Overwintering Sites of *Iris yellow spot virus* and *Thrips tabaci* (Thysanoptera: Thripidae) in Colorado

Author(s): S. Szostek and H. F. Schwartz
Source: Southwestern Entomologist, 40(2):273-290.
Published By: Society of Southwestern Entomologists
DOI: http://dx.doi.org/10.3958/059.040.0204
URL: http://www.bioone.org/doi/full/10.3958/059.040.0204

Overwintering Sites of *Iris yellow spot virus* and *Thrips tabaci* (Thysanoptera: Thripidae) in Colorado

S. Szostek and H. F. Schwartz

Department of Bioagricultural Sciences & Pest Management,
Colorado State University, Fort Collins, CO

Abstract. *Iris yellow spot virus* (family Bunyaviridae, genus *Tospovirus*) and its insect vector, onion thrips, *Thrips tabaci* Lindeman, are of economic concern worldwide in regions where onions (*Allium cepa* L.) are grown. Several weed species have been described as additional hosts and likely green bridges for survival of *Iris yellow spot virus*, however, there is little work regarding the overwintering habits and potential of onion thrips as a source of inoculum during the following season. The results of this work confirm onion thrips and *Iris yellow spot virus* presence near three Colorado onion fields throughout the winter, onion thrips reproduction on six non-allium plant species, and larval acquisition of *Iris yellow spot virus* from two non-allium plant species. Thrips were monitored by sticky traps during the winter months from 2011 to 2013. Thrips activity seemed to cease when the average temperature was cooler than 0°C and resumed once the average temperature warmed above 0°C. Onion cull piles were constructed and were apparently conducive to survival of thrips, but no live thrips were collected from the piles after onion bulbs began to decay. *Iris yellow spot virus* was detected by RT-PCR in live adult and larval thrips from onion; common mallow, *Malva neglecta* Wallr.; dandelion, *Taraxacum officinale* Weber in Wiggers; flixweed, *Descurainia sophia* (L.) Webb. Ex Prantl; prickly lettuce, *Lactuca serriola* L.; and salsify, *Tragopogon dubius* Scop. during the winters from 2010 to 2013. *Iris yellow spot virus* was detected in prickly lettuce and flixweed. The five weed species were grown from seed in a greenhouse and exposed to viruliferous thrips to elucidate their potential as green bridges. Of the five weeds, *Iris yellow spot virus* was detected in eight of 15 salsify leaf samples and in three of six thrips larval samples reared on the plant. Winter annuals play a role in overwintering survival of onion thrips and *Iris yellow spot virus*, providing inoculum the next growing season, and weed management during the winter might be warranted.

Introduction

Iris yellow spot virus (family Bunyaviridae, genus *Tospovirus* (King 2012)) is vectored by onion thrips, *Thrips tabaci* Lindeman, and both can cause significant damage to onion (*Allium cepa* L.) and other *Allium* spp. (Cortes et al. 1998). Feeding by thrips injures onion plants by reducing the photosynthetic capacity which can result in decreased yield, and also creates wounds that allow fungal, viral, and bacterial pathogens access to the plant (Cranshaw 2008, Diaz-Montano et al. 2011,

Dutta et al. 2014). Onion plants can become infected with *Iris yellow spot virus* after viruliferous onion thrips feed on the plants (Cortes et al. 1998).

Onions infected with *Iris yellow spot virus* typically have diamond-shaped straw-colored, chlorotic, or necrotic lesions on the leaves and scapes (Pozzer et al. 1999, Kritzman et al. 2001). Lesions on scapes can coalesce, causing the scape to collapse (Gent et al. 2006), which can negatively impact production of onion seed.

Iris yellow spot virus-infected onions are associated with negative economic consequences. The virus results in fewer colossal- or jumbo-sized onions (Gent et al. 2004, du Toit and Pelter 2005, Shock et al. 2008). *Iris yellow spot virus* can cause significant reduction in yield potential of onion bulbs (Kritzman et al. 2000) and even complete loss of onion bulb and seed crops (Pozzer et al. 1999). Decreased yield of onion seed has been correlated to increased insecticide use to control thrips in an attempt to prevent infection by *Iris yellow spot virus* (Long and Morandin 2011). Economic loss from increased labor might occur when leaves with lesions must be removed from bunching onions before they are deemed acceptable to market (Krauthausen et al. 2012).

Iris yellow spot virus and onion thrips can be introduced into a field by onion transplants (Schwartz et al. 2008, 2014; Hsu et al. 2011), however, it is still unclear how *Iris yellow spot virus* persists during the winter between growing seasons when onions and other host plants are not present. Cull piles have been reported as a source of *Iris yellow spot virus* and have long been considered a source of onion thrips (Horsfall and Fenton 1922). To date, seed is not known to transmit *Iris yellow spot virus* (Kritzman et al. 2001, Robene-Soustrade et al. 2006, Szostek 2014).

Several non-allium species have been reported as additional hosts of *Iris yellow spot virus* and are summarized by Smith et al. (2011). Recent studies investigating non-allium plants as suitable hosts for *Iris yellow spot virus* or onion thrips have focused on either the virus (Hsu et al. 2011) or insect (Smith et al. 2011), and have been done immediately before and after the onion-growing season. Thrips collected from flixweed and western salsify during the spring of 2006 to 2009 were transferred to healthy onion plants that became infected with *Iris yellow spot virus* following feeding by thrips (Schwartz et al. 2014). There are few studies published regarding the overwintering habits of onion thrips that include sampling fields in the winter, and those that do exist focus on crop plants. Onion thrips were collected from alfalfa (*Medicago sativa* L.), clover (*Trifolium pretense* L.), grass sod, and onion during the winter from 1945 to 1950 in southern Idaho (Shirck 1951). During the winters of 1952 and 1953, onion thrips were found on red clover, alfalfa, winter wheat (*Triticum aestivum* L.), and oat (*Avena sativa* L.) stubble in southwestern Ontario, Canada (Boyce and Miller 1953). Onion thrips were found during the winter of 1982 to 1984 on wheat, alfalfa, oat, cabbage (*Brassica oleracea* L.), barley (*Hordeum vulgare* L.), and weeds in New York (North and Shelton 1986). The studies found that onion thrips overwinter on plant material where winter temperatures often fall below freezing. No surveys seem to connect onion thrips, *Iris yellow spot virus*, and non-allium host plants during the winter.

After identifying additional host plants of *Iris yellow spot virus*, Hsu et al. (2011) wondered if thrips would complete a life cycle on plant hosts of *Iris yellow spot virus*. They pointed out that if adult onion thrips were unable to oviposit on or larvae were unable to acquire *Iris yellow spot virus* from a plant, the plant would not be a source of *Iris yellow spot virus* and would be considered a “dead-end”. Thrips were reared on six non-allium plant species in a greenhouse and *Iris yellow spot virus* status of the plants and thrips larvae developing on the plants were monitored.

The objectives were to study survival of *Thrips tabaci* and *Iris yellow spot virus* during the winter and evaluate weed species to better understand their role as potential hosts of *Iris yellow spot virus* and/or onion thrips.

Materials and Methods

Thrips activity was monitored during two winters. Onion cull piles and weeds were investigated as sources of thrips, *Iris yellow spot virus*, or both. Living thrips were located on several plant species and the *Iris yellow spot virus* status of the plants and thrips was determined. Thrips were reared on weed species in a greenhouse, and the plants and thrips larvae were evaluated for *Iris yellow spot virus*.

Cull Piles. Cull piles were created at CSU-ARDEC north of Fort Collins, CO, where a research field planted with onions had been maintained annually since 1997. Cull piles are harvested onions discarded after being deemed unsuitable for marketing or processing. The cull piles sampled from January to March 2011 were scattered throughout the onion research field, and consisted of remnants from unrelated experiments. The piles varied in size, but were approximately 0.5 m^3 or smaller. Five onions were removed from five or six piles each month and stored for 1-2 days at 4°C before they were examined. Onions were sliced into quarters and visually examined layer by layer for thrips larvae and adults. Thrips were removed with a paintbrush and preserved in 95% ethanol for later use. If green neck tissue was present, each leaf was also visually examined for the presence of thrips. Onions collected from cull piles in subsequent years were similarly stored and examined.

In March 2011, onions standing in the field from the previous growing season were collected in addition to onions from cull piles. Live thrips collected from the onions and cull piles were transferred to healthy onion plants in a greenhouse to determine if the thrips could transmit *Iris yellow spot virus*. Thrips were left on the plants in isolation for 1 month at which time one leaf was removed from each pot of plants and tested for *Iris yellow spot virus* by DAS-ELISA as modified from Gent et al. (2004). Symptomatic leaves were preferentially selected, followed by leaves with the most visible damage by thrips. Leaves were cut into 0.2-g sections and stored at -80°C until analyzed by DAS-ELISA.

In October 2011, three cull piles each approximately 1.5 m^3 were constructed in the onion research field where onions from the previous season remained until spring 2012. Temperature probes (Watchdog data logger, Spectrum Technologies, Inc, Aurora, IL) were fastened to a pole 0, 10, 20, and 30 cm above the soil at the center of each pile. Temperature was recorded hourly from October 2011 to February 2012. Nine onions were collected from each pile (three each from the top, middle, and bottom) at the beginning of each month until February when the onions were too decayed to separate and examine.

In October 2012, three cull piles were constructed the same as the piles of 2011 to 2012 and were in an empty field approximately 750 m northwest of the 2012 onion research field. Five onions were collected from the middle of each pile at the beginning of each month through February when the onions were too decayed to sample. Temperature was recorded hourly from October 2012 to May 2013.

Thrips Activity. Thrips were monitored with 23 x 28 cm yellow sticky traps stapled to stakes about 1.2 m off the ground from November 2011 to June 2012 and

October 2012 to June 2013. In 2011 to 2012, the sticky traps were initially placed near each cull pile, but later transferred to each corner of the field after the cull piles were moved with a front-end loader to the perimeter to facilitate spring field preparation and planting. In 2012 to 2013, yellow sticky traps were placed at each corner of the field. Traps were collected and replaced at approximately 2-week intervals. Thrips were counted in a laboratory and averaged over the number of traps. Traps were occasionally blown away by wind and often partially impacted and covered with soil and debris. Air temperatures (1.5 m above ground level) were recorded from nearby CoAgMet Hourly Data Access (http://www.coagmet.colostate.edu/hourlysum_form.php) site FTC03 – CSU – ARDEC.

Additional Plant Hosts. Eleven plant species typically considered weeds were collected within 175 m of onion fields from January to March during 2012 and 2013 (Table 1). Although each species is commonly found in the vicinity of onion fields, not all plants were available at each site during the collection period. Occasionally, snow prevented collection on a particular date at a site.

From January to March 2012, a wide range of green plants was collected at ARDEC. Additional plants were collected in April 2012 from the CSU Horticulture Research Farm (Hort Farm) approximately 11 km northeast of Fort Collins, and from a commercial onion field approximately 32 km north of Fort Collins in Larimer County, Colorado. Thrips and *Iris yellow spot virus* were observed at ARDEC and the Larimer County field during the previous growing season. During the 2011 growing season, slightly more than six adult thrips were found per onion plant and *Iris yellow spot virus* incidence was about 60% at ARDEC. *Iris yellow spot virus* incidence was 10% at the Larimer County field, with fewer than two adult thrips per onion plant. Few onions were planted previously at the Hort Farm, and it is not known if onions or *Iris yellow spot virus* were present during the 2011 growing season.

Table 1. Plant Species Collected in Northern Colorado and Examined for Thrips and *Iris yellow spot virus* during 2012 and 2013

Scientific name	Common name	Location[a] and year collected
Bromus inermis Leyss.	Smooth brome	ARDEC (2012)
Chorispora tenella (Pall.) DC.	Blue mustard	Larimer, Weld (2013)
Convolvulus arvensis L.	Bindweed	ARDEC, Hort Farm, Larimer (2012)
Descurainia sophia (L.) Webb. Ex Prantl	Flixweed	ARDEC (2012, 2013), Hort Farm (2012), Weld (2013), Larimer (2013)
Erodium cicutarium (L.) L'Her. Ex Ait.	Redstem filaree	ARDEC (2012)
Helianthus sp.	Sunflower	ARDEC (2012)
Lactuca serriola L.	Prickly lettuce	ARDEC (2012, 2013), Hort Farm (2012), Weld (2013)
Malva neglecta Wallr.	Common mallow	ARDEC (2012, 2013), Hort Farm (2012)
Taraxacum officinale Weber in Wiggers	Dandelion	ARDEC, Hort Farm, Larimer (2012)
Tragopogon dubius Scop.	Salsify	ARDEC (2012), Larimer (2013)
Triticum aestivum	Winter wheat	ARDEC (2012)

[a]ARDEC: CSU Agricultural Research and Development Center; Hort Farm: CSU Horticulture Research Farm; Larimer: commercial onion field in Larimer County, Colorado; Weld: commercial onion field in Weld County, Colorado.

Results from 2012 led to collection of a more focused range of plants from two commercial onion fields (one each in Larimer and Weld counties, Colorado) in addition to ARDEC during 2013. The Larimer County field was approximately 18 km north and the Weld County field was approximately 77 km southeast of Fort Collins, CO. Thrips and *Iris yellow spot virus* were observed in each field during the previous growing season (Schwartz and Szostek, unpublished). During the 2012 growing season, thrips density per onion plant reached 65.5 at ARDEC, 51.3 in the Larimer County field, and >200 in the Weld County field. *Iris yellow spot virus* incidence in each field reached 78, 87, and 20%, respectively. Thrips numbers and *Iris yellow spot virus* incidence at each location were obtained through collaborators with the Onion IPM PIPE project (http://www.alliumnet.com/IPMPipe.html).

Plants were stored in resealable plastic bags at 4°C until inspection for thrips, usually within 5 days of collection. Each plant was visually inspected for thrips with the aid of a dissecting microscope. Thrips were removed from the plants with a fine-tipped (#1, white bristle) artist's paintbrush and preserved in 95% ethanol until prepared for RT-PCR. Larvae were separated from adults. Once thrips had been removed from the plants, the leaves were rinsed and blotted dry, and 10 0.1-g samples were stored at -80°C until RT-PCR analysis.

Larval Acquisition of *Iris yellow spot virus*. To determine if thrips could complete their life cycle on the collected plants and to confirm that larvae could acquire *Iris yellow spot virus* from other potential host plants, common mallow, flixweed, dandelion, prickly lettuce, salsify, and winter wheat (cultivar 'Byrd') were grown from seed in the presence of viruliferous thrips in a greenhouse. Weed seeds were collected from ARDEC and the area around the CSU University Greenhouses, and winter wheat seed was obtained from the CSU wheat-breeding project. Seeds were started in flats and transplanted into 3.8-liter pots when the roots outgrew the individual cells. When possible, four plants were transplanted into each of five pots for a total of 20 plants per species. Approximately 10 adult thrips from symptomatic onion plants were transferred to each pot before covering to limit thrips movement between pots. The cover was a plastic cylinder that fit inside the pot. The top and four ventilation holes (5-cm diameter) were covered in a thrips-proof mesh glued to the outside of each opening. Adult thrips from symptomatic onion plants were also collected and tested as a check for *Iris yellow spot virus*. After 4 weeks, thrips larvae were removed from each plant to be tested for *Iris yellow spot virus* by RT-PCR. One leaf from each plant was removed, rinsed, and blotted dry. Sections of each leaf with damage by thrips were excised. Leaf excisions were pooled, so a single sample contained 0.1 g of plant material from two leaves. Samples were stored at -80°C until further use.

RNA Extraction and cDNA Synthesis. Total RNA was extracted using the Spectrum Plant Total RNA kit with the 750-µl binding solution option according to instructions by the manufacturer (Sigma-Aldrich, St. Louis, MO). Total RNA was measured with a NanoDrop 1000 (Thermo Scientific, Waltham, MA). Aliquots of RNA were treated with DNase I according to instructions of the manufacturer (Fermentas, Glen Burnie, MD). M-MLV reverse-transcriptase was used according to the instructions of the manufacturer to synthesize cDNA from total RNA for use with random primers (Invitrogen, Carlsbad, CA). No more than 1.0 µg total RNA was used in each 20-µl reaction. When possible, cDNA was brought to a final concentration of 10 ng/µl before use in PCR.

RT-PCR Conditions. RT-PCR for each sample was used to confirm the presence or absence of *Iris yellow spot virus*. The *Iris yellow spot virus*

nucleoprotein gene was amplified by RT-PCR with primers designed by Coutts et al. (2003), and primers specific to plant NADH dehydrogenase ND2 subunit (Thompson et al. 2003) were used as a check. Primers were designed with PrimerQuest software (IDT, Coralville, IA) against the cytochrome oxidase subunit I genes for *Thrips tabaci* (GenBank accession number AM932043) and western flower thrips, *Frankliniella occidentalis* (Pergande) (GenBank accession number AM932029) as checks for thrips. The primers for onion thrips were 5' ATAAAGAAGGAGCGGGAACGGGAT 3' (forward) and 5'ATAGCTCCCGCTAACACTGGCAAA 3' (reverse). The primers used against *F. occidentalis* were 5' TGCGGGAACGGGATGAACAGTTT 3' (forward) and 5'-CTCCTCTCGGATCTAAGAAGGATGT -3' (reverse). Primers were not developed for other, less frequently encountered thrips species.

Reverse-transcriptase PCR (RT-PCR) reactions were done on a MJ Research PTC-200 thermocycler (Waltham, MA) with an initial step of 94°C for 3 minutes; followed by 40 repeats of a three-step sequence of 94°C for 45 seconds, 55°C for 30 seconds, and 72°C for 90 seconds; and a final step of 72°C for 10 minutes. Each 20-µl reaction consisted of 20 mM Tris-HCl pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 0.15 mM dNTP mix, 1 unit Taq DNA polymerase (New England Biolabs, Ipswich, MA), 20 ng cDNA, and water.

Reaction products were run in either 1 or 1.5% agarose gels depending on the expected product sizes and stained with ethidium bromide in 1X TAE buffer (Bio-Rad Laboratories, Inc., Hercules, CA). A 100 bp molecular weight marker (Fermentas, Glen Burnie, MD) was included on each gel.

DAS-ELISA. Double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) were done according to instructions of the manufacturer (Agdia, Inc., Elkhart, IN) with the following modifications. Plant tissue was ground in liquid nitrogen as described by Gent et al. (2004). A 0.2-g sample of plant tissue was typically used with 1.0 ml of extraction buffer. All PBST rinses were four short rinses and one longer rinse that remained on the plate for 5 minutes. Positives were 2X background.

Identification of Thrips. Adult thrips were identified to species by J. Hardin, Research Associate at CSU, using the keys, *Thysanoptera: An Identification Guide* (Mound and Kibby 1998) and *Thrips of California* (Hoddle et al. 2012).

Results

Cull Piles. Live thrips were collected from the scattered cull piles during early 2011. Five adults and one larva were found in January, six adults and two larvae in February, and 24 adult thrips in March. Species were onion thrips and western flower thrips. Onion and western flower thrips were identified by RT-PCR individually or from pooled samples. Additional species might have been present in the pooled samples, but were not detected. Subsequently, the proportion of all species in the 2011 pooled samples is not known. *Iris yellow spot virus* was detected in a pool of four adult thrips (onion and western flower thrips were in the pool) collected in January 2011, in a single adult onion thrips, and a pool of three adult thrips (onion thrips was in this pool) collected in February 2011. Western flower thrips was not detected by RT-PCR in the samples collected during February 2011. The live thrips collected in March were transferred to seven healthy onion plants in the CSU greenhouse, and *Iris yellow spot virus* was subsequently detected in five of the seven onion plants 1 month after exposure to field-collected thrips.

No live thrips were recovered from the constructed cull piles during the winters of 2011 to 2012 or 2012 to 2013. Onions in the cull piles did not sprout as the onions in the smaller piles of early 2011 did, but began to decay. Although live thrips were not found in the piles, other small, live arthropods were noted.

Temperatures in the constructed cull piles at each location were similar between cull piles in 2011 to 2012 and 2012 to 2013 (Fig. 1). Temperatures in the

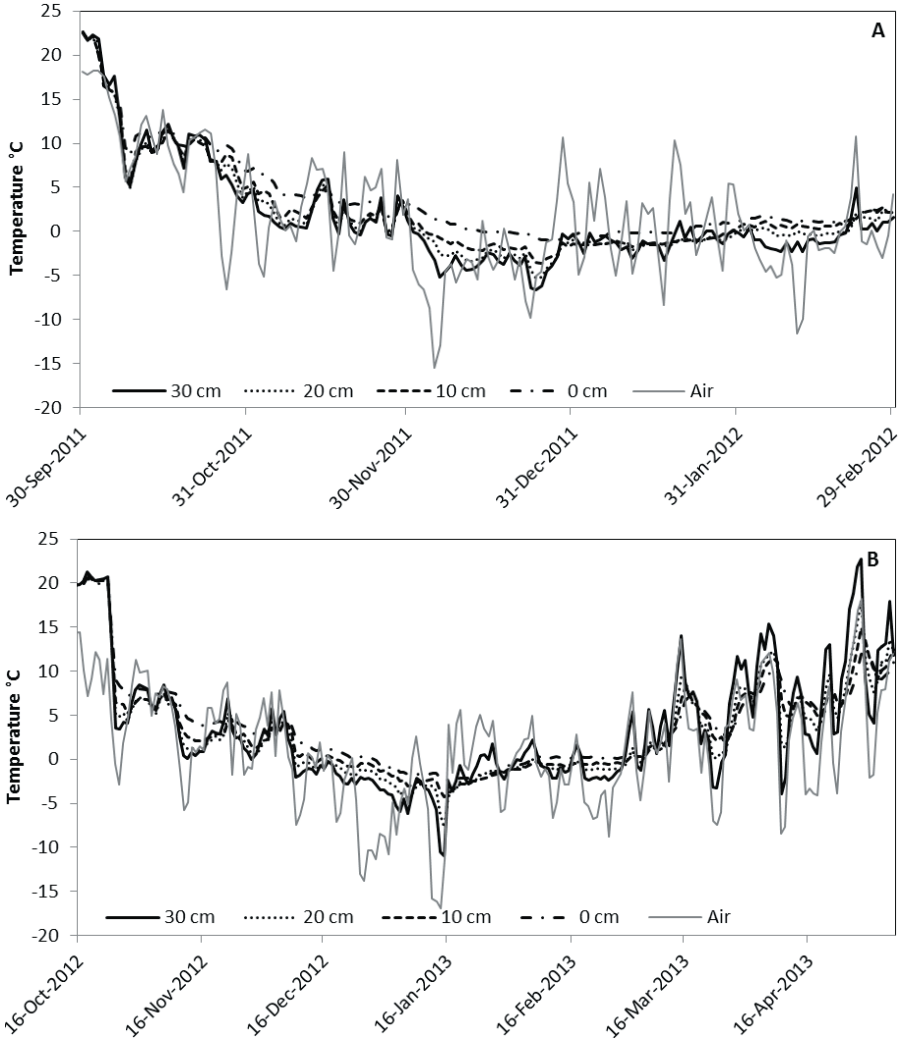

Fig. 1. Average daily temperatures of the three cull piles at each of four locations (0, 10, 20, or 30 cm above the soil) within the three cull piles compared to the average daily air temperature. A: average temperatures for 2011 to 2012; B: average temperatures for 2012 to 2013.

cull piles did not fluctuate as much as the surrounding air temperature did. The temperature probes in the center pile (2011 to 2012) and in the south pile (2012 to 2013) occasionally recorded erroneous temperatures and these temperatures were omitted. Temperatures at the bottom of each pile (0 cm) fluctuated least, while temperature fluctuation increased toward the top of each dome-shaped pile where the insulating layer of onions was not as deep. Each pile was exposed to temperatures below freezing for several weeks and the onions in the piles eventually froze.

Thrips Activity. In both years, the number of thrips caught on sticky traps decreased as air temperature decreased and increased as air temperature increased (Fig. 2). Thrips were not trapped during periods when the average temperature remained below 0°C. A single thrips was trapped in January 2012 when the average temperature was warmer than 0°C for several days. Once average temperatures warmed above 0°C, thrips began to be caught again despite daily low temperatures colder than 0°C. The average temperature from February to April 2012 was warmer and more thrips were caught than during this period in 2013. Numbers of thrips further increased after onions were transplanted into the field.

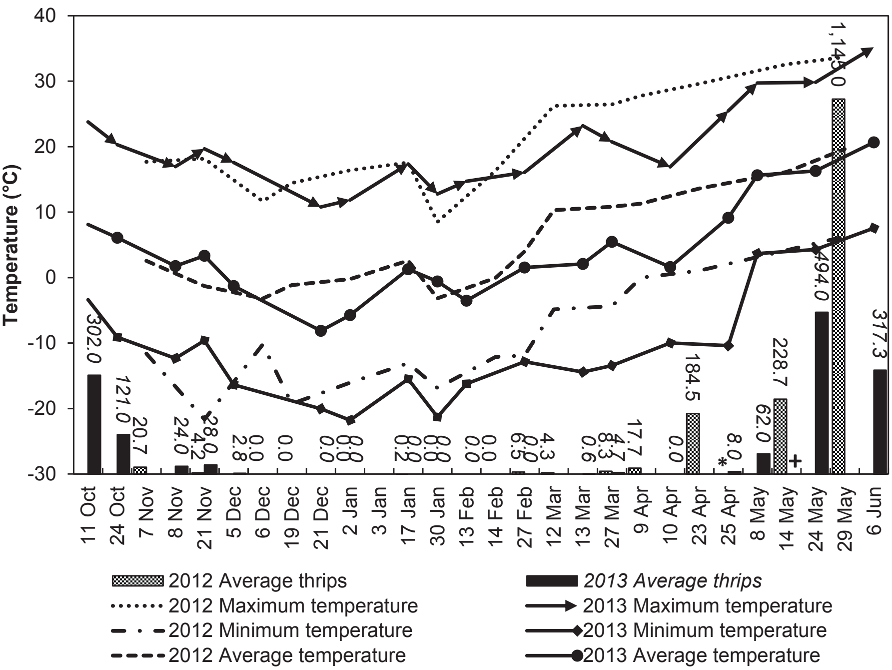

Fig. 2. Temperatures averaged over the approximately two-week intervals that sticky traps were in the field. Thrips numbers are averages of the number of traps remaining in the field at the end of each interval. The 2012 onion planting date is marked with *; the 2013 planting date with +.

Additional Plant Hosts. Live adult and larval thrips were collected from 12 plant species during two winters (Table 2). *Iris yellow spot virus* was detected in field-collected blue mustard (*Chorispora tenella* (Pall.) DC.), flixweed (*Descurainia sophia* (L.) Webb. Ex Prantl), prickly lettuce (*Lactuca serriola* L.), salsify (*Tragopogon dubius* Scop.), smooth brome (*Bromus inermis* Leyss.), sunflower (*Helianthus annuus* L.), and winter wheat. The onion plants were not tested because they are a known host of *Iris yellow spot virus*. *Iris yellow spot virus* was detected in adult thrips collected from common mallow, dandelion, flixweed, onion, prickly lettuce, and salsify. *Iris yellow spot virus* was detected in thrips larvae from onion, but not in larvae from any other plant. Thrips larvae were not found on any plant in which *Iris yellow spot virus* was detected. Adults and larvae from the same plant did not always both have *Iris yellow spot virus*; for example, *Iris yellow spot virus* was detected in adults but not larvae collected from common mallow in January 2012. Likewise, the *Iris yellow spot virus* status of the plant and thrips on the plant was not always identical; for example, *Iris yellow spot virus* was detected in blue mustard and flixweed (March 2013), but not in adult thrips from those plants. *Iris yellow spot virus* was detected in flixweed and prickly lettuce collected in March 2012 and in adult thrips from those plants. A greater variety of plant species was available later in the season than in January or February.

Table 2. *Iris yellow spot virus* Status of Plants and Thrips Collected in Northern Colorado during the Winters of 2012 and 2013

Date	Plant collected (IYSV status[a])	Location	Number of thrips[b] (IYSV status[c]) Adult	Larva	Adult thrips species (%)[g]
Jan. 2012	Common mallow (-)	ARDEC	15 (+)	2 (-)	
	Onion (n.t.)	ARDEC	23 (+)	22 (+)	
Feb. 2012	Common mallow (-)	ARDEC	6 (+)	3 (-)	*Thrips tabaci* (67%) *Frankliniella occidentalis* (33%)
	Prickly lettuce (+)	ARDEC	0	0	
	Winter wheat (+)	ARDEC	4 (-)	0	*T. tabaci* (100%)
Mar. 2012	Bindweed (-)	ARDEC	0	0	
	Dandelion (-)	ARDEC	53 (+)	1 (-)	*T. tabaci* (92%) *F. occidentalis* (5%) *F. fusca* (3%)
	Flixweed (+)	ARDEC	3 (+)	0	*T. tabaci* (100%)
	Prickly lettuce (+)	ARDEC	1 (+)	0	*T. tabaci* (100%)
	Redstem filaree[d]	ARDEC	1 (-)	100 (-)	*F. occidentalis* (100%)
	Salsify (-)	ARDEC	3 (+)	17 (-)	*T. tabaci* (100%)
	Smooth brome (+)	ARDEC	1[e]	0	
	Sunflower (+)	ARDEC	1[f]	0	*Microcephalothrips abdominalis* (100%)
	Winter wheat[b]	ARDEC	0	11 (-)	

Apr. 2012	Bindweed (-)	Hort Farm	2 (-)	1 (-)	
	Bindweed (-)	Larimer	0	6 (-)	
	Common mallow (-)	Hort Farm	0	24 (-)	
	Dandelion (-)	Hort Farm	2 (-)	33 (-)	
	Dandelion (-)	Larimer	2 (-)	17 (-)	
	Flixweed (-)	Hort Farm	2 (-)	1 (-)	
	Prickly lettuce (-)	Hort Farm	0 (-)	1 (-)	
Jan. 2013	Common mallow (-)	ARDEC	23 (-)	7 (-)	*T. tabaci* (33%) *F. occidentalis* (67%)
	Flixweed (-)	ARDEC	23 (-)	35 (-)	*T. tabaci* 67%) *F. occidentalis* (33%)
	Flixweed (-)	Weld	2 (-)	0	*T. tabaci* (100%)
	Prickly lettuce (-)	ARDEC	5 (-)	11 (-)	*T. tabaci* (33%) *F. occidentalis* (67%)
	Prickly lettuce (-)	Weld	0	0	
Feb. 2013	Blue mustard (-)	Weld	1 (-)	0	*T. tabaci* (100%)
	Flixweed (-)	Larimer	10 (-)	9 (-)	*T. tabaci* (100%)
	Flixweed (-)	Weld	0	1 (-)	
	Prickly lettuce (-)	Weld	0	0	
	Salsify (-)	Larimer	2 (+)	0	*T. tabaci* (100%)
Mar. 2013	Blue mustard (+)	Larimer	3 (-)	0	*T. tabaci* (100%)
	Blue mustard (-)	Weld	0	0	
	Common mallow (-)	ARDEC	4 (-)	2 (-)	*T. tabaci* (25%) *F. occidentalis* (75%)
	Flixweed (-)	ARDEC	13 (-)	7 (-)	*T. tabaci* (83%) *F. occidentalis* (17%)
	Flixweed (-)	Weld	0	0	
	Flixweed (+)	Larimer	36 (-)	0	*T. tabaci* (88%) *F. occidentalis* (12%)
	Prickly lettuce (-)	Weld	0	0	

n.t., not tested.

[a]Positive status if *Iris yellow spot virus* was detected in at least one of 10 0.1-g plant leaf tissue samples.

[b]Number collected from all bulked plants of the particular plant species on that collection date.

[c]*Iris yellow spot virus* status of the pooled adult or larval thrips collected from the corresponding plant.

[d]Samples would not amplify by RT-PCR.

[e]Misplaced, not tested.

[f]Slide mounted for identification, not tested.

[g]Calculated from the speciated portion of the sample.

Iris yellow spot virus was detected in thrips collected from plants in January, February, and March 2012, while in 2013, it was detected only in thrips in March. *Iris yellow spot virus* was not detected in plants or thrips collected in April 2012 from the Hort Farm or the commercial field in Larimer County, nor was *Iris yellow spot virus* detected in any plant or thrips from the Weld County field in 2013.

The primers designed to detect onion and western flower thrips confirmed the morphological identification of the species (Fig. 3). Results presented in Fig. 3 are a representative example, and the additional samples in the original image have been removed for simplicity. The thrips represented in Fig. 3 were removed from common mallow collected in January 2013 from ARDEC. Larvae were separated from adult thrips, and adult thrips were identified and separated by species. Onion and western flower thrips were the only adult thrips species in the sample. RT-PCR of thrips identified as *T. tabaci* produced an amplicon between 200 and 300 bp (predicted size 262 bp), and an amplicon of approximately 300 bp (predicted size 308 bp) was produced from thrips identified as *F. occidentalis*. Both amplicons were produced from the larval sample, indicating both species were present as larvae. *Iris yellow spot virus* was not detected in the thrips. No amplification occurred in the negative checks. The primers designed for one species did not produce amplicons when incubated with cDNA of a different species.

During two winters, only four thrips species were found on the collected plants (Table 2). Onion and western flower thrips were the most abundant species at each location. Most plants were infested with more than one species.

Larval Acquisition of *Iris yellow spot virus*. Each plant species (common mallow, dandelion, flixweed, prickly lettuce, salsify, and winter wheat) grown from seed in the greenhouse was damaged by thrips feeding, and larvae were found on each plant species. *Iris yellow spot virus* was detected by RT-PCR in adult thrips from symptomatic onion plants, thrips larvae from salsify (three of six larval samples), and thrips larvae from dandelion (one of five larval samples). *Iris yellow spot virus* was detected in eight of 15 salsify leaf samples and one of 25 prickly lettuce leaf samples from different plants. Dandelion samples did not amplify. *Iris yellow spot virus* was not detected in common mallow, flixweed, or wheat, nor in the thrips larvae from those plants.

Fig. 3. Representative agarose gel visualization of amplicons obtained by RT-PCR of RNA extracted from thrips species removed from common mallow. Lanes 1 to 3: mixed larvae; lanes 4-6: adult *F. occidentalis*; lanes 7 to 9, adult *T. tabaci*; lanes 10 to 12: -RT negative check; lanes 13 to 15: water only negative check. Lane M: 100 bp molecular weight marker; lanes 1, 4, 7, 10, 13: *Iris yellow spot virus*; lanes 2, 5, 8, 11, 14: *T. tabaci*; lanes 3, 6, 9, 12, 15: *F. occidentalis*.

Discussion

Onion thrips, the vector of *Iris yellow spot virus*, was among the thrips species found overwintering on a variety of plants at several locations (Table 2). *Iris yellow spot virus* was detected in six non-allium plant species and in thrips from five non-allium plant species. Dandelion (Hsu et al. 2011) and prickly lettuce (Sampangi et al. 2007) have been reported as hosts of *Iris yellow spot virus*, and common mallow and dandelion have been reported as plants on which onion thrips reproduce (Smith et al. 2011). Blue mustard, flixweed, and salsify were recently reported as potential hosts of *Iris yellow spot virus* (Schwartz et al. 2014) because *Iris yellow spot virus* was detected in the plant species by DAS-ELISA, but the results were not confirmed with RT-PCR. Sunflower and winter wheat have not been reported as potential hosts of *Iris yellow spot virus*.

Plants and the thrips associated with them did not always share the same *Iris yellow spot virus* status (Table 2). The discrepancy in the status suggests that onion thrips infected with *Iris yellow spot virus* survive on plants that are either not hosts or not yet infected, or onion thrips might not have acquired *Iris yellow spot virus* from infected plants during the winter, and/or the amount of plant material tested was insufficient to detect *Iris yellow spot virus*. It is difficult to find *Iris yellow spot virus* in the landscape, and *Iris yellow spot virus* might be more prevalent than is apparent. If an infected plant leaf was not sampled, the virus might have been missed. *Iris yellow spot virus* is distributed unevenly within onion plants (Nischwitz et al. 2007, Schwartz et al. 2008) and distribution within other plant species might also be uneven.

The results of this study suggest that onion thrips infected with *Iris yellow spot virus* survive the winter (from harvest one season to onions planted the next season) in low numbers on any available plants. Sticky traps seemed to indicate that thrips were absent once the average temperature cooled below freezing, however, live thrips were found on plants during the same period. Temperatures might have been too cold for flight. Thrips associated with onions survived as long as 6 weeks at 0 to 1°C on a non-preferred food source, although numbers did decrease over time (Yokoyama and Miller 2000). With access to a source of food, female western flower thrips can survive as many as 40 days at an average temperature of 0°C (Tsumuki et al. 2007). During the two winters of this study, the average temperature did not remain below freezing for more than 14 days. Thrips in the field experience temperature fluctuations that might allow a longer lifespan than under experimental conditions. Western flower thrips freeze at -26°C, but, the coldest air temperature during the study was -21.8°C and might have been warmer in the plant spaces occupied by thrips. The freezing point of onion thrips has not been reported. Details about the specific microclimate in each plant in which thrips were found are unknown, but microclimates might influence survival and development.

Although thrips larvae were found throughout the winter, it is unknown if they continually emerged or had emerged earlier when the temperature was warmer then prevented from further development by cooler temperatures. Edelson and Magaro (1988) found that development of onion thrips ceased when cooler than 11.5°C, and Horsfall and Fenton (1922) reported that onion thrips required longer to progress from one life stage to the next at cooler temperatures (20 compared to 29°C). The observation of increased adult thrips activity when the average

temperature warmed above 0°C and the noticeable increase of adult thrips caught on sticky traps once the average temperature warmed above 11.5°C (Fig. 2) suggest adult thrips already present might become active at a cooler temperature than development occurs. Insects capable of overwintering adapt to local conditions and acclimate themselves to the changing seasons (McDonald et al. 2000). It is possible that thrips in Colorado have adapted to develop at colder temperatures than thrips in Texas where the 11.5°C developmental threshold was observed.

Iris yellow spot virus was present in adult onion thrips collected during the winter, but it is unknown in what season or at which life stage the thrips acquired the virus. It is generally accepted that onion thrips must acquire *Iris yellow spot virus* as a larva to transmit the virus as an adult (Gent et al. 2006). It is reasonable to assume that at least some overwintering adult thrips acquired *Iris yellow spot virus* as larvae because healthy onion plants in a greenhouse became infected with *Iris yellow spot virus* after exposure to thrips collected in March 2011 from green onion leaf tissue, suggesting the adult thrips were viruliferous. The possibility that onion thrips already in the greenhouse were responsible for transmitting *Iris yellow spot virus* to the healthy onion plants, despite insect-proofing the pots, must also be considered. *Iris yellow spot virus* was detected in thrips larvae from onions in January 2012, suggesting the larvae acquired *Iris yellow spot virus* when days were warm before overwintering, or that they acquired *Iris yellow spot virus* during winter. Thrips larvae were not on any field-collected non-allium plants in which *Iris yellow spot virus* was detected, and it is not known if the adult thrips collected acquired *Iris yellow spot virus* as larvae or as adults. There seem to be no studies on the effect of temperature on onion thrips acquisition of *Iris yellow spot virus* from plants, or of any other plant-tospovirus-thrips complex. Tsumuki et al. (2007) found that adult western flower thrips walked less after exposure to -5°C, which prevented them from reaching their food source. The larvae collected in this study were already in contact with their food source, but it is possible that the movements required for feeding could become inhibited by cold temperatures. It would be interesting to learn whether temperature affects the ability of a plant to host or of thrips to acquire *Iris yellow spot virus*.

Larvae were found on each of the plant species grown in a greenhouse indicating onion thrips can reproduce on the plant species. Not all of the plants or larvae associated with them had *Iris yellow spot virus*. The larval acquisition experiments in the greenhouse showed that thrips larvae could acquire *Iris yellow spot virus* from dandelion and salsify. It is unlikely that thrips larvae moved from *Iris yellow spot virus*-infected onions in the greenhouse onto the weeds grown in isolation chambers because, onion thrips larvae are not capable of flight and do not travel far from the plant on which they emerge (Hsu et al. 2011).

This work did not attempt to determine competency of any of the weed species to host *Iris yellow spot virus*, or the efficiency that thrips could acquire *Iris yellow spot virus* from any of the potential host plants. However, it would appear that the acquisition efficiency of *Iris yellow spot virus* from plant to insect might vary by plant species because *Iris yellow spot virus* was detected in half of the larval samples from salsify, but in only 1 of 5 larval samples from dandelion. While there is information regarding transmission of *Iris yellow spot virus* from onion thrips to plants (Kritzman et al. 2001, Inoue et al. 2010, Nischwitz et al. 2012), information is lacking on how efficiently onion thrips acquire *Iris yellow spot virus* from different plant species. Some plant species become systemically infected with *Iris yellow*

spot virus, while others develop only local lesions (Bag and Pappu 2009). This difference in virus distribution throughout a plant might affect acquisition by thrips, and it is possible that *Iris yellow spot virus*-infected plants in this study were only locally infected, and the thrips larvae had not fed in a leaf area with *Iris yellow spot virus*. The possibility of variable acquisition of *Iris yellow spot virus* by onion thrips is supported by similar work on other plant-thrips-tospovirus complexes (Chatzivassiliou et al. 2007, Okuda et al. 2010).

The plants collected in this study seemed to support *Iris yellow spot virus* overwintering in two ways: as a direct host of *Iris yellow spot virus* (dandelion, flixweed, prickly lettuce, salsify) or as a host for *Iris yellow spot virus*-infected onion thrips (common mallow). As a winter annual and/or biennial plant (Whitson 2004), common mallow seems to be an indirect green bridge supporting *Iris yellow spot virus*-infected thrips. *Iris yellow spot virus*-infected onion thrips that overwinter on common mallow could move to *Iris yellow spot virus*-susceptible plants once they are available.

Thrips larvae from dandelions in the greenhouse had *Iris yellow spot virus*, but because RT-PCR of the dandelion samples did not produce any amplicons, the *Iris yellow spot virus* status of the plants could not be confirmed. The plants were assumed to have *Iris yellow spot virus* because the larvae acquired it. The perennial nature of dandelion (Whitson 2004) makes it a good green bridge. As a biennial or winter annual (Whitson 2004), prickly lettuce is another good green bridge. Results presented here confirm earlier reports of prickly lettuce as a host of *Iris yellow spot virus* (Sampangi et al. 2007). While prickly lettuce can host *Iris yellow spot virus* and thrips, it is not known whether thrips can acquire *Iris yellow spot virus* from prickly lettuce because the virus was not detected in any of the larvae from prickly lettuce.

As a winter annual (Whitson 2004), flixweed would need to be inoculated with *Iris yellow spot virus* after germination in autumn. Cold temperatures inhibiting thrips movement might prevent or reduce inoculation of flixweed and is a reasonable explanation of why *Iris yellow spot virus* was not detected in more flixweed samples from the field. However, this does not explain the lack of *Iris yellow spot virus* in greenhouse-grown flixweed. Many larvae were observed on the greenhouse-grown flixweed, and were on flixweed from the field in contrast with the findings of Smith et al. (2011) who did not find thrips larvae on flixweed. Flixweed might support thrips through the winter or increase the number of thrips early the next growing season.

Iris yellow spot virus was detected in more greenhouse-grown salsify (eight of 15 samples) and in larvae associated with them (three of six larvae) than in other plant/thrips combinations. These results and those of Schwartz et al. (2014) suggest that salsify could be a more important overwintering host of *Iris yellow spot virus* than the other non-allium plants in this study, and that salsify plays a role in the persistence of *Iris yellow spot virus* in the landscape. As a biennial (Whitson 2004), salsify could become infected with *Iris yellow spot virus* during its first year and remain infective during its second year, although it is not known if this occurs.

Iris yellow spot virus was detected in only one sample of field-collected winter wheat but not in any greenhouse-grown wheat nor in the larvae from them. The cultivar of winter wheat in the field was not known, so varietal differences are possible. Additional cultivars of winter wheat should be studied to determine if they host *Iris yellow spot virus*. Because onion thrips overwinter on wheat (Boyce and Miller 1953, North and Shelton 1986) it may serve as a green bridge for the vector,

if not the virus; this could be problematic if overwintering thrips were already infected. The sunflower in which *Iris yellow spot virus* was detected was a newly sprouted volunteer of a domesticated type and not present until mid-March. While native sunflowers are common in Colorado, they are not green during the winter and are unlikely to be an important overwintering site of *Iris yellow spot virus* or onion thrips; therefore, sunflower was not further studied as a host of *Iris yellow spot virus*.

Most thrips positive for *Iris yellow spot virus* were adults collected at ARDEC in 2012 when onions were still in the field. It is unknown when thrips arrived at the non-allium plants. Thrips from non-allium plants at ARDEC could have been migrating from the onions; however, few adult thrips were caught in the sticky traps between January and March 2012 and 2013. Onion thrips prefer onions (Doederlein and Sites 1993) but feed on other plants when necessary. It seems unlikely that onion thrips would leave a preferred plant in favor of a less preferred plant. In contrast, onions from the previous growing season had been removed from the Larimer County field in 2013, yet *Iris yellow spot virus* was in the thrips on salsify at that site. This suggests *Iris yellow spot virus* can persist in the absence of previously infected onions (e.g., volunteer or cull).

Onion cull piles would seem to be a logical place for *Iris yellow spot virus* and onion thrips to overwinter because the preferred food source is readily available. However, sampling onion cull piles in Ontario, Canada during winter (Boyce and Miller 1953) did not yield any onion thrips, and in this work live thrips were not found in the deliberately constructed cull piles, but were found in the field cull piles left from the 2010 growing season. The most reasonable explanation for this is that many onions in the early 2011 cull piles had started to sprout and green leaf tissue was where thrips were most often found. The onions in the constructed cull piles began to decay rather than sprout, and there was no green tissue. This (with the results of the non-allium host survey) suggested that overwinter survival of onion thrips might be linked to the availability of green plant tissue. Thrips are known to feed on the contents of epidermal, palisade, and spongy mesophyll cells, and to develop faster on new, young leaves than on bulbs (Lewis 1997). Frozen onion bulbs might not be an optimal source of nutrition or site for development. However, onion cull piles *may* be a source of *Iris yellow spot virus* if the onions within them sprout, thus providing onion thrips with food and shelter.

This work suggests several areas for further research. It would be useful to know how far from an onion field *Iris yellow spot virus*-infected plants and thrips persist, to better target weed control and crop rotation recommendations. Boyce and Miller (1953) found onion thrips were plentiful in an alfalfa field 45 but not 400 m from an onion field. It would be interesting to know if *Iris yellow spot virus* incidence decreased with increasing distance from an onion field. Likewise, a formal study examining effectiveness of control strategies would be useful to onion growers. As discussed previously, winter wheat should be further evaluated as a potential source of *Iris yellow spot virus*, especially because it is often grown adjacent to or in rotation with onions in Colorado and elsewhere. It would be interesting to know if thrips oviposited in any of the overwintering plants, and if there is any relationship between ovipositing and *Iris yellow spot virus* status of the plant. This information could be useful to prioritize which weeds should be controlled.

The results of this study suggested some strategies that might reduce the incidence of *Iris yellow spot virus* and/or thrips. To prevent overwintering by thrips and *Iris yellow spot virus* onions should not be left in the field between growing

seasons, because they can support *Iris yellow spot virus*-infected adult and larval onion thrips. Onion culls should be destroyed to prevent volunteers in cull piles. Green weeds, particularly salsify, should be removed from the vicinity of onion fields. Horsfall and Fenton (1922) recommended burning weedy areas along roads, creeks, and windbreaks to eliminate hibernating thrips. However, eradicating all weeds or plants from the vicinity of an onion field might not be practical and might conflict with other IPM goals or practices such as maintaining refugia for beneficial organisms or preventing soil erosion.

Acknowledgment

We wish to thank and acknowledge those assisting in the research of this project: Janet Hardin, Whitney Cranshaw, Mark McMillan, Kris Otto, Matt Miller, and Robin Ward. Supporting funds for this study were received from the Colorado Onion Association, Colorado State University Agricultural Experiment Station, and USDA-NIFA-SCRI Project 2010-01193.

References Cited

Bag, S., and H. R. Pappu. 2009. Symptomatology of *Iris yellow spot virus* in selected indicator hosts. Plant Health Progress, Plant Management Network. doi:10.1094/PHP-2009-0824-01-BR.

Boyce, K. E., and L. A. Miller. 1953. Overwintering habits of the onion thrips, *Thrips tabaci* Lind. (Thysanoptera: Thripidae), in southwestern Ontario. Annual Report of the Entomological Society of Ontario: 82-86.

Chatzivassiliou, E. K., D. Peters, and N. I. Katis. 2007. The role of weeds in the spread of *Tomato spotted wilt virus* by *Thrips tabaci* (Thysanoptera : Thripidae) in tobacco crops. J. Phytopathol. 155: 699-705.

Cortes, I., I. C. Livieratos, A. Derks, D. Peters, and R. Kormelink. 1998. Molecular and serological characterization of *Iris yellow spot virus*, a new and distinct tospovirus species. Phytopathol. 88: 1276-1282.

Coutts, B. A., L. A. McMichael, L. Tesoriero, B. C. Rodoni, C. R. Wilson, A. J. Wilson, D. M. Persley, and R. A. C. Jones. 2003. *Iris yellow spot virus* found infecting onions in three Australian states. Australasian Plant Pathol. 32: 555-557.

Cranshaw, W. S. 2008. Thrips, pp. 89-91. *In* H. F. Schwartz and S. K. Mohan [eds], Compendium of Onion and Garlic Diseases and Pests, 2nd ed. APS Press, St. Paul, MN.

Diaz-Montano, J., M. Fuchs, B. A. Nault, J. Fail, and A. M. Shelton. 2011. Onion thrips (Thysanoptera: Thripidae): a global pest of increasing concern in onion. J. Econ. Entomol. 104: 1-13.

Doederlein, T. A., and R. W. Sites. 1993. Host-plant preferences of *Frankliniella-occidentalis* and *Thrips-tabaci* (Thysanoptera, Thripidae) for onions and associated weeds on the southern High-Plains. J. Econ. Entomol. 86: 1706-1713.

du Toit, L. J., and G. Q. Pelter. 2005. Susceptibility of storage onion cultivars to Iris yellow spot in the Columbia Basin of Washington, 2004. Biological & Cultural Tests 20 (V006).

Dutta, B., A. K. Barman, R. Srinivasan, U. Avci, D. E. Ullman, D. B. Langston, and R. D. Gitaitis. 2014. Transmission of *Pantoea ananatis* and *P. agglomerans*, causal agents of center rot of onion (*Allium cepa*), by onion thrips (*Thrips tabaci*) through feces. Phytopathol. 104: 812-819.

Edelson, J. V., and J. J. Magaro. 1988. Development of onion thrips, *Thrips-tabaci* (Thysanoptera, Thripidae) Lindeman, as a function of temperature. Southwest. Entomol. 13: 171-176.

Gent, D. H., H. F. Schwartz, and R. Khosla. 2004. Distribution and incidence of *Iris yellow spot virus* in Colorado and its relation to onion plant population and yield. Plant Dis. 88: 446-452.

Gent, D. H., L. J. du Toit, S. F. Fichtner, S. K. Mohan, H. R. Pappu, and H. F. Schwartz. 2006. *Iris yellow spot virus*: an emerging threat to onion bulb and seed production. Plant Dis. 90: 1468-1480.

Hoddle, M. S., L. A. Mound, and D. L. Paris. 2012. Thrips of California. CBIT Publishing, Queensland.

Horsfall, J. L., and F. A. Fenton. 1922. Onion thrips in Iowa. Bulletin Iowa Agricultural Experiment Station 205: 56-68. Agricultural Experiment Station, Iowa State College of Agriculture and the Mechanic Arts, Ames, IA.

Hsu, C. L., C. A. Hoepting, M. Fuchs, E. A. Smith, and B. A. Nault. 2011. Sources of *Iris yellow spot virus* in New York. Plant Dis. 95: 735-743.

Inoue, T., T. Murai, and T. Natsuaki. 2010. An effective system for detecting *Iris yellow spot virus* transmission by *Thrips tabaci.* Plant Pathol. 59: 422-428.

King, A. M. 2012. Virus taxonomy: classification and nomenclature of viruses. Ninth Report Inter. Comm. on Taxonomy of Viruses. Academic Press, London.

Krauthausen, H. J., G. M. E. Leinhos, J. Muller, P. C. Radtke, and J. A. Jehle. 2012. Identification and incidence of *Iris yellow spot virus* in Allium field crops in Southwest Germany. Europ. J. Plant Pathol. 134: 345-356.

Kritzman, A., H. Beckelman, S. Alexandrov, J. Cohen, M. Lampel, M. Zeidan, B. Raccah, and A. Gera. 2000. Lisianthus leaf necrosis: a new disease of lisianthus caused by *Iris yellow spot virus*. Plant Dis. 84: 1185-1189.

Kritzman, A., M. Lampel, B. Raccah, and A. Gera. 2001. Distribution and transmission of *Iris yellow spot virus*. Plant Dis. 85: 838-842.

Lewis, T. 1997. Thrips as Crop Pests. CAB International, Wallingford, UK.

Long, R. F., and L. Morandin. 2011. Low hybrid onion seed yields relate to honey bee visits and insecticide use. Calif. Agric. 65: 155-158.

McDonald, J. R., J. Head, J. S. Bale, and K. F. A. Walters. 2000. Cold tolerance, overwintering and establishment potential of *Thrips palmi*. Physiol. Entomol. 25: 159-166.

Mound, L. A., and G. Kibby. 1998. Thysanoptera: An Identification Guide. CAB International, Wallingford, UK.

Nischwitz, C., S. Mullis, R. Torrance, D. Langston, A. Sparks, and R. Gitaitis. 2007. Distribution of *Iris yellow spot virus* in onion leaves. Phytopathol. 97: S182.

Nischwitz, C., R. Srinivasan, S. Sundaraj, S. W. Mullis, B. McInnes, and R. D. Gitaitis. 2012. Geographical distribution and survival of *Iris yellow spot virus* in spiny sowthistle, *Sonchus asper*, in Georgia. Plant Dis. 96: 1165-1171.

North, R. C., and A. M. Shelton. 1986. Overwintering of the onion thrips, *Thrips-tabaci* (Thysanoptera, Thripidae), in New York. Environ. Entomol. 15: 695-699.

Okuda, M., S. Fuji, S. Okuda, K. Sako, and T. Iwanami. 2010. Evaluation of the potential of thirty two weed species as infection sources of *Impatiens necrotic spot virus*. J. Plant Pathol. 92: 357-361.

Pozzer, L., I. C. Bezerra, R. Kormelink, M. Prins, D. Peters, R. D. Resende, and A. C. de Avila. 1999. Characterization of a tospovirus isolate of *Iris yellow spot virus* associated with a disease in onion fields in Brazil. Plant Dis. 83: 345-350.

Robene-Soustrade, I., B. Hostachy, M. Roux-Cuvelier, J. Minatchy, M. Hedont, R. Pallas, A. Couteau, N. Cassam, and G. Wuster. 2006. First report of *Iris yellow spot virus* in onion bulb- and seed-production fields in Reunion Island. Plant Pathol. 55: 288.

Sampangi, R. K., S. K. Mohan, and H. R. Pappu. 2007. Identification of new alternative weed hosts for *Iris yellow spot virus* in the Pacific Northwest. Plant Dis. 91: 1683.

Schwartz, H. F., K. Otto, S. Szostek, C. Boateng, W. S. Cranshaw, M. A. Camper, and L. Mahaffey. 2008. Thrips and IYSV sources in Colorado onion production systems. National Allium Res. Conf., Savannah, GA.

Schwartz, H. F., D. H. Gent, S. M. Fichtner, K. Otto, C. O. Boateng, S. Szostek, W. S. Cranshaw, and L. A. Mahaffey. 2014. *Thrips tabaci* (Thysanoptera: Thripidae) and *Iris yellow spot virus* associated with onion transplants, onion volunteers, and weeds in Colorado. Southwest. Entomol. 39: 691-704.

Shirck, F. H. 1951. Hibernation on onion thrips in southern Idaho. J. Econ. Entomol. 44: 1020-1021.

Shock, C. C., E. Feibert, L. Jensen, S. K. Mohan, and L. D. Saunders. 2008. Onion variety response to *Iris yellow spot virus*. Hort. Tech. 18: 539-544.

Smith, E. A., A. Ditommaso, M. Fuchs, A. M. Shelton, and B. A. Nault. 2011. Weed hosts for onion thrips (Thysanoptera: Thripidae) and their potential role in the epidemiology of *Iris yellow spot virus* in an onion ecosystem. Environ. Entomol. 40: 194-203.

Szostek, S. A. 2014. Biology and over-winter survival of *Iris yellow spot virus* in Colorado. Doctoral dissertation, Colorado State University, Fort Collins, CO.

Thompson, J. R., S. Wetzel, M. M. Klerks, D. Vaskova, C. D. Schoen, J. Spak, and W. Jelkmann. 2003. Multiplex RT-PCR detection of four aphid-borne strawberry viruses in *Fragaria* spp. in combination with a plant mRNA specific internal control. J. Virol. Methods 111: 85-93.

Tsumuki, H., H. Ishida, H. Yoshida, S. Sonoda, Y. Izumi, and T. Murai. 2007. Cold hardiness of adult western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae). Appl. Entomol. Zool. 42: 223-229.

Whitson, T. D. [ed.] 2004. Weeds of the West. Newark, CA, Western Society of Weed Science in cooperation with the Western United States Land Grant Universities Coop. Ext. Serv.

Yokoyama, V. Y., and G. T. Miller. 2000. Response of omnivorous leafroller (Lepidoptera: Tortricidae) and onion thrips (Thysanoptera: Thripidae) to low-temperature storage. J. Econ. Entomol. 93: 1031-1034.