

Seasonal Dynamics of Thrips (*Thrips tabaci*) (Thysanoptera: Thripidae) Transmitters of Iris Yellow Spot Virus: A Serious Viral Pathogen of Onion Bulb and Seed Crops

SUDEEP BAG,^{1,2} SILVIA I. RONDON,³ KERI L. DRUFFEL,¹ DAVID G. RILEY,⁴
AND HANU R. PAPPU^{1,5}

J. Econ. Entomol. 107(1): 75–82 (2014); DOI: <http://dx.doi.org/10.1603/EC13141>

ABSTRACT Thrips-transmitted Iris yellow spot virus (IYSV) is an important economic constraint to the production of bulb and seed onion crops in the United States and many other parts of the world. Because the virus is exclusively spread by thrips, the ability to rapidly detect the virus in thrips vectors would facilitate studies on the role of thrips in virus epidemiology, and thus formulation of better vector management strategies. Using a polyclonal antiserum produced against the recombinant, *Escherichia coli*-expressed nonstructural protein coded by the small (S) RNA of IYSV, an enzyme linked immunosorbent assay was developed for detecting IYSV in individual as well as groups of adult thrips. The approach enabled estimating the proportion of potential thrips transmitters in a large number of field-collected thrips collected from field-grown onion plants. Availability of a practical and inexpensive test to identify viruliferous thrips would be useful in epidemiological studies to better understand the role of thrips vectors in outbreaks of this economically important virus of onion.

KEY WORDS *Thrips tabaci*, onion thrips, polyclonal antiserum, virus detection, overwintering

Thrips (Thysanoptera: Thripidae) are cosmopolitan insects and act both as a pest and virus vector thus causing serious economic damage to numerous horticultural and field crops in many parts of the world (Montano et al. 2011). For example, onion thrips (*Thrips tabaci* L.) can cause yield losses of >50% in onion (Fournier et al. 1995). It is considered an indirect pest of dry bulb onion as it feeds on leaves rather than bulb. Feeding injury reduces the photosynthetic ability of the host plant (Molenaar 1984; Parrella and Lewis 1997) by destroying the chlorophyll-rich leaf mesophyll (Molenaar 1984), which interferes with nutrient transportation to bulb (Parrella and Lewis 1997). In addition to being a serious pest, it also transmits the Iris yellow spot virus (IYSV) (Bunyaviridae: Tospovirus), a serious viral pathogen of onion (Gent et al. 2006, Pappu et al. 2009). Increasing incidence of IYSV has been reported in the United States and other parts of the world in recent years (Bulajic et al. 2008, Huchette et al. 2008, Pappu and Matheron, 2008, Ward et al. 2008, Pappu et al. 2009, Bag et al. 2009a, Sether et al. 2010, Mandal et al. 2012). IYSV is predominantly

transmitted by onion thrips, *T. tabaci* (Nagata et al. 1999, Kritzman et al. 2001). Recently, tobacco thrips, *Frankliniella fusca* (Hinds), was shown to transmit IYSV albeit with much lower efficiency as compared with onion thrips (Srinivasan et al. 2012). The virus is not transmitted through the egg and there is no evidence of virus transmission through seed. Hence, infected plants as transplants and viruliferous thrips (= transmitters) are the primary source and means of virus spread. At present, there are limited options available for managing IYSV outbreaks (Pappu et al. 2009). The ability to rapidly and accurately detect IYSV in thrips vectors for the purpose of estimating the proportion of viruliferous thrips from the field could potentially provide information that would be useful in managing this pest complex.

The family Bunyaviridae consists of arthropod-borne RNA viruses (Elliott 1990, Nichol et al. 2005) and all but one genus Tospovirus in this family infect animals or humans. Genus Tospovirus, is the only genus in this family whose members infect plants (Moyer 1999, Tsompana and Moyer 2008). Tospoviruses developed a close biological association with their thrips vectors; adult thrips transmits the virus only if the virus was acquired during the larval stage (Sakimura 1962, Ullman et al. 1992a), with majority of thrips becoming viruliferous in the second larval stage (Wijkamp and Peters 1993). The frequency of emergence of viruliferous thrips depends on the duration of feeding by the first instar larvae on infected plants (Wijkamp and Peters 1993) and may be affected by the

¹ Department of Plant Pathology, Washington State University, Pullman, WA 99164.

² Current address: Department of Plant Pathology, University of California, Davis, CA 95616.

³ Department of Crop and Soil Sciences, Hermiston Research and Extension Center, Oregon State University, Hermiston, OR 97838.

⁴ Department of Entomology, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793.

⁵ Corresponding author, e-mail: hrp@wsu.edu.

amount of virus particles acquired by larvae. Even though the virus replicates in its thrips vector, transmission of virus by the adult thrips occurs intermittently for the duration of their lives (Ullman et al. 1992b, Wijkamp et al. 1993). Adult thrips that fed on virus-infected plants, for the first time do not transmit the virus (Wetering et al. 1996, de Assis Filho et al. 2004). However, the relationship between the virus accumulation in the plant and the acquisition rate is not well known.

Control of tospovirus epidemics by managing thrips populations with insecticides has been difficult because of the wide and diverse host range of both tospoviruses and thrips vectors (Cho et al. 1989, Pappu et al. 2009). Still, pest management practitioners recommend the use of insecticide applications or rotating the cropping systems to avoid thrips infestation (Mautino et al. 2012).

The ability to determine the proportion of viruliferous in a population would facilitate a better understanding of the role of a particular thrips species in tospovirus epidemiology may help refine vector management tactics to increase their efficiency and effectiveness. Information on thrips transmitters could be a useful parameter in developing a forecasting system for disease outbreaks. One approach to identify viruliferous thrips population is to conduct a bioassay using plant hosts as indicators (Cho et al. 1989, 1991; Allen and Matteoni 1991). Other methods that were used for the detection of viruliferous thrips include serological methods, such as enzyme linked immunosorbent assay (ELISA; Cho et al. 1989, Ullman et al. 1992c, Bandla et al. 1994), electron microscopy (Ullman et al. 1992a), nucleic acid dot blots (Rice et al. 1990), immunological squash blot (Aramburu et al. 1996), and real-time quantitative polymerase chain reaction (RT-PCR; Boonham et al. 2002). ELISA-based testing of thrips using antiserum against the viral structural protein (nucleoprotein) for determining potential virus transmitters likely overestimates the proportion of transmitters because a given field-originated thrips population may contain both transmitters (that acquired the virus in their larval stage) and those that ingested the virus as adults. Still, use of biological assays to identify the transmitters is effective but expensive and time consuming.

The complete genomes of IYSV L and M RNA were cloned and sequenced (Bag et al. 2009b, 2010). The L-RNA is in negative sense and potentially codes for the RNA-dependent RNA polymerase (RdRp) in virion complementary sense (de Haan et al. 1991, Bag et al. 2010). The M and S RNAs are ambisense in their genome organization and code for two proteins each (Bag et al. 2009b, Cortez et al. 2002). The S RNA of tospoviruses encodes the viral structural nucleocapsid protein (N) and a nonstructural protein (NSs). NSs was found in infected plant cells and thrips transmitters but not in assembled virions or healthy plants (de Avila et al. 1990, de Haan et al. 1990, Kormelink et al. 1991).

A serological assay that can facilitate high throughput testing and is sensitive, rapid, and specific to the virus is more desirable as it is also likely to be cost effective. An ELISA-based assay that would detect a NS of IYSV would be a useful tool. Detection of NSs

would then be indicative of virus replication, suggesting that the thrips that test positive could potentially transmit the virus as opposed to those that carry the virus as a contaminant. Thus, the objective of this research was to develop materials (NSs-specific antibodies) and a methodology (an ELISA-based assay) and apply them in identification of potential thrips transmitters from field collected adult thrips.

Materials and Methods

Onion samples from various commercial fields in the states of California (IYSV-CA), Idaho (IYSV-ID), and New York (IYSV-NY) were collected and tested for the presence of IYSV infection by ELISA using a commercially available ELISA kit (Agdia Inc., Elkhart, IN) or RT-PCR (Pappu et al. 2006). Uninfected, healthy onion plants grown in a growth chamber were used as negative controls.

The NSs gene (1,331 nt) was amplified using gene specific primers 5'-CCT TTT TTT TTT CAT ATG TCT ACC GTT AGG ACT ACG GC-3' (forward primer) and 5'-TTA TGG ATC CTC ACT GCA GCT CTT CTA CA-3' (reverse primer) with NdeI and BamHI restriction sites (underlined) at 5' end of forward and reverse primers, respectively. The amplicon was cloned into TOPO-TA cloning vector (Invitrogen, Carlsbad, CA). Recombinant clones were identified using colony PCR and restriction analysis and the NSs gene was released by restriction digestion with NdeI and BamHI, subcloned into expression vector pET15b containing the N terminal His-tag sequence (Novagen, Darmstadt, Germany). The recombinant pET15b clone was mobilized into the *Escherichia coli* (Rosetta) strain (maintained in laboratory) according to the standard molecular biology protocols (Sambrook and Russell 2001). Transformants were screened and maintained on Luria agar plates containing appropriate antibiotics (50 µg/ml of ampicillin and 34 µg/ml of chloroamphenicol). Expression of fusion protein by 100 mM isopropyl-β-D-1-thiogalactopyranoside and was purified using nickel (Ni) column according to the manufacturer's instructions. Fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970) using 5% stacking gel and 12% resolving gel.

The recombinant NSs protein was purified and emulsified with an equal volume of Freund's incomplete adjuvant and injected intramuscularly (100 µg per animal) into two rabbits. Four additional booster doses were given at weekly intervals. Rabbits were first bled 14 d after every booster dose and the serum was collected and stored at 4°C.

The antiserum was first tested for reactivity against *E. coli* expressed recombinant IYSV-NSs protein. The recombinant protein was electroblotted (30 V for 3 h) onto nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ) using Tris borate-ethylenediaminetetraacetic acid (TBE) buffer. The membrane was kept in blocking solution (3% bovine serum albumin [BSA] in tris-buffered saline) for 1 h and probed with the antiserum produced against the NSs

at 1:4,000 dilutions for 120 min. The bound antibodies were detected with antirabbit IgG alkaline phosphatase conjugate (Sigma, St. Louis, MO; 1:5,000 dilution) and 0.33 mg/ml Nitro blue tetrazolium chloride (Sigma, St. Louis, MO) and 0.175 mg/ml 5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt (Sigma, St. Louis, MO) as substrate.

The antiserum was used in direct antigen-coated (DAC)-ELISA (Clark and Bar-Joseph, 1984) to detect IYSV in various onion plant samples that were previously confirmed to be IYSV-infected. Plant samples infected with impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) were included to rule out cross reactivity of the antiserum to these two viruses. A dilution series of the antiserum, ranging from 1:200 to 1:6,000, was used to determine the optimal titer of the antiserum for use in DAC-ELISA for detecting IYSV.

Further, for the detection of IYSV-NSs protein in thrips, DAC-ELISA (Clark and Bar-Joseph, 1984; Bandla et al. 1994) was used to test thrips collected from three different sources. A fine-tipped brush was used in collecting, transferring, and removal of thrips from plants. Thrips were placed in vials containing 100 μ l of extraction buffer (0.01 M sodium-potassium phosphate buffer, pH 7.4, containing 0.02% sodium azide [wt:vol], 0.8% sodium chloride [wt:vol], 0.05% Tween 20 [vol:vol], and 2% polyvinylpyrrolidone mol wt 40,000 [wt:vol] (Sigma) and stored at -20°C until analysis. Individual thrips were removed from the storage vials with a fine-tipped camel's-hair brush, placed onto separate micro centrifuge tube, triturated in 50 μ l of ELISA extraction buffer with a sterile blunt end plastic pestle. The suspension was transferred to a flat bottom ELISA plate and incubated overnight at 4°C . Plates were washed three times with Phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS-T) and blocked with 75 μ l of 1% BSA for 2 h at 37°C . After washing the plates, 50 μ l of polyclonal anti-NSs antibody diluted (1:4,000) in antigen dilution buffer (0.2% BSA, 2% PVP 40 and 0.02% sodium azide, pH 7.4) were added to the wells, and the plates were incubated at 37°C for 2 h. Plates were washed three times with PBS-T and 50 μ l of goat anti-rabbit IgG-alkaline phosphatase (1:5,000) (Sigma, St. Louis, MO) in antibody dilution buffer was added to each well and the plates were incubated at 37°C for 2 h. Colorimetric reactions were read at A_{405} nm after addition of 0.5 mg/ml substrate (*p*-nitrophenyl phosphate disodium, in 1M diethanolamine buffer containing 0.5 mM MgCl_2 , and 0.02% sodium azide) to each well. Absorbance values (A_{405}) were taken at 1 and 2 h after the addition of substrate on an ELISA plate reader (Bio-Tek Instruments, Winooski, VT).

Collection of *T. tabaci*. To standardize the detection technique in individual thrips, thrips samples were collected from various locations in Georgia. The samples were collected from onion cull piles (TtR), imported onion plants that were tested for IYSV (TtT), and *T. tabaci* maintained under controlled greenhouse conditions (TtC).

To further validate the test, thrips collected from IYSV-infected onion fields were tested. During the

cropping seasons of 2008 and 2009, *T. tabaci* were collected from two different experimental fields: Field A adjacent to an overwintering field and another Field B was located at least 2 km away from any overwintering onion field. Both fields were located at the Oregon State University Hermiston Research and Extension Center, Hermiston, OR. The soil was an Adkins fine sandy loam (coarse-loamy, mixed mesic Xerollic Camborthid). The area was fumigated in the fall with Sectagon applied at 40 gpa. "Vision" onions were seeded on 6 April 2008, 2–30' beds per plot, 34" between beds, 4 rows per bed, with a Monosem vacuum precision planter. On 11 April, Dacthal (dimethyl tetrachloroterephthalate) was broadcast at 6 lb per acre for weed control and fertilizer (10-35-0 N-P₂O₅-K₂O) was banded over the seed rows. Lorsban (chlorpyrifos) was banded over the planting rows at 3 pt per acre to control seed corn maggot. In 2008, poor stand resulted in plots being rototilled and replanted on 4 May. Dacthal and Lorsban were applied at 6 lb per acre and 1 qt per acre, respectively, on 8 May. On June 7 Prowl (pendimethalin) herbicide was applied 1.2 pt per acre, along with Goal 2XL (oxyfluorfen) at 0.5 pt per acre and Bucril (bromoxynil) at 1.2 pt per acre. Additional nitrogen was applied through the center pivot irrigation system. The same procedure was followed in 2009. There were no seed corn maggot issues. The crop was grown according to normal commercial production practices. Beginning early June, 10 plants per field were removed weekly, bagged, and transferred to the laboratory, leaves were examined for damage, and thrips were counted.

Subsamples of thrips were slide-mounted to verify their identification. Ninety-nine percent of the thrips collected were *T. tabaci*, while the remaining 1% was identified as *F. occidentalis* (<http://www.ento.csiro.au/thysanoptera/worldthrips.php>). Thrips were collected at an interval of 7 d from 10 different plants per field, sorted, and stored in 100 ml of 0.1 M phosphate buffer at -20°C until further analysis. *T. tabaci* were identified and tested for the presence IYSV by DAC-ELISA using antiserum specific to the NSs of IYSV.

Results

The antiserum produced to the recombinant fusion protein of NSs was first tested for specificity against the homologous purified protein. The antiserum was specific to *E. coli*-expressed and gel-purified IYSV-NSs, and IYSV-infected onion sample in immune blot (western blot) assay. A band of expected ≈ 50 kDa, which was absent in the healthy control, was detected suggesting the specificity of the antiserum. The antiserum reacted with and detected only the 50 kDa protein and not with any proteins in the uninfected, healthy sample (negative control).

To further validate the specificity of the antiserum, onion samples collected from different parts of the United States were tested. The antiserum could detect IYSV in a wide range of onion samples collected from California, Idaho, New York, and Washington. The antiserum did not react with INSV or TSWV-infected

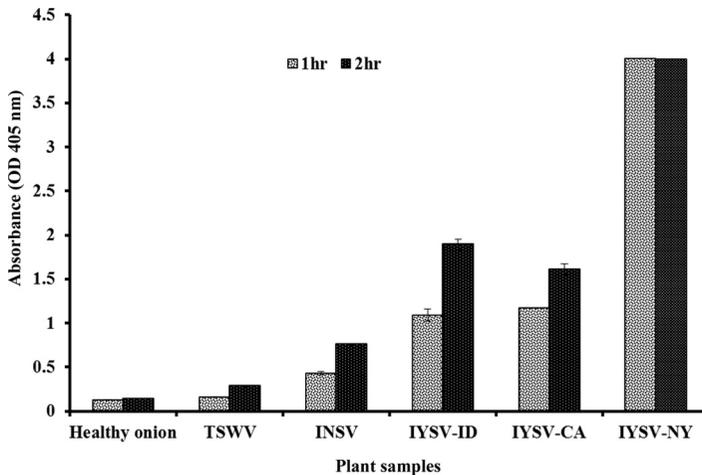


Fig. 1. Reaction of antiserum produced against the NSs protein of IYSV with different tospovirus-infected plant samples in ELISA. Plant samples infected with TSWV, INSV, and IYSV were used. Samples were on the x-axis and the absorbance value (A_{405}) is on the y-axis. Data shown are net absorbance values for means of duplicate ELISA wells after deducting the mean absorbance values for buffer controls. The values for 1 and 2 h were shown with standard deviation error bars.

plant samples suggesting the specificity of the antiserum to IYSV and the lack of cross reactivity to corresponding proteins coded by INSV and TSWV (Fig. 1). The absorbance reading after 1 h of incubation was able to differentiate between healthy and infected samples. Usually, there was a significant increase in the absorbance values after 2 h of incubation (Fig. 1).

Individual thrips were then tested using this ELISA assay for its ability to detect IYSV in adults collected from onion fields in Georgia. The antiserum was able to detect IYSV- NSs protein in individual thrips; thus, potentially differentiating the viruliferous adults from the nonviruliferous (Fig. 2). Of the total thrips tested, 35.7% (5 out of 14) from onion cull piles and 58.9% (33 out of 56) thrips from a colony maintained on IYSV-infected onions tested positive with the NSs antise-

rum. Thrips maintained on healthy plants and those used as negative control did not react to the antiserum (Fig. 2). There was a significant increase in color development after 2 h of incubations, and provided clear differentiation between the viruliferous and nonviruliferous thrips.

In the field plot of Oregon State University, Hermiston, thrips collected from the onion fields were serologically tested to differentiate between viruliferous and nonviruliferous thrips. In 2008, 157 thrips (Field A) and 111 thrips (Field B), and in 2009, 534 thrips (Field A) and 677 thrips (Field B) were tested for the presence of IYSV. In 2008, the mean number of thrips per plant in Field A was (2.3–17.1) and in Field B was (0.1–27.27), while in 2009 Fields A and B had 2.35–27.75 and 3.5–47.65, respectively. In Field A (Fig. 3a

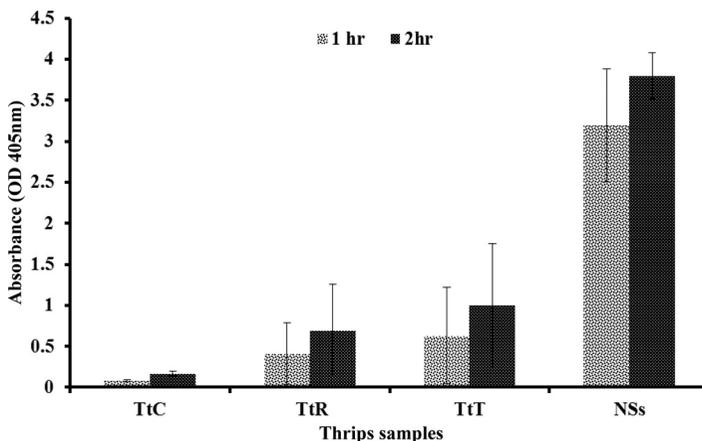


Fig. 2. Detection of NSs protein of IYSV in onion thrips. The absorbance values were taken 1 and 2 h after the addition of the substrate. Various groups of thrips samples were on the x-axis and the absorbance values (A_{405}) on the y-axis. NSs-purified protein; TtC- virus-free adult *T. tabaci* as negative control, TtR- adult *T. tabaci* from onion cull piles, TtT- adult *T. tabaci* collected from imported onion. Data shown are net absorbance values for means of total thrips after deducting the mean absorbance values for buffer controls. The values for 1 and 2 h were shown with standard deviation error bars.

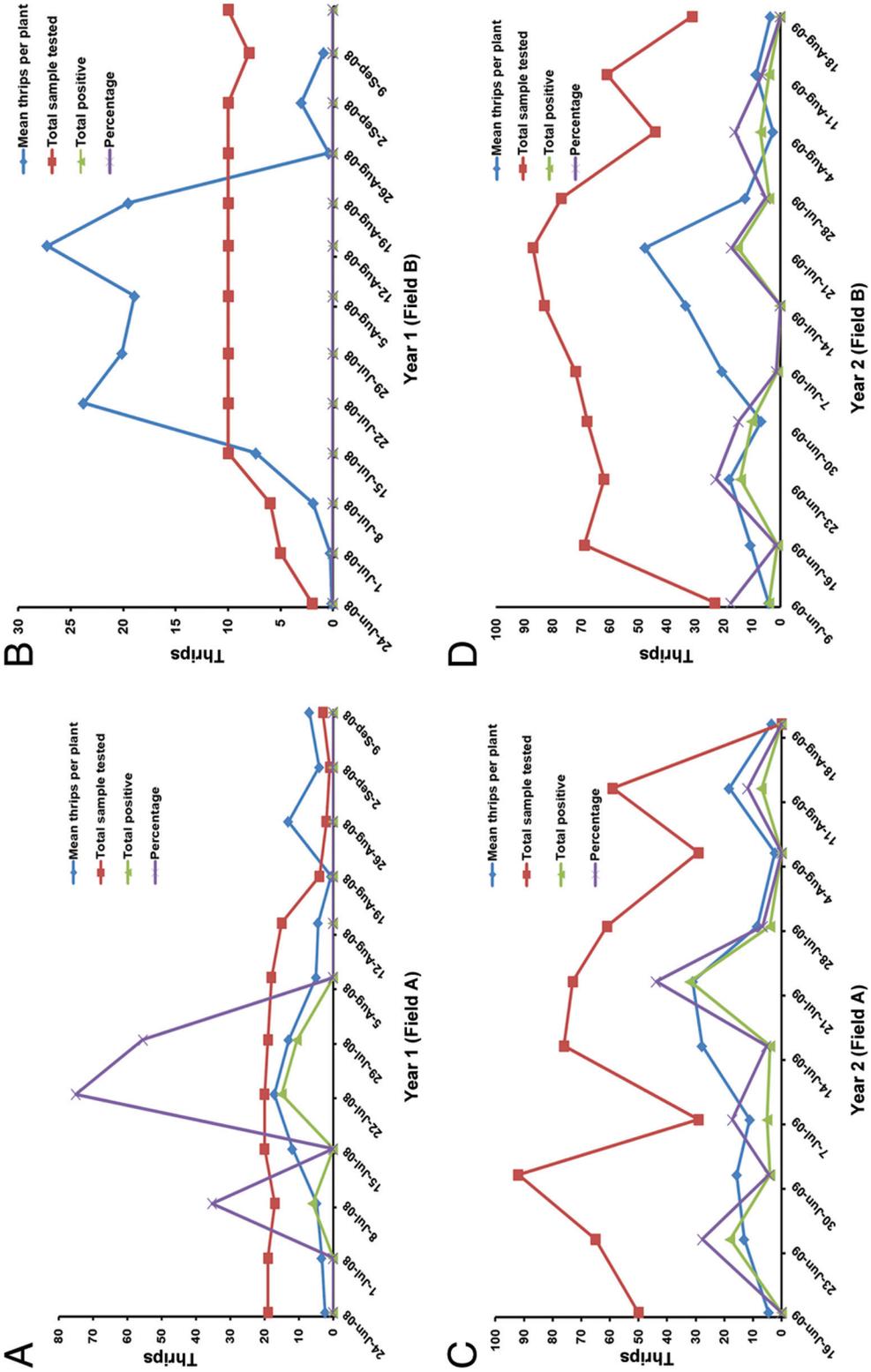


Fig. 3. Seasonal dynamics of *T. tabaci* populations and potential IYSV transmitters from two onion fields near Hermiston, OR. Days of collection on x-axis and mean numbers of thrips per plants, numbers of thrips tested, total number of positive thrips and percentage of viruliferous thrips on y-axis. (A and C) Analysis for thrips collected in year 1 from Field A, which is close to overwintering field. (B and D) Analysis for thrips collected in year 2 from Field B, which is far from any overwintering field.

and c) and Field B (Fig. 3b and d), the highest incidence of thrips was during middle of July, for both years 2008 and 2009. From Field A, the highest proportion of viruliferous thrips (75 and 35%) and the highest thrips incidence were found during 22 July 2008 and 21 July 2009, respectively, (Fig. 3a and c).

For Field B, which was far removed from any overwintering onion field even with the presence of infected onion in 2008, the thrips collected during the experimental period were nonviruliferous as none of the thrips tested were found to be positive to IYSV-NSs (Fig. 3b). However, during the cropping season of 2009, the maximum numbers of viruliferous thrips were present in both early (23 June 2009) and mid-season (21 July 2009; Fig. 3d). The percentage of viruliferous thrips decreased late in the season (Fig. 3a–d). The antiserum was able to detect the virus in individual thrips collected from IYSV-infected onion fields.

Discussion

The ability to quickly identify potential transmitters among field-collected thrips populations could facilitate a better understanding of the vector dynamics in a given cropping system. ELISA using polyclonal antibody raised against the NSs was able to detect IYSV in both onion plant samples and thrips. The antiserum detected the virally-coded protein in individual thrips collected from onion cull piles located in an open field. In a similar study, Bandla et al. (1994) produced a monoclonal antibody to the NSs protein of TSWV and showed that the detection of NSs in adult thrips was correlated with the ability to transmit the virus.

Thrips collected from the two onion fields provided different results. Although the antiserum was able to detect the virus in individual thrips, from Field A, which was close to an overwintering field, a higher proportion of viruliferous thrips were detected in both years, but in Field B, viruliferous thrips were not found in 2008 but detected in 2009. The differences in field condition during subsequent years could be due to different environmental conditions that influence the disease and thrips incidence. Hsu et al. (2010) reported high larval densities per plant during early season mid July and late August.

Monitoring thrips in greenhouses or in the field either by the use of sticky traps or manual counting is an important component of Integrated Pest Management (IPM) strategy for managing thrips populations to reduce their impact both as a pest and virus vector (Morsello et al. 2008, Olatinwo et al. 2011). However, this gives no indication of viruliferous thrips at any given time point. A rapid and cost effective method for the reliable detection of virus in individual thrips would aid in the understanding the factors leading to IYSV epidemics. The ELISA-based method facilitates estimation of IYSV transmitters among thrips populations collected from plants grown either in protected or open field conditions. The antiserum could potentially differentiate transmitters from nontransmitters

because NSs is not part of the virion (Kormelink et al. 1991) and is expressed in plants and thrips vectors only upon virus replication. Thus, detection of NSs in the insect is an indication of the virus replication and only those thrips in which virus had replicated are capable of transmitting the virus.

Results from this study suggested the antiserum produced to a NS of IYSV is effective in detecting the virus in plants and single thrips using ELISA. The *E. coli*-based protein expression systems offer a major advantage in obtaining proteins for antibody production because it is difficult to purify these proteins from virus-infected plant tissue as these proteins are not part of the virions. The antiserum produced was found to be specific to IYSV and it did not react with two other distinct tospovirus species, INSV and TSWV. The ELISA-based assay described here is being used to conduct studies on the seasonal dynamics of IYSV transmitters among onion thrips collected from onion fields.

Information on the seasonal dynamics of viruliferous thrips could be useful in refining thrips management tactics as part of an IPM strategy for reducing the impact of thrips-transmitted IYSV. This along with other control tactics, such as manipulating planting and harvest dates (Hsu et al. 2010), and other cultural practices, such as managing nitrogen levels (Buckland et al. 2013), could enhance the effectiveness of the existing IPM programs for reducing the impact of thrips and IYSV in onion.

Acknowledgments

Funded in part by U.S. Department of Agriculture–National Institute of Food and Agriculture (USDA–NIFA) Specialty Crops Research Initiative, USDA–NIFA Western Region IPM Center Grants Program, and the Washington State Commission for Pesticide Registration, PPNS No. 0632, Department of Plant Pathology, College of Agricultural, Human and Natural Resource Sciences, Agricultural Research Center, Project no. WNPO 0545, Washington State University, Pullman, WA 99164–6430.

References Cited

- Allen, W. R., and J. A. Matteoni. 1991. *Petunia* as an indicator for use by the grower to monitor for thrips carrying the *Tomato spotted wilt virus* in green house. *Plant Dis.* 75: 78–82.
- Aramburu, J., J. Riudavets, J. Arno, A. Lavina, and E. Moriones. 1996. Rapid serological detection of *Tomato spotted wilt virus* in individual thrips by squash-blot assay for use in epidemiological studies. *Plant Pathol.* 45: 367–374.
- Bag, S., K. L. Druffel, T. Salewsky, and H. R. Pappu. 2009a. Nucleotide sequence and genome organization of the medium RNA of *Iris yellow spot virus* (genus *Tospovirus*, family *Bunyaviridae*) from the United States. *Arch. Virol.* 154: 715–718.
- Bag, S., J. Singh, R. M. Davis, W. Chounet, and H. R. Pappu. 2009b. *Iris yellow spot virus* in Nevada and Northern California. *Plant Dis.* 93: 674.
- Bag, S., K. L. Druffel, and H. R. Pappu. 2010. Structure and genome organization of the large RNA of *Iris yellow spot*

- virus (genus *Tospovirus*, family *Bunyaviridae*). Arch. Virol. 155: 275–279.
- Bandla, M. D., D. M. Westcot, K. D. Chenault, D. E. Ullman, T. L. German, and J. L. Sherwood. 1994. Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of *Tomato spotted wilt tospovirus* to identify viruliferous thrips. *Phytopathology* 84: 1427–1431.
- Boonham, N., P. Smith, K. Walsh, J. Tame, J. Morris, N. Spence, J. Bennison, and I. Barker. 2002. The detection of *Tomato spotted wilt virus* (TSWV) in individual thrips using real time fluorescent RT-PCR (TaqMan). *J. Virol. Methods* 101: 37–48.
- Bulajic, A., J. Jovic, S. Krnjajic, M. Petrov, I. Djekic, and B. Krstic. 2008. First report of Iris yellow spot virus on onion (*Allium cepa*) in Serbia. *Plant Dis.* 92: 1247.
- Buckland, K., J. R. Reeve, D. Alston, C. Nischwitz and D. Dorst. 2013. Effect of nitrogen fertility and crop rotation on onion growth and yield, thrips densities, Iris yellow spot virus and soil properties. *Agric. Ecosyst. Environ.* 177: 63–74.
- Cho, J. J., R.F.L. Mau, D. E. Ullman, and D. M. Custer. 1991. Detection of *tomato spotted wilt virus* within thrips. Virus-plant interaction of *Tomato spotted wilt virus*. U.S. Dept. Agric. Workshop ARS 87: 144–152.
- Cho, J. J., R.F.L. Mau, T. L. German, R. W. Hartmann, L. S. Yudin, D. Gonsalves, and R. Providenti. 1989. A multidisciplinary approach to management of *tomato spotted wilt virus* in Hawaii. *Plant Dis.* 73: 375–383.
- Clark, M. F., and M. Bar-Joseph. 1984. Enzyme immunosorbent assays in plant virology, pp. 51–85. In K. Maramorsch, and H. Koprowski (eds.), *Methods in virology*, vol. II. Academic, New York.
- Cortez, I., A. Aires, A. M. Pereira, R. Goldbach, D. Peters, and R. Kormelink. 2002. Genetic organization of Iris yellow spot virus M RNA: indications for functional homology between the G(C) glycoproteins of tospoviruses and animal-infecting bunyaviruses. *Arch. Virol.* 147: 2313–2325.
- de Assis Filho, F.M.C.M. Deom and J. L. Sherwood. 2004. Acquisition of *Tomato spotted wilt virus* by adults of two thrips species. *Phytopathology* 94: 333–336.
- de Avila, A. C., C. Hugent, O. de Resende, E. W. Kitajima, R. W. Goldbach, and D. Peter. 1990. Serological differentiation of 20 isolates of *Tomato spotted wilt virus*. *J. Gen. Virol.* 71:2801–2801.
- de Haan, P., L. Wagemakers, D. Peters, and R. Goldbach. 1990. The SRNA segment of *Tomato spotted wilt virus* has an ambisense character. *J. Gen. Virol.* 71: 1001–1007.
- de Haan, P., R. Kormelink, R.O. Resende, F. van Poelwijk, D. Peters, and R. Goldbach. 1991. *Tomato spotted wilt virus* L RNA encodes a putative RNA polymerase. *J. Gen. Virol.* 72: 2207–2216.
- Elliott, R. M. 1990. *Molecular biology of the Bunyaviridae*. *J. Gen. Virol.* 72: 501–522.
- Fournier, F., G. Boivin, and R. K. Stewart. 1995. Effect of *Thrips tabaci* (Thysanoptera: Thripidae) on yellow onion yields and economic thresholds for its management. *J. Econ. Entomol.* 88: 1401–1407.
- Gent, D. H., L. J. du Toit, S. F. Fichtner, S. Krishna 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.
- Hsu, C. L., Hoeping, C. A., Fuchs, M., Shelton, A. M., and Nault, B. A. 2010. Temporal dynamics of Iris yellow spot virus and its vector, *Thrips tabaci* (Thysanoptera:Thripidae), in seeded and transplanted onion fields. *Environ. Entomol.* 39: 266–277.
- Huchette, O., C. Bellamy, R. Filomenko, B. Pouleau, S. Seddas, and H. R. Pappu. 2008. Iris yellow spot virus on shallot and onion in France. *Plant Health Progress*. doi: 10.1094/PHP-2008-0610-01-BR.
- Kormelink, R., E. W. Kitajima, P. de Haan, D. Zuidema, D. Peters, and R. Goldbach. 1991. The non structural protein (NSs) encoded by ambisense S RNA segment of *Tomato spotted wilt virus* is associated with fibrous structures in infected plant cells. *Virology* 181: 459–468.
- Kritzman, A., M. Lampal, B. Raccach, and A. Gera. 2001. Distribution and transmission of Iris yellow spot virus. *Plant Dis.* 85: 838–842.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the bacteriophage T4. *Nature* 227: 680–685.
- Mandal, B., R. K. Jain, M. Krishnareddy, N. K. Krishna Kumar, K. S. Ravi, and H. R. Pappu. 2012. Emerging Problems of Tospoviruses (*Bunyaviridae*) and their management in the Indian subcontinent. *Plant Dis.* 96: 468–479.
- Mautino, G. C., L. Bosco, and L. Tavella. 2012. Integrated management of *Thrips tabaci* (Thysanoptera:Thripidae) on onion in north-western Italy: basic approaches for supervised control. *Pest Manag. Sci.* 68: 185–193.
- Molenaar, N. D. 1984. Genetics, thrips (*Thrips tabaci* L.) resistance and epicuticular wax characteristics of non-glossy and glossy onions (*Allium cepa* L.). Ph.D dissertation, University of Wisconsin, Madison.
- Montano, J. D., F. 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.
- Morsello, S. C., R. L. Groves, B. A. Nault, and G. G. Kennedy. 2008. Temperature and precipitation affect seasonal patterns of dispersing Tobacco thrips, *Frankliniella fusca*, and Onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) caught on sticky traps. *Environ. Entomol.* 37: 79–86.
- Moyer, J.W. 1999. Tospoviruses (*Bunyaviridae*), pp1803–1807. In A. Granoff and R. G. Webster (eds.), *Encyclopedia of virology*. Academic, San Diego, CA.
- Nagata, T., A.C.L. Almeida, R. O. de Resende, and A. C. de Avila. 1999. The identification of the vector species of Iris yellow spot tospovirus occurring on onion in Brazil. *Plant Dis.* 83: 399.
- Nichol, S. T., B. J. Beaty, R. M. Elliott, R. Goldbach, A. Plyusnin, C. S. Schmaljohn, and R. B. Tesh. 2005. *Bunyaviridae*, pp. 695–716. In C. M. Fauquet, M. A. Mayo., J. Maniloff, U. Desselberger, and L. A. Ball (eds.), *Virus taxonomy, VIIIth Report of the ICTV Elsevier/Academic*, San Diego, CA.
- Olatinwo, R. O., T. Prabha, J. O. Pez, D. G. Riley, and G. Hoogenboom. 2011. The weather research and forecasting (WRF) model: application in prediction of TSWV vector populations. *J. Appl. Entomol.* 135: 81–90.
- Pappu, H. R., and M. E. Matheron. 2008. Characterization of Iris yellow spot virus from onion in Arizona. *Plant Health Progress*. doi:10.1094/PHP-2008-0711-01-BR.
- Pappu, H. R., L.J. du Toit, H. F. Schwartz, and K. Mohan. 2006. Sequence diversity of the Nucleoprotein gene of Iris yellow spot virus (genus *Tospovirus*, family *Bunyaviridae*) isolates from the western region of the United States. *Arch. Virol.* 151: 1015–1023.
- Pappu, H. R., R.A.C. Jones, and R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes gained and challenges ahead. *Virus Res.* 141: 219–236.
- Parrella, M. P., and T. Lewis. 1997. Integrated pest management (IPM) in field crops, pp. 595–614. In: T. Lewis (ed.), *Thrips as crop pests*. CAB International, New York
- Rice, D. J., T. L. German, R.F.L. Mau, and F. M. Fujimoto. 1990. Dot blot detection of *Tomato spotted wilt virus-*

- RNA in plant and thrips tissues by cDNA clones. *Plant Dis.* 74: 274–276.
- Sakimura, K. 1962. The present status of thrips-borne viruses, pp. 33–40. In K. Maramorosch, (eds.), *Biological transmission of disease agents*. Academic, New York.
- Sambrook, J., and D. W. Russell. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbour Laboratory Press, New York.
- Sether, D. M., W. B. Borth, R. S. Shimabuku, H. R. Pappu, M. J. Melzer, and J. S. Hu. 2010. First Report of Iris yellow spot virus in Onion in Hawaii. *Plant Dis.* 94: 1508.
- Srinivasan, R., S. Sundaraj, H. R. Pappu, S. Diffie, D. Riley, and R. Gitaitis. 2012. Transmission of Iris yellow spot virus by *Frankliniella fusca* and *Thrips tabaci* (Thysanoptera: Thripidae). *J. Econ. Entomol.* 105: 40–47.
- Tsompana, M. and J. W. Moyer. 2008. Tospoviruses, pp 157–162, In B.W.J. Mahy and M.H.V. Van Regenmortel (eds.), *Encyclopedia of virology*, vol. 5, 3rd ed. Elsevier Ltd., Oxford, United Kingdom.
- Ullman, D. E., J. J. Cho, R.F.L. Mau, D. M. Westcot, and D. M. Custer. 1992a. A midgut barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. *Phytopathology* 82: 1333–1342.
- Ullman, D. E., J. J. Cho, R.F.L. Mau, W. B. Hunter, D. M. Westcot, and D. M. Custer. 1992b. Thrips-*Tomato spotted wilt virus* interactions: morphological, behavioral and cellular components influencing thrips transmission, pp 195–240. In K. F. Harris (eds.), *Advances in disease vector research*, vol. 9. Springer, New York.
- Ullman, D. E., D. M. Westcot, F. A. Cantone, J. L. Sherwood, and T. L. German. 1992c. Immunocytochemical evidence for Tomato spotted wilt virus (TSWV) replication in cells of the western flower thrips, *Frankliniella occidentalis* (Pergande). *Phytopathology* 82: 1087.
- Ward, L. I., Z. Perez-Egusquiza, J. D. Fletcher, C.F.M. Ochoa, J. Z. Tang, L. W. Liefting, E. J. Martin, B. D. Quinn, H. R. Pappu, and G.R.C. Clover. 2008. First Report of Iris yellow spot virus on *Allium cepa* in New Zealand. *New Dis. Rep.* 17: 38.
- Wetering, F., R. Goldbach, and D. Peter. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology* 86: 900–905.
- Wijkamp, I., and D. Peters. 1993. Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis*, using a novel leaf disk assay. *Phytopathology* 83: 986–991.
- Wijkamp, I., J. van Lent, R. Kormelink, R. Goldbach, and D. Peters. 1993. Multiplication of tomato spotted wilt virus in its insect vector, *Frankliniella occidentalis*. *J. Gen. Virol.* 74: 341–349.

Received 23 March 2013; accepted 9 December 2013.
