Reducing Risks Associated With Viruses Affecting Legumes in the Inland Pacific Northwest

USDA Risk Avoidance and Mitigation Program, Award 2008-51101-04522

Project Duration: Aug. 2008 – Dec. 2012

Progress Report

April 10, 2011

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Overview: The NIFA award has supported the establishment of the ***Legume Virus Project*** **(LVP)** with three goals: 1) *develop virus risk assessment and decision support tools to facilitate effective vector management to reduce impacts of virus diseases, 2) develop varieties of pea and lentil resistant to the most damaging viruses occurring in the region, 3) integrate these technologies to provide sustainable disease management compatible with overall crop production practices in the Palouse region*

The project was authorized to receive funding in Aug. 2008 and funding was received in October of that year. Modifications to the subcontract structure of the project were required after PI Kevin McPhee moved to North Dakota State University, which was accomplished approximately 1 year into the project. PI Larry Smith (UI extension) retired 1 year into the project and has been replaced by Lydia Clayton (UI extension).

This report has been prepared approximately 30 months into the funding period, with 21 months remaining (including the no-cost extension). In overview,

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Part I. Virus Risk Assessment and Management

Objective 1. Develop a virus risk assessment method for cool season legumes

This objective has had four main parts. a. Monitor pea aphid populations throughout the region each year to estimate virus abundance and spread, b. Use climate predictors to model the disease prevalence, c. Monitor pea aphids and virus in the Columbia basin. d. Better assess the identity and variability of *Pea enation mosaic virus* and *Bean leaf roll virus isolates* and the populations of pea aphid in the region. Data from each of these are anticipated to inform a comprehensive model based on weather and monitoring that can be used to forecast the risk of virus in legume crops grown in the Palouse region.
Part a. Monitoring pea aphid populations in the Palouse region to estimate virus abundance and spread

Damon Husebye, PhD Candidate, and Sanford Eigenbrode,

INTRODUCTION

The Palouse region of the Inland Pacific Northwest is the second largest producer of cool season food legumes (CSFL) in North America. This includes green peas, lentils, chickpeas, winter peas, et al. In 2009, (the last available year of combined data), Idaho and Washington had more than 200,000 acres under CSFL production yielding 206,000 metric tons of produce.

Pea Enation Mosaic Virus (PEMV) and *Bean Leaf Roll Virus* (BLRV) are plant viruses in the family Luteoviridae. Both viruses are persistently vectored by the pea aphid *Acythosiphon pisum.* PEMV and BLRV prevalence on the Palouse is annually variable and has the potential to be a major constraint on CSFL production in the region. The pea aphid is unable to successfully overwinter locally on the Palouse, subsequently pea aphid populations and inoculum must re-colonize CSFL fields annually from external sources. Predominant control strategies have relied on direct reduction of pea aphid densities through insecticide application. Within the context of CSFL production on the Palouse, monitoring incoming pea aphid populations offers the potential of enhancing producer control tactics.

Objective: Monitor early immigrants

Continuing work conducted since 2007, a yellow pan trap network was established among pea growing areas on the Palouse. 27 locations were utilized in 2010, forming an approximately 75x35 mile area, (Figure 1.1 below).

Pan traps were in place May 18, 2010. The first observation was recorded May 20th, 2010: one alate pea aphid alate was collected from the Almota site. This site has consistently yielded early season observations in all 4 years of the study.

In addition to the pan traps, beginning on May 20th field sites in the southern part of the study region were monitored for aphids in the crop using sweep nets, (sites further north were insufficiently advanced to allow practical sweep netting). On May 21st aphids were detected in the crop at three sites, all located in the southern stretches of the study area. Fifty percent or more of the aphids collected from sweep netting were alatae. Aphids were not detected with sweep nets at any sites further north of the Beehive site (approximately 46.9° latitude) until June 7th.

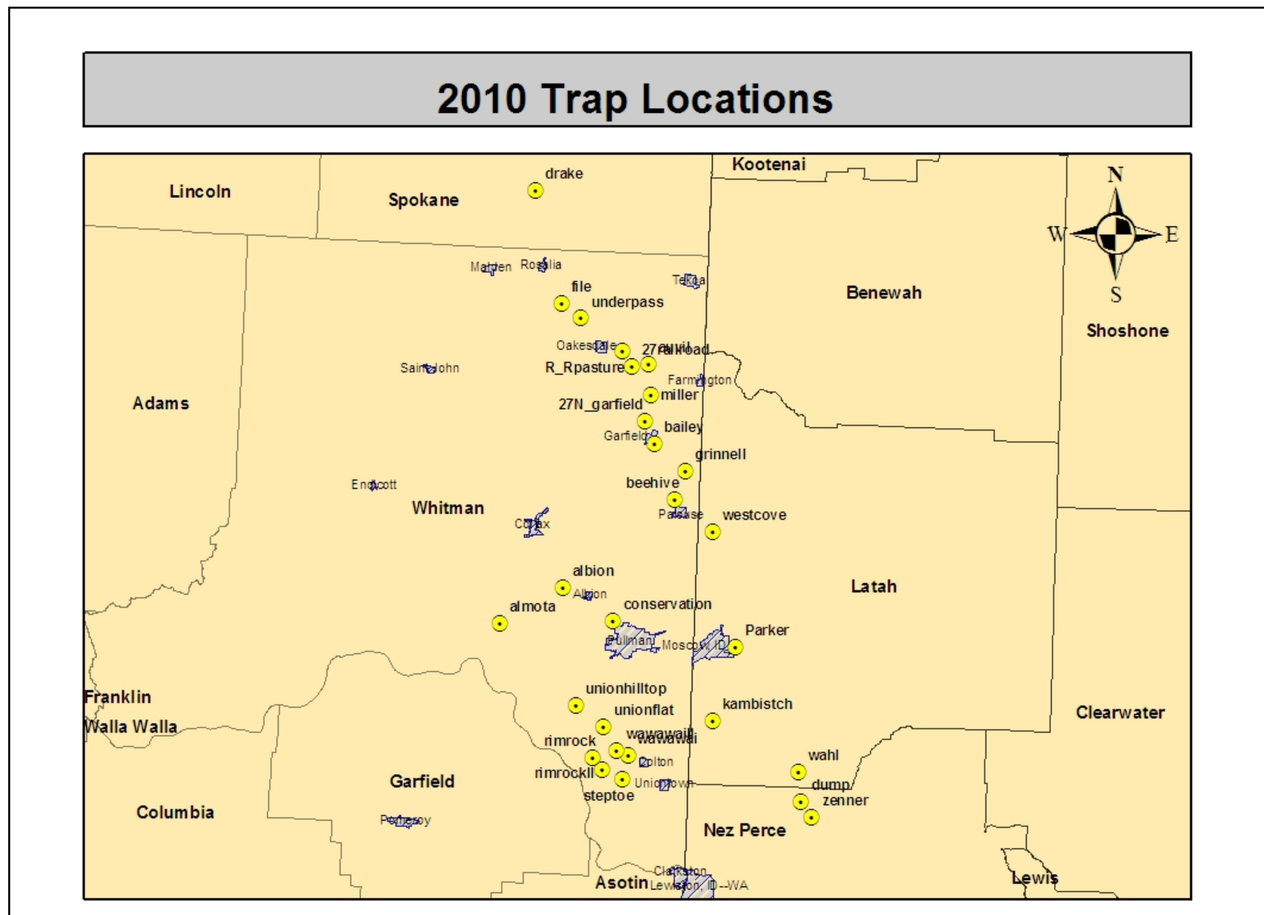

Figure 1.1: 2010 yellow pan trap locations for monitoring aphid immigration

Plant virus was first detected from aphid tissue collected from pan traps at two sites in the southern part of the study region on June 3rd, both were BLRV positive. Less than 1% of all aphids collected from pan traps tested positive for virus over the course of the field season.

Two sites in the southern part of the study region (Union Hilltop, Wawawai II) began to manifest visual symptoms consistent with virus infected plants by early to mid June. Crop tissue was collected from these two sites and tested positive for PEMV.

Plant tissue was collected from *all* field sites twice throughout the season: once in mid-June and again in mid-July. Two sets of transects were conducted, 1) Three, one hundred meter random transects; 2) Three, one hundred meter "targeted" transects that collected symptomatic plant tissue. Each random transect produced 10 plants per transect for a total of 30 plants per site surveyed. Target transects produced a variable number of plants per transect pursuant to the number of plants determined to look symptomatic by the evaluator. The presence of PEMV was confirmed by these surveys, BLRV was not detected in any plant tissue from field surveys despite having been observed in aphid tissue on June 3rd.

With the help of producers, the study this year was able to compare yield figures from field sites with the prevalence of virus detected by the field surveys, (see Figure 1.2). This comparison begins to show a possible trend between disease prevalence and diminished crop yield at the field scale, this is the first time in the four years of the study that georeferenced point source data of virus prevalence has been related with point source data of yield.

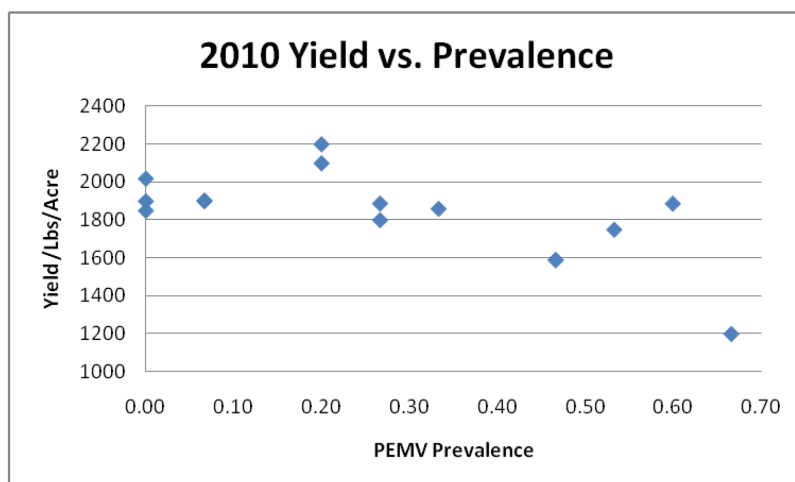

Figure 1.2: 2010 Crop yield to PEMV prevalence in commercial fields

Table 1.1. Summary of surveys conducted prior to and with* RAMP funding

	Year				
	2006	2007	2008	2009*	2010*
Aphid trap-site-days	42	320	475	510	513
Site-days with aphids PEMV-positive (%)	26(61.9)	6(1.8)	2(0.42)	7(1.4)	8(1.5)
Site days with aphids BLRV-positive (%)	3(7.1)	4(1.2)	2(0.42)	8(1.6)	3(0.58)
No. fields sampled at bloom	34	30	26	30	27
Fields positive for PEMV (%)	20(58.8)	3(10)	2(7.6)	7(23)	7(25)
Fields positive for BLRV (%)	7(20.4)	2(6.7)	2(7.6)	6(20)	0
Disease symptoms?	Yes	No	No	Yes	Yes
Fields with disease symptoms (%)	≈5(15)	0(0)	0(0)	≈12(40)	≈11(40)
Clement Index**	1.5	0	0	2	2

** data obtained since project inception,* ** Clement et al. 2010 (see project publications)

Part b. Use climate predictors to model the disease prevalence

Data analysis of putative climate predictors of disease incidence and prevalence outcomes is ongoing. Thus far statistical modeling efforts have been unable to identify a meaningful and reliable forecasting model of risk factors associated with disease outcomes. Utilizing climate variables in Columbia Basin reservoir areas, a regression model has demonstrated a correlation between eventual local aphid *abundance* on the Palouse and variance of temperatures in the basin. Because the pathosystem is dynamic and multidimensional, it is appropriate to further incorporate predictor variables from local conditions on the Palouse into the modeling efforts.
Taken together, and pursuant to more robust quantification, the yellow pan-trap and field survey data are beginning to reveal a non-completely spatially random pattern (CSR). It is a study hypothesis that a disease gradient consistent with a south to north orientation may be at least partially responsible for the observed spread and distribution of inoculum on the Palouse. Utilizing these data it will be possible to generate summary statistics from which expected frequencies can be drawn and used to demonstrate agreement or disagreement with a random null hypothesis. Divergence from a null expectation would strengthen the proposition of a disease gradient and the identification of such a gradient would allow greater characterization of specific location to risk relationships. Quantification of a dispersion gradient could also catalyze the use of climate variables to predict spread and distribution of infection on the landscape and be integrated with economic injury level (EIL) calculations of expected impact of variable aphid densities on yield.

Part c. Monitor pea aphids and virus in the Columbia basin.

Each year of the project, in mid April, a team has surveyed the Columbia basin for pea aphids in leguminous crops and assessed these aphids and the plant tissue for the presence of PEMV and BLRV. d. Better assess the identity and variability of PEMV and BLRV isolates, and the populations of pea aphid in the region. Consistently, aphids are found in alfalfa fields throughout the Columbia Basin and approximately 15% of these fields have tested positive for BLRV. No source of PEMV has yet been identified. Alfalfa has not been found to be a host of PEMV in this region so other legumes have been the focus of our search for PEMV. Aphids have been found on vetch and clover, but these aphids and the crops from which they have been collected have never tested positive for virus. The sampled area has been extensive including most of the basin and the Columbia plateau as far south as Pendleton. We have sampled the riparian zones along the Umatilla River and irrigated fields west of Othello, WA. In 2010 we sampled clover and vetch in the northern Willamette Valley without finding PEMV.
Part d. Better assess the identity and variability of populations of pea aphid and isolates of Pea enation mosaic virus *and* Bean leaf roll virus *isolates in the region.*

Characterizing PEMV and BLRV in the region

Molecular characterization of viruses associated with pea enation mosaic: PEMV-1 and PEMV-2

B. Vemulapati and H.R. Pappu

Overview: *Pea enation mosaic virus* (PEMV, genus: *Enamovirus*) infects several legume crops including chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*) and pea (*Pisum sativum*). *Bean leafroll virus* (BLRV, genus: *Luteovirus*) occurs in Europe, the Middle East, India and the USA infecting legumes that include faba bean, pea, chickpea, cowpea, French bean and lentil. BLRV is phloem-limited and transmitted by aphids in a persistent manner. Thus, aphids play an important role in disease epidemiology. To better understand the role of aphid vectors in virus outbreaks and to develop tools and technologies that would aid in formulating an IPM strategy, several projects were undertaken. These included generating basic information on the structure and sequence diversity of the PEMV and BLRV viral genome, rapid and high throughput ELISA-based methods for detecting the viruses to aid in assessing the virus infection over large areas in PNW and in experimental plots.

Specific Objectives and Results

Genomic Characterization of *Pea enation mosaic virus*-2 from the Pacific Northwestern USA

The genetic diversity of PEMV-2 from two PEMV isolates, one from Idaho (PEMV2-ID) and one from Washington (PEMV2-WA) was determined. The complete genomic ORFs of these two PEMV-2 isolates were sequenced and their sequence properties were determined.

Results:

Each of the sequenced regions of PEMV2-ID and PEMV2-WA isolates contained 4257 nt and 4197 nt spanning four ORFs (1-4) in 5′ to 3′ direction, respectively. The ORF-1 of PEMV2-ID and PEMV2-WA contained 909 nt each that can potentially encode a 302 amino acid polypeptide of 33 kDa of unknown function. ORF-2, along with ORF-1 is expressed as a frame shift fusion polypeptide of ~93 kDa. The RdRp of PEMV2-ID and PEMV2-WA contained 2549 nt and 2540 nt, respectively. Multiple sequence alignment (MSA) of nucleotide and deduced amino acid of the RdRp of PEMV2-ID and –WA, with the available PEMV-2 isolates revealed the presence of nine extra bases in PEMV-ID resulting in three extra codons (ELR) in the RdRp polypeptide. The ORF-3 (long-distance movement protein) in both the isolates was separated from the ORF-2 by an intergenic region (IGR). The IGR in PEMV2-ID contained 216 nt while the PEMV2-WA contained 220 nt similar to the PEMV2-WSG and –UK strains. The PEMV2-ID IGR contained a deletion of four bases (TTTT). The ORF-3 in PEMV2-WA and PEMV2-ID consisted of 693 nt that potentially codes for a polypeptide of 230 amino acids (~26 kDa). The ORF-4 in PEMV-2 overlaps the ORF-3 almost entirely (25 nt downstream of the initiation codon of ORF-3). ORF-4 contained 753 nt potentially encoding a 27 kDa protein of 250 amino acids which function in cell-to-cell movement of PEMV-2. The 3′ untranslated region (UTR) consisting of about 706 nt in PEMV2-ID. The PEMV2-WA partial 3′ UTR contained 671 nt with a 13 nt deletion which was not observed in the rest of the PEMV-2 isolates compared.

The individual ORFs of the PEMV2-ID and PEMV2-WA were compared to the ORFs of other PEMV-2 strains available in the GenBank (Accession #s NC_003853 and AY714213). ORFs 1-4 of PEMV2-ID shared the highest nucleotide and deduced amino acid sequence identities with the PEMV2-UK strain (Table

1.2A). The nucleotide sequence identity of the 3' UTR was 94.6% with the PEMV2-UK. ORFs -1, -2 and 4 of PEMV2-WA shared the highest sequence identity with the PEMV2-WSG while ORF-3 shared the highest identity with PEMV2-WA (Table 1.2B). The 3' UTR had 92.6% identity with the PEMV2-WSG.

Recombination Detection Program (RDP) suggested that PEMV2-WA exhibited a single recombination event spanning 228 nt (nt 42-269) of the ORF-1. There was no evidence of recombination between PEMV2-WA and the other known umbraviruses. The major parent appeared to be PEMV-WSG while the minor parent was PEMV-ID. More isolates need to be characterized to determine if there are additional recombination sites. From the RDP analyses, it appears that the PEMV-WA is a possible recombinant strain of PEMV2-WSG and PEMV2-ID.

Phylogenetic analyses of PEMV-1 isolates from Oregon:

Results:

Two isolates of *Pea enation mosaic virus-1* (PEMV-1), T-1058 (PEMV-OR1) and T-848 (PEMV-OR1) collected from experimental plots from Corvallis were characterized at the molecular level to better understand the sequence diversity. The CP gene was amplified, cloned and sequenced. Sequence analysis revealed that PEMV-OR1 and PEMV-OR2 were closest to the PEMV-ID (Fig 1.3A; Table 1.3A).

Phylogenetic analyses of BLRV from alfalfa and pea from Washington:

Results

Two isolates of *Bean leaf roll virus* (BLRV) isolates designated BLRV-US3 (pea isolate) and BLRV-US4 (alfalfa isolate) collected from Washington were characterized at the molecular level to determine the sequence diversity. The CP gene was amplified, cloned and sequenced. Phylogenetic tree based on CP sequences showed that BLRV-US3 and BLRV-US4 were closest to a BLRV strain 'FRG' from Germany (Fig 1.3B; Table 1.3B).

Progress toward the development of a reliable inoculation protocol for PEMV infection of pea

The present study was initiated to determine the response of various pea varieties and genotypes to mechanical inoculation by PEMV.

Results:
PEMV-WA isolate collected from a pea plant grown in a greenhouse at IAREC, Prosser, WA was used in the study. The presence of PEMV in the source plant was confirmed by DAC-ELISA and RT-PCR. Approximately 100 mg of leaf sample exhibiting mosaic, leaf distortion and enations was ground in 1 ml of 0.05 M potassium phosphate buffer (pH 7.4) containing 0.2% DIECA; 0.2% sodium sulphite . To the homogenate, 30-50 mg Celite was added and gently rubbed on the upper and lower surface of the leaves which were at an unfolded three leaf stage (approximately 10-12 day old seedling). Five cultivars of pea (*Pisum sativum*) were used. ‘EP8221’, ‘Lance’ and ‘Stirling’ were susceptible to PEMV, whereas ‘New Era’ and ‘Lifter’ were rated as resistant to PEMV. Ten plants from cvs. EP8221, Lance and Stirling were tested thrice while five plants each from cvs. New Era and Lifter were tested. Inoculated plants were kept in an insect proof greenhouse maintained at 25 to 30°C, and 12 h light (light intensity 15 to 20 klx) followed by 12h dark period. Inocluated plants were observed for symptom development.

Three cultivars (EP8221, Lance and Stirling) became infected 10-14 days postinoculation (DPI) with leaves showing pin-head chlorotic flecks that gradually increased in size after a 7 day interval. Depending on the cultivar, PEMV produced systemic symptoms like chlorotic flecks, mosaic, enations, leaf deformation, vein banding and vein clearing (Fig. 1.4). Symptom severity on cv. Stirling was more compared to cv. Lance and cv. EP8221. Vein clearing and vein banding was mainly observed accompanied by leaf distortion in the case of cv. Stirling. Cv. EP8221 showed the development of chlorotic flecks which gradually lead to the formation of enations while chlorotic flecks leaf deformation was mainly observed in cv. Lance. Cvs. New Era and Lifter did not get infected. All the three cultivars that got infected exhibited enations on their pods resulting in the reduction in the size of pod and seed. Cv. Stirling showed a higher percentage of infection rates (70%) followed by Lance and EP8221 (60% each) (Table 1.3). The presence or absence of PEMV infection on these inoculated plants (all the five cultivars) was confirmed by DAC-ELISA.

Table 1.2: A) Comparison of percent nucleotide and deduced amino acid sequence identities of ORFs1-4 of *Pea enation mosaic virus*2-ID isolate (PEMV2-ID) with known strains/isolates of PEMV-2. B) *Pea enation mosaic virus*2-WA isolate (PEMV2-WA) with known strains/isolates of PEMV-2.

(A)

PEMV2-ID ORFs	PEMV-2 isolate* % nucleotide identity			PEMV-2 isolate* % amino acid identity		
	WSG	UK	WA	WSG	UK	WA
ORF-1; unknown protein	91.5	92.1	92.4	91.0	91.7	91.0
ORF-2; RdRp	92.7	93.2	92.1	94.3	95.0	93.7
ORF-3; long-distance movement protein	94.2	97.2	95.5	90.6	94.0	90.4
ORF-4; cell-to-cell movement protein	97.0	97.4	95.8	97.2	98.0	96.4
3' UTR	94.4	94.6	88.1	-	-	-

(B)

PEMV2-WA ORFs (nt)	PEMV-2 isolate* % nucleotide identity		PEMV-2 isolate* % amino acid identity	
	WSG	UK	WSG	UK
ORF-1; unknown protein	93.3	93.2	92.7	92.7
ORF-2; RdRp	96.0	94.2	95.5	95.0
ORF-3; long-distance movement protein	96.0	97.2	94.0	94.7
ORF-4; cell-to-cell movement protein	98.6	97.6	98.4	97.6
3' UTR	92.6	89.3	-	-

Table 1.3: Percent deduced coat protein amino acid sequence analyses (A) *Pea enation mosaic virus*-1 (PEMV-1) from Oregon with GenBank sequences. (B) *Bean leafroll virus* (BLRV) from alfalfa and pea from Washington with GenBank sequences.

(A)

PEMV1 Strain/ isolate/ origin	Designation used	GenBank Accession #	% AA identity
WSG	PEMV-WSG	L04573	97.7
AT-	PEMV-AT-	Y09100	97.4
AT-D	PEMV-AT-D	Y09098	96.8
UP58	PEMV-UP58	AY661882	96.4
Germany	PEMV-FRG	Z48507	96.3
SP	PEMV-SP	AF082833	94.2
AT+	PEMV-AT+	Y09099	96.3
ID	PEMV-ID	HM439775	**97.8**
T-1058	**PEMV-OR1**		100

(B)

BLRV Strain/ isolate	Designation used	GenBank Accession #	% AA identity
USA	BLRV-US1	AF441393	99.4
USA	BLRV-US2	U15978	99.4
Germany	BLRV-FRG	X53865	**100.0**
USA, WA, pea	**BLRV-US3**		100.0
USA, WA, alfalfa	**BLRV-US4**		

Table 1.4: Response of five cultivars to *Pea enation mosaic virus*.

Cultivar	Symptoms	Symptoms -DPI (Days)	Number of plants infected (DAC-ELISA positives)/ inoculated Trial 1 (06/01/10)	Trial 2 (06/10/10)	Trial 3 (06/20/10)	Percent infection
Lance	Chlorotic flecks, Leaf deformation	10-14	05(05)/08	05(05)/08	08(08)/14	60
EP-8221	Chlorotic flecks, Leaf deformation	10-14	04(04)/08	06(06)/08	08(08)/14	60
Stirling	Vein clearing, Leaf deformation	10-14	05(05)/08	06(06)/08	10(10)/14	70

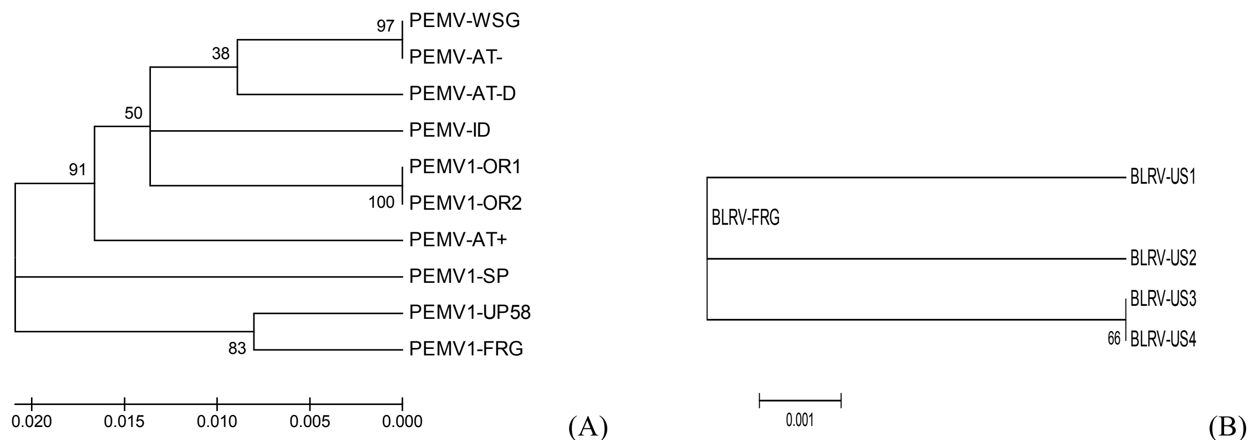

Fig 1.4: Coat protein-based phylogenetic tree of A. *Pea enation mosaic virus*-1 (PEMV-1); B. *Bean leafroll virus* (BLRV).

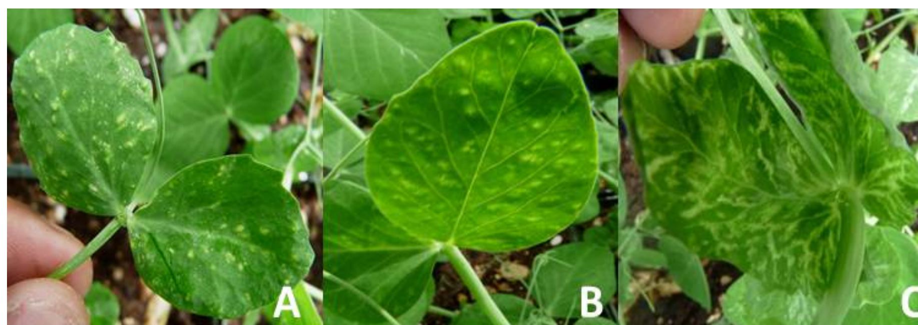

Fig.1.5: Symptoms produced by *Pea enation mosaic virus* (PEMV) infection on three pea cultivars. A. Chlorotic flecks, Enations and leaf deformation on EP8221; B. Mosaic, chlorotic flecks on Lance and, C. - Vein clearing, vein banding and leaf deformation on Stirling.

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Biotyping Pea Aphids to Understand Migration Patterns

Sanford Eigenbrode, Damon Husebye and David Hawthorne

We have collaborated with David Hawthorne, Univ. Maryland, who has genotyped aphids from our regional surveys using a set of approximately 150 AFLP markers. This set is capable of distinguishing among host associated biotypes of the pea aphid. Working with 150 individuals collected in 2009, we have detected evidence for genetic structure in the pea aphid populations. Specifically, aphids collected from alfalfa are genetically distinct from those collected from peas. Within alfalfa there are two genetic types. Pea aphids collected in our pan traps on the Palouse are genetically more diverse than aphids collected from specific host plants. Early season migrants (alates) collected within pea fields are also more diverse than is typical within a single crop type. As the season progresses, variability diminishes and genotypes more typical of those found on pea elsewhere predominate in the Palouse. These patterns appear consistent with the hypothesis that prevailing winds bring a diversity of pea aphid types into the Palouse but that a particular type becomes predominate because of the prevalence of pea and the tendency of one particular biotype to perform best on pea. By inference, the sources of virus inoculum are potentially diverse and include all pea aphid types detectable in the region. An example is shown in Fig. 1.5.

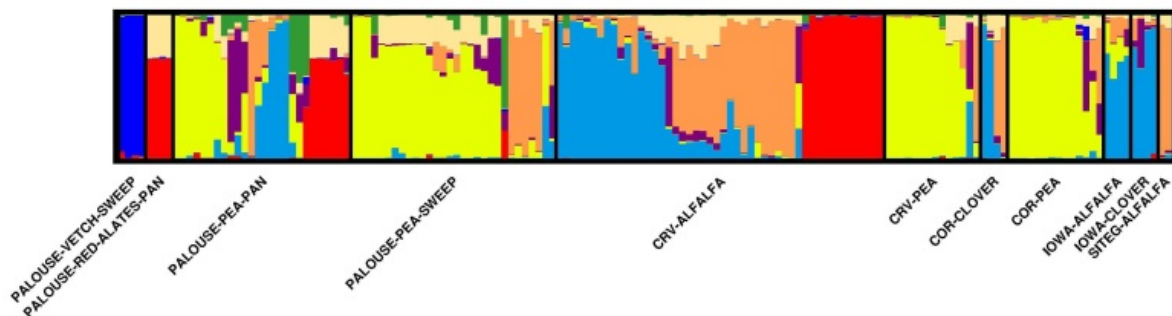

Fig.1.5. Genotypes of pea aphid collected from various parts of the study region, as estimated using the program "Structure". Each vertical line represents a single aphid, genotyped using an AFLP procedure based on 150 markers. All aphids were collected in 2009. Along the X axis are sources: Palouse-vetch-sweep = commercial vetch field, sweep net sample; Palouse-red-alates-pan = red-bodied alates taken from pan traps in the Palouse; Palouse-Pea-Pan = alates captured in pan traps near pea fields; Palouse-Pea-Sweep = taken using sweep netting in commercial pea fields; CRV-Alfalfa = Columbia River Valley (Columbia Basin) sweep net samples from alfalfa; CRV-pea = Columbia Basin sweep net samples from pea; Cor-Clover = Corvallis OR, clover sweep net samples, etc. Principal patterns of interest: genotypes from alfalfa in Columbia Basin and elsewhere differ from those taken from pea across the region. Palouse pan trapped aphids are genetically more diverse than those eventually colonizing the crop (analysis by David Hawthorne, Univ. of Maryland).

Characterization of the laboratory host range of PEMV

Xue Feng and Alexander V. Karasev

The casual agent of Pea enation mosaic disease consists of two distinct, yet dependent viruses: PEMV-1 which belongs to *Enamovirus*, and PEMV-2 which belongs to *Umbravirus.* The genome of each virus consists of single-stranded, plus-sense RNA. PEMV is transmitted by pea aphids in a persistent manner. Nymphs can acquire the virus in 15 minutes and adults in 120 minutes.

Pea enation mosaic disease can be a problem in many legume growing areas worldwide, including sporadic problems in peas and lentils in the Palouse area of Central/Eastern WA and Northern Idaho. Epidemics start in late spring and then spread through the early August. Since PEMV does not appear to be endemic in this area, finding the source of virus should be of great importance. Until now, the local host for PEMV to overwinter is unknown and each year the virus is brought back by pea aphids.

The objective of this study is to find the potential overwintering host for PEMV through a laboratory host range test. The host should fulfill several requirements: (i) it has to support PEMV replication; (ii) it has to be available in Central/Eastern WA, and (iii) it has to be an attractive host for pea aphids. Our working hypothesis is that is likely a wild or cultivated legume.

MATERIALS AND METHODS

Plants, aphids and virus preparation

The plant species we tested include the peas, lentils, Faba beans, alfalfa, lupine, 5 different kinds of clover including the yellow blossom, medium red, patriot, durana white and white clover and 3 kinds of vetch. We got the seeds of peas, lentils and Faba beans from the entomology lab; ordered the seeds of alfalfa, lupine and 5 kinds of clovers (most popular clovers growing in this area) from the commercial seed company and got the seeds of 3 kind of vetch from USDA germ plasmid center. The seeds were planted in the little pots, with soil and fertilizers. 10 pots per species, each pot one or two plants. The pots were kept in green house. Usually, peas, lentils, beans and alfalfa will germinate in about 7-10 days and will be ready for inoculation when they are 5-6 leaves big. Lupine and vetch spent longer time to germinate, often need to wait about 2-3 weeks after planting in the soil. Vetch will be ready for inoculation when they are 6-7 leaves big and lupine will be ready for inoculation 7-10 days after germination. Clovers germinate fast, usually 1 week is enough and 5-7days after that can be used for inoculation.

When seedlings were ready, PEMV infected leaves were collected from the PEMV colony and used for mechanically inoculation. Infected leaves from the colony plants were ground by mortar with PBS buffer and then we took the sap of the plants to do the inoculation. Carborundum powder was firstly used to create the microwound and then the sap was used to rub the leaves again. The PEMV colony was established by entomology lab and we kept testing the leaves collected from the plants in the colony. The result showed the colony was maintained quite well and most leaves were really PEMV-infected leaves. Each time before inoculation, the leaves were tested by PCR.
We had also checked clover plants by aphid transmission. When clover seedlings were ready, aphids' nymph from the PEMV colony were used to inoculate the seedlings. 10 aphids were used in one clip

cage and several clip cages were placed on one clover seedling. The clip cages were stayed for 72h and then the inoculated plants were kept in the green house for testing.

Extraction of RNA from the leaf tissue of plants

The host range test was repeated three times. Each time young growing leaves from the top were collected 7, 14 and 21 days after inoculation. Virus RNA was extracted by Dellaporta I method and then amplified by RT-PCR. The product of PCR was fractionated on the 1.2% TBE-agarose gel and the result was analyzed. PEMV-1 and PEMV-2 was separately amplified by different specific primers.

RESULTS AND DISCUSSION

Of the 13 plant species tested, peas and lentils can be consistently infected by PEMV. From the first repeat (Table.1.6a), one pea out of ten was infected by PEMV and one lentil out of ten was infected by PEMV. From the second repeat (table.1b), two peas out of ten were infected by PEMV and two lentils out of ten were infected by PEMV. From the third repeat (Table.1c), two peas out of ten were infected by PEMV and one lentil out of ten was infected by PEMV. From the separate host range test on clovers by aphid transmission (Table.1.7), pea plant was used as a positive control and one pea out of ten was infected by PEMV and no clovers were found to be infected in the test. We have also tried to correlate the symptom with the result of RT-PCR (Table. 1.8). However, we fail to find any PEMV-infected symptomatic plants during the test. Although sometimes the inoculated plants developed some virus-like symptom, meanwhile the negative control plant also showed the same symptom. It indicated the symptom we observed was not caused by PEMV.

Table.1.6a

Species	1st Repeat		
	7days after inoculation No. of plant infected	14days after inoculation No. of plant infected	21days after inoculation No. of plant infected
Peas	1/10	1/10	1/10
Lentils	1/10	1/10	1/10
Beans	0/10	0/10	0/10
Alfalfa	0/10	0/10	0/10
Lupine	0/10	0/10	0/10
Yellow blossom clover	0/10	0/10	0/10
Medium red clover	0/10	0/10	0/10
Patriot clover	0/10	0/10	0/10
Durana white clover	0/10	0/10	0/10
White clover	0/10	0/10	0/10
Vetch #1	0/10	0/10	0/10
Vetch #2	0/10	0/10	0/10
Vetch #3	0/10	0/10	0/10

Table.1.6b

Species	2nd Repeat		
	7days after inoculation No. of plant infected	14days after inoculation No. of plant infected	21days after inoculation No. of plant infected
Peas	1/10	2/10	2/10
Lentils	1/10	2/10	2/10
Beans	0/10	0/10	0/10
Alfalfa	0/10	0/10	0/10
Lupine	0/10	0/10	0/10
Yellow blossom clover	0/10	0/10	0/10
Medium red clover	0/10	0/10	0/10
Patriot clover	0/10	0/10	0/10
Durana white clover	0/10	0/10	0/10
White clover	0/10	0/10	0/10
Vetch #1	0/10	0/10	0/10
Vetch #2	0/10	0/10	0/10
Vetch #3	0/10	0/10	0/10

Table.1.6c

Species	3rd Repeat		
	7days after inoculation No. of plant infected	14days after inoculation No. of plant infected	21days after inoculation No. of plant infected
Peas	2/10	2/10	2/10
Lentils	1/10	1/10	1/10
Beans	0/10	0/10	0/10
Alfalfa	0/10	0/10	0/10
Lupine	0/10	0/10	0/10
Yellow blossom clover	0/10	0/10	0/10
Medium red clover	0/10	0/10	0/10
Patriot clover	0/10	0/10	0/10
Durana white clover	0/10	0/10	0/10
White clover	0/10	0/10	0/10
Vetch #1	0/10	0/10	0/10
Vetch #2	0/10	0/10	0/10
Vetch #3	0/10	0/10	0/10

Table. 1.7. Results from three repeat host range tests by mechanically inoculation: plants were tested after 7, 14 and 21 days after inoculation. 1a, one pea out of ten was infected by PEMV and one lentil out of ten was infected by PEMV. 1b, two peas out of ten were infected by PEMV and two lentils out of ten were infected by PEMV. 1c, two peas out of ten were infected by PEMV and one lentil out of ten was infected by PEMV.

Species	Aphid Transmission		
	7days after inoculation No. of plant infected	14days after inoculation No. of plant infected	21days after inoculation No. of plant infected
Yellow blossom clover	0/10	0/10	0/10
Medium red clover	0/10	0/10	0/10
Patriot clover	0/10	0/10	0/10
Durana white clover	0/10	0/10	0/10
White clover	0/10	0/10	0/10
Peas	1/10	1/10	1/10

Table. 1.8 Results from the separate host range test on clovers by aphid transmission. Plants were tested after 7, 14 and 21 days after inoculation. Pea plant was used as a positive control and one pea out of ten was infected by PEMV and no clovers were found to be infected in the test.

Host	Symptoms	RT-PCR
Peas	no	yes
Lentils	no	yes
Beans	no	no
Alfalfa	no	no
Lupine	no	no
Yellow blossom clover	no	no
Medium red clover	no	no
Patriot clover	no	no
Durana white clover	no	no
White clover	no	no
Vetch #1	no	no
Vetch #2	no	no
Vetch #3	no	no

No distinct PEMV symptom was found during the whole host range test.

Overall, several conclusions are drawn from above: (i) PEMV can infect peas and lentils under laboratory conditions, (ii) none of the clovers supported PEMV replication (both mechanical and aphid inoculation), (iii) None of the other legumes tested was infected with PEMV in our lab tests. We do not know at the

moment what plant may serve as a host for overwintering of PEMV. In the further study, we would test more legumes or go to the landscape to find potential winter host for PEMV.

Objective 1. ONGOING AND PLANNED ACTIVITIES

We will again deploy a yellow pan-trap network for the 2011 growing season. As in past seasons, trap site placement will seek to maximize consistency of coverage established in past seasons, while also being contingent upon CSFL field availability and producer concerns. These data will further augment the modeling efforts discussed above.

The behavior of individual pea aphids may also have a structuring effect on the dispersion and prevalence of field infections. Bioassays are being undertaken to identify if pea aphids manifest a prevalence for infectious vs. non-infectious host plants. The presence/ absence of a behavioral response to host infection status could have dramatic implications for the quality of secondary spread of infection within and between crop fields. Furthermore, the quality and quantity of any response may exhibit differential outcomes as a function of variable aphid biotype proportions. Preliminary data developed in cooperation with Dr. David Hawthorne's lab at the University of Maryland, suggests the composition of pea aphid populations assort in relation to the dominate host plant matrix on the landscape. Variance of this type of population structure may have an important effect on annual variance of PEMV/BLRV dispersion on the Palouse, especially if there is differential infectivity associated with behavioral responses.

This year we have also begun to develop a smart-phone application using the "Epicollect" software platform. This is a freeware web application that utilizes Google AppEngine for the development of mobile data collection projects. This has the potential to expand the monitoring network and increase the speed of information dissemination between producers, and can be operated and maintained by producers with minimal cost. The application is based on freeware developed at Imperial College London and is funded by the Wellcome Trust. A beta-testing workshop is being developed with producers and will eventually be posted from the Aphid-tracker website for downloading and use by producers.
In 2011, the search for PEMV sources will extend south to Corvallis OR.

Host range of PEMV will be assessed again using different inoculation techniques and detection methods.

Objective 2. Develop tools for improved virus vector management

Economic injury levels and thresholds: Quantification of crop damage to dry peas (*Pisum sativum* 'Aragorn') caused by viruliferous and non-viruliferous pea aphids (*Acyrthosiphon pisum* Harris)

Bradley S. Stokes, Edward J. Bechinski and Sanford D. Eigenbrode

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris), is a pest of dry peas, *Pisum sativum*, in the Palouse agricultural area of eastern Washington and northern Idaho. Pea aphids indirectly reduce crop yields by vectoring two economically important virus diseases: Bean Leaf Roll Virus (BLRV) and Pea Enation Mosaic Virus (PEMV) (Figs. 2.1 and 2.2, respectively). Because both BLRV and PEMV are circulative and persistent in their transmission by pea aphids, it may be possible for commercial growers to reduce disease incidence by applying insecticides that kill the aphid vectors before they transmit these viruses (Perring et al. 1999, Mowry 2001, Latham & Jones 2004, Jeger et al. 2004, Andret-Link & Fuchs 2005, Hooks & Fereres 2006). Plant infestation by a single viruliferous pea aphid can initiate BLRV or PEMV disease. Hence, we hypothesize that the yield impacts of aphids as virus vectors depends primarily on the time when plants are infected, rather than on aphid density or duration of infestation. The relationship between timing of infection and crop yield is unknown for BLRV and PEMV in dry peas.

Figure 2.1. BLRV symptoms

Figure 2.2 PEMV symptoms

In addition to causing indirect crop yield loss, pea aphids also can directly damage dry peas by consuming phloem-translocated photosynthates (Figure 2.3). The relationship between aphid density and yield loss is unknown in the Palouse. Earlier studies in Canada (Maiteki and Lamb 1985) suggested an economic threshold value of 2-to-3 pea aphids per stem during the reproductive growth stages of dry peas.

Figure 2.3. Aphid-infested pea plant

Figure 2.4. Study site

Materials and Methods

Small-plot field studies were conducted June through August during 2009 and 2010 at the University of Idaho Parker Farm near Moscow, Idaho (Fig. 2.4). The study site was seeded with *Pisum sativum* 'Aragorn', a semi-leafless variety that is the regional commercial standard. Plot management followed industry practice, except for the absence of insecticide applications.

Indirect-Damage Field Experiments

Experimental design was a randomized complete block with two treatments: (1) BLRV or PEMV infected aphids, and (2) time of plant inoculation with viruliferous aphids. Each treatment was replicated 10 times. Replicates consisted of 10 plants in a single row, typically ranging from 25-40 cm in length (Fig. 2.5). Replicates were isolated 3-m linearly and 3-m side-to-side.

Studies began as soon as plants were large enough to support a leaf clip-cage. Three viruliferous aphids (positive for either PEMV or BLRV) from greenhouse colonies were transferred to each leaf clip-cage (Fig. 2.6) and immediately transported to the field where one cage was clipped to each plant within a replicate. Each clip cage was placed randomly on the median node stipule (from soil level to meristem). Encaged aphids remained on each plant for a 72-hour inoculation access period, after which cages were removed and plants were sprayed to runoff with insecticidal soap to eliminate the aphids. Treatment densities and access periods were based on pilot field studies during 2008 (Eigenbrode), which had demonstrated virus transmission to plants.

Each year plant inoculation with viruliferous aphids began approximately 10 days after plant emergence. Inoculations continued every 3rd or 4th day until pea plants reached maturation and senescence (approximately 60 days after emergence) for a total of 14 inoculation dates each year. Yield parameters were measured at crop maturity by clipping stems at the soil level, placing foliage within paper bags, then into drying ovens for 48-hrs at 90 degrees Fahrenheit. Seeds were obtained by hand-threshing seed pods. Response parameters were seed weight per replicate and total biomass per replicate. For statistical analysis, seed weight for each virus was re-expressed as standardized yield by dividing mean weight for each inoculation date (i.e., mean of 100 plants per inoculation date for each virus) by the

mean seed yield weight for that virus from the last inoculation date. Seeds also were sieved through standard 11/64"x3/4" oblong and 16/64" round grading pans to determine commercial yield grade, but those data have yet to be analyzed and are not presented. Regression analyses and ANOVA were run using SAS 9.1.

Figure 2.5. A single PEMV replicate

Figure 2.6. Clip cage containing 3 aphids

Direct-Damage Field Experiments

Experimental design was a randomized block with two treatments: aphid density and plant growth stage at infestation. There were six density treatments, each with four replicates during 2009 and five replicates during 2010: (1) 0 aphids, (2) 4 aphids, (3) 9 aphids, (4) 18 aphids, (5) 90 aphids, and (6) 270 aphids released from greenhouse colonies into A-frame field cages for a 17-day infestation period. We intended that these treatments generate a wide range in realized aphid densities rather than replications of these specific levels. The 2009 experiment was limited to infestations imposed during reproductive plant growth beginning 46 days after plant emergence. Experiments during 2010 were conducted during two plant growth stages: late vegetative growth beginning 46 days after plant emergence and early reproductive growth beginning 32 days after plant emergence.

Replicates consisted of 18 pea plants in a single row (96cm long) encaged within A-frame screened cage (Fig. 2.7). Replicates were isolated 3m linearly and 3m laterad. The middle 10 pea plants of each replicate were reserved for seed yield measurement at the end of the season, while the remaining plants on both row ends (North and South) were saved for destructive sampling to determine realized aphid density. Two randomly selected plants per replicate (one from the North, one from the South) were cut at the soil surface and examined for pea aphids on days 3, 10 and 17 following initial infestation. The Day 3 census provided an estimate of aphid cohort mortality while the Day 10 and 17 censuses measured subsequent population growth. We assumed that aphid density on plants sampled were representative of densities on the center 10 plants reserved for seed yield analysis.

A-frame cages were removed on Day 17 and aphid infestations were terminated by spraying plants with insecticidal soap; replicates were kept free of subsequent natural infestations by applying insecticidal soap as needed until plants matured and senesced. Seed yield was measured as described in the

indirect-damage experiment and regression models were computed for each experiment between yield and various expressions of aphid density. When sufficiently dry, plants were hand harvested (Fig. 2.8)

Figure 2.7. A-frame cages in field

Figure 2.8. Replicate ready for harvest

Results

Indirect-Damage Field Experiments (Fig. 2.9)

1. Yield loss was most severe the earlier plants were inoculated with viruliferous aphids.

2. There was considerable variation in seed-yield dry weight among plants within replicates on each inoculation date for both BLRV and PEMV during 2009 and 2010.

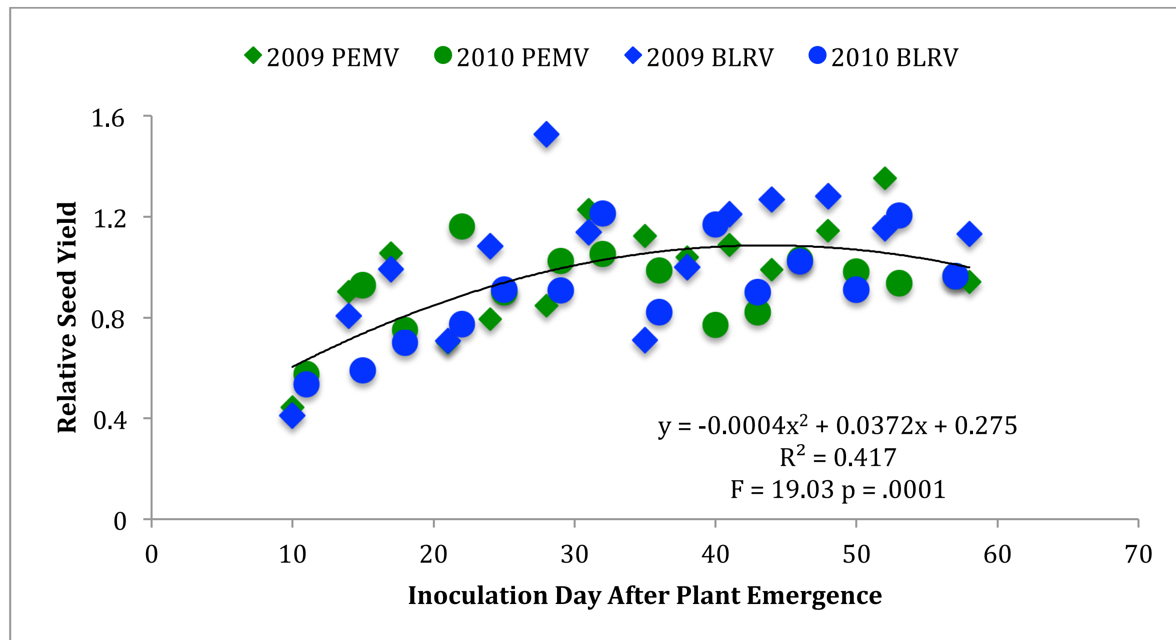

Figure 2.9. Relative seed yield during 2009 and 2010 as a function of the day after plant emergence on which replicates were inoculated with clip cages containing 3 aphids positive for either *Pea enation mosaic virus* (PEMV) or Bean *leaf roll virus* (BLRV). Each data point is the mean seed yield response of 10 replicates (100 plants). Mean seed yield for each replicate was standardized to the mean seed weight from the last inoculation period each year.

3. Simple linear models and linear threshold models with a yield plateau were poor descriptors of the relationship of seed yield and date of viruliferous aphid infestation.
4. Curvilinear models best described the relationship between seed yield and date of viruliferous aphid infestation, but accountability of statistical models was low-to-moderate.

<u>Direct-Damage Field Experiments</u> (Figs. 2.10-2.13)

1. The 2009 experimental protocol successfully generated the desired large range of realized aphid densities (Fig. 2.10).

The linear model was a poor descriptor of yield loss during 2009. It especially failed to account for dramatic declines in seed yield among plants infested with 1 to 100 aphids.

Relative yield best was described statistically as a curvilinear function of aphid density from the Day 3 post-infestation census date. The best-fit model suggests that per capita damage declines as a function of aphid density.

2. Fig. 2.11 was derived from Fig. 2.10. It incorporated only those data points less than 100 aphids per plant because Fig. 2.10 showed that economic decreases in seed yield (i.e. >5% loss) began at low pest densities. A simple linear model best described this relationship. We used this model to calculate economic injury levels for pea aphids on dry peas.

3. There were no statistically significant relationships between seed yield and realized aphid density during 2010 for neither the late-vegetative plant growth stage infestation (Fig. 2.12) nor the reproductive plant growth stage infestation (Fig. 2.13). Realized aphid densities during 2010 were substantially smaller than those from our 2009 experiments.

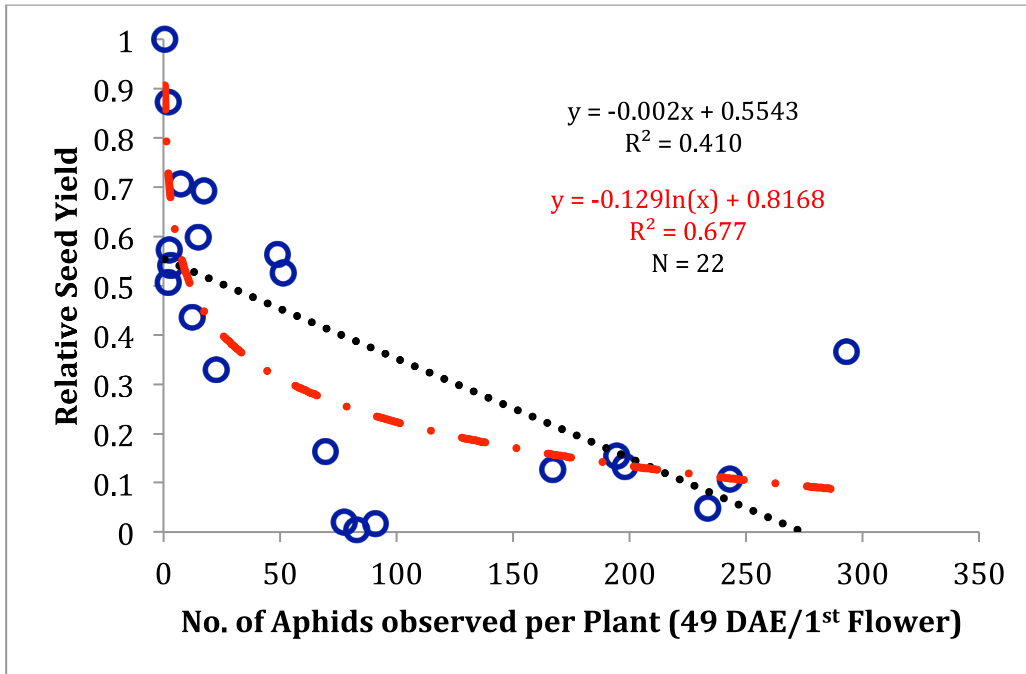

Figure 2.10. Relative seed yield from plants inoculated with aphids for a 17-day infestation period during the reproductive stages of crop growth in 2009. Aphid density is the mean of two plants from the Day 3 census; seed yield is the mean of one replicate (10 plants). Mean seed yield for each replicate was standardized to the highest yielding control plot.

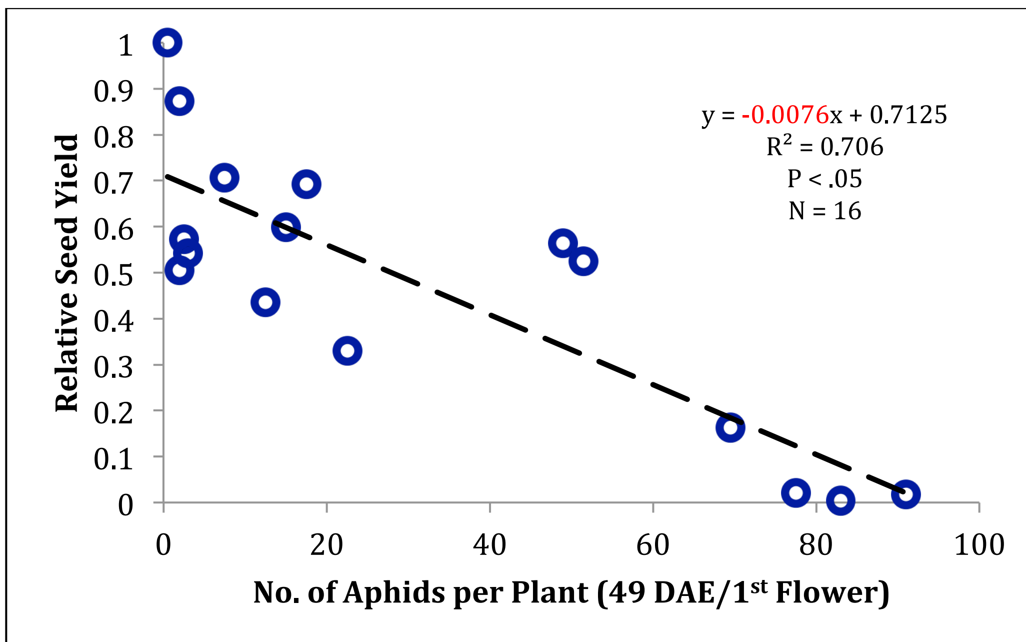

Figure 2.11. Relative seed yield from plants inoculated with aphids for a 17-day infestation period during the reproductive stages of crop growth in 2009. Figure manipulated from Fig. 2.10 to show data points fewer than 100 aphids per plant.

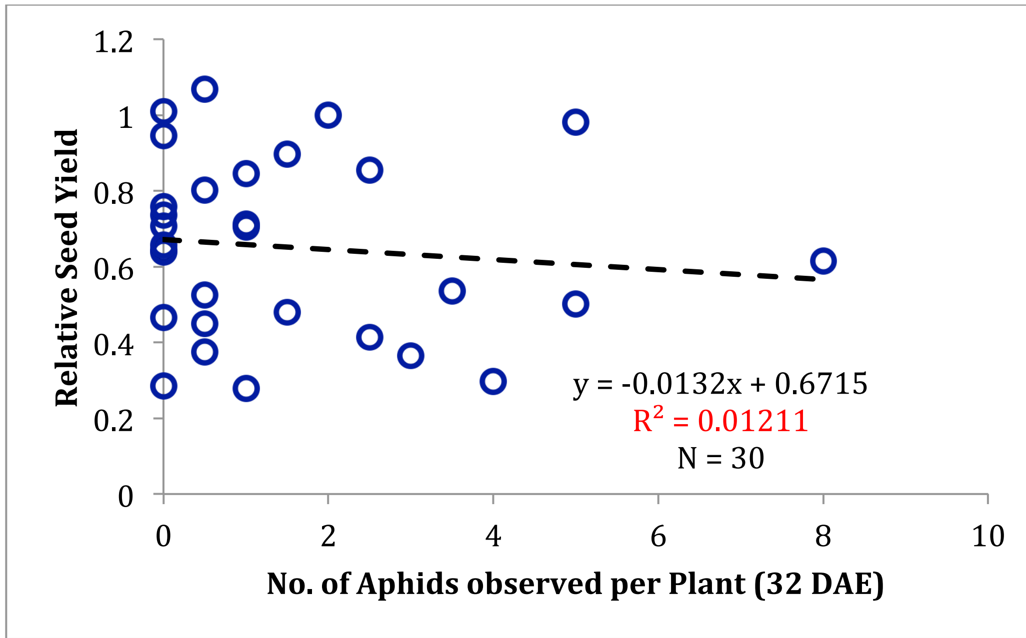

Figure 2.12. Relative seed yield from plants inoculated with aphids for a 17-day infestation period during the late vegetative stages of crop growth in 2010. Aphid density is the mean of two plants from the Day 3 census; seed yield is the mean of one replicate (10 plants). Mean seed yield for each replicate was standardized to the highest yielding control plot.

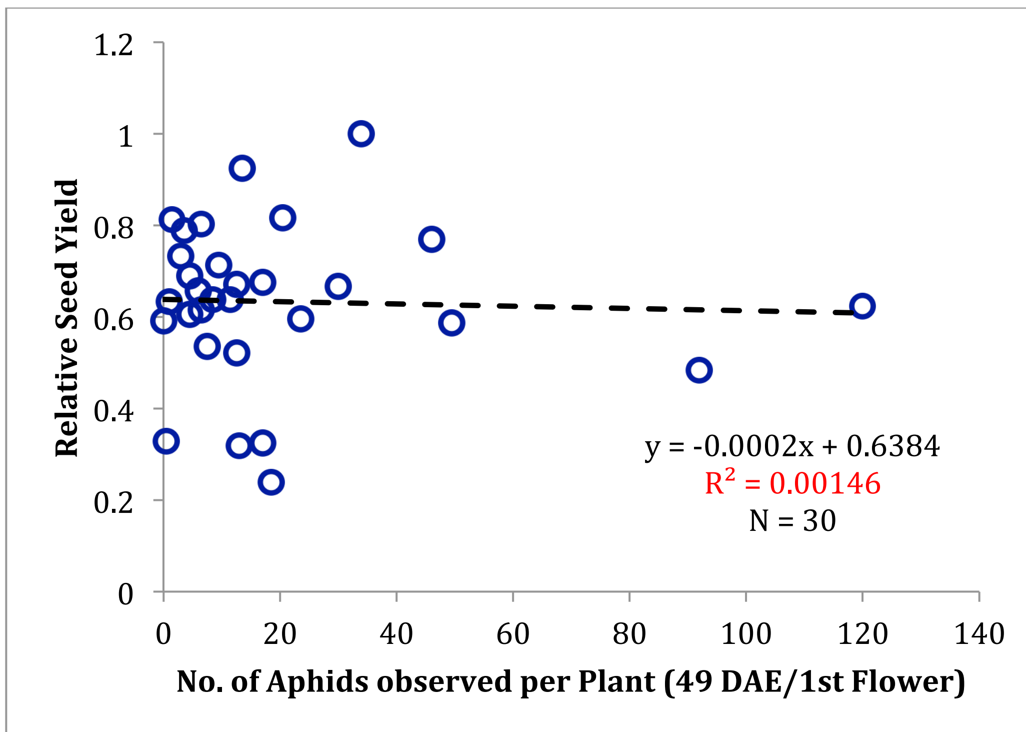

Figure 2.13. Relative seed yield from plants inoculated with aphids for a 17-day infestation period during the reproductive stages of crop growth in 2010. Aphid density is the mean of two plants from the Day 3 census; seed yield is the mean of one replicate (10 plants). Mean seed yield for each replicate was standardized to the highest yielding control plot

Discussion

Indirect-Damage Field Experiments

Pea yield loss from both viruses was economically catastrophic when plants were inoculated with viruliferous aphids immediately after plant emergence. But statistical models show that early-season BLRV caused more severe losses than early-season PEMV. In particular, regression models for 2009 and 2010 predict an average 48% decrease in seed yield if pea plants are inoculated 10-days after emergence with aphids **viruliferous** for BLRV; but a 30% loss if plants are inoculated with aphids viruliferous for PEMV. Differential yield impacts between BLRV and PEMV essentially disappear when pea plants are inoculated 40-days or more after crop emergence.

Given current crop market values, regional crop yield potentials and insecticide purchase and application expenses, commercial producers of dry peas in the Palouse region can tolerate up to 5% seed yield reduction without incurring economic loss; if expected crop yield loss is more than 5%, growers can **more** than recoup the cost of insecticide application. The 2009 and 2010 BLRV regression models predict that economic loss occurs if plants are first colonized by viruliferous aphids within 28-days of crop emergence; the 2009 and 2010 PEMV models similarly predict economic loss if pea plants are first colonized by viruliferous aphids within 23-days of crop emergence.

We combined the 2009 and 2010 data from both virus inoculation experiments into a single analysis so as to increase **model** robustness. Results are presented in a simple graphical decision aid (Fig. 2.14) that commercial growers can use to predict categories of yield loss and determine the need for insecticides applied as a foliar spray to kill colonizing pea aphids. Growers can conservatively assume that colonizing aphids are positive for either BLRV or PEMV. Alternatively, growers can await on-line results from regional University of Idaho *APHIDTRACKER* pan-trap survey network for real-time data about the incidence of viruliferous aphids.

Direct-Damage Field Experiments

The regression model from the 2009 reproductive stage plant growth experiment has sufficient explanatory **power** (i.e., $R^2 = 0.706$) to compute economic injury levels (EIL) that pea producers can use to decide if infestations of non-viruliferous aphids justify insecticide application in commercial fields. We calculated values with the formula from Pedigo et al. (1986):

$$EIL = C/(DYVK)$$

where C is control cost ($/acre for insecticide purchase and application), D is per capita damage coefficient (proportion crop yield loss per aphid per plant), Y is historic crop yield potential (cwt/acre), V is anticipated crop market value ($/cwt), and K is killing power, the proportional reduction in pest density following insecticide application. Here the value for D is given by the slope term from the regression model, $y = -0.0076x + 0.7125$, where D is equal to .0076. The resulting EIL values (Table 1) can be used by growers to make quick management decisions.

Our methods for both 2010 direct-damage experiments were essentially the same as 2009, except for the application of insecticidal soap, which was heavily applied to pea plants 2 days before the initial infestation of non-viruliferous aphids. Weather also was quite different in 2010; more rain and colder temperatures may have reduced survival of the initial infesting aphid population survival.

Decision Support Tools Developed from this Research:

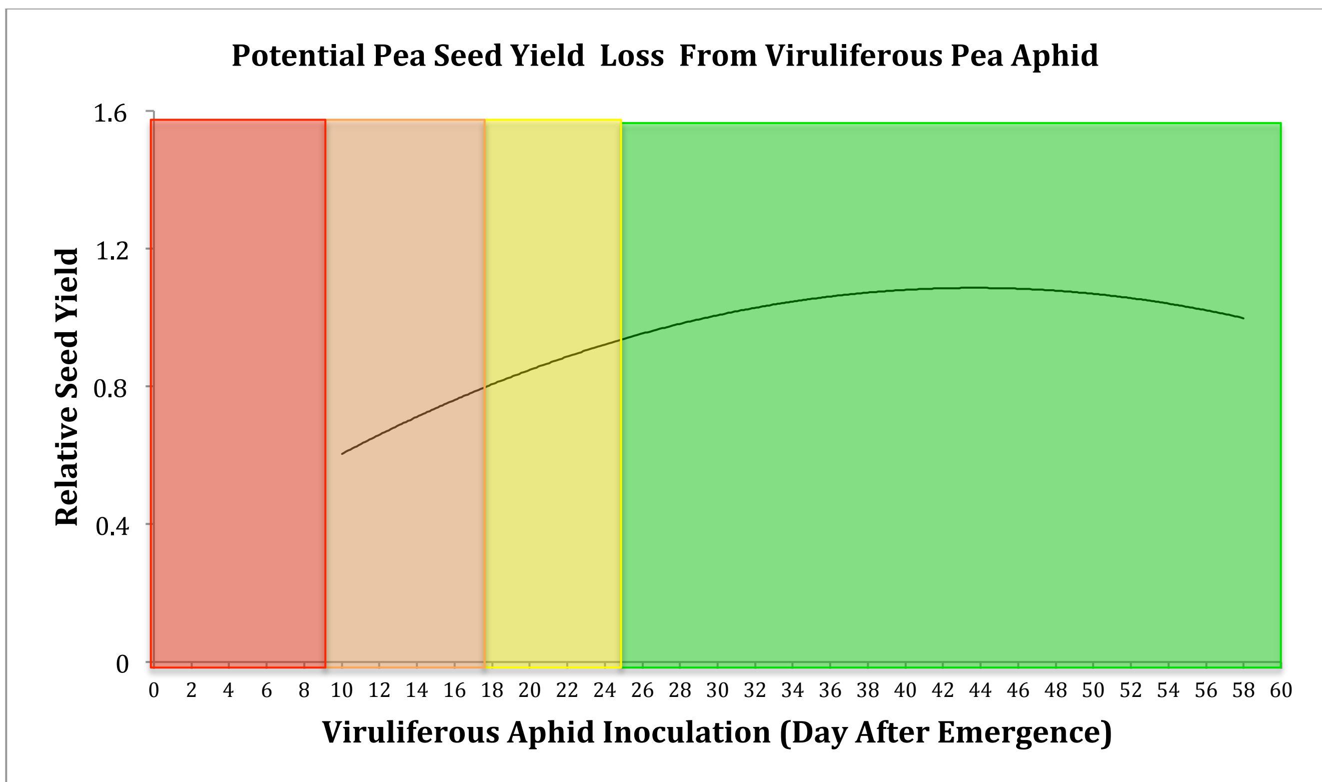

Figure 2.14. Potential seed yield loss as a function of colonizing viruliferous aphids in days after crop emergence (Red = >40% loss, Orange = 21-39% loss, Yellow = 5-20% loss, Green = < 5% loss)

Table 2.1. Economic injury levels for non-viruliferous pea aphids. EIL values are the number of aphids per plant based on our EIL formula: EIL = C/(DVYK), assuming K = 1 and Y = 18.5 cwt/acre.

Control Cost ($/acre)	Crop Value ($/cwt)			
	$12	$16	$20	$24
$15	9	7	5	4
$20	12	9	7	6
$25	15	11	9	7
$30	18	13	11	9

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Assessing Insecticide Seed Treatments for Reduction of Virus Diseases in Peas

Sanford Eigenbrode and Ying Wu

Introduction

Viral diseases of crops, and the economic injury they cause, can be reduced by limiting spread of viruses by their vectors (Latham and Jones 2004, Jeger et al. 2004, Andret-Link and Fuchs 2005, Hooks and Fereres 2006). Insecticidal treatments have been employed successfully to reduce vector populations in many crops (Perring et al. 1999, Mowry 2001, Hook and Fereres 2006). *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus (*BLRV) both are persistently aphid-transmitted viruses (Irwin and Thresh 1990, De Zoeten and Skaf 2001), which makes them good candidates for insecticide-based management. Generally, virus infection of the plants early in crop development causes substantially more injury than later infections (when seed treatment efficacy is likely to have eroded) (Jones et al. 2007). Preliminary research shows that infection of pea plants by PEMV earlier in the season (up to 20 days after emergence) causes more severe injury in terms of yield than later infections (Eigenbrode et al. unpublished data from 2008 field season). A systematic comparison of available insecticides to manage PEVM and BLRV viruses in legumes has not been undertaken. Therefore, the field experiments were conducted to compare the efficacy of selected insecticides to limit the spread of disease-causing viruses BLRV and PEMV to legumes by the pea aphid. Seed treatments like Gaucho and Cruiser, are increasing in adoption by legume producers in the region. Different seed treatment rates were assessed, and compared with standard field rate of foliar insecticide dimethoate and untreated control. The rates for these materials represent typical rates for aphid control, a lower rate typical for pea leaf weevil control, and a still lower rate (1/2 of pea leaf weevil rate).

Hypotheses of the research

1) Commercial insecticides, including seed treatments, differ in effectiveness in reducing transmission and spread of PEMV and BLRV in pea.
2) Some commercial insecticides are as effective as or more effective than dimethoate for reducing transmission and spread of PEMV and BLRV in pea.
3) The effects in (1) and (2) are rate dependent.

Objectives of the research

To compare the effectiveness of selected foliar and seed treatment insecticides for reducing spread of BLRV and PEMV in the field.

Material and methods

The field experiments (PEMV and BLRV) were conducted at the University of Idaho plant Science Farm, near Moscow Idaho. Each included nine treatments: Cruiser 5FS at 0.05 lb, 0.075 lb and 0.10 lb AI/cwt seed; and Gaucho at 0.0625 lb, 0.0975 lb and 0.125 lb AI/cwt seed, two foliar treatments of dimethoate used at standard field rate ('dimeth3x was sprayed three times on 23 June 23, 30 June and 8 July, and dimeth1x was sprayed once on 30 June), and an untreated control. Plots were planted on 13 May 2010 and each treatment was replicated five times within a complete randomized block design. The variety was Banner. Plot dimensions were 4x4m and the plots were separated by a one-meter buffer in all sides to allow aphid densities in the plots to develop independently, with little interference across the buffers. Each plot was inoculated by transplanting one 6" pot with four plants, infected with BLRV and PEMV from laboratory colonies of these viruses. The pots with infected plants were buried flush with the soil surface at the center of the plots. These 'spreader plants' were approximately 3-4 nodes ahead of the plants in the field at the time of transplant.

Aphid populations were monitored four times during the season (22 June, 28 June, 6 July, 14 July) by counting all the aphids on ten plants in each plot. The plants to be sampled were selected randomly within zones to ensure coverage: 6 plants were sampled adjacent to radii and near the center of the plot, 4 plants were sampled in the corners (Fig. 2.15).

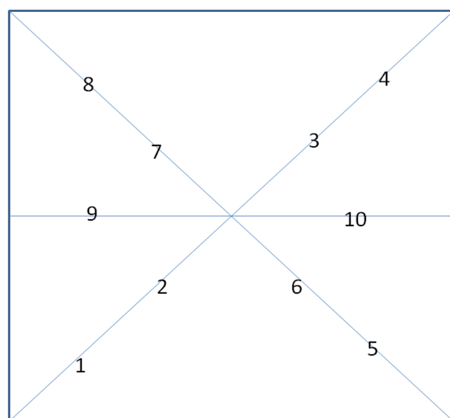


Figure 2.15. Illustration of aphid counting method

Virus symptoms were monitored twice during the season on 30 June and 15 July using a sample of 80 plants from each plot. Twenty plants were selected at random along each of 4 transects. (Fig. 2.16). Plant position was recorded so that the spatial pattern of disease symptoms could be assessed within the plot on each sample date. Disease symptoms were scored as 1 = healthy, no disease symptoms, 2 =

mild, evidence of virus disease, distortion chlorosis, enations (for PEMV) on some parts of the plant, 3 = severe, symptoms over most of the plant.

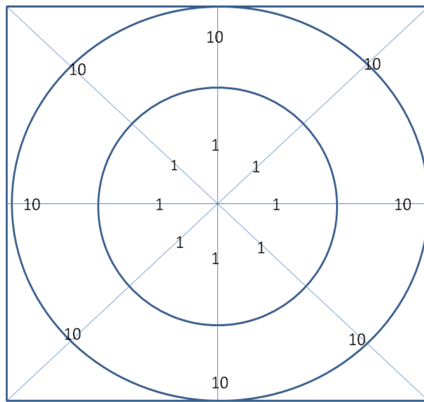


Figure 2.16. Illustration of disease scoring method

Virus presence in the plants was assessed with ELISA. Ten plant tissue samples were collected from each plot at bloom stage and samples were taken along transects. Samples were tested using ELISA.

All the plots were harvested using a Hege research plot combine (model number) on 16 August. Yields were converted to pounds per acre for each plot before analysis.

Analysis

All data were analyzed with ANOVA, with a blocking factor, and LSD for means comparisons, using SAS version 9.2, PROC GLM.

Results

I. PEMV

Aphid counts per plant differed among the treatments on all dates (Table 2.2). Gaucho treatments showed a dose response effect for aphid suppression. The highest rate (3.2 oz) had significantly fewer aphids than the control on all sample dates, as did the intermediate rate (1.6 oz) on all dates but the final sample date (14 July). The lowest rate (0.8 oz) was never different from the untreated control. In contrast (Table 2), Cruiser appeared to have no effect on aphid densities, which were not different from controls at any rate on any date. Dimethoate suppressed aphids on all dates after treatment and the triple treatment was significantly more effective than the single spray. Single spray suppressed aphids significantly below untreated controls. Both dimethoate treatments were more effective than any seed treatment. The plots treated with Dimethoate had significantly lower aphid counts compared to other treatments on June 28, July 6 and July 14 counts. However, the plots treated only once on June 30 had higher aphid counts on July 14 then July 6.

Average disease scores on 30 June and on 15 July were different significantly among the treatments (Table 2.2), mainly due to higher scores in the controls and lower scores in the two dimethoate sprayed treatments. Gaucho scores tended to be lower than Cruiser scores, but this difference was not significant, and there was a trend towards a dose response for Gaucho on average disease score.

There were significant differences between treatments in yields (Table 2.2). Plots sprayed with Dimethoate (1x and 3x) had the highest yields compare to other treatments. The plots treated with higher rates of Gaucho resulted in significantly higher yields than any seed treatment. There was a significant dose response in yield for Gaucho.

Figure 3 and 4 show the disease scores from center of the plot to the edge on 30 June and 15 July. The first scoring indicates that plants closer to the center of the plot had higher disease appearance then outer edge. However, the scoring made two weeks later, indicates the appearance of disease is more evenly distributed throughout the plot. Figure 2.17 also shows that plots treated with higher rates of Gaucho and sprayed with Dimethoate had lower disease scorings.

Table 2.2. PRMV aphid counts (number/per plant); disease scores on different dates and the yield.

	Total Aphid Counts				**Disease Scores**		**Yield** (lbs/acre)
Treatments	**22-Jun**	**28-Jun**	**6-Jul**	**14-Jul**	**30-Jun**	**14-Jul**	
Gaucho 0.8 oz/cwt	2.2[a]	18[ab]	60[ab]	270[ab]	1.3	2.5	181.3[e]
Gaucho 1.6 oz/cwt	0.6[b]	11[bc]	37[bc]	220[ab]	1.4	2.2	318.2[d]
Gaucho 3.2 oz/cwt	0.3[b]	7[c]	24[c]	110[b]	1.3	2.1	614.5[c]
Cruiser 0.32 oz/cwt	2.5[a]	18[ab]	74[a]	402[a]	1.4	2.7	50.0[f]
Cruiser 0.64 ox/cwt	2.0[a]	13[ab]	60[a]	329[a]	1.5	2.5	89.8[ef]
Cruiser 1.28 oz/cwt	2.2[a]	15[ab]	54[ab]	270[ab]	1.3	2.5	185.7[e]
Dimethoate triple spray	2.2[a]	3[d]	2[e]	1[d]	1.4	1.7	995.6[a]
Dimethoate single spray	4.0[a]	22[a]	8[d]	37[c]	1.3	1.9	849.4[b]
Control	4.0[a]	24[a]	67[a]	364[a]	1.6	2.7	70.5[ef]
P values	<0.001	<0.001	<0.001	<0.001			<0.001

Table 2.3. Contrast between different treatments in PEMV trial.

		P-values			
Contrast	**df**	**23-Jun**	**29-Jun**	**6-Jul**	**14-Jul**
Trt. 7 vs others	1	0.4711	<0.0001	<0.0001	<0.0001
Trt. 9 vs other	1	0.0067	0.0023	<0.0001	0.0009
Trt 9 vs trt. 1, 2, 3	1	<0.0001	0.0038	0.0043	0.0910
Trt. 9 vs trt. 4, 5, 6	1	0.0861	0.0863	0.7485	0.8088
Trt. 1, 2, 3 vs trt. 4, 5, 6	1	0.0002	0.0656	0.0005	0.0419

Note: Trt. 1, 2, 3 are Gaucho at 0.8, 1.6 and 3.2 oz/cwt; trt. 4, 5, 6 are Cruiser at 0.32, 0.64 and 1.28 oz/cwt; trt. 7 is dimethoate triple spray and trt.7 is untreated control.

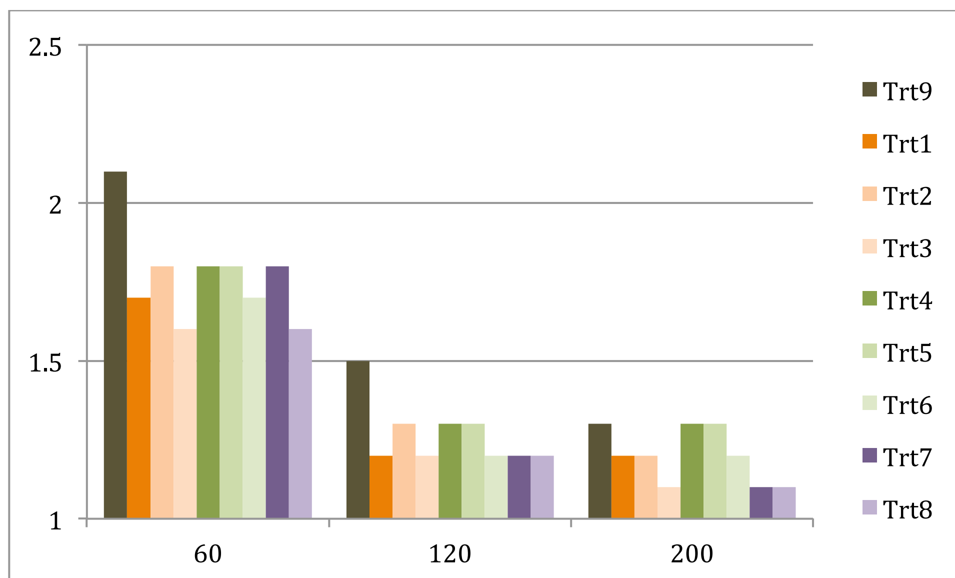


Figure 2.17. PEMV disease scores on 30 June, 2010 (Gaucho treatments (1-3): orange bars; Cruiser treatments (4-6): green bars, dark purple is triple dimethoate spray is dark purple; single dimethoate is light purple, and untreated control is dark brown).

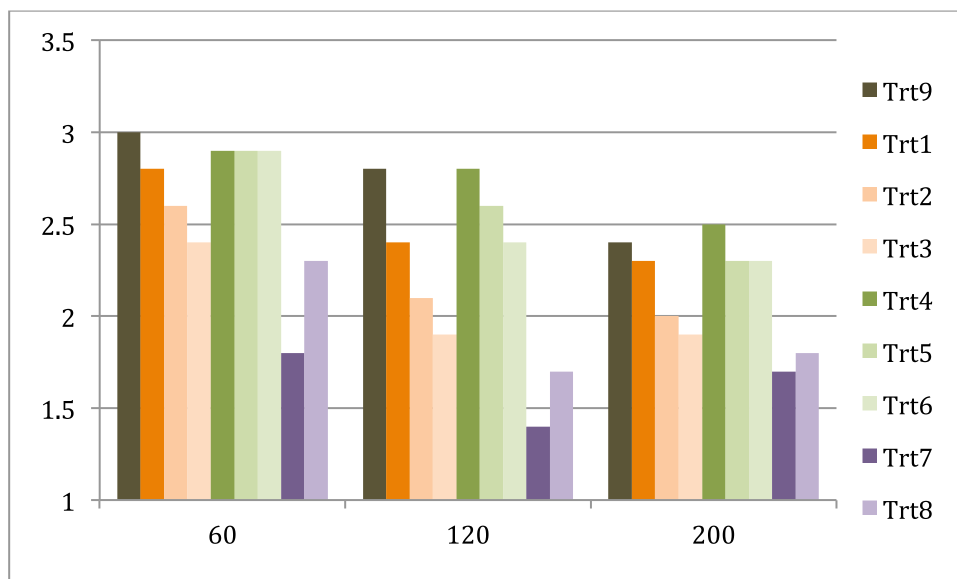


Figure 2.18. PEMV disease scores on 15 July, 2010 (bar colors are same as Figure 1).

II. BLRV

Aphid counts per plant were different between treatment on 29 June, 6 July and 14 July (Table 2.4). Gaucho treatments also showed a dose response effect for aphid suppression. The highest rate had significantly fewer aphids than the control on all dates. In contrast (Table 2.5), Cruiser appeared to have little effect on aphid densities on all three dates except at highest rate (1.28 oz) on 14 July. Dimethoate suppressed aphids on all dates after treatment and the triple treatment was significantly more effective than the single spray. Gaucho at rate of 3.2 oz had similar aphid counts with single Dimethoate spray treatment on 4 July and 14 July.

Average disease score on 15 July were significantly different among treatments mainly due to higher scores in control and lower scores in the dimethoate sprayed treatments. There were significant differences in yield between treatments (Table 2.4). Gaucho at highest rate produced the highest yield, followed by Dimethoate treatments.

Figure 5 and 6 show the disease scorings from center of the plot to the edge on 30 June and 15 July. The first scoring made on 30 June indicates that plants closer to the center of the plot had higher disease appearance then the outer edge. However, the scoring made two weeks later, indicates the appearance of disease is more evenly distributed throughout the plot.

Table 2.4. BLRV aphid counts (numbers per plant), disease scores (1 is healthy, 2 is somewhat sick and 3 is sick), and the yield.

	Total Aphid Counts				Disease Scores		Yield (lbs/acre)
Treatments	**23-Jun**	**29-Jun**	**6-Jul**	**14-Jul**	**30-Jun**	**15-Jul**	
Gaucho 0.8 oz/cwt	0.6	9^{bc}	27^{ab}	200^{ab}	1.2	1.9	513.4^{de}
Gaucho 1.6 oz/cwt	0.3	7^{dc}	16^{b}	99^{b}	1.2	1.7	683.0^{cd}
Gaucho 3.2 oz/cwt	0.2	4^{d}	7^{c}	47^{c}	1.1	1.6	1229.2^{a}
Cruiser 0.32 oz/cwt	1.0	18^{a}	49^{a}	244^{a}	1.1	2.0	322.4^{e}
Cruiser 0.64 oz/cwt	0.8	11^{abc}	37^{a}	244^{a}	1.1	1.9	345.9^{e}
Cruiser 1.28 oz/cwt	0.6	8^{bc}	33^{ab}	110^{b}	1.2	1.9	521.2^{de}
Dimethoate triple spray	1.8	4^{d}	1^{d}	3^{d}	1.1	1.7	1159.8^{ab}
Dimethoate single spray	1.1	15^{ab}	5^{c}	33^{c}	1.2	1.8	852.0^{bc}
Control	1.4	13^{ab}	40^{a}	244^{a}	1.2	2.0	306.1^{e}
P values	No	<0.001	<0.001	<0.001			<0.001

Table 2.5. Contrast between different treatments in BLRV trial.

		P-values			
Contrast	**df**	**23-Jun**	**29-Jun**	**6-Jul**	**14-Jul**
Trt 7 vs others	1	0.0841	0.0014	<0.0001	<0.0001
Trt 9 vs other	1	0.2232	0.0499	0.0002	0.0002
Trt 9 vs trt 1, 2, 3	1	0.0315	0.0034	0.0014	0.0059
Trt 9 vs trt 4, 5, 6	1	0.3944	0.6533	0.9975	0.4576
Trt 1, 2, 3 vs trt 4, 5, 6	1	0.0588	0.0006	<0.0001	0.0039

Note: Trt. 1, 2, 3 are Gaucho at 0.8, 1.6 and 3.2 oz/cwt; trt. 4, 5, 6 are Cruiser at 0.32, 0.64 and 1.28 oz/cwt; trt. 7 is dimethoate triple spray and trt. 7 is untreated control.

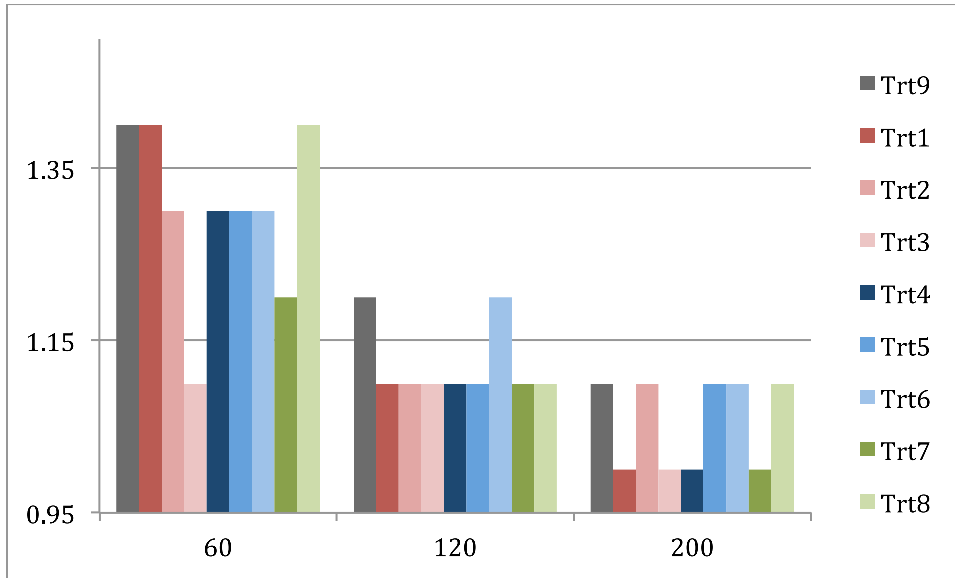


Figure 2.19. BLRV disease scores on 30 June, 2010 (Gaucho treatments (1-3): red bars; Cruiser treatments (4-6): blue bars, dark purple is triple dimethoate spray is dark green; single dimethoate is light green, and untreated control is dark brown).

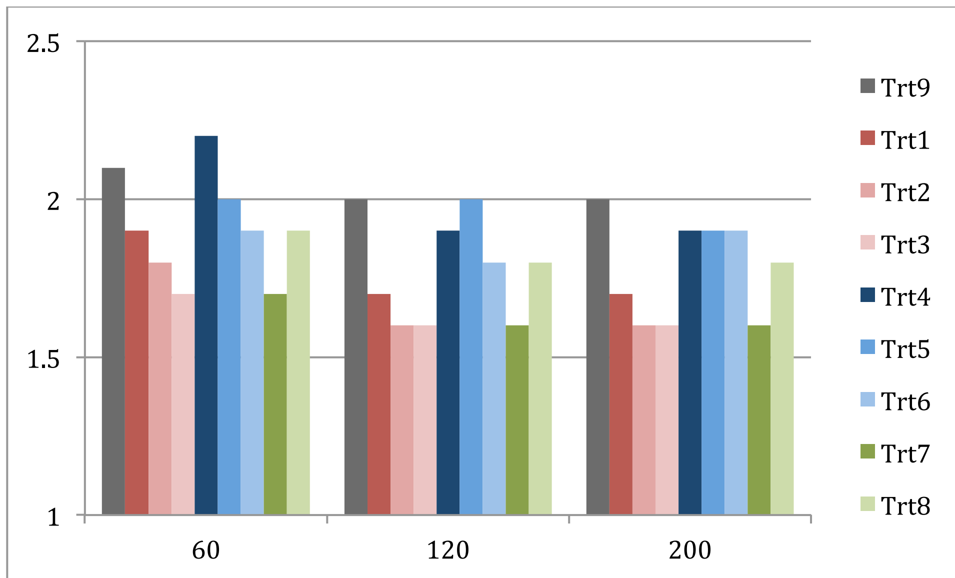


Figure 2.20. BLRV disease scores on 15 July, 2010 (bar colors are same as Figure 5).

Discussion

Gaucho seed treatments suppressed aphids more effectively than Cuiser, which appeared no better than the untreated control. Aphid suppression reduced virus incidence within the plot and this reflects in disease scores and the yields. The rate of spread outward from the spreader plants appeared to be similar among all treatments, but overall the mean severity differed among treatment.

The yield impact of PEMV appears to be greater than BLRV in this experiment, although the level of infection achieved for PEMV was much higher based on visual scores and ELISA results. Nonetheless, yield loss as a function of proportion of infected plants appears to be greater for PEMV. A single dimethoate spray on June, which is similar to current grower practices improved yield by 1105% in the presence of PEMV and 178% in the presence of BLRV.

Part II: Breed for Virus-Resistant Cool Season Legumes

Objective 3. Develop varieties of pea and lentil with resistance to PEMV and BLRV and use marker-assisted selection to augment the breeding process.

Objective 4. Increase marker density in the genomic region of *En* and *lr* for resistance to PEMV and BLRV, respectively.

Shalu Jain and Kevin McPhee

Increase marker density and finding linked markers to the PEMV resistance gene

Finding a marker closely linked to a resistance gene is a crucial step for marker assisted selection (MAS) and subsequent screening of segregating populations when developing resistant varieties. It is important that the recombination frequency between the resistance gene and the linked marker be as low as possible to improve the efficiency of MAS. Saturation of the consensus *Pisum* genetic map (Loridon et al. 2005), has made it possible to identify new closely linked flanking markers to specific genes of interest. Resistance to Pea Enation Mosaic Virus (PEMV) in pea is conferred by a single dominant gene, *En*, located on linkage group III (Gritton and Hagedorn 1980; Marx et al. 1985). The *En* locus was mapped between *Uni* and *Adh1* which are ~10 cM apart on LG III using 64 RILs derived from the susceptible parent 'Alaska' and resistant parent 'B880-221' by Yu et al. (1995). Dr. Norman Weeden at Montana State University, Bozeman, MT, identified gene-based markers Prx1, Cngc and LKA2 which are very closely linked to *En*. These markers segregate in PRIL5, a F_7-derived recombinant inbred population ('Lifter'/'Radley') comprised of 393 lines. Lifter is resistant while Radley is susceptible to PEMV. Prx1 and Cngc show length polymorphism of the PCR product while LKA2 shows polymorphism following restriction digestion of a ~400bp PCR product with *HinfI*, also referred to as a Cleaved Amplified Polymorphic Site (CAPS) (Fig. 4.1). LKA2 is currently the most closely linked marker.

PRIL-5 was screened for reaction to PEMV resistance at Corvallis, OR, in 2010. Field data indicate that *En* segregates in the expected 1:1 ratio. After incorporating field data with LKA2 marker data, only 0.1% recombination frequency was found, suggesting LKA2 is a good candidate marker for MAS. Screening through mechanical inoculation of PEMV is being conducted at Prosser, WA. All 393 PRIL-5 lines will be screened for virus again in 2011 at Corvallis, OR.

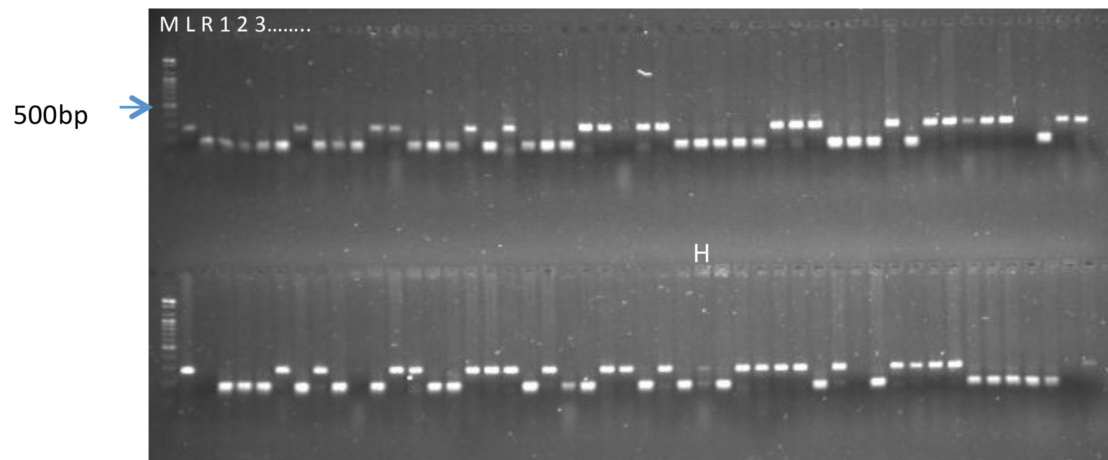


Figure 4.1. Pattern of LKA2 polymorphism in PRIL-5 lines 1 through 94 on 2% agarose after digestion with *HinfI*. M, molecular weight standard; L, Lifter; R, Radley; 123.., RILs; H, heterozygotes.

Generation of Near Isogenic Lines (NILs)

Mapping genes of interest in segregating populations often suffers from low resolution. Near isogenic lines (NILs) are genetic lines, which differ only in a small region of the genome associated with a gene of interest and, thereby, allow more precise placement of genes in that region on the genetic map. We have chosen three RILs from PRIL5 to select heterozygotes for LKA2 to generate NILs. Since all the other loci have been fixed during selfing in previous generations, only the LKA2 region will segregate among progenies of these lines after selfing. Currently, 80 plants from three PRIL-5 lines (PRIL-5-232, PRIL-5-250, PRIL-5-352) are growing in the greenhouse and are being tested for segregation of the LKA2 marker. Once NILs are developed they will be used for genetic and phenotypic characterization of virus resistance.

Increase marker density and identifying markers linked to the BLRV resistance gene, *lr*

Resistance to *Bean leaf roll virus* (BLRV) is conferred by the recessive gene, *lr*, but lack of a reliable screening protocol has limited progress toward identifying closely linked markers for use in MAS. Mechanical transmission is not possible for BLRV and an efficient protocol for aphid transmission is not available. According to preliminary work done by Ludmila Krokhmal (1994) for her graduate thesis, there are two independent recessive genes for BLRV, designated lrv_1 and lrv_2; however, the presence of two genes has not been confirmed. The first gene, lrv_1, is loosely linked (33% recombination frequency) to the gene for cotyledon color (*i*) on LG I in pea. We have begun to saturate the *Pisum* genetic map with additional markers in this region based on this putative placement. The additional markers are being developed using syntenic comparison with the *Medicago truncatula* genome.

Medicago truncatula is a model legume crop and its genome has been sequenced (www.mediacgo.org). Linkage group I of pea is syntenic to chromosome 5 of *M. truncatula*. We developed a set of 75 gene-based markers using the sequence from *M. truncatula* following the strategy outlined in Fig. 4.2.

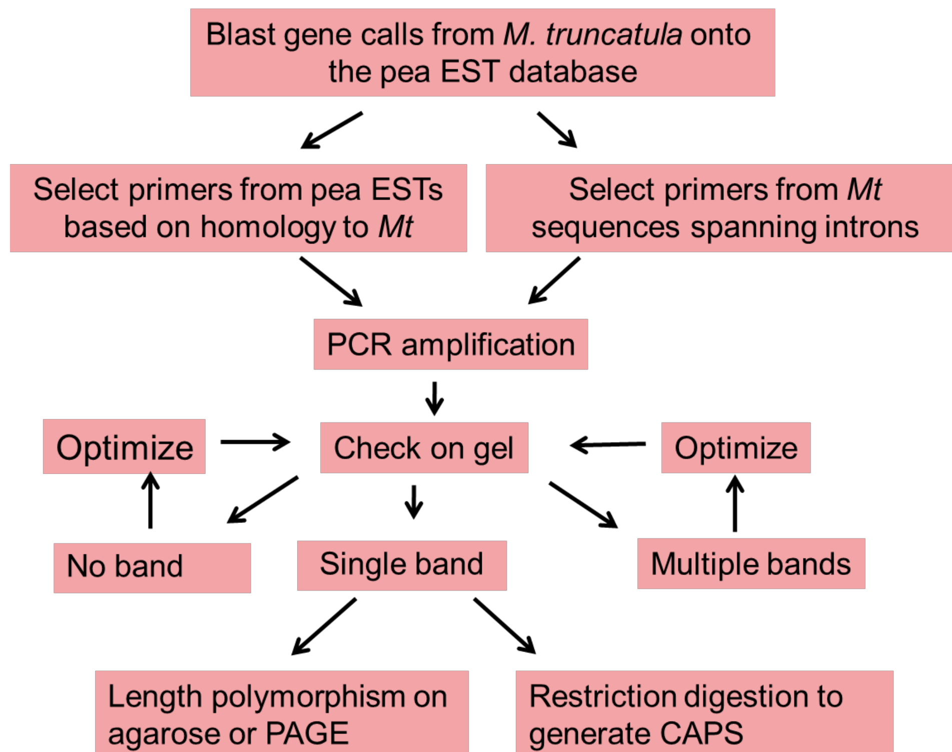


Figure 4.2. Method of gene-based marker development.

These novel markers were run on the parents of thirteen RILs and showed either length or CAPS polymorphism. To determine the position of these markers on the pea linkage map, 15 polymorphic markers were run on three RIL populations (PRIL-5, PRIL-9 and PRIL-12) along with several anchor markers from LG I. Results indicate that all the markers tested are located on LG I and show macrosynteny with *M. truncatula* chromosome 5 with few exceptions (Fig. 4.3).

Two additional RIL populations, PRIL-7 (susceptible parent PI179449-1 X resistant parent 'Green Arrow') and PRIL-9 (susceptible parent 'Sparkle' X resistant parent 'JI 73'), segregate for BLRV resistance. Genetic mapping of these populations are in progress. Parental screening for polymorphism with 400 SSR/CAPS markers has been completed and 48 polymorphic markers have been run on these populations. We will be able to confirm the genomic position of the BLRV resistance gene as determined with PRIL-5 when phenotypic data for virus reaction becomes available for these populations. The set of novel gene-based markers and others in the genomic region can then be used for MAS and to accelerate the development of resistant varieties.

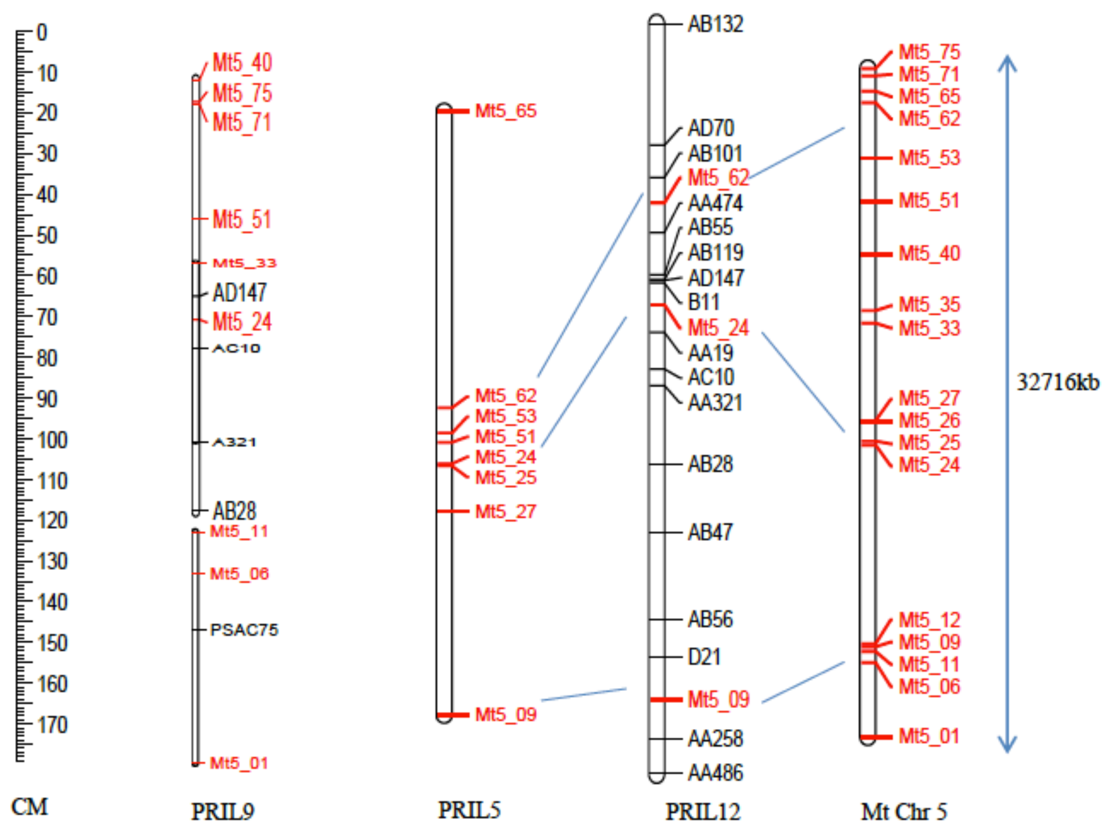


Figure 4.3. Mapping of novel gene-based markers on linkage group I and evidence of macro-synteny with chromosome 5 of *M. truncatula*.

Lentil with resistance to PEMV and BLRV

Earlier screening of lentil germplasm (Aydin et al. 1987; Short 1995) identified resistance to PEMV in lentil germplasm. We selected a set of 39 lines to screen for PEMV resistance under natural conditions at Corvallis, OR, in 2010 (Table 4.1). Tissue samples obtained from the field were tested for the presence of virus through ELISA. Phenotypic symptoms and ELISA results indicated that few lines are truly resistant to virus. We selected six resistant PI lines (highlighted in green in Table 4.1) and ten susceptible named varieties (highlighted with yellow in Table 4.1) to use as parents to develop genetic mapping populations. Crosses were made in the greenhouse in Fargo, ND, in the fall of 2010.

Table 4.1: Phenotype and ELISA results for lentil germplasm screened at Corvallis, OR, in 2010.

	Accession number	Physical symptoms	ELISA Results	PEMV (OD)
1	PI178971	Susceptible	+	0.232
2	PI193547	Resistant	+	0.225
3	PI207492	Resistant	+	0.242
4	PI212100	Resistant	+	0.266
5	PI212610	Resistant	+	0.367
6	PI250156	?	+	0.272
7	PI298122	Resistant	+	0.157
8	PI299351	Resistant	+	0.173
9	PI429838	Resistant	+	0.092
10	PI431622	Resistant	+	0.129
11	PI431663	Resistant	_	0.07
12	PI431666	Susceptible	_	0.055
13	PI431712	Susceptible	+	0.365
14	PI431714	Susceptible	+	0.409
15	PI431717	Susceptible	+	0.234
16	PI431728	Resistant	+	0.219
17	PI431753	Susceptible	+	0.09
18	PI432002	Resistant	+	0.187
19	PI432033	Resistant	+	0.128
20	PI432071	Resistant	+	0.431
21	PI432124	Resistant	+	0.252
22	PI472137	Susceptible	+	0.161
23	PI472590	Susceptible	+	0.172
24	PI533693	Resistant	+	0.219
25	PI289079	Resistant	+	0.241
26	PI431613	Susceptible	+	0.467
27	PI431624	Susceptible	+	0.501
28	PI432028	Resistant	_	0.056
29	PI533691	Resistant	+	0.242
30	PRECOZ	Susceptible	+	0.09
31	TEKOA	Susceptible	+	0.183
32	BREWER	Susceptible	+	0.403
33	BENEWAH	Susceptible	+	0.636
34	CHILEAN78	Susceptible	+	0.146
35	CRIMSON	Susceptible	+	0.186
36	EMERAL	Susceptible	+	0.181
37	ESTON	Susceptible	+	0.364
38	LAIRD	Susceptible	+	0.227
39	PALOUSE	Susceptible	+	0.158

Six lentil germplasm lines, ILL 74 (PI612871), ILL 85 (PI612872), ILL 213 (PI612873), ILL 214 (PI612874), and ILL 6816 (PI612875) have been identified with resistance to bean leaf roll virus by the International Center for Agricultural Research in the Dry Areas (ICARDA), located at Aleppo, Syria (Makkouk et al. 2001). These lines were used as resistant parents in crosses with the same virus susceptible lines mentioned above (Table 1). The germplasm lines from Table 1 along with the six ILL lines listed above will be screened at Corvallis, OR, in 2011 to confirm virus resistance and susceptibility.

F_1 plants from 45 crosses were grown in the greenhouse from December 2010 - March 2011. Leaf tissue was collected from all F_1 plants for DNA extraction and used in PCR to confirm crosses using polymorphic markers. F_2 seeds obtained from F_1 plants (highlighted in green in Table 2) represent potential mapping populations to locate the gene responsible for PEMV resistance and are currently being grown in the greenhouse. We have also begun to screen the lentil parents and populations in the greenhouse using PEMV infected tissue and mechanical inoculation based on the protocol provided by Drs. Hanu Pappu and Murthy.

Table 4.2: Pedigree and number of F_2 seed for mapping populations in lentil.

Female Parent	Male Parent	No. of F_2 seed
Eston	PI429838	145
Chilean78	PI429838	23
Chilean78	PI431663	44
Chilean78	PI533691	30
Tekoa	PI429838	94
Tekoa	PI431622	20
Tekoa	PI431663	22
Tekoa	PI431666	32
Emerald	PI432028	20
Crimson	PI429838	25
Crimson	PI431622	38
Crimson	PI431663	13
Crimson	PI612871	21
Palouse	PI432028	23
PI533691	Emerald	22
PI533691	Benewah	18
Brewer	PI429838	166
Brewer	PI432028	54
Brewer	PI533691	44
Brewer	PI431666	94
Brewer	PI612870	26
PI612870	Eston	102
PI612872	Tekoa	55
PI612873	Eston	49

Objective 5. Establish reliable, high throughput diagnostic tools to characterize and verify virus incidence and disease reaction

B. Vemulapati and H.R. Pappu

ELISA tests for BLRV and PEMV

Development of an antigen-coated plate (ACP) or direct antigen-coating (DAC)-ELISA
Results:

Polyclonal antisera raised against the bacterially expressed CP of BLRV and PEMV were used to develop an antigen-coated plate (ACP) or direct antigen-coating (DAC)-ELISA and its application in identification of these two viruses in alfalfa and pea samples.

Symptomatic pea and alfalfa samples collected from different commercially grown fields in Washington and Idaho were used in this study. Uninfected pea plants grown in an insect proof green house were used as healthy negative controls. Alfalfa samples collected from Idaho that were RT-PCR confirmed for BLRV and pea plants collected from Washington State that were RT-PCR confirmed for PEMV were used to standardize the ELISA assays. DAC-ELISA was performed in polystyrene plates with the sap preparation included grinding infected leaves at 1:10 dilution (w/v) in coating buffer followed by centrifugation at 5000 rpm for 5 min. Anti-BLRV serum was tested in dilutions of 1:200, 1:500, 1:1000 and 1:2000 while anti-PEMV serum was tested in dilutions of 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200 and 1:6400 in PBS containing 0.05% Tween-20 and 0.5% non-fat dry milk. Anti-rabbit IgG alkaline phosphatase conjugate was used at 1:5000 dilution. Colorimetric reactions at 405 nm were read one hour and overnight after the addition of substrate (*p*-nitro phenyl phosphate, 0.5 mg/ml) by using an ELISA plate reader.

Results indicated that the BLRV antiserum was specific to BLRV in DAC-ELISA in up to 1:1000 dilutions. However the difference in the absorbance values between healthy and infected samples was more distinctive in the case of 1:500 antiserum dilution (Fig. 5.1). Anti-PEMV serum was able to detect the virus in up to 1:6400 dilutions (Fig. 5.2). The mean of the absorbance values obtained for the PEMV infected samples was found to be more than twice obtained for the healthy in all the antiserum dilutions up to 1:3200 dilutions.

a. Detection of BLRV and PEMV from transects collected during June to August 2010 (Damon Husebye's Experiment)

The following experiment was conducted in different months a) from 06/24/2010 to 06/28/2010 (batch-1; Table 5.1A) and b) from 08/01/2010 to 08/08/2010 (batch-2; Table 5.1B) to determine the spread of BLRV and PEMV in pea plants collected from the following Whitman and NezPerce county's field plots: 27 Rail Road, Rail Road underpass, Drake, Auvil, Beehive, West Cove, N. Garfield, Bailey Road, Miller Road, Grinnell, Albion, Wahl Road, Almota, Steptoe Grade, Rimrock 1, Union Hill top, Kambistch, Rimrock 2, Dump Road, Zenner, Wawawai, Union Flat, File Road, Wawawai 2, Rail Road Pasture. Samples were tested by DAC-ELISA for BLRV and PEMV. Samples were ground in 1:10 dilutions of antigen: buffer and antiserum raised against PEMV was tested in a 1:1000 (antiserum: Buffer, PBS-TPO) while it was 1:500 for BLRV.

Results:

Results on DAC-ELISA from batch-1 samples indicated the presence of PEMV infection only from Almota (Table 5.2). BLRV was not detected in any of these samples. In batch-2, a total of 177 pea samples were randomly collected from the above field plots. Each plot contained 3 batches of 5 plants each totaling 15 samples from each plot). Initially each batch was tested on the whole and positives obtained from each batch were individually tested for the viruses. Results on the detection of PEMV showed that 89 (20 plots) samples from a total of 177 (24 plots) were positive. However the total number of samples infected with PEMV in these plots varied among plots. Union Hilltop (10/15; 10 out of 15 plants); Almota (09/15); Albion (07/15) and Grinnel (08/15) had the most number of infected plants that were collected randomly (Table 5.3). BLRV was not found in any of these plants.

Detection of BLRV and PEMV from spreader experiment (Dr. Sanford Eigenbrode's experiment)

A total of 900 pea samples (450 samples each for the detection of PEMV and BLRV). Each of the 450 samples was randomly collected from 45 experimental plots numbered 1 to 45. Each experimental plot had a PEMV or a BLRV-infected plant in the center with aphids and healthy plants were grown around the infected plant. Ten plants were collected from each experimental plot and were numbered from 1 to 10 (plants numbered 1 to 5; and 6 to 10 were either collected from inner circle or outer circle surrounding the infected plant). These samples were randomly collected during 10 July and tested by DAC-ELISA (07/14/2010 to 07/21/2010). Antiserum raised against PEMV was tested in a 1:1000 and 1:500 (antiserum: Buffer, PBS-TPO) for BLRV.

Results

Detection of PEMV: 37 plots from a total of 45 had samples infected that were numbered 6 to 10. A total of 14 plots from 45 plots had samples infected from serial no. 1 to 5. 13 plots from a total of 45 had samples infected from serial no.1 to 10. Two plots from a total of 45 had samples infected from serial no. 1 to 5. [Avg. infected sample reading: 0.60 (ELISA plates 1 to 5) and 0.40 (ELISA plates 6 to 10); Avg. healthy sample reading: 0.20 (ELISA plates 1 to 5) and 0.15 (ELISA plates 6 to 10)].

Detection of BLRV: 15 plots from a total of 45 had samples infected from serial no. 1 to 5. 04/45 had samples infected from serial no. 6 to 10. 03 plots from 45 had samples infected from serial no.1 to 10. One plot from 45 had samples infected from serial no. 6 to 10.

Table 5.1A: DAC-ELISA on transects (batch-1). Positives are indicated in color.

SAMPLE	PEMV	SAMPLE	PEMV
1-1 27 Rail Road	0.10	18-1 Rimrock 2	0.13
1-2	0.11	18-2	0.13
1-3	0.05	18-3	0.12
2-1 Rail Road underpass	0.10	19-1 Dump Road	0.13
2-2	0.10	19-2	0.12
2-3	0.10	19-3	0.12
3-1 Drake	0.10	20-1 Zenner	0.12
3-2	0.10	20-2	0.12
3-3	0.10	20-3	0.13
4-1 Auvil	0.05	21-1 Wawawai	0.11
4-2	0.10	21-2	0.12
4-3	0.10	21-3	0.12
5-1 Beehive	0.10	22-1 Union Flat	0.12
5-2	0.10	22-2	0.13
5-3	0.05	22-3	0.13
6-1 West Cove	0.10	23-1 File Road	0.11
6-2	0.10	23-2	0.11
6-3	0.10	23-3	0.12
7-1 27N. Garfield	0.10	24-1 Wawawai 2	0.12
7-2	0.10	24-2	0.12
7-3	0.10	24-3	0.12
8-1 Bailey Road	0.10	25-1 Rail Road Pasture	0.13
8-2	0.10	25-2	0.11
8-3	0.10	25-3	0.11
9-1 Miller Road	0.10	SYMPTOMATIC SAMPLES	
9-2	0.10	26 sick plot (Union Hilltop)	**4.00**
9-3	0.10	27	0.13
10-1 Grinnell	0.10	28	**1.80**
10-2	0.10	29	**3.00**
10-3	0.10	30	0.11
11-1 Albion	0.10	31 Alfalfa	0.11
11-2	0.10	32 Alfalfa	0.11
11-3	0.10	33 Alfalfa	0.11
12-1 Wahl Road	0.10	34 Wawawai 2	**2.00**
12-2	0.10	35	**1.7**
12-3	0.10	36 Albion	**0.72**
13-1 Almota	**2.65**	37	0.11
13-2	0.12	38	0.18
13-3	0.13	39	**0.92**
13-4	**2.00**	40	**2.45**
14-1 Steptoe Grade	0.18	61	**2.15**
14-2	0.12	62 Wawawai	**2.25**
14-3	0.12	63 Albion	**2.38**
15-1 Rimrock 1	0.12	64 West Cove	0.11
15-2	0.13	65 Albion	**1.55**
15-3	0.13	66 Almota	**2.45**
16-1 Union Hill top	0.13	67	0.12
16-2	0.13	68 Bailey Road	0.11
16-3	0.13	69	0.12
17-1 Kambistch	0.12	70 Wahl Road	0.11
17-2	0.12	71 Steptoe Grade	**2.60**
17-3	0.12	72	**3.2**
		73 Dump Road	0.22
		74 Union Flat	**0.45**
		PEMV POSITIVE SAMPLE	**2.00** (avg)

Table 5.1B: DAC-ELISA on transects (Round 2). Total number of samples: 177; Total number of samples positive for *Pea enation mosaic virus* (PEMV): 89; (Positives: indicated by '+'; Negatives: indicated by '-'); Total number of samples positive for *Bean leafroll virus* (BLRV): Nil.

Sample	ELISA result PEMV (each batch)			Sample	ELISA result PEMV (each batch)		
	1	2	3		1	2	3
Steptoe (07/15)				Beehive (01/15)			
1	+	-	+	1			-
2	-	-	-	2			-
3	+	+	-	3	-	-	+
4	+	+	-	4			-
5	+	-	-	5			-
Wahl Rd (02/15)				Garfield (01/05)			
1	+			1	+		
2	-			2	-		
3	-	-	-	3	-		
4	+			4	-		
5	-			5	-		
Kambistch (03/15)				27 Rail Rd (03/15)			
1		+	-	1	+		-
2		-	-	2	+		-
3	-	+	-	3	-	-	-
4		-	+	4	-		-
5		-	-	5	-		+
Union Hilltop (10/15)				27 N.Garfield (00/15)			
1	-	+	-	1			
2	+	+	+	2			
3	-	-	+	3	-	-	-
4	+	+	+	4			
5	+	-	+	5			
Miller (00/15)				Wawawai (02/10)			
1				1	-	+	
2				2	-	-	
3	-	-	-	3	-	-	
4				4	-	-	
5				5	+	-	
Wawawai-2 (07/15)				Almota (09/15)			
1	+	+	-	1	+	+	-
2	+	+	+	2	+	+	-
3	-	-	+	3	+	+	+
4	-	-	-	4	+	-	+
5	+	-	-	5	-	-	-
Albion (07/15)				File Rd (00/15)			
1	+	-	+	1			
2	+	-	-	2			
3	+	+	+	3	-	-	-
4	-	+	-	4			
5	-	-	-	5			
Wawawai-1 (06/10)				Auvil (05/15)			
1	+	-		1	+	+	-
2	+	+		2	-	-	+
3	+	+		3	+	-	-
4	-	-		4	-	-	-
5	-	+		5	-	-	+
Rail Rd Pasture (03/15)				Conservation (05/15)			
1		+	-	1	+	-	+

2		-	+	2	+	-	-
3	-	-	+	3	-	+	-
4		-	-	4	-	+	-
5		-	-	5	-	-	-
Rimrock (01/15)				RailRdPass (04/15)			
1			+	1	+	+	-
2			-	2	+	-	-
3	-	-	-	3	-	-	-
4			-	4	-	-	-
5			-	5	-	-	+
Zenner (02/15)				Bailey (00/15)			
1			-	1			
2			+	2			
3	-	-	+	3	-	-	-
4			-	4			
5			-	5			
Union Flat (04/15)				WestCove (00/15)			
1	-	-	-	1			
2	-	+	+	2			
3	-	-	+	3	-	-	-
4	+	-	-	4			
5	-	-	-	5			
Rimrock-2 (00/15)				Drake (00/15)			
1				1			
2				2			
3	-	-	-	3	-	-	-
4				4			
5				5			
Grinnel (08/15)							
1	+	+	+				
2	+	-	-				
3	-	-	-				
4	+	+	-				
5	-	+	+				

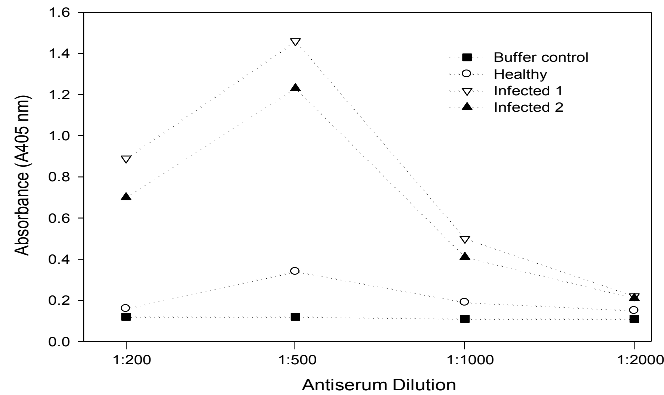
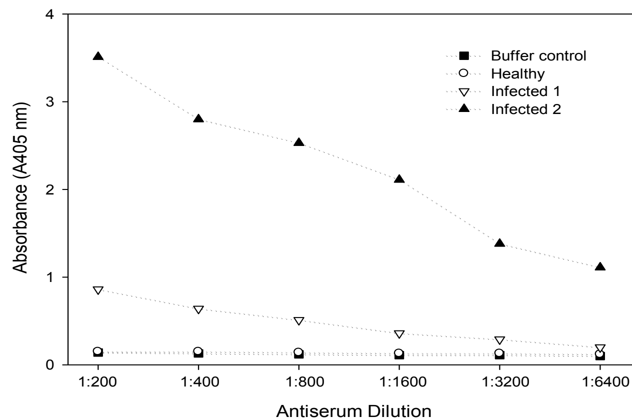


Fig. 5.1. Results of DAC-ELISA using different dilutions of *Bean leafroll virus* (BLRV) polyclonal antiserum. Healthy and BLRV infected alfalfa samples 1 and 2 (1:10 w/v) were tested. A405 values shown overnight after the addition of substrate is shown on Y-axis with the antiserum dilutions shown in the X-axis.



5.2. Results of DAC-ELISA using different dilutions of *Pea enation mosaic virus* (PEMV) polyclonal antiserum. Healthy and two PEMV infected pea samples (1 and 2) were tested. A 405 values shown overnight after the addition of substrate is shown on Y-axis with the antiserum dilutions shown in the X-axis.

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Part III: Develop and Extend Crop Protection Practices from Outcomes of I and II

Goal - *To ensure project findings are communicated to growers and industry and that project activities are responsive to grower interests.*

Specific Objectives

6. Develop an advisory group of farmer cooperators…………………………………………
7. Educate growers about aphid management and use of project tools…………………….
8. Assess farmer learning and adoption of aphid management tools from the project
9. Disseminate project information through various media………………………………..

Accomplishments

6. Advisory group
We recruited a farmer advisory group and we met with them twice to get feedback on the project, the mechanisms for communicating with them, including web site and listserve.

7. Educate Growers
A summary of the educational events that comprise the outreach and extension of the project is included as an appendix. These included 22 talks reaching a total audience of more than 900 growers, crop advisors and other industry professionals. We published three articles in industry magazines. We included basic educational materials about aphids, viruses and their management on the web page for the project, which receives approximately 800 hits per month in the growing season. We conducted three tours or field presentations, one each summer, to present project concept and demonstrate virus injury to growers and other professionals.

Our project website is also a forum for presenting information about all aspects of the project and for displaying 'real time' data on aphid movements and virus incidence in our region. The website URL – http://www.cals.uidaho.edu/aphidtracker/index.asp. Example screen shots are presented in an appendix to this report.

8. Assess farmer learning
At the outset of the project in the fall of 2008 we surveyed participants (farmers and field consultants) at 3 grower workshops:

- The Spokane County Crop Improvement Association
- The Western Pea and Lentil Growers Annual Meeting
- A workshop at Nez Perce, ID
-

This survey (with responses from 78 participants) will form a baseline for the project. At the conclusion of the project we will survey participants to measure learning and adoption of practices taught. The survey ad response summary is included as an appendix to this report.

9. Dissemination
Articles, web site and presentations, listed in Appendix to this report.

Additional sections of the report cover efforts adopted to meet overall project goal

Project Management

The project holds annual meetings in February at which participants make presentations of research and extension efforts and planning is carried out for the coming year.
The project also holds ad hoc meetings of specific working teams as needed.
A midsummer meeting has been held each year with partial participation.

Connections with Other Projects

The project is now linked with a recently funded USDA NIFA CAP on Climate Change in PNW Cereal Production Systems. Some of the approaches to monitoring aphid movements and virus will be applicable, although the focus is on wheat rather than legumes. The project has collaborated closely with a WIPM – funded project on the economics of virus forecasting in legumes. This has resulted in presentations at Agricultural Economics meetings and a published paper (listed in outputs section). The project's focus on aphids and virus have allowed some cross fertilization with an ongoing NIFA AFRI project on aphids and viruses affecting potatoes and wheat (Eigenbrode et al.)

Plans for Remainder of Project

- The project continues until Dec. 2012. The following activities are planned.
- Continue monitoring aphids immigration and virus disease incidence and prevalence for two more field seasons.
- Relate the overall patterns to weather as basis for forecasting.
- Carry out direct and indirect injury studies on lentils, to complement studies completed in pea.
- Complete a second season of work on seed treatments for reducing virus spread.
- Carry out more complete biotyping of pea aphids from across the region.
- Establish grower-based aphid and virus monitoring using the Epicollect smart-phone application.
- Determine mechanism to sustain maintenance of project website for the industry.
- Complete characterization of the PEMV and BLRV isolates from the region.
- Develop virus resistant breeding lines of pea and lentil.
- Produce a comprehensive bulletin that summarizes project work, for posting to university web sites.
- Continue presentations and field days for producers.
- Complete end-of-project survey to assess grower knowledge gains.
- The team will continue to communicate project findings to growers and industry though appropriate outlets including:
    - Grower Meeting and Schools
    - Field Tours
    - A research preview at the Western Pea and Lentil Growers annual meeting in 2011
    - A research review at the Western Pea and Lentil Growers annual meeting in 2012
- Publish scholarly articles on aphid movements and virus forecasting (3), thresholds for pea and lentil (4), pea aphid biotypes and movement in the region (2), characterization of viruses (2), host range of PEMV (1), pea aphid behavior and virus spread (1), breeding for virus resistance (3) – total of 16 anticipated

Publications and Presentations Resulting from the Project

Popular press

Radio - *Aphids Spreading Viruses* Today's Idaho Ag News Date: November 18, 09

Legume virus sneaks up on pea producers, Capital Press, July 30, 2009

New pea lines could combat virus outbreaks, Capital Press, Dec. 17, 2009

Extension related publications

Eigenbrode, S. D., Bechinski, E., Clayton, L., Husebye, D. Jain, S., Karasev, A., Larsen, R.., McPhee, K., Pappu, H. Porter, L., Roberts, D., Stokes, B., Vemulapati, B. **E**yes on Legume Viruses in the Palouse Region. *Take Your Pulse*, US Dry Pea and Lentil Council; Vol. I, No. 1; November 2010.

Eigenbrode, S.. D., Husebye, D., Porter, L. V is for Virus; *Pulse Pipeline*, US Dry Pea and Lentil Council; Vol. X, No. 4; July 23, 2010.

Refereed journal articles appearing or accepted for publication

Vemulapati, B., Druffel, K. L., Eigenbrode, S. D, Karasev, A, and H. R. Pappu. 2010. Molecular Characterization of *Pea enation mosaic virus* and *Bean leafroll virus*: Two viruses in the Family *Luteoviridae. Archives of Virology* 155:1713-1715.

Elbakidze, L., Lu, L., Eigenbrode, S. D. 2011. Evaluating Vector-Virus-Yield Interactions for Peas and Lentils under Climatic Variability: A Limited Dependent Variable Analysis. American Journal of Agricultureal Economics, *accepted for publication*

Journal articles submitted

Vemulapati, B., Druffel, K. L., Eigenbrode, S. D., Karasev, A, and H. R. Pappu. 2011. Antigen-coated Plate ELISA Assays for Rapid and Reliable Detection of Two Members of Luteoviridae: Pea enation mosaic virus (genus Enamovirus) and Bean leaf roll virus (genus Luteovirus). Submitted to Journal of Virological Methods.

Vemulapati, B., Druffel, K. L., Eigenbrode, S. D., Karasev, A, and H. R. Pappu. 2011. Genomic Characterization of Pea enation mosaic virus-2 from the Pacific Northwestern USA. Submitted to Archives of Virology.

Journal articles in preparation

- Husebye et al. patterns of pea aphid immigration…
- Husebye et al. weather and aphid outbreaks during a 25 year period in the Palouse region
- Stokes et al. new economic injury levels for pea aphid in pea
- Stokes et al. PEMV and BLRV injury as a function of date of infection in pea
- Eigenbrode et al. – (for the Journal of Integrated Pest Management) – the Legume Virus Project
- Jain et al. – Improved genetic markers for BLRV and PEMV resistance in pea

Book chapters

Clement, S. L., D. S. Husebye, and S. D. Eigenbrode. 2010. Ecological Factors Influencing Pea Aphid Outbreaks in the US Pacific Northwest, pp. 108-128. In P. Kindlmann, A. F. G. Dixon and K. Houdková [eds.], Global warming and aphid biodiversity: patterns and processes. Springer, Dordrecht.

Presentations at conferences

Jain, Shalu, Kasia Kinzer, Sanford Eigenbrode, Kevin McPhee Screening of Lentil Germplasm for Pea Enation Mosaic Virus (PEMV), North Dakota EPSCoR

Vemulapati, B., Druffel, K. L., Eigenbrode, S. D., Karasev, A, and H. R. Pappu. 2009. Antigen-coated Plate ELISA Assays for Rapid and Reliable Detection of Two Members of Luteoviridae: Pea enation mosaic virus (genus Enamovirus) and Bean leafroll virus (genus Luteovirus). WLPGA meeting, 9th December, Moscow, ID.

Vemulapati, B., Druffel, K. L., Eigenbrode, S. D., Karasev, A, and H. R. Pappu. 2010. Molecular characterization and ELISA based detection of Bean leafroll virus and Pea enation mosaic virus from the Pacific Northwestern U.S.A. APS annual meeting, 7-11th August, Charlotte, NC.

Elbakidze, L. Liang Lua, Eigenbrode, S. D. Evaluating vector-virus-plant interaction in regional supply of peas and lentils: a limited dependent variable analysis. Agricultural & Applied Economics Association 2010 AAEA, CAES, & WAEA Joint Annual Meeting, Denver, Colorado, July 25-27, 2010

Husebye, D. S., S.D. Eigenbrode, E.Bechinksi, Alex V. Karasev, Stephen L. Clement, Bhadramurthy Vemulapati, Hanu PappuHusebye, D. Monitoring and Forecasting Virus Diseases In Legumes in the Palouse Region of the Inland Pacific Northwest, USA. Joint Meetings Plant Virus Epidemiology Symposium and Plant Virus Ecology Network. Ithaca, New York, June 20-24, 2010. Poster presentation.

Stokes, B.S., Bechinski, E.J., and Eigenbrode, S.D. Economic Injury Levels for Pea Aphid on Dry Peas. Entomological Society of America Pacific Branch Conference. Boise, Idaho. April 12, 2010. 10-minute graduate student paper competition.

Stokes, B.S., Bechinski, E.J., and Eigenbrode, S.D. Viruliferous Aphid Damage During Specific Growth Stages of Dry Peas. Entomological Sociey of America National Conference. San Diego, California. December 12-15, 2010. Graduate student poster competition. (2nd place)

Jain, S. Eigenbrode, S. and McPhee, K. A gene based marker set for linkage group I of pea (*Pisum sativum* L.)". Plant and Animal Genome Conference XIX, San Diego, California, US on Jan 15-19, 2011.

Clement, S., Eigenbrode, S.D., Husebye, D. Pea Aphid Outbreak Cycles in the U.S. Pacific Northwest, Annual Meeting of the Entomological Society of America, Indianapolis Indiana, Dec. 12-15, 2009

Husebye, D., Eigenbrode, S.D., Clement. S. Forecasting and monitoring pea aphid (*Acyrthosiphon pisum*) pressure on the Palouse. Annual Meeting of the Entomological Society of America, Indianapolis Indiana. Dec. 12-15, 2009

Invited presentations

Eigenbrode, S. D. 2010. Chemical ecology of insect-vectored plant viruses. Department of Entomology, University of Florida, Gainesville, Sep. 17, 2009.

Eigenbrode, S. D. 2009. Chemical ecology of insect-vectored plant viruses. Department of Entomology, University of Florida, Gainesville, Sep. 17, 2009.