a 1:10 (wt/vol) ratio (Zhou and Tzanetakis 2013). The infectious sap was manually rubbed onto the first true leaves dusted with 600 mesh carborundum. Inoculated plants were rinsed with tap water after approximately 5 min.

Thrips inoculations were performed on five plants randomly arranged in the growth chamber for each cover crop and specialty crop species (Table 1). Five viruliferous adult thrips were placed onto the first true leaves of each plant using a small paint brush and aspirator. Thrips were contained within a small clear plastic bag sealed around the leaf petiole during the 72-h transmission period. The individual plastic bags were removed from the leaf petioles after the transmission period, and the leaves were examined for signs of feeding. Leaves with signs of feeding were marked for later analysis, after which each plant was covered with a clear plastic bag or a cup until sampling was performed 14 to 30 days post-inoculation (dpi).

SVNV symptoms were observed on all thrips-inoculated buckwheat and melon. Buckwheat leaves first developed areas of gray-ringed melting necrosis, and then dark-brown stem lesions appeared, resulting in total plant necrosis (Fig. 1B), similar to symptoms described for systemically infected *N. benthamiana* (Zhou and Tzanetakis 2013). Inoculated leaves on melon exhibited small sunken gray lesions 2 to 3 mm in diameter (Fig. 1C). Symptoms of SVNV infection were not observed on any other thrips-inoculated plant species nor any of the mechanically inoculated plants.

ELISA Virus Detection

To further test for the presence of SVNV in symptomatic and asymptomatic plants, ELISA assays were performed. The original inoculated first true leaf and young newly emerging leaves were sampled for broadleaf species at 30 dpi, and grass species were sampled at 14 dpi, based on preliminary studies (Khatabi et al. 2012). Both mechanically inoculated and thrips-inoculated leaves and new foliar growth were tested for the presence of SVNV in all 25 species using ELISA. Inoculated leaves and new growth tissue were tested separately to allow for assessment of local and systemic infection, respectively. The SVNV reagent set and positive control were used for ELISA according to the manufacturer's recommendations (Agdia).

Both the new growth and inoculated leaves tested positive for SVNV in all five thrips-inoculated buckwheat plants. Positive ELISA results for SVNV were obtained for inoculated leaves from three of five symptomatic thrips-inoculated melon plants, but the virus was not detected on new growth. None of the mechanically inoculated plant species, which were asymptomatic, tested positive for SVNV by ELISA.

Polymerase Chain Reaction (PCR) Virus Detection

Nested reverse-transcriptase PCR (RT-PCR) was performed on SVNV-inoculated and noninoculated buckwheat and melon to confirm positive ELISA results. SVNV-infected soybean leaves were used as positive controls. After RNA was extracted from individual plants, samples were pooled by plant species (buckwheat and melon), tissue type (inoculated leaves versus new growth), and ELISA results (positive versus negative). Approximately 100 mg of leaf tissue was flash frozen in liquid nitrogen and stored at –80°C until RNA extraction. Frozen leaf tissues were pulverized using a 1600 MiniG tissue homogenizer (SPEX SamplePrep, Metuchen, NJ), and total RNA was extracted using the Qiagen Plant RNeasy kit (Qiagen, Germantown, MD). Total RNA quality was assessed using the Nanodrop 2000 (ThermoFisher Scientific, Waltham, MA) and quantified using the Qubit 3.0 Fluorometer (ThermoFisher Scientific). Pooled samples (based on plant species, tissue type, and ELISA results) were cleaned up using the Qiagen RNeasy MinElute cleanup kit. First-strand cDNA was synthesized using the RevertAid First-Strand cDNA Synthesis Kit (ThermoFisher Scientific) following the manufacturer's instructions. The first-strand cDNA was quantified using a Qubit 3.0 Fluorometer.

PCR amplicons of the SVNV S-segment (Groves et al. 2016) and plant reference genes for each species were assessed by nested RT-PCR. Existing primer sequences were used for the buckwheat reference genes (Demidenko et al. 2011), melon (Sestili et al. 2014), and soybean (Jian et al. 2008) (Table 2). SVNV nested primers from Groves et al. (2016) were used for nested RT-PCR (Table 2). Nested RT-PCR was performed using 20 ng of cDNA as a template for detection of SVNV S-segment according to the method of Groves et al. (2016). As internal controls for each plant species, PCR was performed using species-specific reference gene primers. Nested RT-PCR was performed in 10-μL reactions for plant reference genes as follows: 20 ng of cDNA as template, 10 mM dNTP, 10 μM gene-specific forward and reverse primers, and iproof high fidelity DNA polymerase (Bio-Rad, Hercules, CA). A C1000 Touch thermal cycler (Bio-Rad) was programmed as follows: 30 s at 98°C, with 35 cycles of 5 s at 98°C, 30 s at 58°C, and 45 s at 72°C, followed by 10 min at 72°C. The resulting PCR fragments were visualized following electrophoresis in a 2% agarose gel containing SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA).

The amplicons for housekeeping genes were detected for all buckwheat, melon, and soybean samples (Fig. 2, odd numbered lanes). Because all buckwheat plants tested positive by ELISA,

TABLE 1
Plant species assessed for two separate studies[a]

				Study 1		Study 2			
				Virus host range[b]		Host preference of thrips[c]			
Use	Family	Species	Common name	Detected using ELISA	Detected using PCR[d]	Plants with adults	Plants with juveniles	Plants with feeding	Feeding damage[e]
Cover crops	Brassicaceae	*Brassica napus*	Canola	0	NA	0	0	0.5	Low
		Brassica sp.	Mustard	0	NA	0.75	0	1	Medium
		Brassica rapa	Turnip	0	NA	0.5	0	1	Medium
		Camelina sativa	False-flax	0	NA	0	0	0	None
		Raphanus sativus	Radish	0	NA	0	0	0.25	Low
	Fabaceae	*Medigo sativa*	Alfalfa	0	NA	1	1	1	High
		Pisum sativum	Winter pea	NA	NA	0	0	0.25	Low
		Trifolium incarnatum	Crimson clover	0	NA	1	0.75	1	High
		Trifolium pretense	Red clover	0	NA	1	0.75	1	High
		Vicia villosa	Hairy vetch	0	NA	0	0	0.25	Low
	Poaceae	*Avena sativa*	Oat	0	NA	0	0	0.5	Low
		Pennisetum glaucum	Pearl millet	0	NA	0.5	0	0.75	Low
		Lolium persicum	Ryegrass	0	NA	0	0	0.25	Low
		Secale cereal	Rye	0	NA	0	0	0	None
		Sorghum bicolor	Sorghum	0	NA	0	0	0.5	Low
		Triticosecale	Triticale	0	NA	0	0	0.25	Low
		Triticum	Wheat	0	NA	0	0	0.25	Low
	Polygonaceae	*Fagopyrum esculentum*	Buckwheat	5	Positive	1	1	1	High
Specialty crops	Amaranthaceae	*Amaranthus tricolor*	Amaranth	0	NA	NA	NA	NA	NA
	Asteraceae	*Helianthus annuus*	Sunflower	0	NA	NA	NA	NA	NA
	Cucurbitaceae	*Cucumis melo*	Melon	3	Positive	NA	NA	NA	NA
	Lamiaceae	*Ocimum basilicum*	Basil	0	NA	NA	NA	NA	NA
	Solanaceae	*Capsicum annuum*	Pepper	0	NA	NA	NA	NA	NA
		Solanum lycopersicum	Tomato	0	NA	NA	NA	NA	NA
		Solanum melongena	Eggplant	0	NA	NA	NA	NA	NA

[a] In study 1, a virus host range study, five plants per species were thrips-inoculated with *Soybean vein necrosis virus* (SVNV). The virus was detected using ELISA and PCR. In study 2, a host preference of the thrips vector, the mean number of plants (a highest possible mean of 1 [4 total]) observed with adult insects, juvenile insects, and presence of plant feeding damage and a qualitative feeding damage assessment is presented. NA = not assessed.

[b] Five plants each of 24 candidate virus hosts were inoculated using SVNV-infected thrips vectors. None of the mechanical inoculations developed symptoms or tested positive for SVNV using ELISA.

[c] The host preference study of thrips consisted of two flats of 18 plants each containing one cover crop plant, repeated once for a total of four observatory flats. To assess host preferences of soybean thrips, means of data from each experiment (two replicates) were averaged.

[d] Nested RT-PCR was conducted only on plant species showing positive results from ELISA.

[e] The level of feeding on each plant species was classified as follows: none = 0, low = 1 (<25% of leaves with at least one area of feeding), medium = 2 (25 to 50% of leaves with at least one area of feeding), and high = 3 (>50% of leaves with at least one area of feeding).

samples were divided by tissue type. The SVNV S-segment was not amplified from the pooled inoculated buckwheat leaf samples (Fig. 2, lane 4), but a bright 845-bp amplicon was observed from the new growth leaf samples (Fig. 2, lane 6). The inoculated melon leaves that tested positive for SVNV by ELISA also produced the expected 845-bp amplicon (Fig. 2, lane 10). The inoculated melon leaves that were symptomatic but tested negative by ELISA also produced the 845-bp amplicon, indicating that SVNV was present. Because ELISA did not detect SVNV in new growth, all new growth melon samples were pooled; a faint SVNV S-segment band was detected (Fig. 2, lane 14). Noninoculated and SVNV-inoculated soybean controls yielded the expected absence and presence of the 845-bp amplicon, respectively (Fig. 2, lanes 16 and 18).

Preferential Thrips Feeding Assay

Feeding preferences of adult soybean thrips were compared among 18 cover crop species (Table 1) to identify plants that may provide habitats for soybean thrips before and after the soybean growing season. Two replicate plastic nursery flats were placed into thrips-proof cages measuring 38 × 55 × 38 cm inside a growth chamber, as previously described for our host range study. Three-week-old plants, the age when all plants had developed true leaves, were used. In each flat, the 9-cm pots were arranged to ensure that every plant species was placed on both an outside edge and an inside row. This arrangement accounted for potential effects of edge positions on thrips feeding preference. Four open vials, each containing five adult soybean thrips, were placed at the corners of the cage, for a total of 20 adult thrips per flat. After 3 weeks, each plant was examined for the presence of adults and larvae and for signs of feeding caused by thrips, regardless of insect stage.

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

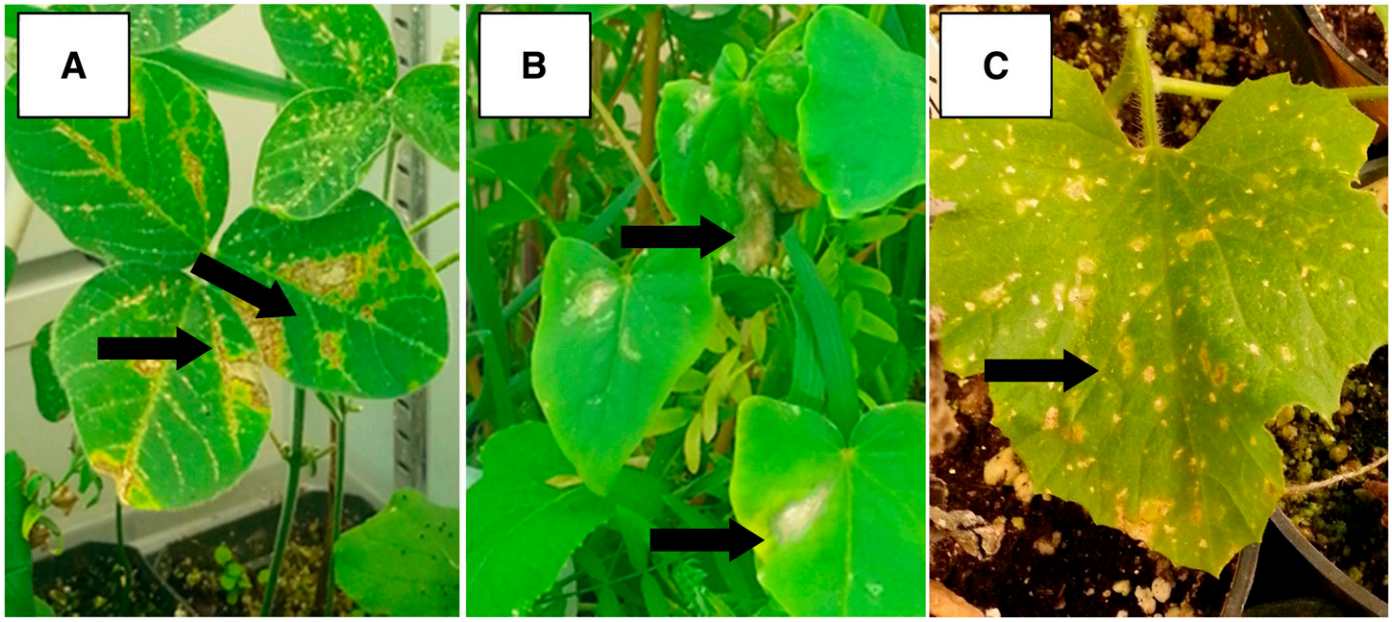

FIGURE 1

Symptoms of *Soybean vein necrosis virus* (SVNV) infection after inoculation with viruliferous thrips. Visible SVNV symptoms are indicated by black arrows on soybean (**A**), buckwheat (**B**), and melon (**C**).

TABLE 2
***Soybean vein necrosis virus* (SVNV) S-segment and housekeeping primers used for RT-PCR amplification**

Target	GenBank	Reference gene	Forward primer	Reverse primer	Amplicon length (bp)
Buckwheat[a]	AT5G46630	CACS	AAGACAGTCAGTTTCGTGCCACCT	TCCATGCGTGTTCTACCCAACTCCTT	125
Melon[b]	XM_008462685	L2	GAAGGCCAGTTTCATCTCCA	TCACCAAGCAGCATCTTGAC	300
Soybean[c]	TC224926[d]	CYP2	CGGGACCAGTGTGCTTCTTCA	CCCCTCCACTACAAAGGCTCG	154
SVNV[e]	JF808207.1	Nucleocapsid	AGATATAAAGTTGAGACACTATC	TGCAACACATCCGGAACTCTG	939
SVNV	JF808207.1	Nucleocapsid	CCTGAATTCATGCCACAAACAGCAGG	TTAGCGGCCGCTAAACAGAAAACTCC	845

[a] Demidenko et al. (2011).
[b] Sestili et al. (2014).
[c] Jian et al. (2008).
[d] Soybase accession.
[e] Khatabi et al. (2012) and Groves et al. (2016).

specks on the leaf (Huckabn and Coble 1991). The level of feeding on each plant species was classified as follows: none = 0, low = 1 (<25% of leaves with at least one area of feeding), medium = 2 (25 to 50% of leaves with at least one area of feeding), and high = 3 (>50% of leaves with at least one area of feeding). There were two independent replications of the experiment for a total of four observatory flats. To assess host preferences of soybean thrips, means of data from each experiment (two replicates) were averaged.

Mean feeding damage after 3 weeks of exposure to thrips was high for alfalfa, buckwheat, crimson clover, and red clover (Table 1), and these were also the only species on which juvenile thrips were present on the leaves. Adult soybean thrips were present on turnip and mustard, and medium feeding damage was observed. Low feeding damage was observed on 10 species, and adult soybean thrips were observed only on pearl millet (Table 1). Rye and false-flax had no feeding damage, and no soybean thrips were present.

Conclusions

Our study provides the first report of SVNV in melon and buckwheat. Of the 25 plant species tested, only buckwheat and melon were found to be hosts of SVNV. Buckwheat may be of potential concern to soybean farmers, because we showed it was a preferred host of soybean thrips and it is sometimes grown as a cover and food crop (USDA 2012), which may place it in proximity to soybean fields.

Buckwheat is a quick growing, frost-tender, and short-lived annual dicot. It can be grown later in the season as the second crop in a double-cropping system or as a cover crop during crop rotations (USDA 2012). Its main benefits as a cover crop are quick soil coverage and improvement of soil aggregate stability, and it is an excellent attractor of beneficial insects (Björkman and Shail 2014). In the United States during 2012, over 13,000 ha were planted in buckwheat, producing over 20,000 metric tons (USDA 2012). However, acreage does not appear to be on an upward trajectory, since 11,366 ha were planted in 2016 (USDA 2016). Buckwheat flour is sold in health food markets and is grown commercially for both domestic and export sale (Myers and Meinke 1994). In the United States, soybean farmers should be aware of buckwheat's potential as an alternative SVNV host. The ability of SVNV to cause severe damage to a buckwheat crop is expected to be equally important for farmers growing buckwheat.

Our results suggest that melon may be partially resistant or slow to succumb to a whole plant infection, because ELISA results and symptoms indicated a local infection and PCR detected systemic infection. Cantaloupe is a popular fruit in the United States with 53,000 acres planted in 2015 (USDA 2016). Commercially produced melons are in mainly produced 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 there is small amount of acreage planted to cantaloupe, and most of it is distant from soybean fields. Melon growers may benefit from knowledge about the effects of SVNV on melon yield and plant health.

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 the plant species that are susceptible to SVNV infection and 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. Part two is identifying plant species that may harbor the virus. Plants that are susceptible to SVNV infection are potential sources of inoculum. It all comes together when a plant species is both infected with SVNV and is preferred by soybean thrips. These plants are expected to have the greatest 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. Although our data on winter pea were inconclusive, it may harbor nonsystemic local infections of SVNV. Regardless, as the least preferred plants by the soybean thrips, any potential influence on the spread of SVNV is expected to be minimal.

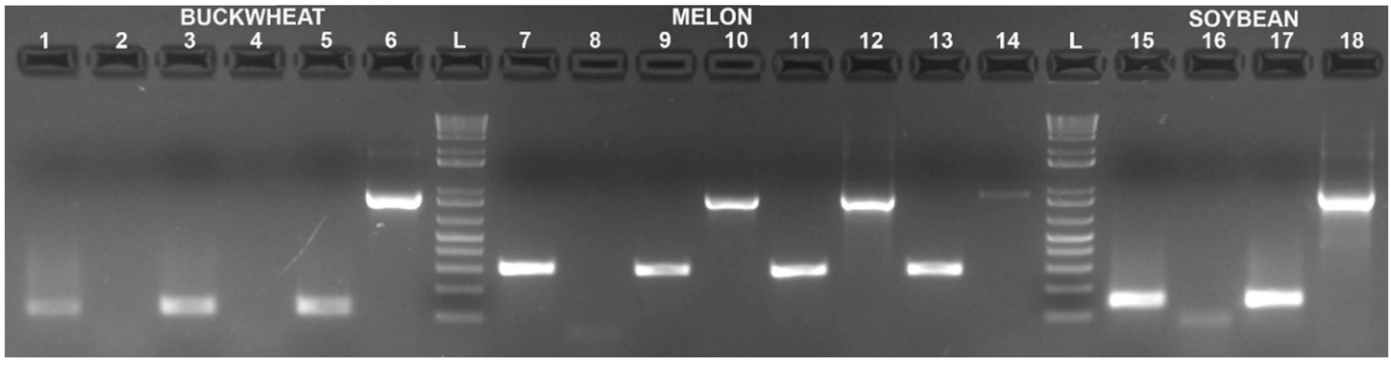

FIGURE 2

Gel electrophoresis (2% agarose) image of reverse-transcriptase PCR products for the detection of *Soybean vein necrosis virus* (SVNV) in buckwheat (lanes 1 to 6) and melon (lanes 7 to 14). Noninoculated and inoculated soybean provided negative and positive controls (lanes 15 to 18). Lanes denoted as L are 1-kb-plus DNA ladders. Odd-numbered lanes 1, 3, 5, 7, 9, 11, 13, 15, and 17 show housekeeping gene amplicons for buckwheat, melon, and soybean. Lanes 2, 8, and 16 show the SVNV S-segment amplicon products from noninoculated buckwheat, melon, and soybean, respectively. Lanes 4 and 6 show the SVNV S-segment amplicon products of buckwheat inoculated leaves and new growth, respectively. Lane 10 shows the SVNV S-segment amplicon product from inoculated melon leaves with positive ELISA results. Lanes 12 and 14 show SVNV products from inoculated leaves and new growth, respectively, with negative ELISA results.

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