Pacific Northwest Vegetable Association

TITLE: Epidemiology and Management of Thrips-Transmitted *Iris yellow spot virus* in Onion Bulb and Seed Crops

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Problem Description

The disease caused by *Iris yellow spot virus* (IYSV), first found in the Treasure Valley of Idaho in the early 1990s, has spread rapidly across the western United States. Since 2000, the disease started to cause economic impact on both bulb and seed onion production. Incidences of near total crop loss were reported both in seed and bulb crops in ID, OR, and WA. The rapid spread of the virus can best exemplified by what happened in Washington State: It was first confirmed in one field in Grant County, WA in 2002 and by 2005, the disease has spread to all the onion-growing counties in the state including Walla Walla County, becoming a significant constraint to bulb and seed production. Most recent reports of IYSV were from New York and Ontario, Canada. IYSV outbreaks were recorded in southern California's Antelope Valley, Imperial Valley in 2006 and we confirmed IYSV in onion crops in northern California in summer 2007 and in Mason Co., Nevada in the summer of 2008. Considered as a rapidly spreading and re-emerging disease, little or no information is available on its epidemiology and the role of thrips vectors. While ongoing research on thrips control practices show some promise, the effect of thrips control on reducing the virus incidence remains to be investigated.

Thrips vectors play a critical role in IYSV spread as the virus is not known to be transmitted through seed. However, the role of thrips vectors and their population dynamics are not known. Preliminary data on the viral strains showed that there could possibly be two distinct virus populations present in the US (Pappu et al., 2006. *Archives of Virology*). A pdf version of the publication is available at http://plantpath.wsu.edu/people/faculty/pappu/pappu.htm

The ongoing research, started this year (2008), would address the above gaps and could potentially lead to an improved understanding of the factors that contribute to the outbreaks (alternate hosts, and the role of thrips vectors) which could lead to development of novel control options. Other deliverables include improved virus detection tools and techniques (IYSV-specific antisera, and a real-time PCR-based detection method). By developing sustainable and environmentally friendly control tactics, the productivity and profitability of the region's onion production can be enhanced.

IYSV, transmitted by thrips, may result in losses of onions from negligible to substantial. The value of fresh bulb onions is approximately $7000 per acre with approximately 20,000 acres in production in Washington State. Seed onion is produced on over 1,000 acres in the state and close to 50% of the seed is exported. Total crop loss due to the disease has been reported in the Columbia Basin (Oregon and Washington) and the Treasure Valley (southwest Idaho and southeast Oregon) in recent years. The Pacific Northwest Vegetable Association has ranked IYSV and thrips as top priorities for research to develop control options.

Project Description:

Strategy: Virus outbreaks are due to the presence of virulent strains, thrips vectors and virus-infected (reservoir) hosts (crops and/or weeds). Improved understanding of the nature of this virus-thrips complex could lead to effective control strategies.

Objectives:

1. Determine the seasonal dynamics of onion thrips vectors in onion seed and bulb crops. Justification: Knowledge on the nature and role of onion thrips in IYSV spread is useful in developing effective thrips management protocols.
2a. Identify and characterize severe strains of IYSV and develop descriptors for virus strain characterization. Justification: Knowledge on the nature and existence of severe strains is essential for developing virus resistant cultivars that are durable and effective.
2b. Screening onion varieties and germplasm for virus resistance. Justification: Growing virus resistant varieties will be the most effective practice to reduce the impact of IYSV. Toward developing resistant varieties, onion varieties and germplasm will continue to be screened under natural field conditions as part of an ongoing collaborative effort with other scientists in WA, OR and CA. Information generated will be useful in identifying varieties with resistance or tolerance to IYSV.

Methodology and Procedures

Objective 1: We propose to determine the seasonal dynamics of the thrips vectors and their role in virus outbreaks. This information will be useful in developing better timing for applying thrips control practices. *T. tabaci* will be monitored with labeled yellow sticky cards (4" x 6") deployed on stakes 1 meter above the ground; this standard thrips monitoring technique will be complemented with whole plant counts. We started this work in summer of 2007, a project funded by WSCPR. Onion fields will be monitored with sticky cards; cards will be replaced weekly during the growing season. Cards will be examined under a microscope and *T. tabaci* adults will be collected and tested individually or in groups for the presence of IYSV using ELISA. We have produced antiserum to the nonstructural gene product, NSs, of IYSV. This antiserum will be used in a serological assay (ELISA) to determine the proportion of viruliferous thrips in selected onion fields. This data will be used to develop the seasonal dynamics of virus transmitters in onion thrips.

Objective 2a: A set of indicator hosts that respond by producing a specific host reaction following mechanical inoculation with IYSV were identified (Bag and Pappu. 2009. Symptomatology of Iris yellow spot virus in selected indicator hosts. *Plant Health Progress* doi:10.1094/PHP-2009-0824-01-BR). These host species produce either localized infection (the virus is able to infect/replicate and produce symptoms in the inoculated leaves but fails to move to younger, uninoculated leaves (=systemic infection). Using this set of IYSV-susceptible, differential indicator hosts, several field-collected IYSV isolates from CA, GA ID, OR, and WA will be screened to identify biologically distinct strains that differ in their disease severity. Preliminary data suggests that there are strains that differ in their ability to colonize and cause systemic infection in infected plants. Simultaneously, efforts to develop an efficient mechanical

inoculation method to infect onion with IYSV will be attempted. We were able to reproduce IYSV symptoms in mechanically inoculated onion plants. IYSV-infected *N. benthamiana* plants appear to be a better source of inoculum to establish IYSV in onion. Existing protocols provided relatively low rates of infection (10 to 20%). A highly efficient mechanical inoculation procedure will facilitate rapid screening of onion varieties and germplasm under controlled greenhouse conditions. In the absence of this method, current evaluations are being done under field conditions where disease incidence varies from year to year.

Objective 2b: Continue evaluation of onion varieties for IYSV resistance. Forty to sixty commercial or experimental onion cultivars will be grown and evaluated for virus presence, virus symptoms, single centeredness, yield and grade in Malheur Experiment Station, Ontario OR. Virus presence will be determined by ELISA from leaf samples. Virus symptoms in each plot will be evaluated subjectively at the time of leaf sampling for ELISA. As part of an ongoing study funded by USDA, onion single centeredness will be determined by cutting 25 bulbs from border rows in each plot. Yield and grade for each plot will be determined after 3 months of storage. An additional replicate of all cultivars will be used for demonstration purposes at a grower field day. Some of the onion varieties with possible IYSV tolerance to be compared with Vaquero, the industry standard: Harmony, Evolution, Granero, Vaquero, Joaquin, Charismatic.

Anticipated benefits:
Proposed research has a high probability of success as the PIs and collaborators have several years of experience working on IYSV and onion. Expected outcomes include increased understanding of the epidemiology of the disease, dynamics of thrips transmitters, information on response of various onion varieties to IYSV infection.

Personnel:
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Likelihood of Success
The above objectives can be accomplished during the proposed time period. PI and the cooperator have the required experience in virus and thrips studies and have the necessary laboratory, greenhouse and field facilities to carry out the proposed research and bring it to a successful completion. PI has already produced antiserum to the viral gene products for their use in ELISA. PI has excellent and very productive working relationships with researchers in the WA State as well those in CA, CO, ID, and OR.

Project Budget

Expenditure	WSCPR (Request)	Co-funding (CASH or IN-KIND)*			TOTAL COST
		Source: Amount (CASH)	Source: Amount (IN-KIND)	Source: Amount (IN-KIND TIME)	
Salaries[1]	15,410	15,410			30820
Employee Benefits	992	992			1984
Temporary or hourly workers					
Travel[2]		2,000			2000
Equipment					
Other (specify)[3]	4,000	3,098			7098
Other (specify)					
Total	20,402	21,500*			41902

[1] Specify the type of position: 0.25 FTE PhD research assistantship is requested.
[2] Provide a brief justification for travel funding requested. All travel must be directly related to the project: travel to research plots in PNW
[3] Materials and supplies: laboratory supplies such as chemicals, restriction enzymes, ELISA reagents, greenhouse supplies (soil, pots, seed).

*Co-funding: Cash from $3,000 from the Pacific Northwest Vegetable Association; $5,000 from Nevada Onion Commission; and 13,500 from the California Garlic and Onion Research Advisory Board

Has this budget been reviewed for accuracy? Yes By Whom? Mary Lou Bricker

Projected Expenditures (by quarter)

Time Period	Jan-Mar 2009	Apr-Jun 2009	Jul-Sept 2009	Oct-Dec 2009	Jan-Mar 2010	Apr-Jun 2010
WSCPR Funds	3400	3400	3400	3400	3400	3402
Total Funds	6983	6983	6983	6983	6983	6987

Has this project been funded previously by WSCPR? __Yes_____

If so, for how long and with what progress? Please summarize progress in less than 200 words. Summary is on the following page.

A set of indicator hosts that respond by producing a specific host reaction following mechanical inoculation with IYSV were identified (Bag and Pappu. 2009. Symptomatology of Iris yellow spot virus in selected indicator hosts. *Plant Health Progress* doi:10.1094/PHP-2009-0824-01-BR). Using this set of IYSV-susceptible, differential indicator hosts, several field-collected IYSV isolates are being screened to identify biologically distinct strains that differ in their disease severity. Preliminary data suggests that there are strains that differ in their ability to colonize and cause systemic infection in infected plants. Efforts to develop an efficient mechanical inoculation method to infect onion with IYSV are ongoing. In 2008 and 2009, 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. Preliminary data showed 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%). Each week, at least 20 onion thrips were collected from each field and from each sampling site and are being tested for IYSV.

Refereed Journal Publications based on research supported by WSCPR. Where possible, funding from WSCPR was acknowledged.

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.