

Western IPM Center Project Report Form

How to submit: Please submit this completed form electronically, as an attached Microsoft Word file, to Frank Zalom at fgzalom@ucdavis.edu. **Content:** Complete each section below, and include responses to as many of the questions listed in Attachment A as are relevant to your project. *These are guidelines.* Provide your readers with enough detail that someone who is not familiar with your project can understand what you were trying to achieve, how you went about it, and what you accomplished, but please keep it concise.

A. Report Data

Date: 10/30/2009

Reporting Period: final

Report Type (please check one):

Progress Report Final Report

B. Grant Data

- Grant Agreement #: 2007-03622
- Title: Developing a Monitoring Program for Thrips-Iris
- Yellow Spot Virus Complex: Adding a Novel Management Component to the IPM Program in Bulb and Seed Onion Crop
- Grant Type: Research
- Lead investigator:
 - Name: H.R. Pappu
 - Title: Associate Professor and Chair
 - Institution: Washington State University
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- Team members (name, title, institution): Dr. Silvia Rondon, Assistant Profesor, Oregon State University, Hermiston, OR
- State(s) involved: OR, WA

C. Nontechnical Summary. An overview of the project, briefly outlining the problem(s), how your project addresses them, and your results, *written to a lay audience*. (500 words)

Thrips-transmitted Iris yellow spot virus (IYSV) is one of the major constraints to bulb and seed onion production in the western US. The disease affects both the yield and grade. Total crop losses in seed crops were reported from Columbia Basin in WA and OR and in southern Idaho. Limited control options are available. Since thrips vectors play a critical role in virus spread, tactics to identify viruliferous thrips and their seasonal dynamics would help devise more effective and environmentally friendly disease management strategies. A rapid and practical ELISA assay was developed that would identify potential transmitters among onion thrips populations. Using this assay, seasonal dynamics of thrips carrying the virus were identified. Using serological and molecular tools, new natural hosts of IYSV were identified. The potential role of these new weed hosts in virus epidemiology needs further investigation.

D. Objectives and Progress. List your objectives and describe your progress for each objective.

Objective 1. Develop and utilize tools to determine the role of thrips vectors in disease spread (produce antisera to the non-structural (NSs) protein and develop an ELISA-based assay to determine the proportion of thrips transmitters).

Progress: One of the prerequisites for a better understanding of the epidemiology and for the development of a management program is the ability to detect the virus in plants and thrips vectors. Polyclonal antiserum was produced to the recombinant, *E. coli*-expressed nonstructural protein (NSs) coded by the small (S) RNA of IYSV. The recombinant fusion protein was obtained in the insoluble fraction, purified using the nickel column, and was used in immunization to produce a high-titred polyclonal antiserum in rabbits. When used in an antigen-coated plate ELISA, the antiserum, diluted up to 1:4000, could detect the virus in single adult thrips and in plants. Availability of antiserum to a non-structural protein of IYSV would be useful in epidemiological studies to better understand the role of thrips vectors in outbreaks of this important virus.

Objective 2. Utilize the tools from #2 to develop a monitoring program for the viruliferous thrips

Progress: In 2008, onion thrips were monitored in two field plots on a weekly basis using full plant counts technique. This research was conducted at OSU's Hermiston Agricultural Research and Extension Center (HAREC). Preliminary data shows that onion fields planted next to overwintering onions, a potential source of onion thrips for the following season, did not increase the mean number of onion thrips per plant per week in the field planted adjacent to it. However, numbers of symptomatic leaves were higher in field planted next to overwintering onion plots (25%) as compared to the field planted on the bare area (4%). Leaves will be tested later this year to confirm this observation. Samples were taken from July 8 until September 9. In field A, a steady increase on onion thrips mean numbers was observed week after week. A peak was reached on August 5; with a consequent decrease thereafter. August 5, 17.10 ± 1.3 onion thrips per plant per week were counted. In field B, two peaks were observed on July 22 and August 12, reaching a maximum number of 27.25 ± 8 onion thrips per plant per week on August 12. Each week, at least 20 onion thrips were collected from each field and from each sampling site. All those samples were preserved on PBS and will be tested for IYSV late 2008- early 2009. These thrips samples are being tested for the presence of IYSV using an ELISA-based assay to determine the proportion of viruliferous thrips. Similar study was conducted during 2009. Data is being analyzed.

Objective 3. Identify weed hosts and evaluate the role of volunteer onions in IYSV epidemiology.

Garlic was confirmed as a naturally susceptible host of IYSV. While IYSV is an important constraint to onion bulb and seed production in several onion growing regions of the United States, there was no information on the status of garlic as a host of IYSV. Garlic was reported to be infected with IYSV in Réunion Island, but there have been no confirmed reports of natural infection of garlic in the USA. Garlic plants showing near-diamond shaped lesions were found in August 2008 in Marion County, Oregon. The one-acre field plot consisted of various true-seeded garlic varieties and was adjacent to three onion fields which showed IYSV symptoms. Symptoms were observed on 5% of the garlic plants with most of the symptomatic plants displaying small and diffuse straw-colored spots. Seven of these symptomatic plants were selected for testing. Of these, two showed characteristic diamond-shaped, elongated straw-colored lesions on garlic scapes. However, the lesions were more diffuse with less defined edges compared to the characteristic diamond-shaped lesions that are often associated with IYSV infection. All the symptomatic plants were positive for IYSV when tested by DAS-ELISA using a commercially available kit (Agdia Inc., Elkhart, IN). To verify IYSV infection, total nucleic acid extracts from the symptomatic parts of the leaves were prepared and tested for the presence of IYSV using reverse transcription-PCR (RT)-PCR with primers 5'TAA AAC AAA CAT TCA AAC AA 3' and 5'CTC TTA AAC ACA TTT AAC AAG CAC 3' which flank the nucleocapsid (N) gene coded by the small RNA of IYSV. An amplicon of the expected ca. 1.2 kb was obtained from all symptomatic plants which was cloned and sequenced. Nucleotide sequence comparisons using BLAST showed that a consensus of three clones derived from the amplicon from garlic (Accession No. FJ514257) was 85% to 99% identical with IYSV sequences available in GenBank (AF001387, AB180918, AB286063) confirming the identity of IYSV. This is the first report of natural infection of IYSV infection of garlic in the USA. Additional surveys and testing are needed to obtain a better understanding of IYSV incidence in garlic in order to evaluate its impact on garlic production.

Besides onion and other susceptible crops such as leek, chives, and several ornamentals, weeds could be serving as potential reservoirs and as sources of virus inoculum. There are reports of several weeds found naturally infected with IYSV. However, there is no report of IYSV infection of a grass species. Leaves of green foxtail (*Setaria viridis* (L.) Beauv.) were collected from naturally occurring plants in a weed trial conducted in commercial onions grown in Box Elder Co., UT in September, 2008. Leaves displayed a range of symptoms that included streaking, purpling, chlorotic and necrotic lesions along leaf margins oriented along the axis of longitudinal venation. IYSV infection was confirmed by ELISA, RT-PCR and sequencing of the amplicon.

E. Outputs. List your project’s outputs, which might include publications, information, data, meetings held, attendance at meetings held, etc.

Refereed Journal Publications

Bag, S., K.L. Druffel and H.R. Pappu. 2009. Completion of the molecular characterization of the multipartite RNA genome of Iris yellow spot virus (IYSV, genus Tospovirus, family Bunyaviridae): Structure and genome organization of the large RNA of IYSV. Archives of Virology. Accepted.

Bag, S., K.L. Druffel, T. Salewsky, and H.R. Pappu. 2009. Nucleotide sequence and genome organization of the medium RNA of Iris yellow spot virus (genus Tospovirus, family Bunyaviridae) from the United States. Archives of Virology 154:715-718.

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

Bag, S., P. Rogers, R. Watson, and H.R. Pappu. 2009. First report of natural infection of garlic (*Allium sativum*) with Iris yellow spot virus in the United States. Plant Disease 93:839.

Bag, S., J. Singh, R.M. Davis, W..Chounet, and H.R. Pappu. 2009. Iris yellow spot virus in Nevada and Northern California. Plant Disease 93:674.

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 that lie ahead. Virus Research 141:219–236.

Evans, C.K., S. Bag, E. Frank, J. Reeve, C. Ransom, D. Drost, and H.R. Pappu. 2009. Natural infection of Iris yellow spot virus in Twoscale saltbush (*Atriplex micrantha*) growing in Utah. Plant Disease 93:430.

Evans, C.K., S. Bag, E. Frank, J. Reeve, C. Ransom, D. Drost, and H.R. Pappu. 2009. Green foxtail (*Setaria viridis*), a naturally infected grass host of Iris yellow spot virus in Utah. Plant Disease 93:670.

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.

F. Impacts and Potential Impacts. The “impacts” and “potential impacts” sections of your report will help the Western IPM Center highlight the value of IPM research and education by detailing the real-world impacts of Center-funded projects. We will use the information in news articles, reports, and informational brochures to showcase the impacts of projects that our program supports. *See Attachment A at end of form for questions to assist you in describing the impacts of your project.*

1. Impacts. Describe any impacts of your work. *Impacts* are specific changes in condition for those affected by your work. Impacts include adoption of technology, creation of jobs, reduced cost to the consumer, less pesticide exposure to farmers, access to more nutritious food, and a cleaner environment and healthier communities.

1. It is too early to document impacts

2. Potential impacts. Describe your project's potential impacts. *Potential impacts* are the ways that your project's outputs could directly lead to changes in condition that will unfold in the future.

The ability to detect viruliferous thrips would provide important information on the seasonal dynamics of thrips transmitters which could be targeted for control thus reducing the number of sprays to reduce the thrips-mediated spread of IYSV
2. Identificaiton of weed hosts that could be serving as reservoirs for IYSV would be useful in formulating weed control strategies to reduce the virus inoculum

G. Appendices

1. With your report, please attach *at least two (2) photographs* that illustrate your project. Please describe the photo and indicate the name and institution of the person who took the photo. (If you submit more than two photographs, please include those additional descriptions and photo credits under "H. Additional Information," below.)

Photo #1 description:

Photo #1 credit (photographer's name and institution):

Photo #2 description:

Photo #2 credit (photographer's name and institution):

2. Also attach any printed fact sheets or other publications resulting from your work that will enhance our understanding of your project and its impacts. Please provide a description of each attached publication below.

Document #1 description:

reprints of refereed journal publications are attached

Document #2 description:

Document #3 description:

H. Additional Information

Credit: Some of the language about impacts and potential impacts was adapted from a PowerPoint presentation by H. Michael Harrington, Executive Director, Western Association of Agricultural Experiment Station Directors, Colorado State University.

Attachment A

Questions to Help in Reporting Impacts and Potential Impacts

Below are some questions that will guide you in assessing and then describing the impacts and potential impacts of your project. The relevance of each question may vary depending on whether yours is a research or extension project. Please answer as many as you can to the best of your ability, and feel free to describe any additional types of impacts not mentioned below. Remember to identify any potential impacts.

1. Innovations in IPM:

Are there new IPM practices that have been (impacts) or could be (potential impacts) adopted as a direct result of your project? What is the total number of acres (or homes, schools, greenhouses, nurseries) on which these practices could realistically be implemented?

2. Safeguarding human health and the environment:

- a. Has the project reduced risk (or could it potentially do so) by changing the use of pesticides on farms, in homes, in schools, etc.? For example, could it result in fewer sprays per season or a switch to lower-risk pesticides? If possible, quantify the changes in condition. (Since there is no unanimous definition of *high* and *low risk*, investigators selecting this indicator are asked to categorize the pesticides they are reporting on as *high* or *low risk* according to the particular situation [e.g., lower risk to natural enemies]).
- b. Are there any other impacts or potential impacts on human health or the environment as a result of your project?

3. Economic benefits:

- a. What is (or could be) the economic benefit (e.g., dollars saved) for clientele who adopt IPM strategies and systems you studied? Do you envision potential commercialization or mass production of these systems?
- b. How many clients are satisfied with IPM results (such as improved yield, improved quality of yield, reduced pest populations, more effective pest control, greater preservation of nonpest species)?
- c. Are there other financial benefits that might be realized (potential impact) as a result of your project?

4. Implementation of IPM:

- a. How many IPM strategies and systems have been validated through this project (e.g., through on-farm trials, large plot tests, or other methods used to confirm efficacy)?
 - b. How many educational materials were delivered? To whom? And what are the impacts or potential impacts?
 - c. What is the number of growers/personnel trained? And what are the impacts or potential impacts?
 - d. For a Web site, what volume of traffic and type of use has the site experienced? (For example, number of visitors per day or month; number of page views; number of unique user sessions; change in volume during growing season; average viewing time.) And what are the impacts or potential impacts?
 - e. How many more people adopted IPM practices as a direct result of your project, or how many people adopted new IPM practices?
 - f. Are there other ways in which your work will result in improved use or increased implementation of IPM strategies in your region or across the West?
5. Has your project or study increased collaboration among stakeholders interested in the development and implementation of improved IPM strategies and systems?