Aphid Pests and Aphid-Transmitted Viruses in Fall-Sown Dry Pea (*Pisum sativum*) in the Inland Pacific Northwest Region

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Authorization to Submit Thesis

This thesis of Jake Hennessey, submitted for the degree of Master of Science with a Major in Entomology and titled “Aphid Pests and Aphid-Transmitted Viruses in Fall-Sown Dry Pea (*Pisum sativum*) in the Inland Pacific Northwest Region,” has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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ABSTRACT

Spring-sown dry pea, *Pisum sativum*, are important rotational crops grown with wheat in the Palouse region of eastern Washington and northern Idaho. In the lower rainfall regions of the Palouse, spring-sown dry pea are not viable rotational crops. New varieties of dry pea adapted for germination in the fall have proved to be a better alternative to spring-sown pea as rotational crops with wheat in the lower rainfall regions of the Palouse. Pea aphids, *Acyrthosiphon pisum* (Harris), are a major pest to spring-sown dry pea, in the Palouse region of eastern Washington and northern Idaho. Pea aphids present a threat to dry pea in the region through direct injury from feeding or indirect injury by transmitting viruses, predominantly *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus* (BLRV). The threat of pea aphid to fall-sown dry pea is not known. The general objectives of this thesis were to assess the threat of pea aphid colonization and virus infection in fall-sown dry pea in the Palouse region.

The first objective was to determine if pea aphid abundance and virus status in fall-sown pea differed from those in spring-sown pea locations. Virus prevalence within pea plants was also compared between the two crops. In a two-year field survey, pea aphids were trapped at both fall-sown and spring-sown pea fields and were tested for virus. Aphid abundance and virus status at the end of the growing season did not differ between fall and spring-sown pea fields, nor did virus prevalence in plants. Pea aphids were collected in pan traps adjacent to fall-sown

pea during the fall of both years of the study but were aviruliferous. Nonetheless, virus was detected in plant samples collected in the fall from fall-sown pea plants.

The second objective of this study was to determine the relationship between the timing of viruliferous (PEMV) pea aphid inoculations and yield parameters of fall-sown pea. Previous studies indicated that plants inoculated earlier in their development are more prone to yield loss than plants inoculated at later development stages. Experiments took place at the University of Idaho Parker Farm and the University of Idaho Kambitsch Farm and a greenhouse experiment was conducted at the University of Idaho Manis Laboratory. A similar experiment was performed using spring-sown pea for comparison at the Kambitsch Farm. Periodical inoculations were performed on pea plants in the field and in the greenhouse. Three inoculations were performed before winter and three inoculations were performed after winter. The timing of inoculation was measured as growing degree days (GDD). Inoculations of fall-sown pea in the field experiments took place after plants had experienced 41.5, 42, 44 (before winter), 187.5, 280 and 387.5 (after winter) GDD after emergence. In order to compare with fall-sown pea, Inoculations of spring-sown pea took place after plants had experienced about 44, 187.5 and 280 GDD.

The field experiments in this research supported the hypothesis that plants inoculated at early growth stages will exhibit greater yield losses. Additionally, spring-sown pea plants that were inoculated at similar growth stages as fall-sown pea plants exhibited less yield loss than did fall-sown pea. Regression analysis expressing yield parameters as a function of the timing of inoculation (GDD) resulted in statistically significant ($p > 0.05$) models for total plant biomass per replicate and mean U.S. #1

grade weight per plant in the field experiments at the Kambitsch Farm and the Parker Farm. All inoculations before winter were pooled and compared to the pooled inoculations that took place after winter, revealing that plants that are inoculated before winter exhibit a significant decrease in yield parameters compared to plants that are inoculated after winter.

Results of these experiments indicate that fall-sown pea is subject to greater yield loss if inoculated with virus in the fall than in the spring. However, results from the two-year field survey demonstrated that pea aphid presence in the fall is very low, and virus infection of plants in the fall is very low as well. Therefore, based on this research, it can be concluded that virus risk in fall-sown pea in the fall is not large enough for fall-sown pea to require additional management steps other than the pea aphid monitoring, attention to numeric thresholds and regional forecasts used for pea aphid management in spring-sown pea. It remains possible in the future, that virus injury in fall-sown pea could be substantial in the fall on the Palouse or in the more arid production zones of eastern Washington, justifying management action. Because of the potential threat of excessive virus injury in the fall and projected climate change, the need for continued work is absolute.

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Dedication

I dedicate this Thesis work to my parents, Tom and Shana Hennessey for always providing me with the support system and framework needed to keep myself on the right path moving forward. I would also like to recognize my grandmother, Alice Hennessey, for being an exceptional role model and for her unparalleled editing assistance in helping me complete this thesis.

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INTRODUCTION

In central Washington and north-central Oregon, a monoculture of winter wheat and summer fallow has been the predominant cropping system for 140 years (Schillinger 2017). Over time, monocultures are susceptible to the accumulating pressure from diseases and weeds that are best managed with a break-crop (Nelson 2017), but there are few viable options for the region because of the low precipitation. In contrast, in the higher precipitation areas of the Palouse region of eastern Washington and northern Idaho, dry, edible spring pea has been a successful rotation crop with wheat, providing opportunities for grassy weed control, limiting wheat diseases (Schillinger 2017). Recently, edible fall-sown or winter pea varieties have been developed as an alternative to spring pea in the Palouse region. Winter pea is viable in the lower rainfall regions of Washington and Oregon, and there is considerable interest in adopting it there for its value as a rotation crop.

Dry peas are commonly affected by several insect pest species (O'Neal 2017). Unfortunately for producers, scientific studies on the entomology of winter peas are lacking. I set out to study the effects of pea planting dates (fall vs. spring) on populations and phenology of pea aphid, *Acyrthosiphon pisum* Harris, one of the most important pests of pea due to its direct injury through feeding and as a vector of injurious plant viruses. I placed pan traps charged with antifreeze on the field margins of both fall-sown and spring-sown pea fields to compare trapped aphids between

treatments. Additionally, field studies were conducted to examine fall-sown pea yield quantity and quality as a function of the time when plants were inoculated with *Pea enation mosaic virus*. Field studies were also conducted to compare the rest of the insect fauna inhabiting the canopy in fall-sown peas and spring-sown peas.

CROP OVERVIEW

In the dryland farming region of the inland Pacific Northwest (PNW), winter wheat is the dominant cash crop. This region consists of more than 24 million hectares of dryland farming in Washington, Oregon, and Idaho in areas that average less than 50 centimeters annual precipitation (McGee et al. 2017). The region receives the majority of its precipitation from October to March followed by hot, dry summers (Nelson 2017, Black et al. 2000).

In areas that receive less than 40 centimeters annual precipitation, a two-year rotation of winter wheat-summer fallow is typically practiced (McGee et al. 2017, Schillinger and Young 2014). In areas that receive 40-50 centimeters annual precipitation, a three-year rotation of winter wheat-spring wheat or barley-summer fallow is typically used (McGee et al. 2017). A fallow period helps maintain moisture in the seed zone, allowing the following winter wheat crop planted in early fall to become established before going dormant during winter months (Nelson 2017). Over time, this monoculture system accumulates disease and weed problems. Growers can correct these issues with break crops. Unfortunately, there have been few if any viable break crops available for the low rainfall zones of the inland PNW. An economically viable broadleaf rotational crop for winter wheat is much needed where summer fallow is practiced and no suitable alternative crop is available.

In higher rainfall zones of the PNW, spring-sown pulse crops are commonly grown in rotation with winter wheat. Historically, pea (*Pisum sativum*) and lentil (*Lens culinaris*) have predominated (Guy & Cox 2002). Recently, chickpea (*Cicer arietinum*) has become predominant, largely due to market forces. These crops are harvested as dry seed and marketed for human consumption (Guy & Cox 2002). A typical rotation includes a fall-planted and spring-planted cereal (wheat or barley), with the pulse crop every third year (Guy & Cox 2002). Most of this so-called annual production occurs in the Palouse region of eastern Washington and northern Idaho which boasts highly favorable conditions for dry peas (Freeman 1943). The pulse crops provide multiple agronomic benefits. As do all legumes, pulses form a symbiotic relationship with *Rhizobium* bacteria, enabling them to fix atmospheric nitrogen, therefore reducing the need for inorganic nitrogen fertilizer in the cropping system (Strydhorst et al. 2015). Planting pulses with wheat and barley breaks up weed, insect, and disease cycles in the wheat crop while also improving soil structure and richness by increasing phosphorus, potassium, and sulfur availability (Strydhorst et al. 2015). Dry pea also uses less water than cereal crops and may leave more moisture for subsequent crops (Strydhorst et al. 2015).

There is considerable interest in the development of edible dry pea varieties adapted for fall planting with tolerance to cold temperatures. Fall-sown peas can provide the same advantages as do spring peas or other pulses in rotation with cereals; they interrupt weed, disease, and insect cycles, use *Rhizobium* bacteria to fix atmospheric N, and are manageable with existing farm equipment (McGee et al. 2017). Additionally, in higher rainfall zones, yields of fall-sown varieties are 150% -

200% greater than those of spring planted varieties (McGee et al. 2017), making them more profitable and just as beneficial when in rotation with wheat. Spring-sown pulses, especially peas, in contrast are often highly variable and not economically viable because they mature in conditions of heat stress and terminal drought (McGee et al. 2017). Fall-sown peas are also viable in lower rainfall zones of the PNW (Nelson 2017, Schillinger 2017, McGee et al. 2017) providing the much-needed rotational crop for these monocultures.

Food grade varieties of fall-sown pea are available, and more are in the process of development. Varieties such as Whistler, Specter, Windham, Koyote, Lynx, and Blaze are currently available and frequently used (McGee et al. 2017, Nelson 2017). One of the most important characteristics of these varieties is their winter hardiness. Newly released varieties Lynx and Blaze have tolerance to about -15°C (Nelson 2017). Recent market forces have driven the popularity of chickpea in the PNW, but the advantages offered by fall-sown pea have earned it considerable interest across the region, especially in low rainfall areas where spring-sown legumes do not survive.

PEA APHID AND VIRUS OVERVIEW

Research documenting the insect pest pressure and injury in fall-sown pea is very limited. The insect pests that infest spring-sown pea are likely present in fall-sown pea as well. These pests include pea weevil (*Bruchus pisorum*), pea leaf weevil (*Sitona lineatus*), *Lygus* spp. bugs, and pea aphid (Dosdall et al. 2011). These pests are all seasonal, and migrate into the crop when temperatures rise. Fall sown-peas are well established during typical migrations of these pests and may be more

tolerant to infestation than spring-sown pea. Conversely, due to the fact that fall-sown peas are present earlier, they may be infested earlier in the season. Pest numbers could build up more than they do in the shorter spring-sown pea season.

Pea Aphid Biology and Ecology

The pea aphid is an important pest of leguminous plants worldwide. In North America, the pea aphid ranges from northern Mexico to Canada and infests legume crops such as pea, alfalfa, chickpea, clover, lentil, vetch, and others (Capinera 2001). Adult pea aphids can be wingless (apterous) or winged (alate) and have two color morphs, green and pink (Blackman and Eastop 2006). The pea aphid apterous morph has a soft, teardrop-shaped body 2.3 to 3.5 mm long (Blackman and Eastop 2006), with black bands on the tibia, tarsi, and tip of the cornicles signifying the genus *Acyrthosiphon* (Pike et al. 2003). Other characteristics of the pea aphid include long antennae, legs and cauda, and a U-shaped head (Pike et al. 2003).

The pea aphid lifecycle alternates seasonally between asexual and sexual reproduction, categorizing aphids as holocyclic organisms. Each spring, eggs hatch into a fundatrix, which is an asexual wingless female morph (Stokes 2012). Fundatrices tend to be very large and are viviparous (Stokes 2012). Fundatrices give birth to several generations of morphs called viginiferae, some of which may be alate (winged) forms that disperse to colonize other leguminous hosts (Dixon, 1977).

An adult female pea aphid can give live birth to 7 to 20 nymphs per day (Kraft and Pfleger 2001). Nymphs can reach adulthood after 4 molts, which can take from 9 to 15 days under optimal conditions (Kraft and Pfleger 2001). Because of this, aphid

populations can reach extremely high levels in a very short time. At the end of the season, virginiferae give live birth to the last generation of the year: wingless oviparae (female sexual reproductive morphs) and reproductive male morphs. This generation reproduces sexually to produce eggs that will overwinter until warmer temperatures return (Stokes 2012).

In the PNW, pea aphids overwinter as eggs on perennial legumes, such as alfalfa. When eggs hatch in the spring, aphids migrate to annual legume hosts such as peas. In areas with severe winters, such as the Palouse region, aphids and eggs are unable to overwinter. Instead, aphid eggs overwinter in lower elevations and newly emerged adults migrate to the Palouse in the spring after hatching (Stokes 2012). Clement (2006) proposed the idea that pea aphids migrate to the Palouse on winds blowing from southwestern Washington. In the spring, winged pea aphids colonize dry peas shortly after the crop emerges where they continue the cycle of repeated asexual, all-female generations of both winged and wingless progeny.

Pea aphids can damage peas either directly or indirectly. Direct damage is caused by aphids feeding on phloem sap. Pea aphids have piercing-sucking mouthparts and feed by sucking accumulated photosynthates from phloem tissue. The stylet of the pea aphid is composed of modified mandibles and maxillae that form a single elongate piercing-sucking structure. The labium forms a protective sheath that encloses the stylet bundle (Chapman 1998). The maxillary stylets interlock forming grooves that create the salivary and food canals for the injection of saliva and uptake of nutritious material.

Phloem sap is composed of high concentrations of sugars and low concentrations of amino acids, giving it a high C:N ratio (van Emden & Harrington 2017). In order to survive on such a carbohydrate-rich and amino-acid poor diet, aphids rely on an endosymbiotic relationship with the proteobacteria *Buchnera* spp. to synthesize essential amino acids needed for aphid development (van den Heuvel et al. 2007). *Buchnera aphidicola* is the primary endosymbiont within pea aphids. *Buchnera* is found within eukaryotic host cells, known as bacteriocytes, located within the aphid body cavity (Brinza et al. 2009). *Buchnera* is transmitted vertically to offspring and is responsible for providing the amino acids that the aphid cannot find in its phloem limited diet (Brinza et al. 2009).

Indirect damage caused by aphids to plants comes in the form of viruses that aphids transmit to their host plants. 190 of the 4700 described aphid species are known to transmit plant viruses (Nault 1997), and these aphids are responsible for transmitting 50% of all insect-vectored plant viruses (Nault 1997). Aphid virus transmission involves four stages: i) acquisition—the process by which aphids acquire the virus from an infected plant; ii) retention—the association of virus particles within the vector; iii) latency—the time period required after acquisition before the aphid can transmit the virus to a different plant; iv) inoculation—the release of virus particles into a new plant (Stokes 2012). Based on the time required for these four processes, virus transmission can be broken up into three categories: non-persistent transmission, persistent transmission, and semi-persistent transmission.

Non-persistent transmission is characterized by a very short infectious time (1-30 minutes) with no latent period (Ng et al. 2006). Non-persistently transmitted viruses are also called stylet-borne viruses because the aphid acquires the virus in the stylet during feeding or probing behavior, and the virus does not leave the stylet and enter the body cavity of the aphid. Virions remain in the stylet until the aphid finds a new plant and probing behavior releases the virions into the new plant (Gray et al. 1999). Non-persistently transmitted viruses are characterized as non-circulative because the virus does not enter the body cavity and circulate within the hemolymph, and so these viruses are lost during the molting of the aphid (Stokes 2012).

Persistent transmission involves a long infectious time, usually more than 24 hours. Persistently transmitted viruses are circulative, meaning the virus enters the body cavity of the aphid, crosses the mid or hindgut wall to circulate in the hemolymph until the virus eventually reaches the salivary glands where it will be released into the next plant with the saliva during probing behavior (Gray et al. 1999). Due to the circulative nature of persistently transmitted viruses, the viruses last through the molting of the aphid.

Semi-persistent transmission involves an infectious time in between that of non-persistent and persistent transmission; 30 minutes to 24 hours (Stokes 2012). Just like non-persistently transmitted viruses, semi-persistently transmitted viruses are non-circulative. The virions are typically ingested into the foregut, but they do not enter the hemolymph of the aphid (Stokes 2012).

On the Palouse, pea aphids are known to transmit four different viruses: *Pea enation mosaic virus* (PEMV), *Bean leaf roll virus* (BLRV*), Pea streak virus* (PeSV),

and *Alfalfa mosaic virus* (AMV). Out of these four, PEMV and BLRV are the most important economically (Clement 2006).

Pea enation mosaic virus is in the Luteoviridae family of plant viruses and is the only virus in this family that is not phloem limited (Liu et al. 2010). PEMV is transmitted in persistent circulative manner by more than 10 aphid species including the pea aphid (Stokes 2012). The acquisition time for PEMV is up to 15 minutes in pea aphid nymphs and 1 to 2 hours in adults (de Zoeten and Skaf 2001). The latency period of PEMV is temperature dependent and ranges from 4 to 70 hours (de Zoeten and Skaf 2001). Aphids can successfully transmit PEMV into a healthy plant in a time frame of 7 seconds to 2 minutes (de Zoeten and Skaf 2001). Symptoms of PEMV include chlorosis, mosaic coloring, stunting, and the development of enations on the leaves (Stokes 2012).

Bean leaf roll virus is also in the family Luteoviridae, but unlike PEMV, BLRV is strictly phloem limited. BLRV is transmitted in a persistent circulative manner by its two primary vectors: the pea aphid and the green peach aphid (*Myzus persicae*) (Stokes 2012). The acquisition time for BLRV is reported to be 2 hours or less with a latency time ranging from 16 to 20 hours (Grünwald 2004). Transmission of BLRV can occur in under 60 minutes (Grünwald 2004). Symptoms of BLRV include chlorosis, leaf rolling, short internodes, and stunting (Stokes 2012).

Management of insect-vectored viruses requires an understanding of the economic importance of a virus to a crop as well as an understanding of the effectiveness of each control measure provided by field experiments (Makkouk and Kumari 2009). Control measures are targeted either at decreasing the virus source or

preventing the spread of the virus through the crop (Makkouk and Kumari 2009). Management of insect-vectored viruses in field crops differs between non-persistently and persistently transmitted viruses. Non-persistently transmitted viruses can spread throughout a field much faster than persistently transmitted viruses; therefore, management is very difficult and often impractical (Perring et al. 1999, Nault 1997, Ng and Falk 2006, Ng and Perry 2004). Persistently transmitted viruses such as PEMV and BLRV are slower to spread through a field because they require more time to acquire and transmit; therefore management of these viruses is much more feasible using insecticides (Perring et al. 1999, Nault 1997, Ng and Falk 2006, Ng and Perry 2004).

In order to determine the best management practices for the pea aphid as a virus vector it is important to understand how the virus affects the plant, as mentioned previously. This includes gaining a better knowledge of when plants are most vulnerable to virus infection. Previous work has been done in spring-sown pea to determine the effects of the timing of infection on yield (Stokes 2012). In this study, plants were infected with BLRV-infected or PEMV-infected aphids from the early vegetative growth stages through the last reproductive growth stages (Stokes 2012). Viruliferous aphids were raised in insectary colonies. Results showed that inoculated plants suffered greater economic yield loss when introduced to virus during the earlier vegetative growth stages, as opposed to the later growth stages (Stokes 2012).

The total number of seeds and seed weight both increased as non-linear plateau functions of the timing of viruliferous aphid inoculation (Stokes 2012). Stokes

(2012) proposed this result could be due to the notion that if the plant is inoculated early, the virus has more time to replicate, increasing the amount of virus particles in the plant and causing a greater impact on the vascular system of the plant. Another biological hypothesis for the decreasing damage over time is that the plant is entering different growth stages and possibly becoming more resilient to both aphids and virus (Stokes 2012). The non-linear plateau model developed by Stokes predicts that viruliferous aphid infestations before 32.49 days after emergence (DAE) will result in some proportional yield loss. Therefore, if aphids arrive on or before 32.49 DAE, then control action is economically justified (Stokes 2012).

A similar study done by Paudel et al. (2018) studied the effects of the timing of infection of BLRV and PEMV in lentils. BLRV-infected or PEMV-infected pea aphids were applied to plants to infect them at different stages of development (Paudel et al. 2018). The research showed that economic yield caused by PEMV and BLRV infection is highest soon after plant emergence from the soil and becomes negligible by 50 DAE (Paudel et al. 2018). Paudel et al. (2018) suggested that controlling pea aphid in lentil as a virus vector is not economically justified after 36 DAE.

Studies assessing the effects on yield of plant age at time of infection have not been conducted in fall-sown pea. The pattern that plants are more vulnerable at younger growth stages seen in spring-sown pea and lentil is not known to be true for fall-sown pea. Fall-sown pea poses new questions considering the effects of plant age at the time of infection because fall-sown pea emerges in the fall and overwinters above ground. It remains to be known if plants infected in the fall carry the virus through the winter, thus expressing virus symptoms early in the spring before pea

aphids have arrived. Judging from previous studies, plants infected in the fall will potentially carry the virus for a very long period of time, assuming the virus stays in the plant the entire winter, and therefore will suffer a larger yield loss than plants inoculated later in the spring. The answers to these questions remain to be resolved. These studies are warranted considering their results could mean that fall-sown pea would require drastically different management practices than spring-sown pea.

LITERATURE CITED

Black, A. E., E. Strand, P. Morgan, J.M. Scott, R.G. Wright, and C. Watson. (2000). Biodiversity and Land-Use History of the Palouse Bioregion: Pre-European to Present. Page http://biology.usgs.gov/luhna/index.html in U. S. G. Survey, editor. Land Use History of North America. United States Geological Survey.

Blackman, R. L., Eastop, V. F. (2006). Aphids on the World's Herbaceous Plants and Shrubs:Volumes 1 & 2. San Francisco, California: John Wiley & Sons Ltd.

Brinza, L., Viñuelas, J., Cottret, L., Calevro, F., Rahbé, Y., Febvay, G., ... & Fayard, J. M. (2009). Systemic Analysis of the Symbiotic Function of *Buchnera Aphidicola*, the Primary Endosymbiont of the Pea Aphid *Acyrthosiphon pisum*. Comptes Rendus Biologies, 1034-1049.

Capinera, J. (2001). Handbook of Vegetable Pests. Elsevier.

Chapman, R. F. (1998). The Insects: Structure and Function. Cambridge University Press. Cambridge UK.

Clement, S. L. (2006). Pea Aphid Outbreaks and Virus Epidemics on Peas in the US Pacific Northwest: Histories, Mysteries, and Challenges. Plant Health Progress, 1099-1108

de Zoeten, Gustaaf A., and Skaf, Jihad S. (2001). Pea Enation Mosaic and the Vagaries of a Plant Virus. Advances in Virus Research, 323-350.

Dixon, A. F. G. (1977). Aphid Ecology: Life Cycles, Polymorphism, and Population Regulation. Annual Review of Ecology and Systematics, 329-353.

Dosdall, L. M., Cárcamo, H., Olfert, O., Meers, S., Hartley, S., & Gavloski, J. (2011). Insect Invasions of Agroecosystems in the Western Canadian Prairies: Case Histories, Patterns, and Implications for Ecosystem Function. Biological Invasions, 1135-1149.

Freeman, O. (1943). The Pacific Northwest Pea Industry. Economic Geography, 118-128.

Gray, Stewart M., Banerjee, Nanditta. (1999). Mechanisms of Arthropod Transmission of Plant and Animal Viruses. Microbiology and Molecular Biology Reviews, 128-148.

Grünwald, N. J., Chen, W., and Larsen, R. C. (2004). Pea Diseases and their Management. Diseases of Fruits and Vegetables. Ed. Naqvi S.A.M.H. Kluwer Academic Press, Dordrecht, The Netherlands. Pg. 301-331.

Guy, & Cox. (2002). Reduced Tillage Increases Residue Groundcover in Subsequent Dry Pea and Winter Wheat Crops in the Palouse Region of Idaho. Soil & Tillage Research, 69-77.

Hamon, N., Bardner, R., Allen-Williams, L., & Lee, J. B. (1987). Flight Periodicity and Infestation Size of *Sitona lineatus*. Annals of Applied Biology, 271-284.

Kraft, J. M., & Pfleger, F. L. (2001). Compendium of Pea Diseases and Pests (No. Ed. 2). American Phytopathological Society (APS Press).

Liu, Sijun., Sivakumar, S., Sparks, Wendy O., Miller, Allen W., and Bonning, Bryony C. (2010). A Peptide that Binds the Pea Aphid Gut Impedes Entry of Pea Enation Mosaic Virus into the Aphid Hemocoel. Virology, 107-116.

Makkouk, K. M., and S. G. Kumari. (2009). Epidemiology and Integrated Management of Persistently Transmitted Aphid-Borne Viruses of Legume and Cereal Crops in West Asia and North Africa. Virus Research, 209-218.

McGee, R. J., Eigenbrode, S., Nelson, H., & Schillinger, W. (2017). Re-Inventing Austrian Winter Pea Towards Developing Food Quality Winter Peas. Crops and Soils, 4-46.

Nault, L.R. (1997). Aphid Transmission of Plant Viruses: A New Synthesis. Annals of the Entomological Society of America, 521-547.

Nelson, Howard (2017). Fall Planted Pea Production Guidelines. Central Washington Grain Growers Inc.

Ng, J.C.K. and Falk, B.W. (2006). Virus-Vector Interactions Mediating Nonpersistent and Semipersistent Transmission of Plant Viruses. Annual Review of Phytopathology, 183-212.

Ng, J. C. K., and K. L. Perry. (2004). Transmission of Plant Viruses by Aphid Vectors. Molecular Plant Pathology, 505–511.

O'Neal, S. D. (2017). Pest Management Strategic Plan for Pulse Crops (Chickpeas, Lentils, and Dry Peas) in the United States. Western Integrated Pest Management Center, https://ipmdata.ipmcenters.org/documents/pmsps/2016PulsePMSP_FINAL.pdf

Paudel, S., Bechinski, E. J., Stokes, B. S., Pappu, H. R., & Eigenbrode, S. D. (2018). Deriving Economic Models for Pea Aphid (Hemiptera: Aphididae) as a Direct-Pest and a Virus-Vector on Commercial Lentils. Journal of economic entomology.

Perring, T. M., N. M. Gruenhagen, and C. A. Farrar. (1999). Management of Plant Viral Diseases through Chemical Control of Insect Vectors. Annual Review of Entomology, 457-481

Pike, K., Boydston, Leslie L, Allison, David W, & Washington State University. Extension Service. (2003). Aphids of Western North America North of Mexico: With Keys to Subfamilies and Genera for Female Alatae. Prosser, Wash.]: Washington State University Extension [and] College of Agricultural, Human, and Natural Resource Sciences.

Schillinger, W. F. (2017). Winter Pea: Promising New Crop for Washington's Dryland Wheat-Fallow Region. Frontiers in Ecology and Evolution, 43.

Schillinger, W. F., and D. L. Young. (2014). Best Management Practices for Summer Fallow in the World's Driest Rainfed Wheat Region. Soil Science Society of America Journal, 1707-1715.

Stokes, B.S. (2012). Bioeconomics of Pea Aphids*, Acyrthosiphon pisum* (Harris), on Commercial Dry Peas, *Pisum sativum* 'Aragorn'. M.S. thesis, University of Idaho.

Strydhorst, S., Olson, M. A., Vasanthan, T., McPhee, K. E., McKenzie, R. H., Henriquez, B., ... & Coles, K. (2015). Adaptability and Quality of Winter Pea and Lentil in Alberta. *Agronomy Journal*, *107*(6), 2431-2448.

U.S. climate data. usclimatedata.com/climate/moscow/idaho. Web. 10 September 2018.

van den Heuvel, J.F.J.M. et al. (2007). The N-Terminal Region of the Luteovirus Readthrough Domain Determines Virus Binding to *Buchnera* GroEL and is essential for virus persistence in the aphid. Journal of Virology, 7258-7265.

Van Emden, H. F., & Harrington, R. (Eds.). (2017). Aphids as Crop Pests. Cabi.

Chapter 2. The Relationship Between Pea Planting Dates and Pea Aphid (*Acyrthosiphon pisum*) Populations and Phenology

ABSTRACT

In the Palouse region of eastern Washington and northern Idaho, spring-sown dry pea, (*Pisum sativum* L.), is commonly grown in rotation with wheat (*Triticum aestivum* L.). In the lower rainfall regions of the Palouse, spring-sown dry pea is not a viable rotational crop. New varieties of dry pea adapted for germination in the fall have proved to be a better alternative to spring-sown pea as rotational crops with wheat in the lower rainfall regions of the Palouse. The pea aphid, *Acyrthosiphon pisum* (Harris), presents a threat to spring-sown pea in this region. Pea aphids can injure a plant directly from feeding or indirectly by transmitting two important viruses, *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus* (BLRV). The threat of pea aphid to fall-sown dry pea is not known. Pea aphid arrival, abundance and virus status were measured in four fall-sown dry pea locations and compared with those in four spring-sown dry pea locations in the Palouse region during the 2016-2017 and 2017-2018 growing season. Virus prevalence within plant samples from fall-sown pea was also measured throughout each growing season and compared with the virus prevalence in spring-sown pea plant samples. Pea aphids were collected in fall-sown pea in the fall of both years, but not determined to be carrying virus. Nonetheless, plant samples collected from fall-sown pea locations tested positive for PEMV or BLRV, confirming that viruliferous pea aphids visited these plants in the fall. In the spring of both years, the average number of trapped aphids summed over the entire growing season and

the percentage of these trapped aphids found to be carrying virus were similar for fall-sown and spring-sown pea. The proportion of virus-infected plants in fall-sown pea and spring-sown pea at the end of the growing season of both years was also similar. These results occurred even though aphids were collected from fall-sown pea fields before traps had been placed in spring-sown pea fields and virus was detected in plant samples from fall-sown pea before spring-sown pea plants were sampled for virus. Thus, in the two years of this study, greater exposure of fall-sown pea to virus pressure, both in the fall and early in the spring, as compared with spring-sown pea, did not result in higher end-of-season virus incidence.

INTRODUCTION

In the dryland farming region of the Pacific Northwest (PNW) known as the Palouse, dry pea (*Pisum sativum* L.) is an important rotational crop with wheat (*Triticum aestivum* L.) because it can break insect and disease cycles, provide grassy weed control and fix nitrogen (Nelson 2017). This region of northern Idaho and eastern Washington receives 40-50 centimeters of precipitation per year (McGee et al. 2017). Dry peas in this region are sown from late March to mid-May. Consistently low prices ($0.20/kilogram) make the crop marginally profitable. The inclusion of the crop in rotation with wheat is justified by the significant increases in wheat yields following a pea crop (Sieling and Christen 2015, St Luce et al. 2015). Farther west of the Palouse, in central Washington and north-central Oregon where less than 40 centimeters of precipitation are common, spring-sown dry pea is not a viable rotational crop with wheat due to the lower amounts of rainfall (McGee et al. 2017).

Development of new varieties of dry pea adapted for germination when planted in the fall and for tolerance to cold temperatures is proceeding rapidly (McGee et al. 2013). Agronomic research on fall-sown pea indicates it can be a viable alternative in crop rotations in regions as far west as the arid zones of central Washington state where yields of 5000 lbs/hectare have commonly been obtained (Schillinger 2016). These considerably high yields have earned tremendous interest among growers (producers at the Western Pulse Growers Association meeting, Dec. 2016).

In the PNW, spring-sown dry pea yield is often affected by pressure from insect pests, including the pea aphid, *Acrythosiphon pisum* (Harris). The pea aphid damages plants directly by feeding on the phloem sap of the plant or indirectly by transmitting viruses to the plant. Historically, *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus* (BLEV) are economically the most damaging viruses transmitted by pea aphids (Clement 2006) in this region.

In the PNW, pea aphids migrate to the Palouse in the spring from lower elevations to their annual leguminous hosts where they spend the summer on pea plants or other annual legumes (Clement 2006). The source of these immigrants is the Columbia River Basin, which is situated upwind, to the west of the Palouse (Eigenbrode et al. 2016). Clement (2006) proposed the idea that pea aphids migrate to the Palouse on winds blowing from southwestern Washington. Pea aphids recolonize the Palouse region each spring following extirpation due to severe winter conditions (Clement 2006, Clement et al. 2010). Fall-sown peas are potentially exposed to viruliferous pea aphids in the fall before cold winter temperatures set in

and early in the spring before spring-sown peas emerge. The abundance and virus status of pea aphids that arrive in fall-sown pea in the fall after sowing, or in the spring prior to typical emergence dates of spring-sown pea, are unknown. Current management recommendations for fall-sown pea are based on studies done in spring-sown pea. These recommendations may not necessarily be accurate for fall-sown pea, considering their differing phenology from spring-sown pea.

In this paper, to anticipate the potential of fall-sowing, we aimed to determine the abundance and virus status of pea aphids within fall-sown pea. Pea aphid abundance was monitored at fall-sown pea locations and compared to pea aphid abundance at spring-sown locations. All fall-sown pea locations were experimental plots, and all spring-sown pea were commercial fields. The virus status of aphids trapped at locations of each planting regime were compared for differences. The abundance and virus status of pea aphids in fall-sown pea during the fall and early in the spring before spring-sown pea had emerged were also determined. Virus prevalence among plants within the field of fall-sown pea locations was monitored in the fall, early spring, and later summer before harvest. Virus prevalence in fall-sown pea fields at the end of the growing season was compared to virus prevalence in spring-sown pea fields at the end of the growing season. Pea aphid abundance and virus status, as well as virus prevalence within the crop of fall-sown pea was hypothesized to not differ from spring-sown pea.

MATERIALS AND METHODS

Pea aphids and crop plant tissue were sampled at fall-sown and spring-sown pea locations during the 2016-2017 and 2017-2018 growing seasons. Fall-sown pea

locations were sampled beginning in the fall as soon as seeds were planted, and sampling continued until temperatures dropped below 0°C. Sampling in fall-sown pea resumed the following spring after plant green-up and continued until harvest. Spring-sown pea was sampled as soon as possible after planting in the spring, and continued until harvest. Each location was sampled weekly for aphid arrival and virus prevalence. Plant tissue samples were also taken at each pea crop location to determine virus prevalence. Fall-sown pea locations were sampled for plant tissue once in the fall, once in early spring, and once in the summer before the plants began to dry. Spring-sown pea locations were sampled for plant tissue once in the summer before plants began to dry.

Aphid Sampling

Aphid sampling was conducted by placing three pan traps along the field margin, near the crop and out of the way of farming equipment. Each pan trap was composed of a 19-liter black bucket and a brown plant saucer 31 cm in diameter with a yellow paper plate glued to it. The bucket is filled with soil to within 2 inches of the rim to keep it in place. The plant saucer and yellow plate are placed on top of the bucket. Three matching holes are drilled in the sides of the plant saucer and the bucket. The plant saucer and bucket are then attached by gardening twist-ties woven through each hole. The trap is then “charged” by pouring Prestone LowTox® Antifreeze/Coolant (Antifreeze/Coolant PN: AF555) into the yellow plate. Enough antifreeze to fill the plate halfway to the rim is poured in. The antifreeze traps the aphids and does not evaporate, allowing the preservation of specimens for several days.

Pan traps were checked on a weekly basis. After pea aphid specimens had been removed from the trap, the used antifreeze containing the remaining arthropod bycatch was poured into an empty antifreeze jug and submitted to Environmental Health and Safety personnel for proper disposal. Fresh antifreeze was poured into the plate, “recharging” the trap for another week of sampling. The traps typically captured a large number of insects from many species. Pea aphids were identified immediately during trap servicing and collected into 2.5 mL freestanding microcentrifuge tubes and preserved in ethyl alcohol (ETOH) 95%. The location and date of collection of each aphid were recorded. For this study all aphids were shipped to the Diagnostics Laboratory at the University of Idaho Parma Research and Extension Center in Parma, Idaho. After arrival, aphids were tested for *Pea enation mosaic virus* and *Bean leaf roll virus* using a PCR method as outlined below. When a large number of aphids (> 10) was collected, a subsample of 10 aphids was shipped for testing. For each date, the total number of aphids testing positive for either virus was used to calculate the proportion of aphids found to be carrying virus on each date.

Aphid sampling in fall-sown pea and spring-sown pea did not occur at consistent locations from year to year. Some of the locations used for the 2017 growing season were geographically close to some of the locations used in the 2018 growing season, but only two fall-sown pea locations were the same for both years of data collection. Sampling locations for pea during 2016-2018 were very scarce due to the low prices of pea as compared with chickpea. This made it nearly impossible to sample the same locations two years in a row. The intent of this sampling was also to

cover a larger geographical area, but the limited number of pea locations available did not allow this, therefore limiting the power of this study. Within any year, fall and spring-sown pea fields were not sited as neighboring pairs, but were up to 56 km from each other.

Plant Tissue Sampling

Each year, plant tissue samples were taken from each location where aphids were trapped. Additional locations dry pea locations deemed too distant for weekly aphid sampling were sampled for plant tissue as well. At fall-sown pea locations, plant samples were taken as late as possible in the fall before temperatures dropped below 0°C, again after green-up in the spring, and a final time before plants began to dry down. In the spring-sown pea, plant tissue was not available for sampling in the fall or during the first sample date in the spring, so plant tissue was only sampled once before plants began to dry down. To sample plant tissue, three separate 100-m transects were measured and samples were taken at intervals along the transect (see below). Each sample consisted of about 1 gram of leaf material plucked by hand. Twenty total samples were taken from each location, seven along each of two transects and six along the third transect. Samples were taken every 14 m on the first two transects and every 17 m on the third transect at each site. Tissue sampled from each individual plant was placed in an individual plastic bags, packed in an insulated package, and shipped to the Diagnostic Laboratory in Parma to be tested individually for PEMV and BLRV, using a similar PCR method to that which was used to test aphids for virus.

Real-time PCR (TaqMan®) was carried out in 96 well plates using the QuantStudio 3 Real-time PCR system. For aphid material, TaqMan® Fast Virus 1-Step Master Mix (Thermo Fisher, Waltham, MA) was used. For plant material, Luna universal one step RT-qPCR kit (New England Biolabs, Ipswich, MA) was used. Primers and probes (Eurofins Genomics) were added to a final concentration of 300 nM and 100 nM respectively, with the remaining volume made up with water to 20ul. Cycling conditions consisted of 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The cycle threshold (Ct) value for each reaction was assessed using the automatic threshold determined by the QuantStudio 3 software. Each sample was tested in two replicates. An average (mean) Ct was calculated each time. Target DNA in samples was quantified by including at least five DNA standards on each PCR run. The standards consisted of synthetic DNA Gene Strands (Eurofins Genomics, Louisville, KY) which was designed to correspond to the appropriate primer and probe sequence. The Gene Strand (Eurofins Genomics, Louisville, KY) was of known concentration and used to produce a dilution series of five, ten-fold dilutions. The amount of viral nucleic acid present was then determined by linear regression.

Primers and probes were either the PEMV1 primers from Timmerman-Vaughan et al. (2009) or recently designed primers with specificity towards BLRV (James Woodhall, personal communication). RNA was extracted using the SV total RNA system with the DNase steps omitted. Aphid samples were homogenized using a Precellys Evolution (Bertin Instruments, Montigny-le-Bretonneux, France) tissue homogenizer. Leaf samples were homogenized in BioReba (Bioreba Agro-

Diagnostics, Reinach, Switzerland) extraction bags in conjunction with a hand model homogenizer.

Fall 2016

During the fall of 2016, five fall-sown pea locations (sites #1- #5, Table 2.1) were sampled for pea aphid presence and virus prevalence. Sites did not have an equal number of sampling dates because traps were not placed in the field until seeds were planted and knowledge of field location was gained. Site #3 was sampled eight times before winter. Sites #1 and #2 were each sampled seven times before winter. Sites #4 and #5 were planted later in the fall, resulting in only 3 sampling dates of these locations. All traps were removed from the field by 30 November 2016. All pea aphid samples were shipped to the University of Idaho Parma Research and Extension Center to be tested for virus.

At the end of the fall on the dates of 29 November 2016 and 30 November 2016, plant tissue samples were taken from each of the five fall-sown pea locations using the above described method. Additionally, plant tissue was sampled from two other fall-sown pea sites (sites #6 and #7). All plant samples were shipped to the University of Idaho Parma Research and Extension Center for virus analysis, concluding the sampling for the fall of 2016.

Spring/Summer 2017

Pan traps were placed back in fall-sown pea fields on 22 March 2017. No traps were placed at site #5 near Ferdinand, ID (Table 2.1) because of herbicide application error in the spring of 2017, killing a majority of the plants in the plot. Pan

traps were sampled for the first time on 17 May 2017 and subsequently sampled on a weekly basis until 26 July 2017 when they were checked for a final time and removed from the fields before harvest. Plant tissue was sampled from each of the fall-sown pea sites (including the discontinued site #5) on the dates of 15 May 2017 and 16 May 2017 to be tested for virus. Plant tissue samples were also taken from the sites near Lind, WA and Dayton, WA on 18 May 2017.

On 2 June 2017 pan traps were placed at eight spring-sown pea locations (Table 2.1). Spring-sown pea locations were provided by Jerry Mraz of the Pacific Northwest Farmers Cooperative. Traps remained in spring-sown pea fields until 26 July 2017 when traps were removed in preparation for harvest. All pea aphid samples were shipped to the University of Idaho Parma Research and Extension Center during the same week of their collecting to be analyzed for virus. Comparisons in the average number of aphids trapped per site per date and the proportion of trapped aphids carrying virus per site per date in fall-sown and spring-sown pea locations were done using only the collection dates when traps had been placed in both fall-sown and spring-sown pea locations. These included the dates from 14 June 2017 to 26 July 2017 for a total of seven collection dates (Figure 2.2).

Plant tissue samples were taken from fall-sown pea fields for the third and final time on the dates of 11 July 2017 – 13 July 2017. Tissue samples were also taken from an additional fall-sown pea field (site #17) (Table 2.1). Tissue samples were taken from spring-sown pea fields for the first and only time of the season on 12 July 2017. All plant samples were shipped to the University of Idaho Parma Research and

Extension Center for virus analysis, concluding the sampling for the 2017 growing season.

Fall 2017

The same methods for aphid sampling were repeated for the 2018 growing season. In the fall of 2017, pan traps were placed at four fall-sown pea locations (table 2.2) to sample pea aphid presence and virus prevalence. Traps were checked on a weekly basis until their removal before winter on 30 November 2017. All pea aphid samples were identified and shipped to the University of Idaho Parma Research and Extension Center for virus analysis. Similar to 2016, traps were placed at the locations on different dates, and were sampled a different number of times. Site #3 was sampled nine times, Site #4 was sampled eight times, and sites #1 and #2 were sampled seven times (Table 2.2).

At the end of the fall on the dates of 10 November 2017 and 11 November 2017, plant tissue samples were taken for the first time from each of the four fall-sown pea locations. Additionally, plant samples were taken from five other fall-sown pea locations where aphid traps were not placed (sites #5-9). All plant samples were shipped to the University of Idaho Parma Research and Extension Center for virus analysis at the end of November, concluding sampling for the fall of 2017.

Spring/Summer 2018

Pan traps were set at fall-sown pea locations on 10 April 2018. Traps were checked on a weekly basis from 17 April 2018 to 23 July 2018 until their removal before harvest. for a total of 15 collection dates (Table 2.2). On the dates of 30 April

2018 and 1 May 2018, plant tissue samples were taken for the second time from all fall-sown pea locations (Table 2.2).

On 24 May 2018, pan traps were placed at five spring-sown pea locations (Table 2.2). Once again, spring-sown pea locations were provided by Jerry Mraz of the Pacific Northwest Farmers Cooperative. These traps remained in the field until their removal on 24 July 2018 in preparation for harvest. Comparisons of the average number of aphids trapped per site per date and the proportion of trapped aphids carrying virus per site per date in fall-sown and spring-sown pea locations were again done using only the collection dates when traps had been placed in both fall-sown and spring-sown pea locations. These included the dates from 28 May 2018 to 23 July 2018 for a total of nine collection dates (Figure 2.2).

Plant tissue samples were taken from all fall-sown pea locations for the third and final time during the week of 9 July 2018 – 13 July 2018 (Table 2.2). The five spring-sown pea locations and two additional spring sown pea locations near Moscow, ID (sites #15 & #16) were sampled for plant tissue for the first and only time during the same week. All tissue samples were shipped to the University of Idaho Parma Research and Extension Center for virus analysis, thus concluding the sampling for the 2018 growing season.

Statistical analyses

The goal of this experiment was to determine if the aphid density in traps and the percentage of viruliferous aphids differed significantly between fall-sown and spring-sown pea. Each sampling date generated two summary statistics: mean

number of aphids trapped per site and mean proportion of viruliferous aphids. I hypothesized there would be no differences in these two metrics between fall and spring-sown fields on the same seasonal sampling dates.

For the 2017 growing season, pea aphid parameters were compared between fall-sown pea and spring-sown pea using only the dates when traps were present in both fall-sown and spring-sown pea fields (14 June 2017 - 26 July 2017, Table 2.1). Only four fall-sown pea fields were being monitored at this point, so only four spring-sown pea fields were used for statistical analyses (sites #8, 11, 12, 13, Table 2.1). As mentioned previously, the lack of study sites did not allow for specific requirements to be made for choosing sites for comparison, so the spring-sown pea sites used for statistical analyses were chosen based on their closer proximity to the fall-sown pea sites compared to the other spring-sown pea sites. Although they were not used for statistical analysis, the remaining spring-sown pea sites (sites # 9, 10, 14, 15, 16, Table 2.1) were still monitored for pea aphids on a weekly basis.

For the 2018 growing season, pea aphid parameters were once again compared between fall-sown pea and spring-sown pea using only the dates when traps were present in both fall-sown pea fields and spring-sown pea fields (28 May 2018 – 24 July 2018, Table 2.2). Once again, only four fall-sown pea locations were monitored, so only four spring-sown pea locations were used for statistical analyses (sites #10, 11, 12, 13, Table 2.2). Spring-sown pea sites used for statistical analyses were again chosen by their closer proximity to the fall-sown pea locations. Site #14 was not used for statistical analysis, but it was still monitored on a weekly basis.

To test for differences in each summary statistic between fall-sown pea sites and spring-sown pea sites, a Welch's two-sample *t*-test (RStudio Team 2016) was used. The Welch's two-sample *t*-test is used when the two samples being compared have unequal sample sizes and unequal variances, making it the most logical statistical test to determine a difference in the average number of pea aphids trapped in two different treatments as well as the average percentage of viruliferous pea aphids trapped in two different treatments.

Additionally, I sought to determine if a difference in virus prevalence within plants in the field was detectable in fall and spring-sown pea. To do this, the proportion of all plants testing positive for virus from all fall-sown pea sites was compared to the proporiton of all plants testing positive for virus from all spring-sown pea sites using a Welch's two-sample *t*-test (RStudio Team 2016). The data from supplemental sites (Table 2.1 & 2.2) that were not monitored with pan traps were used in this analysis. I compared for differences in the proportion of infected plants between fall-sown and spring-sown pea for both years using the plant samples taken at the end of summer when plant samples could be taken from both fall and spring-sown pea locations. I also tested for a yearly effect. This means I tested for differences in the proportion of infected plants between the 2017 and 2018 growing seasons. Thus, the proportions of infected plants at fall-sown pea locations in the fall of 2016, spring of 2017 and summer of 2017 were compared to the corresponding proportions of infected plants at fall-sown pea locations in the fall of 2017, spring of 2018 and summer of 2018 respectively. Similarly, the proportion of infected plants at

spring-sown pea locations in the summer of 2017 was compared to the proportion of infected plants at spring-sown pea locations in the summer of 2018.

RESULTS

Fall 2016

During the fall of 2016, only three pea aphids were collected during the entire fall 2016 trapping season (Figure 2.1). All three aphids came from site #2 on 26 October 2016. All three of the trapped pea aphids were tested for virus and all tested negative for both PEMV and BLRV (Figure 2.2).

Plant tissue samples collected from fall-sown pea fields (14) at the end of the fall revealed 18% of the total number of plant samples (140 samples) tested positive for either PEMV or BLRV (Table 2.3). Due to the small percentage of BLRV (a total of 4%), it was combined with the percentage infected with PEMV to create a total proportion of plants infected with virus. Although infection with BLRV was rare across both years, an exception occurred in fall of 2016 at the location near Lind, WA (47.001467 N, 118.563105 W), in which 19 out of 20 samples tested positive for BLRV. This proportion accounted for 14% out of the total 18% of infected plants for the fall of 2016 (Table 2.3)

Spring/Summer 2017

The mean number of pea aphids trapped per site per date at fall-sown and spring-sown pea locations did not differ at the end of the 2017 growing season ($p=0.9579$, Figure 2.1). The number of aphids trapped also did not differ between spring-sown and fall-sown pea on any individual date, except for 26 July 2017

(p=0.006) when pea aphids were only collected from fall-sown pea locations. The percentage of trapped aphids carrying virus per site did not differ between fall-sown and spring-sown pea locations during the entire 2017 growing season (p=0.4228, Figure 2.2) or on any single date. (Figure 2.2). The average percentage of aphids testing positive for virus in fall-sown pea and spring-sown pea was 22% and 17% respectively.

Plant tissue sampling of fall-sown pea in the early spring of 2017 resulted in 19% of the plant samples testing positive for virus, a very slight increase from the fall of 2016 (Table 2.3). Plant tissue samples collected from both fall-sown and spring-sown pea locations in the summer of 2017 did not differ, as 30% and 33% of plant samples tested positive for virus in fall-sown and spring-sown pea, respectively (Table 2.3). These percentages did not differ (p=0.776).

Fall 2017

Two pea aphids were collected during the entire fall season of 2017 (Figure 2.1), one from site #1 and the other at site #2. Both aphids were collected on 26 October 2017 and neither aphid tested positive for virus (Figure 2.2). Plant tissue samples were collected at the end of the fall, and 1% of the samples tested positive for virus (Table 2.3).

Spring/Summer 2018

The average number of pea aphids trapped per site per date did not differ between fall-sown and spring-sown pea locations at the end of the 2018 growing season (p=0.6531, Figure 2.1) or on any individual collection date. The percentage of

trapped aphids carrying virus per site did not differ between fall-sown and spring-sown pea locations during the 2018 growing season (p=0.6052, Figure 2.2) or on any single collection date (p > 0.05). The average percentage of aphids testing positive for virus in fall-sown pea and spring-sown pea was 7% and 11% respectively.

Of the plant tissue samples collected at fall-sown pea locations in the spring of 2018, 3% tested positive for virus (PEMV or BLRV), again a slight increase from the fall of 2017 (Table 2.3). Of the tissue samples collected from fall-sown and spring-sown pea locations in the summer of 2017, 36% and 38% of plants tested positive for virus (Table 2.3). These percentages did not differ between the two planting regimes (p=0.6452, Table 2.3).

Plant Tissue Infection Comparison Between Years

There was no year vs. year effect on the proportion of plants testing positive for virus. The proportion of fall-sown pea plants testing positive for virus did not differ in the fall (p = 0.3194), early spring (p = 0.4566), or summer (p = 0.2006) of both years (Table 2.4). The proportion of plants infected with virus also did not differ between spring-sown pea plants in the summer of 2017 vs. the summer of 2018 (p=0.2266, Table 2.4). Lastly, the proportion of fall-sown and spring-sown pea plants infected by virus across the entire 2017 and 2018 seasons did not differ. (p=0.2563, Table 2.4).

DISCUSSION

This study provides information on the timing and rate of infestation of colonizing pea aphids as well as aphid-vectored virus prevalence in fall-sown pea in

the Palouse region of eastern Washington and northern Idaho. Prior to this research, no data had been gathered on the threat of pea aphid infestation and aphid-borne virus infection to fall-sown pea in the fall after sowing or in the early spring before spring-sown pea has emerged. In the fall, few aphids were collected in pan traps and none were determined to be carrying virus in either year of the study, suggesting a very minimal threat of aphid infestation in the fall. Nonetheless, plant tissue samples collected from fall-sown pea fields at the end of the fall each year included samples testing positive for PEMV or BLRV. Since PEMV and BLRV are not seedborne and are obligately transmitted by aphids (Liu et al. 2010, de Zoeten and Skaf 2001, Grünwald 2004), these infected plants must have been visited by and infected by viruliferous aphids during the 4 weeks from emergence until the plant samples were taken. Virus prevalence within plant samples confirms that pea aphid is a potential threat to fall-sown pea as a virus vector in the fall in the PNW.

Other fall-planted crops in the Palouse are vulnerable to virus infection prior to onset of winter. Fall flights of cereal aphids, especially *Rhopalosiphum padi* present a risk as vectors of *Barley yellow dwarf virus* in many regions where wheat and barley are sown in the fall (Halbert and Pike 1985, Fabre et al. 2003). Information about fall flights of *R. padi* has been used to model risk of virus in cereals (Thackray et al. 2009). Fall-planted cereals can be inoculated with mite-transmitted *Wheat streak mosaic virus* (Hadi et al. 2011) with implications for management of this virus. Fall-planted forage legumes or pulses are also known to acquire viruses prior to winter in other locations (Ashby 1980, McLaughlin 1983), so our finding that this is the case in PNW pulses is not unexpected. In suction trap samples taken across the PNW from

1985 to 2003, small numbers of pea aphids were taken into October in the Palouse most years (unpublished suction trap data provided by K. Pike, A. Rashed, S. Halbert). Fall-sown pea in the PNW may require monitoring for aphids and virus prior to winter to assess the risk and manage the viruses that can be introduced by fall flights of pea aphids. The few aphids trapped during fall in this study were taken in late October (26 Oct in both years), possibly indicating a period when fall flights of pea aphids from lower elevations occur. For fall-sown cereal crops vulnerable to virus, later planting is sometimes recommended to reduce risks of BYDV infection (Murray et al. 1984, Makkouk et al. 2009, Perry et al. 2000). This recommendation is difficult to follow because of other constraints on the timing of fall planting cereal crops (Ray Mosman, personal communication). Similarly, delayed planting of fall-sown pea to avoid inoculation might be indicated, but would be very difficult to implement, especially if viruliferous aphids are arriving throughout October (Murray et al. 1984).

Pea aphids migrate to the Palouse in the spring from lower elevations to colonize pea plant hosts and return again to lower elevations before winter (Clement et al. 2010, Eigenbrode et al. 2016). This migration pattern explains the typical infestation of pea aphids in spring-sown pea on the Palouse. Although this study and prior work documents fall flights of pea aphid, their origins are unknown. Presumably, these aphids arrive on prevailing westerly winds similar to those carrying pea aphid flights in spring. Sources of virus in the spring are apparently cultivated alfalfa (BLRV), vetch, pea, or clover (PEMV and BLRV) (Eigenbrode et al. 2016). By the end of the summer, it is possible that other cultivated host plants or wild hosts become

reservoirs for virus and sources for immigrating pea aphids (McWhorter & Cook 1958, Hampton & Weber 1983). If so, this may have implications for the severity or frequency of virus infestations in fall-sown pea. As with spring planted pea (http://legumevirusproject.org), longer term records of virus incidence in fall-planted pea will be needed to determine these risks.

In the spring of both years, the average number of trapped aphids and the percentage of these trapped aphids found to be carrying virus were similar for fall-sown pea and spring-sown pea. This occurred even though aphids were collected from fall-sown pea fields before traps had been placed in spring-sown pea fields, albeit at barely detectable densities, and in 2017 a high percentage of these aphids trapped early in fall-sown pea were found to be carrying virus. Nonetheless, by the end of both growing seasons, the average proportion of plant samples testing positive for aphid-vectored virus from fall-sown and spring-sown pea locations did not differ. Thus, in the two years of this study, greater exposure of fall-sown pea to virus pressure, both in the fall and early in the spring, as compared with spring-sown pea, did not result in higher overall incidence of infection. Fall-sown pea is exposed to virus pressure longer than spring-sown pea, and based on this study can become infected during the time that spring-sown pea is unavailable. Studies have shown that pea aphids prefer to settle on plants infected with BLRV or PEMV compared with healthy plants (Wu et al. 2014). If immigrating pea aphids orient towards virus-infected fall-sown pea instead of healthy, newly emerged spring-sown pea, this could elevate the presence of virus in a landscape in which both types of pea are grown extensively, assuming aphid flights then occur among these crops, potentially

carrying virus. During this study, fall planted pea were extremely rare in the inland PNW so virus movement between the crops would be negligible at best. Based on the data in this study, virus infestation continued during the spring, obscuring differences between spring and fall-sown pea that occurred due to the longer exposure of fall-sown pea to flights of infectious aphids.

Although the proportion of plants infected was similar in fall-sown and spring-sown pea, the timing of potential infection differed. Virus was detected in fall-sown pea plant samples early in the spring of both years, before plant tissue was sampled from spring-sown pea fields, and in the fall before the onset of winter. Some of these plants might have been infected earlier in development than is typical in spring-planted pea. Since pea plants are not known to recover from virus infection once infected, they must remain infected through the winter, with unknown effects on pea plant health and yield. Stokes (2012) determined that spring-sown pea yield is less affected by virus infection as the plant matures and Paudel et al. (2018) reported a similar effect for lentils. Maturity or ontogenic tolerance to plant pathogen infection occurs for other systems (Develey-Rivière & Galiana 2007). This means that the plants infected at early growth stages exhibit greater detrimental effects on yield as compared to plants infected at later growth stages. Fall-sown pea plants infected early in development could be more injured that those infected later. Yields of spring and fall-sown pea were not compared in this study, but the greater yield potential of fall-sown pea (McGee et al. 2017) would potentially confound differences between spring and fall-sown pea due to virus infections. In order to determine the most effective management practices for pea aphid in fall-sown pea, it is important to

understand the age-related effects of virus inoculation on yield in fall-sown pea. A controlled experiment for the effect of infection timing has not been conducted in fall-sown pea (see Chapter 3, this Thesis).

Pea aphids found in spring and summer months in the PNW are genetically diverse (Eigenbrode et al. 2016), as they are elsewhere where they have been investigated (Peccoud et al. 2008, Peccoud et al. 2009, Peccoud and Simon 2010). This has implications for what are their primary or preferred host plants and which viruses they may carry into the crop (Davis et al. 2017). Fall migrants have never been examined for their genetic diversity or similarity to spring migrants. It is possible that fall migrants may also be genetically diverse, and this possibly merits investigation. That would require continued sampling to obtain sufficient numbers of aphids for analysis.

This study had several limitations. Sampling locations for both fall-sown pea and spring-sown pea were very limited due lack of fields as a result of low prices of pea as compared to chickpea. The scarcity of pea locations made it nearly impossible to sample the same locations two years in a row as well as cover a large area geographically, thus decreasing the power of this study. Additionally, sampling locations did not all receive the same number of collection dates because they were not planted at the same time or I was not informed of their location at the same time. In both years, aphid traps were not placed at spring-sown pea locations until 14 June 2017 and 28 May 2018.

In summary, pea aphids were captured in fall-sown pea locations in the fall and early spring, but by the end of the growing season, the average number of pea

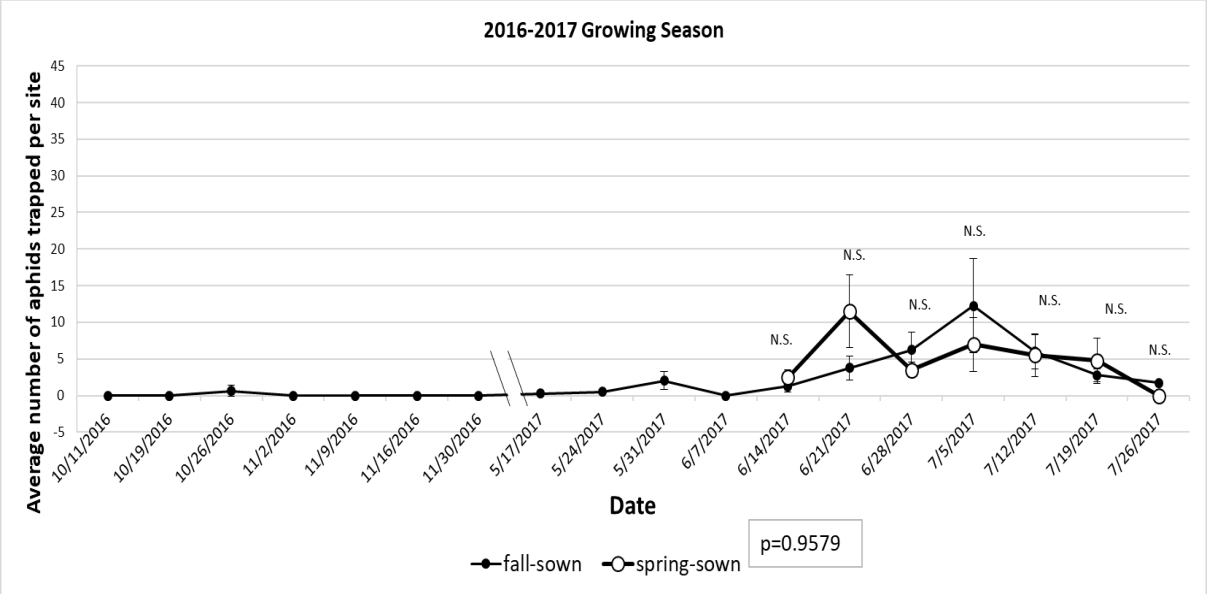
aphids trapped per site per date did not differ between fall-sown pea and spring-sown pea, suggesting that fall-sown pea is not more or less vulnerable to aphid infestation compared to spring-sown pea, but just as vulnerable. Virus was detected in plant tissue in fall-sown pea in the fall and early spring, but by the end of the growing season, the proportion of infected fall-sown pea plants and infected spring-sown pea plants did not differ, suggesting that fall-sown pea is at least as vulnerable to aphid vectored virus infection as is spring-sown pea. The results of this study suggest that the same treatment thresholds and management practices of pea aphid that have been developed for spring-sown pea are appropriate to implement in fall-sown pea. Chapter 3 of this thesis describes additional work needed to validate this conclusion and to determine proper management practices.

Table 2.1 – Locations for fall-sown and spring-sown pea that were sampled for the prevalence of virus in plant tissue and for the arrival and virus status of aphids using field-side pan traps in 2016-2017.

		FALL 2016		
Name	**Coordinates**	**Description**	**Sample Start**	**Sample End**
Site #1	47.032921 N, 117.033265 W	11.1 km northeast of Garfield, WA	10-19-16	11-29-16
Site #2	46.693925 N, 117.138842 W	5 km south of Pullman, WA	10-19-16	11-30-16
Site #3	46.87777 N, 116.947137 W	19.3 km south of Moscow, ID	10-11-16	11-29-16
Site #4	46.727697 N, 116.955698 W	3.2 km east of Moscow, ID	11-9-16	11-29-16
Site#5	46.158974 N, 116.422030 W	2.9 km west of Ferdinand, ID	11-8-16	11-30-16
Site #6	47.001467 N, 118.563105 W	5.5 km northeast of Lind, WA	11-30-16	Plant tissue sampled only
Site #7	46.392315 N, 118.055179 W	16.6 km northwest of Dayton, WA	11-30-16	Plant tissue sampled only
		SPRING/SUMMER 2017		
Name	**Coordinates**	**Description**	**Sample Start**	**Sample End**
Site #1	47.032921 N, 117.033265 W	11.1 km northeast of Garfield, WA	3-29-17	7-26-17
Site #2	46.693925 N, 117.138842 W	5 km south of Pullman, WA	3-29-17	7-26-17
Site #3	46.87777 N, 116.947137 W	19.3 km south of Moscow, ID	3-29-17	7-26-17
Site #4	46.727697 N, 116.955698 W	3.2 km east of Moscow, ID	3-29-17	7-26-17
Site#5	46.158974 N, 116.422030 W	2.9 km west of Ferdinand, ID	Discontinued in the spring/summer	NA
Site #6	47.001467 N, 118.563105 W	5.5 km northeast of Lind, WA	5-18-17 & 7-12-17	Plant tissue sampled only
Site #7	46.392315 N, 118.055179 W	16.6 km northwest of Dayton, WA	5-18-17 & 7-12-17	Plant tissue sampled only
Site #8	47.361737 N 117.102705 W	6.9 km southeast of Fairfield, WA	6-14-17	7-26-17
Site #9	47.372867 N 117.109997 W	5.9 km southeast of Fairfield, WA	6-14-17	7-26-17
Site #10	47.391597 N 117.255901 W	8.9 km west of Fairfield, WA	6-14-17	7-26-17
Site #11	46.586676 N 117.187143 W	19.8 km south of Pullman, WA	6-14-17	7-26-17
Site#12	46.607488 N 117.206171 W	18.2 km south of Pullman, WA	6-14-17	7-26-17
Site #13	46.610778 N 117.241623 W	18 km south of Pullman, WA	6-14-17	7-26-17
Site #14	46.589918 N 117.216111 W	21.4 km south of Pullman, WA	6-14-17	7-26-17
Site #15	46.573245 N 117.237750 W	27.8 km south of Pullman, WA	6-14-17	7-26-17
Site #16	46.549638 N 117.181676 W	27.4 km south of Pullman, WA	6-14-17	7-26-17
Site #17	46.242187 N, 117.059244 W	15.9 km south of Asotin, WA	7-13-2017	Plant tissue sampled only

Table 2.2 - Locations for fall-sown and spring-sown pea that were sampled for the prevalence of virus in plant tissue and for the arrival and virus status of aphids using field-side pan traps in 2017-2018.

		FALL 2017		
Name	**Coordinates**	**Description**	**Sample Start**	**Sample End**
Site #1	46.727697 N, 116.955698 W	3.2 km east of Moscow, ID	10-20-17	11-30-17
Site #2	46.87777 N, 116.947137 W	19.3 km south of Moscow, ID	10-19-17	11-30-17
Site #3	46.518191 N 116.826716 W	10.1 km east of Genesee, ID	10-6-17	11-30-17
Site #4	47.117940 N 117.542154 W	6.9 km northwest of St. John, WA	10-11-17	11-30-17
Site #5	47.001467 N, 118.563105 W	5.5 km northeast of Lind, WA	11-11-17	Plant tissue sampled only
Site #6	46.392315 N, 118.055179 W	16.6 km northwest of Dayton, WA	11-11-17	Plant tissue sampled only
Site #7	46.693925 N, 117.138842 W	5 km south of Pullman, WA	11-10-17	Plant tissue sampled only
Site #8	47.032921 N, 117.033265 W	11.3 km northeast of Garfield, WA	11-1017	Plant tissue sampled only
Site #9	46.548365 N, 116.909372 W	1.3 km east of Genesee, ID	11-10-17	Plant tissue sampled only
		SPRING/SUMMER 2018		
Name	**Coordinates**	**Description**	**Sample Start**	**Sample End**
Site #1	46.727697 N, 116.955698 W	3.2 km east of Moscow, ID	4-17-18	7-23-18
Site #2	46.87777 N, 116.947137 W	19.3 km south of Moscow, ID	4-17-18	7-23-18
Site #3	46.518191 N 116.826716 W	10.1 km east of Genesee, ID	4-17-18	7-23-18
Site #4	47.117940 N 117.542154 W	6.9 km northwest of St. John, WA	4-17-18	7-23-18
Site #10	46.657486 N 117.314420 W	15.9 km southwest of Pullman, WA	5-28-17	7-23-18
Site #11	46.626410 N 117.237584 W	16.9 km south of Pullman, WA	5-28-17	7-24-18
Site #12	46.610778 N 117.241623 W	18 km south of Pullman, WA	5-28-17	7-24-18
Site #13	46.587633 N 117.587633	21.9 km south of Pullman, WA	5-28-17	7-24-18
Site #14	46.541442 N 117.195458 W	28.8 km south of Pullman, WA	5-28-17	7-24-18
Site #5	47.001467 N, 118.563105 W	5.5 km northeast of Lind, WA	5-1-18 & 7-12-18	Plant tissue sampled only
Site #6	46.392315 N, 118.055179 W	16.6 km northwest of Dayton, WA	5-1-18 & 7-12-18	Plant tissue sampled only
Site #7	46.693925 N, 117.138842 W	5 km south of Pullman, WA	5-1-18 & 7-12-18	Plant tissue sampled only
Site #8	47.032921 N, 117.033265 W	11.3 km northeast of Garfield, WA	5-1-18 & 7-12-18	Plant tissue sampled only
Site #9	46.548365 N, 116.909372 W	1.3 km east of Genesee, ID	5-1-18 & 7-12-18	Plant tissue sampled only
Site #15	46.727697 N, 116.955698 W	3.2 km east of Moscow, ID	7-13-18	Plant tissue sampled only
Site #16	46.87777 N, 116.947137 W	19.3 km south of Moscow, ID	7-13-18	Plant tissue sampled only



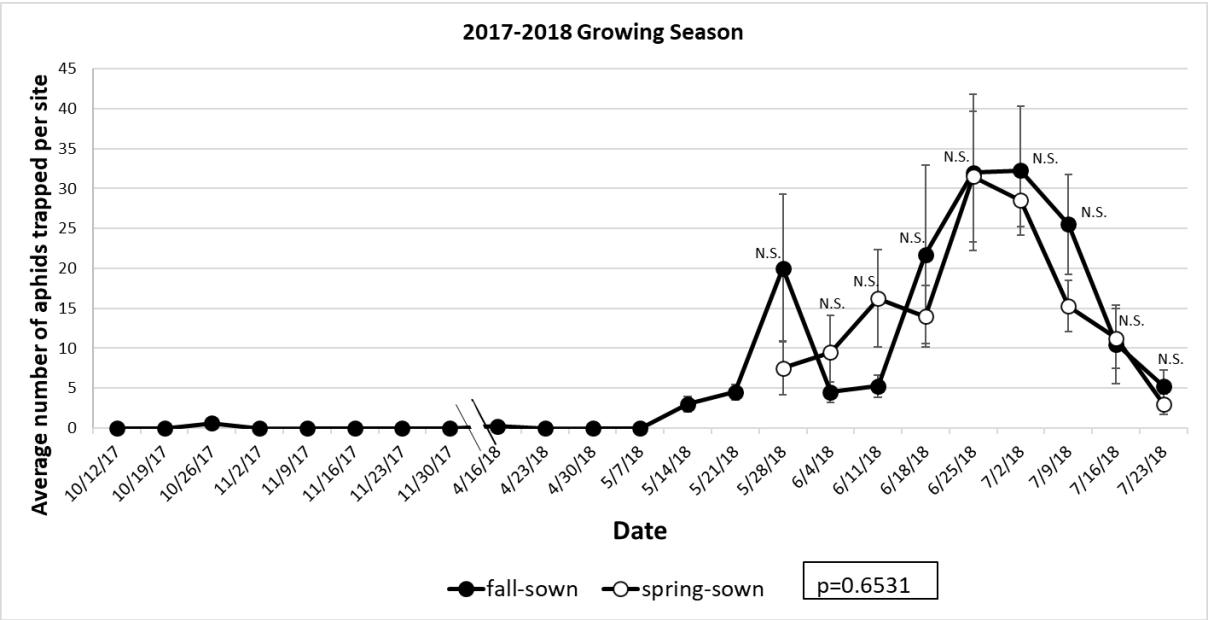
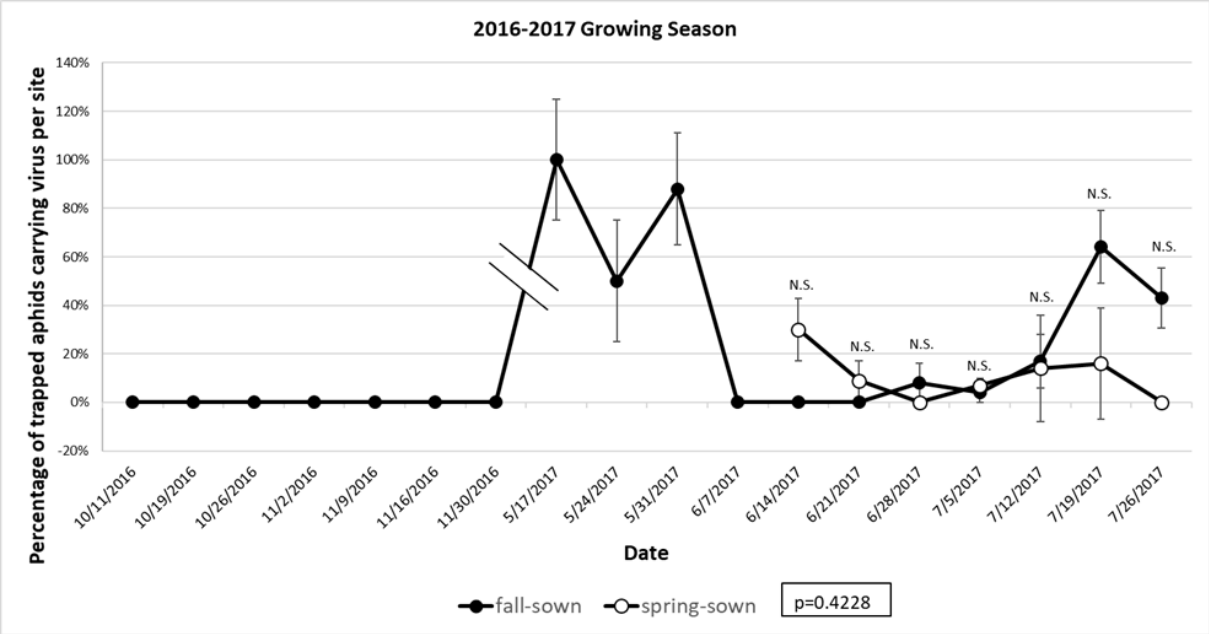


Figure 2.1 - The mean number of aphids trapped per site per date in both fall-sown and spring-sown pea locations for the 2016-2017 and 2017-2018 growing seasons. Each data point is the mean of four fall-sown or four spring-sown pea locations. The numbers trapped were compared between the planting regimes on each date. NS = p > 0.05 unless a p value is given (7-26-17; p=0.006). The average number of aphids trapped per site per date during the entire growing season did not differ between fall-sown and spring-sown pea location during both years of study (p=0.9579 & p=0.6531 respectively).



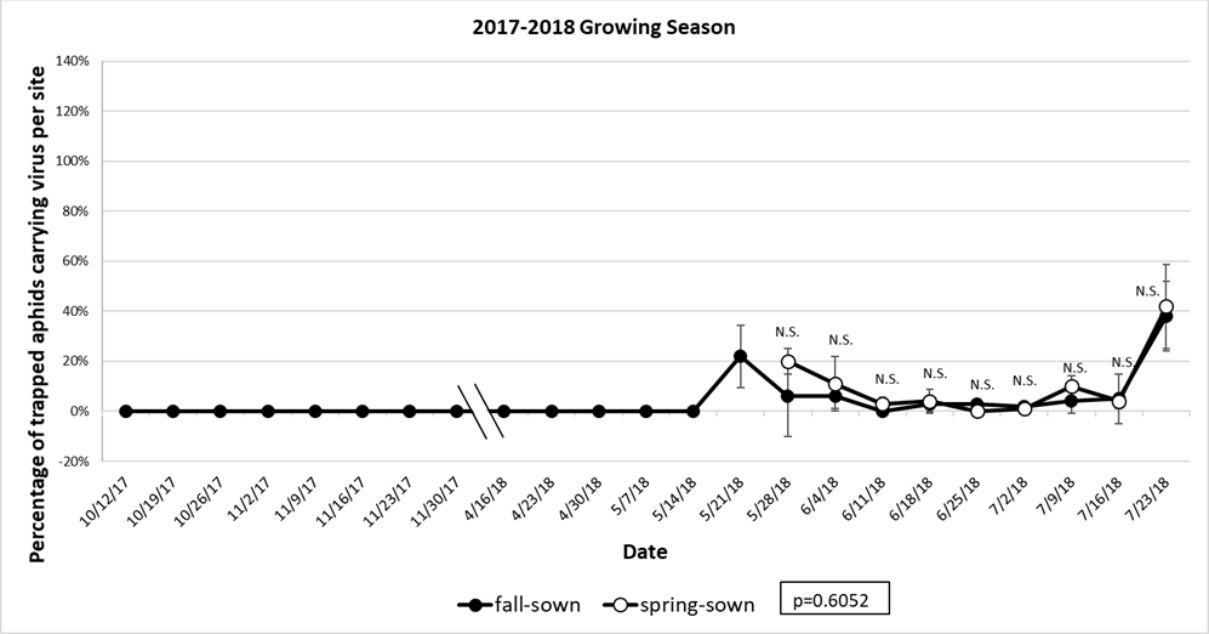


Figure 2.2 - The average number of trapped aphids carrying virus per site per date in both fall-sown and spring-sown pea locations for the 2016-2017 and 2017-2018 growing seasons. Each data point is the mean number of aphids found to be carrying virus from four fall-sown or four spring-sown pea locations on the given date. The numbers trapped were compared between the planting regimes on each data. NS = p > 0.05 unless a p value is given. The average number of trapped aphids carrying virus per site per date during the entire growing season did not differ between fall-sown and spring-sown pea locations during both years of study (p=0.4228 & p=0.6052 respectively).

Table 2.3 - The mean proportion of plants infected with virus (PEMV or BLRV) during the 2017 and 2018 growing season. The proportion of plants infected with BLRV was combined with the proportion of plants infected with PEMV because the proportion of BLRV was so small (a total of 4% in the 2017 growing season and a total of 0.1% in the 2018 growing season).

2016-2017	Fall	Spring	Summer
Fall-sown pea			
Mean proportion infected:	0.18	0.19	0.30
Variance:	0.12	0.02	0.04
Standard deviation:	0.34	0.15	0.21
Spring-sown pea			
Mean proportion infected:	N/A	N/A	0.33
Variance:	N/A	N/A	0.05
Standard deviation:	N/A	N/A	0.21
2017-2018	Fall	Spring	Summer
Fall-sown pea			
Mean proportion infected:	0.01	0.03	0.36
Variance:	0.0005	0.007	0.02
Standard deviation:	0.02	0.08	0.13
Spring-sown pea			
Mean proportion infected:	N/A	N/A	0.38
Variance:	N/A	N/A	0.007
Standard deviation:	N/A	N/A	0.09

Table 2.4 - The p-values from the comparisons of the mean proportions of virus-infected pea plant samples from different planting regimes collected at three different times (fall, spring and summer) compared between different growing seasons (2016-2017 and 2017-2018) The p-value from the comparison of the total proportion of virus-infected pea plants combined between both planting regimes compared between both years.

	2016-2017 vs. 2017-2018
Fall-sown pea (sampled in the fall)	P = 0.3194
Fall-sown-pea (sampled in the spring)	P = 0.4566
Fall-sown pea (sampled in the summer)	P = 0.2006
Spring-sown pea (sampled in the summer)	P = 0.2266
Total infected plants (sampled in the summer)	P = 0.2563

LITERATURE CITED

Ashby, J. W. (1980). Virus Diseases of Annual Legume Crops. Proceedings Agronomy Society of New Zealand 10, 77-80.

Clement, S. L. (2006). Main Content Area Pea Aphid Outbreaks and Virus Epidemics on Peas in the US Pacific Northwest: Histories, Mysteries, and Challenges. Plant health progress, 1099-1108.

Clement, S. L., D. S. Husebye, and S. D. Eigenbrode. (2010). Ecological Factors Influencing Pea Aphid Outbreaks in the US Pacific Northwest. Aphid Biodiversity Under Environmental Change: Patterns and Processes, 108-128

Davis, T. S., Y. Wu, and S. D. Eigenbrode. (2017). The Effects of Bean Leafroll Virus on Life History Traits and Host Selection Behavior of Specialized Pea Aphid (Acyrthosiphon pisum, Hemiptera: Aphididae) Genotypes. Environmental Entomology 46, 68-74.

de Zoeten, Gustaaf A., and Skaf, Jihad S. (2001). Pea Enation Mosaic and the Vagaries of a Plant Virus. Advances in Virus Research. 57, 323-350.

Develey-Rivière, M. P., & Galiana, E. (2007). Resistance to Pathogens and Host Developmental Stage: A Multifaceted Relationship within the Plant Kingdom. New Phytologist, 175(3), 405-416.

Eigenbrode, S. D., T. S. Davis, J. R. Adams, D. S. Husebye, L. P. Waits, and D. Hawthorne. (2016). Host-Adapted Aphid Populations Differ in their Migratory Patterns and Capacity to Colonize crops. Journal of Applied Ecology 53, 1382-1390.

Fabre, F., C. A. Dedryver, J. L. Leterrier, and M. Plantegenest. (2003). Aphid Abundance on Cereals in Autumn predicts Yield Losses caused by Barley Yellow Dwarf Virus. Phytopathology 93, 1217-1222.

Grünwald, N. J., Chen, W., and Larsen, R. C. (2004). “Pea Diseases and Their Management.” Diseases of Fruits and Vegetables, 301-331.

Hadi, B. A. R., Langham, M. A. C., Osborne, L., & Tilmon, K. J. (2011). Wheat Streak Mosaic Virus on Wheat: Biology and Management. Journal of Integrated Pest Management, 2(1), J1-J5.

Halbert, S. E., and K. S. Pike. (1985). Spread of Barley Yellow Dwarf Virus and Relative Importance of Local Aphid Vectors in Central Washington. Annals of Applied Biology 107, 387-395.

Hampton, R. O., & Weber, K. A. (1983). Pea Streak and Alfalfa Mosaic Viruses in Alfalfa: Reservoir of Viruses Infectious to Pisum Peas. Plant Disease, 67(3), 308-310.

Liu, Sijun., Sivakumar, S., Sparks, Wendy O., Miller, Allen W., and Bonning, Bryony C. (2010). A Peptide that Binds the Pea Aphid Gut Impedes Entry of Pea Enation Mosaic Virus into the Aphid Hemocoel. Virology 401, 107-116.

Makkouk, K. M., & Kumari, S. G. (2009). Epidemiology and Integrated Management of Persistently Transmitted Aphid-Borne Viruses of Legume and Cereal Crops in West Asia and North Africa. Virus Research, 141(2), 209-218.

McGee, R. J., Eigenbrode, S., Nelson, H., & Schillinger, W. (2017). Re-Inventing Austrian Winter Pea Towards Developing Food Quality Winter Peas. *Crops and Soils*, *50*(4), 4-46.

McGee, R. J., H. Nelson, and K. E. McPhee. (2013). Registration of 'Lynx' Winter Pea. Journal of Plant Registrations 7, 261-264.

McLaughlin, M. R. (1983). Viruses Infecting Forage Legumes in Tennessee. Plant Disease, 67(5), 490-492.

McWhorter, F. P., & Cook, W. C. (1958). The Hosts and Strains of Pea Enation Mosaic Virus. Plant Dis. Rep, 42(1), 51-60.

Murray, G. A., Swensen, J. B., & Auld, D. L. (1984). Influence of Seed Size and Planting Date on the Performance of Austrian Winter Field Peas. Agronomy Journal, 76(4), 595-598.

Paudel, S., Bechinski, E. J., Stokes, B. S., Pappu, H. R., & Eigenbrode, S. D. (2018). Deriving Economic Models for Pea Aphid (Hemiptera: Aphididae) as a Direct-Pest and a Virus-Vector on Commercial Lentils. Journal of economic entomology, 111(5), 2225-2232.

Peccoud, J., A. Ollivier, M. Plantegenest, and J.-C. Simon. (2009). A Continuum of Genetic Divergence from Sympatric Host Races to Species in the Pea Aphid Complex. Proceedings of the National Academy of Science 106, 7495-7500.

Peccoud, J., and J. C. Simon. (2010). The Pea Aphid Complex as a Model of Ecological Speciation. Ecological Entomology 35, 119–130.

Peccoud, J., C. C. Figueroa, A. X. Silva, C. C. Ramirez, L. Mieuzet, J. Bonhomme, S. Stoeckel, M. Plantegenest, and J.-C. Simon. (2008). Host Range Expansion of an Introduced Insect Pest through Multiple Colonizations of Specialized Clones. Molecular Ecology 17, 4608–4618.

Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., & Ohm, H. (2000). Yield Effects of Barley Yellow Dwarf Virus in Soft Red Winter wheat. Phytopathology, 90(9), 1043-1048.

Schillinger, W. F. (2016). Seven Rainfed Wheat Rotation Systems in a Drought-Prone Mediterranean Climate. Field Crops Research 191, 123-130.

Sieling, K., and O. Christen. 2015. Crop Rotation Effects on Yield of Oilseed Rape, Wheat and Barley and Residual Effects on the Subsequent Wheat. Archives of Agronomy and Soil Science 61, 1531-1549.

St. Luce, M., C. A. Grant, B. J. Zebarth, N. Ziadi, J. T. O'Donovan, R. E. Blackshaw, K. N. Harker, E. N. Johnson, Y. T. Gan, G. P. Lafond, W. E. May, M. Khakbazan, and E. G. Smith. (2015). Legumes can Reduce Economic Optimum Nitrogen Rates and Increase Yields in a Wheat-Canola Cropping Sequence in Western Canada. Field Crops Research 179, 12-25.

Stokes, B.S. (2012). Bioeconomics of Pea Aphids*, Acyrthosiphon pisum* (Harris), on Commercial Dry Peas, *Pisum sativum* 'Aragorn'. M.S. thesis, University of Idaho.

Thackray, D. J., A. J. Diggle, and R. A. C. Jones. (2009). BYDV Predictor: A Simulation Model to Predict Aphid Arrival, Epidemics of Barley Yellow Dwarf Virus and Yield Losses in Wheat Crops in a Mediterranean-Type Environment. Plant Pathology 58, 186-202.

Wu, Y., Davis, T. S., & Eigenbrode, S. D. (2014). Aphid Behavioral Responses to Virus-Infected Plants are Similar Despite Divergent Fitness Effects. Entomologia Experimentalis et Applicata, 153(3), 246-255.

Chapter 3. The Effects on Yield Parameters of Fall-Sown Dry Pea after Periodical Inoculations of Pea Enation Mosaic Virus (PEMV)

ABSTRACT

Relationships between the timing of inoculations of PEMV viruliferous pea aphid, *Acyrthosiphon pisum* (Harris), and yield parameters of fall-sown dry pea, *Pisum sativum* were quantified in field experiments at the University of Idaho Parker Farm, the University of Idaho Kambitsch Farm, and in a greenhouse experiment at the University of Idaho Manis Laboratory. A similar experiment was performed using spring-sown pea for comparison at the Kambitsch Farm. Pea aphids were applied to plants via clip cages throughout the fall of 2017 and the 2018 growing season for an inoculation access period of 72-hours. Three inoculations were performed before winter and three inoculations were performed after winter. The timing of inoculation was based on pea plant growing degree days (GDD) above 5°C. Inoculations of fall-sown pea in the field experiments took place after plants had experienced 41.5, 42, 44 (before winter), 187.5, 280 and 387.5 (after winter) GDD after emergence. In order to compare with fall-sown pea, Inoculations of spring-sown pea took place after plants had experienced about 44, 187.5 and 280 GDD. Regression analysis expressing yield parameters as a function of the timing of inoculation (GDD) resulted in statistically significant ($p > 0.05$) models for total plant biomass per replicate and mean U.S. #1 grade weight per plant in the field experiments at the Kambitsch Farm and the Parker Farm. All inoculations before winter were pooled and compared to the pooled inoculations that took place after winter, revealing that plants that are inoculated before winter exhibit a significant decrease in yield parameters compared to plants that are

inoculated after winter. Spring-sown pea receiving inoculations after experiencing a similar amount of GDD as did fall-sown pea showed increased yield parameters over fall-sown pea. Assumptions, limitations, and practical implementations of the experimental approach and model are further discussed.

INTRODUCTION

In the Palouse region of eastern Washington and northern Idaho, spring-sown pulse crops are commonly grown in rotation with wheat (Guy & Cox 2002). Planting pulses with wheat and barley breaks up weed, insect, and disease cycles in the wheat crop while also improving soil structure and richness by increasing phosphorus, potassium and sulfur availability (Strydhorst et al. 2015). In areas of the Palouse that receive less than 40 centimeters of precipitation annually, spring-sown pulse crops are not viable (McGee et al. 2017), leaving a monoculture of winter-wheat.

New varieties of edible dry pea adapted for fall planting with winter tolerance have gained considerable interest. Fall-sown peas can provide the same advantages as do spring peas or other pulses in rotation with cereals; they interrupt weed, disease, and insect cycles, use *Rhizobium* bacteria to fix atmospheric N, and are manageable with existing farm equipment (McGee et al. 2017). Additionally, fall-sown peas are viable in the lower rainfall regions of the PNW (Nelson 2017, Schillinger 2017, McGee et al. 2017) providing a rotational crop for the monocultures in these areas.

Pea aphids are a common pest of commercially grown legumes in the Palouse region. Pea aphids overwinter in lower elevations of the PNW and emigrate to the

Palouse in the spring to infest legumes such as dry pea and lentil (Clement 2006). Aphids damage plants directly from feeding or indirectly as vectors of plant viruses. On the Palouse, pea aphids are known to transmit four different viruses: *Pea enation mosaic virus* (PEMV), *Bean leaf roll virus* (BLRV), *Pea streak virus* (PeSV), and *Alfalfa mosaic virus* (Clement 2006). Predominantly, PEMV and BLRV are the two most economically important viruses (Clement 2006).

Both PEMV and BLRV are in the Luteoviridae family of plant viruses and are transmitted in a non-propagative circulative persistent manner (Andret-Link and Fuchs 2005, ICTVdB 2006, Vemulapati et al. 2010, Liu et al. 2010). Symptoms of PEMV include chlorosis, mosaic, stunting, vein clearing and enations, while symptoms of BLRV include chlorosis, leaf rolling, shortened internodes and stunting (ICTVdB 2006). Both viruses can cause yield damage by forming pathological tubules in plasmodesmata, ultimately reducing photosynthetic potential of the plant (Hull 2002, Waigmann et al. 2004).

When virus outbreaks occur on the Palouse, commercial producers can experience drastic economic losses. On average, aphid outbreaks result in about a 5% reduction in pea yields under adopted pest management activities (Elbakidze et al. 2011). Previous work has quantified the yield loss of spring-sown dry peas as a function of when viruliferous aphids arrive and transmit BLRV and PEMV and thus has developed a decision support system for producers to help them manage the aphids and limit diseases caused by these (Stokes 2012). Stokes (2012) conducted experiments measuring the effects on yield due to inoculations of either BLRV or PEMV to pea plants at different growth stages. The results showed that plants

inoculated at earlier growth stages exhibited greater yield losses to disease caused by each virus. A similar study by Paudel et al. (2018) found a very similar pattern in spring-sown lentil. In both studies, plants displayed maturity tolerance or resistance to the viruses they were exposed to. Maturity related tolerance or resistance is not uncommon for plant pathogens and can have implications for disease management (Difonzo et al. 1994, Lindblad & Sigvald 2003, Fabre et al. 2003). For example, Difonzo et al. (1994) found that susceptibility to potato leaf roll virus (PLRV) decreased as inoculation age increased in three different potato cultivars. In an experiment with wheat dwarf virus (WDV) in winter wheat, Lindblad and Sigvald (2003) found that infection rates quit increasing as crops reached the end of stem elongation, and plants became resistant to infection at growth stage DC 31 (first node detectable).

Such a study has never been done in fall-sown pea. Similar studies previously have been conducted using *barley yellow dwarf virus* (BYDV) and winter wheat (Riedell et al. 1999, Fabre et al. 2003), and each showed that virus infection in the autumn when plants are immature resulted in yield loss. The phenology of fall-sown pea allows it to be available and vulnerable to aphid-transmitted virus infection in the fall and early spring before spring-sown legumes are present. Pea aphids have been found to be present on the Palouse and airborne in the fall. Using suction traps, K. Pike, A. Rashed, S. Halbert captured airborne pea aphids on the Palouse into late October (Unpublished data). Although these suction traps were not specifically located at fall-sown pea locations, they showed that aphids were moving in the air column and could potentially colonize host plants if present. Pea aphids have been

found to be present at fall-sown pea locations, and aphid-transmitted viruses have also been detected in fall-sown pea plants in the fall (Chapter 2 of this thesis). However, pea aphid and virus incidence in the crop were found to be very low in the fall (Chapter 2 of this thesis). The effects of early virus inoculation on yield of fall-sown pea are unknown. This is important because if a severe virus outbreak does occur in the fall, it is necessary to understand the effect it can have on yield.

The objective of this research was to quantify the yield loss of fall-sown dry pea following virus inoculation at differing growth stages in the fall and early in the spring. This study focused on PEMV only. In prior work (Stokes 2012 and Paudel et al. 2018) the relationship between yield loss and inoculation timing for PEMV and BLRV in spring-sown pea and spring-sown lentil were nearly identical, so a focus on PEMV seemed reasonable. Also, in most years, PEMV is the predominant virus in the Palouse region (Chapter 2 of this thesis, https://www.legumevirusproject.org) and the most economically important (Clement 2006). Pea aphids are present in fall-sown pea in the fall, and virus has been detected in fall-sown pea plants in the fall; thus, it is possible that the virus may stay within the plant through the winter until maturity. I hypothesized that plants inoculated during early growth stages would be more susceptible to virus damage, showing that maturity tolerance or resistance occurs in the crop. This would be similar to the results seen in spring-sown pea and lentil. To examine this, I conducted two replicated field trials and one greenhouse experiment with staged inoculations and measured the effects on yield of a cultivated pea developed for fall planting.

MATERIALS AND METHODS

Field inoculation studies in fall-sown pea with viruliferous pea aphids from an insectary colony positive for *Pea enation mosaic virus* (PEMV) were conducted during the 2018 growing season and preceding fall. Separate studies were conducted in the field at the University of Idaho Kambitsch Farm (19.3 km south of Moscow, ID) and the University of Idaho Parker Research Farm (3.2 km east of Moscow, ID), and in the greenhouse at the Hubert C. Manis Entomological Laboratory (Moscow, ID) (Manis Laboratory). A similar study was also conducted in spring-sown pea at the Kambitsch Farm to compare results with the study conducted in fall-sown pea at the Kambitsch Farm. Aphids came from a colony maintained in the Manis Laboratory in a growth chamber at 20°C with a 16:8 hours (L:D) photoperiod.

The study at Kambitsch Farm was conducted using ‘Koyote’ dry peas. Seeds were treated with standard commercial rates of the fungicides Maxim, ApronXL, Vibrance, and the fertilizer sodium molybdate. The study at Parker Farm was conducted using ‘Specter” dry pea and no fertilizer or fungicides. The experimental designs of the studies in fall-sown pea at the Kambitsch Farm and Parker Farm were similar. Each study included six different treatment groups in which plants were inoculated with PEMV at different times after crop emergence, and a non-inoculated control. Each treatment group and control consisted of 7 replicates each with 10 plants. Each plant in every replicate, excluding plants in control replicates, was exposed to five pea aphids from a PEMV-infectious colony, attached to the plant with a clip cage for an inoculation period of 72 hours. The clip-cages (2 cm in height and 1.5 cm in diameter) were screened on one side using BioQuip No-see-um Netting

(BioQuip, www.bioquip.com). After the 72-hour inoculation access period, the clip-cages were removed from the plants, and all aphids were removed by hand. The number of viruliferous aphids and the length of the inoculation access period were chosen based on previous studies in which pea plants were inoculated with PEMV in the field (Stokes 2012, Paudel et al. 2018).

The independent variable between the treatments was the timing of the inoculation relative to emergence of the plants. The six inoculation treatment points included three inoculation dates in the fall, before onset of winter, and three inoculation dates in the spring. The first inoculation in the fall took place one week after plant emergence. The next two inoculations were performed at weekly intervals following the first inoculation (Table 3.1). The first inoculation in the spring was conducted as soon as the first pea aphid was collected in a pan trap at the Kambitsch Farm and again at weekly intervals for the following two inoculations (Table 3.1). The number of growing degree days (GDD) that plants had experienced from emergence was recorded. GDD were calculated by subtracting the base or threshold temperature for pea (5°C; Bourgeois et al. 2000) from the mean daily air temperature. Daily air temperature was measured using a Campbell Scientific CR10X data logger in a weather station box (Campbell Scientific, Logan, Utah).

At the Kambitsch Farm, all fall-sown pea treatments were covered with 89cm x 82cm x 38cm PVC pipe cages with No-See-Um Mosquito Netting (Seattle Fabrics, Inc., seattlefabrics.com) to prevent outside inoculation while mimicking the natural surroundings as best as possible. Cages were placed on top of crop rows before plant emergence. As mentioned previously, each replicate consisted of 10 plants, but

because cages were placed before plants emerged, sometimes 10 plants were not available inside the cage space to inoculate for the first three inoculations; therefore, some replicates consisted of less than 10 plants (Table 3.1). All cages were removed from each treatment once plants had been inoculated. At the same time, aphids were removed from plants after the 72-hour access period. The screens remained on the remainder of the replicates from the treatment groups that had not experienced an inoculation until they were removed on 30 November 2017 for the winter. In early March, before aphid flights commenced, screening was replaced on cages of treatment groups that had not been inoculated during the fall. Screens were removed from the replicates of the last three inoculation treatment groups in the spring after inoculation. Screens remained on the replicates from the control treatment group for the entire experiment. No screens were used in the study at the Parker Farm.

Plant tissue samples were collected from all plants, including plants from controls, and tested for virus infection following the completion of all inoculations on 23 July 2018. Plant samples were tested for virus using a double anti-body sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Nano Diagnostics, Inc., nanodiaincs.com). Plants that had been inoculated, but tested negative for virus inoculation based on the DAS-ELISA, were removed from the analysis. Six percent of plants were removed from the Kambitsch experiment, and 3% of plants were removed from the Parker experiment based on this criterion.

Plants were harvested on 31 July 2018 by clipping at soil level after they had reached maturity and dried down. Pea seeds were manually threshed from pods. Response parameters measured were total above ground biomass (g dry wt.), 100-

seed weight (HSW) per rep (g), and mean U.S. #1 grade dry pea weight per plant (USWPP). Mean USWPP was determined by sieving seeds through a standard commercial pea seed grading pan with 11/64-inch holes (Seedburo Equipment Company, www.seedburo.com); pea seeds that passed through the sieve did not meet U.S. grade minimum size standard for #1 dry peas, while those that were retained on the sieve constituted economic yield (USDA United States Standards for Whole Dry Peas, Split Peas, and Lentils 1999).

The experiment conducted in the greenhouse at the Manis Laboratory was designed to mimic the two field experiments with fall-sown pea at the Kambitsch and Parker Farms as closely as possible. The experiment was conducted using ‘Koyote’ dry peas. Seeds were treated with standard commercial rates of the Maxim, ApronXL, Vibrance, and sodium molybdate. Pea plants were grown in a greenhouse maintained at temperatures of 18.3°C at night and 26.7°C during the day with a photoperiod of 16:8 hours (L:D) and relative humidity of 40-50% (ambient). The study consisted of six treatment groups and a control treatment group. Each treatment group consisted of 12 separate plants inoculated with PEMV by encaging viruliferous aphids from insectary colonies in clip cages. The independent variable was the time of virus inoculation after plant emergence. The first inoculation took place one week after emergence, and the next two inoculations took place at weekly intervals thereafter (Table 3.1). Plants were then moved into a walk-in cooler one week after the third inoculation to expose plants to simulated “winter” conditions. The cooler was maintained at a constant temperature of 0°C with a 16:8 (L:D) photoperiod. Plants remained in the cooler for 42 days; this was considered to be the minimum amount of

time needed for dormancy. Plants were removed from the cooler and returned to the same greenhouse. After four days, the fourth inoculation took place, and the final two inoculations occurred at weekly intervals thereafter (Table 3.1). Five of the twelve plants from the control treatment did not survive the time in the cooler, so the control treatment only consisted of 7 plants (Table 3.1).

Plants in the greenhouse experiment were harvested by hand on 25 August 2018 after all the plants had dried down. Seeds were threshed by hand. Response parameters included total biomass, total pod count, HSW, and USWPP, determined as for the field experiments.

To compare the response of fall-sown pea to spring-sown pea at different ages of inoculation, a field study was conducted at the Kambitsch Farm in spring 2018. Pea plants were planted on 27 April 2018, and plant emergence occurred on 10 May 2018. Again, ‘Koyote’ dry peas were used, and seeds were treated with standard commercial rates of the Maxim, ApronXL, Vibrance, and sodium molybdate. To facilitate comparison, inoculations in spring-sown pea took place approximately when the plants had experienced the same number of GDD after emergence as had the fall-sown pea plants at the Kambitsch farm on the third, fourth, and fifth inoculation dates (Table 3.1). In particular, the first inoculation of spring-sown pea took place on 14 May 2018, when plants had experienced 56.5 GDD, roughly the same as the GDD experienced by fall-sown pea plants inoculated in the third inoculation treatment group at the Kambitsch Farm (44 GDD, Table 3.1). The second inoculation took place on 21 May 2018, when plants had experienced 198.5 GDD, roughly the same as the GDD experienced by fall-sown pea plants that were

inoculated in the fourth inoculation treatment group at the Kambitsch Farm (187.5 GDD, Table 3.1). The third and final inoculation took place on 25 May 2018, when plants had experienced 288.5 GDD, roughly the same as the GDD experienced by fall-sown pea plants that were inoculated in the fifth inoculation treatment group at the Kambitsch Farm (280 GDD, Table 3.1). Screen cages were not placed over spring-sown pea plants at the Kambitsch Farm.

Plant tissue samples were gathered from all plants, including plants from control replicates, and tested for virus infection following the completion of all inoculations on 24 July 2018. Plant samples were tested for virus using DAS-ELISA. Plants that had been inoculated, but tested negative for virus inoculation, were removed from analysis.

Plants were harvested on 2 August 2018 by clipping plants at soil level. Pea seeds were manually threshed from pods. Response parameters included total biomass, HSW and mean USWPP, determined as in previous experiments in this study.

Data Analysis

Relationships between pea seed yield parameters and timing of inoculation were quantified across the different experiments. All data were examined for conformation with parametric assumptions prior to analysis. In the experiments with fall-sown pea at the Kambitsch Farm, Parker Farm and greenhouse, relationships between timing of inoculation and average plant biomass, USWPP and HSW were quantified by computing linear regression analysis (Microsoft Excel). Because plants

were inoculated at very similar growth stages in the fall, fall-sown pea plants from the experiments at the Kambitsch Farm, Parker Farm and greenhouse that received inoculations before winter were pooled to represent “before winter” inoculations, and their responses were compared to those from the remaining plants in the experiments, pooled to represent “after winter” inoculations using a Welch’s two-sample *t*-test (Rstudio team, 2015). A Welch’s two-sample *t*-test was used to test for differences in average plant biomass, mean USWPP and HSW because of differing variances between treatments. Yield parameters for fall and spring-sown peas inoculated at similar GDD of maturity were compared by comparing the slope of the regression lines by means of analysis of covariance (ANCOVA) (Rstudio team, 2015).

The results from the Kambitsch and Parker Farms were standardized by expressing yield as possible yield attained relative to the maximum yield attainable (yield produced by the controls). The standardized relative yields from the Kambitsch and Parker Farms were combined. Linear regression analysis was computed on the combined relative yield to determine relationships between timing of inoculation and total above ground plant biomass, mean USWPP and HSW.

RESULTS

Linear regression analyses expressing yield parameters as a function of the timing of inoculation (GDD) resulted in statistically significant models for two of the three dependent variables ($p < 0.05$) from the experiment in fall-sown pea at the Kambitsch Farm (Table 3.2, Figure 3.1a-b). Only HSW was unaffected by inoculation

timing ($p > 0.05$) (Figure 3.1c). Total plant biomass and mean USWPP were statistically significant ($p < 0.05$) (Table 3.2, Figure 3.1a & b).

Similar results were obtained from linear regression analyses expressing yield parameters as a function of the timing of inoculation (GDD) of the fall-sown pea from the Parker Farm; only HSW was unaffected by inoculation timing ($p > 0.05$) (Table 3.3, Figure 3.2c), while total plant biomass and mean U.S. #1 grade dry pea weight per plant were statistically affected ($p < 0.05$) (Table 3.3, Figure 3.2a-b).

Linear regression analyses on the data from the greenhouse experiment resulted in statistically significant models for the pod-count per plant and U.S. #1 grade weight per plant ($p < 0.05$) (Table 3.4, Figure 3.3b-c). Although these models were significant, the trendline produced by both models had a negative slope. These results contradict the results from the significant models produced from the field studies at Kambitsch Farm and Parker Farm. Plant biomass and HSW were unaffected by inoculation timing in the greenhouse study ($p > 0.05$) (Table 3.4, Figure 3.3a & d).

Linear regression analyses on the relative combined biomass, U.S. #1 grade weight and 100-seed weight from the experiments at the Kambitsch Farm and Parker Farm resulted in statistically significant models for biomass and mean U.S. #1 grade weight per plant ($p < 0.05$) (Table 3.5, Figure 3.4a-b), but not for HSW ($p < 0.05$) (Table 3.5, Figure 3.4c).

For the experiment in fall-sown pea at Kambitsch Farm, a Welch's Two-sample *t*-test yielded significant differences between the pooled "before winter"

inoculations and the pooled “after winter” inoculations for biomass and mean USWPP ($p < 0.05$) (Figure 3.5a-b). HSW did not differ between the “before winter” inoculations and the “after winter” inoculations ($p > 0.05$) (Figure 3.5c). In the field experiment at the Parker Farm, biomass, mean USWPP and 100-seed weight differed between the pooled “before winter” inoculations and the pooled “after winter” inoculations ($p < 0.05$) (Figure 3.6a-c). In the greenhouse experiment, biomass, pod-count per plant, USWPP and HSW differed between the pooled “before winter” inoculations and the pooled “after winter” inoculations ($p < 0.05$) (Figure 3.7a-d).

Linear regressions were calculated for yield, HSW and biomass of fall-sown and spring-sown pea plants that had received virus inoculations after experiencing a similar amount of GDD at the Kambitsch Farm. The regression for spring-sown pea was significant for HSW only ($p < 0.05$) (Table 3.6, Figure 3.8c). Biomass and USWPP were both unaffected by the timing of inoculation ($p > 0.05$) (Table 3.6, Figure 3.8a-b). The regressions for fall-sown pea were significant for biomass and mean USWPP ($p < 0.05$) (Table 3.6, Figure 3.8a-b), but not for HSW ($p > 0.05$) (Table 3.6, Figure 3.8c). ANCOVA comparing yield parameters for fall and spring-sown peas inoculated at similar GDD of maturity revealed a significant difference in mean USWPP between the two groups for biomass and mean USWPP ($p < 0.05$) (Table 3.7, Figure 3.8a-b), but not for total plant biomass or HSW ($p > 0.05$) (Table 3.7 Figure 3.8c). A Welch’s two-sample *t*-test revealed that all three inoculation dates of spring-sown pea (44 GDD, 187.5 GDD, and 280 GDD) yielded greater relative biomass and relative USWPP than fall-sown pea inoculated after experiencing the same amount of GDD ($p < 0.05$) (Table 3.8). HSW did not differ between any of the

spring-sown pea plants and fall-sown pea plants that were inoculated after experiencing similar GDD ($p > 0.05$) (Table 3.8).

DISCUSSION

In the field and greenhouse experiments, PEMV-viruliferous aphids were shown to affect dry pea yield parameters. In both field experiments at the Kambitsch Farm and Parker Farm, total plant biomass and mean USWPP showed significant reductions when inoculated at earlier growth stages as compared to later growth stages. This same pattern was seen when yield results from both the Kambitsch Farm and Parker Farm were standardized and combined. The first three inoculations that took place before winter were performed at weekly intervals, but in terms of GDD, these plants were inoculated at very similar growth stages (41, 42.5 and 44 GDD respectively). This was not the case for the plants inoculated in the spring. Those inoculations also took place at weekly intervals, but the GDD experienced by these plants were much more spread out (187.5, 280 and 387.5 GDD respectively).

Plants from both field experiments from the “before winter” inoculations showed a significant reduction in biomass and mean USWPP compared to plants inoculated after winter. At the Parker Farm, there was also a significant difference in HSW between the pooled plants inoculated before winter and plants inoculated after winter.

In the greenhouse experiment, pod-count per plant and USWPP were the only two yield parameters affected by timing of inoculation. The direction of the effect was opposite that observed in the field experiments; plants inoculated at later growth

stages showed a greater reduction in pod-count per plant and USWPP. When plants were pooled into “before winter” inoculations and “after winter” inoculations, those with “after winter” inoculation exhibited significant reductions in biomass per plant, pod-count per plant, USWPP, and HSW compared with those inoculated “before winter”.

When comparing spring-sown pea and fall-sown pea, results showed decreased biomass and mean USWPP in inoculated fall-sown pea compared to spring-sown pea that were inoculated after experiencing similar GDD. Early inoculations in fall-sown pea appeared to be more damaging than early inoculations in spring sown pea, despite the fact that the same variety of dry pea was used for fall-sown and spring-sown pea. The first inoculation in spring-sown pea was performed when plants had experienced about 44 GDD since emergence, matching up with the third and final inoculation before winter in the fall-sown pea. The second inoculation in spring-sown pea took place when plants had experienced about 187.5 GDD, matching up with the fourth inoculation (first inoculation after winter) in the fall-sown pea. The difference was that in spring-sown pea, the inoculation at 187.5 GDD took place only seven days after the inoculation at 44 GDD, whereas in fall-sown pea, the inoculation at 187.5 GDD took place over 5 months after the inoculation that took place at 44 GDD. This could potentially explain why yield loss was more drastic in early inoculations in fall-sown pea, compared to early inoculations in spring-sown pea.

The results from the field experiments in fall-sown pea support the hypothesis that plants inoculated earlier in their development are more susceptible to PEMV

inoculation. Many plants show increases in resistance to viral, bacterial, and fungal pathogens correlated with increasing stages of plant development (Panter & Jones 2002). Younger plants may be infected more easily and may be more prone to damage than older plants (Lindblad & Sigvald, 2004, Smith 1963). A similar study involving spring-sown pea (Stokes 2012) showed that as plants mature to a certain age, they become more resilient to virus infection.

This pattern could also be explained by the amount of virus within the tissue. When the virus enters a plant cell, it replicates and infects new cells (Stokes 2012). This takes time. The virus also has a latency period that halts replication within the plant for a period of time (Grünwald 2004, de Zoeten and Skaf 2001). The virus has more time to replicate after the latency period within plants that received earlier inoculations. In this case, plants inoculated in the fall give the virus several months to replicate and increase the amount of virus particles within the plant, causing a greater impact on the plant's vascular system.

The results from the greenhouse experiment did not support this hypothesis. It is likely that this effect is an artifact rather than an effect of the virus inoculation alone. Eleven out of 12 plants from the fourth inoculation (first inoculation after "winter") and nine out of 12 plants from the fifth inoculation (second inoculation after "winter") developed symptoms of powdery mildew two weeks after removal from the freezer. Based on the appearance of these plants, their decreased yields as compared to plants that received inoculations at earlier growth stages probably were due to powdery mildew.

Overall, yield results were much larger at the Parker Farm than the Kambitsch Farm. The average total biomass of the control replicates at the Parker Farm was 239 g compared to 122 g at the Kambitsch Farm. The average mean USWPP of the control replicates at the Parker Farm was 107 g compared to 25 g at the Kambitsch Farm. The average HSW of the control replicates at the Parker Farm was 15 g compared to 14 g at the Kambitsch Farm. The experiment at the Parker Farm used 'Specter' dry peas with no fertilizer or inoculum added. The experiment at the Kambitsch Farm used 'Koyote' dry peas treated with Maxim, ApronXL, Vibrance, and sodium molybdate. The difference in variety of pea and chemical applications could cause such a difference in yield results. These differences may also be caused by biotic factors such as soil flora and fauna. Abiotic factors such as geographic location, soil content (nutrient uniformity) and composition (sand, silt, clay) could also be influential. The Kambitsch experiment was conducted using cages with screens, and this may have contributed to these differences as well. The cages may have inhibited plant growth by restricting lateral expansion of the plant while also restricting sunlight available to the plant. Regardless of the differences in overall yield, the pattern of damage due to infection remained the same across both experiments. Total plant biomass and USWPP were both significantly affected by the timing of inoculation at the Parker Farm and the Kambitsch Farm. The pooled results at both farms showed that plants inoculated with PEMV before winter experienced a greater reduction in yield than plants inoculated after winter.

Based on this research, fall-sown pea is more susceptible to yield loss if infected in the fall as compared to plants infected in the spring. These results assume

that each plant is colonized with five viruliferous aphids and a method of control is executed 72 hours after aphid colonization. These assumptions are not realistic because aphid arrival is randomly scattered throughout the field, and movement throughout the field is random as well. The extent of injury to the crop depends upon secondary (within field) spread (Elbakidze et al 2011, Linblad & Sigvald 2004, Kennedy 1976), and secondary spread was not examined in this research; therefore these models predict yield loss that occurs only due to primary infection.

PEMV has been very prevalent on the Palouse over the last 10 years (https://www.legumevirusproject.org) and historically is one of the most important insect-vectored viruses in the Palouse (Clement 2006). BLRV is another important aphid-vectored virus in legumes on the Palouse. These experiments were all done with PEMV. Stokes (2012) and Paudel et al. (2018) showed that PEMV and BLRV have the same age-related tolerance in spring-sown pea and spring-sown lentil. Nonetheless, the effects of fall inoculation by BLRV have not been measured, and it is possible that BLRV virus has different effects on fall-sown pea than does PEMV.

This study has shown that if inoculated with PEMV in the fall, pea plants will show a significantly greater reduction in yield than if the fall crop is inoculated in the spring. Most importantly, USWPP was consistently affected by timing of inoculation. Economic quality seed weight is the component of yield directly associated with the income and profit of growers in the Palouse region. Although inoculation with PEMV in the fall can be more injurious than inoculation in the spring, the frequency of inoculation in the fall is quite low (see Chapter 2), such that the overall risk of injury by virus in fall-sown peas is small. Based on these results, it can be concluded that

risk of injury in fall-sown pea, particularly to PEMV, does not differ from spring-sown pea and does not require additional steps to manage virus risk.

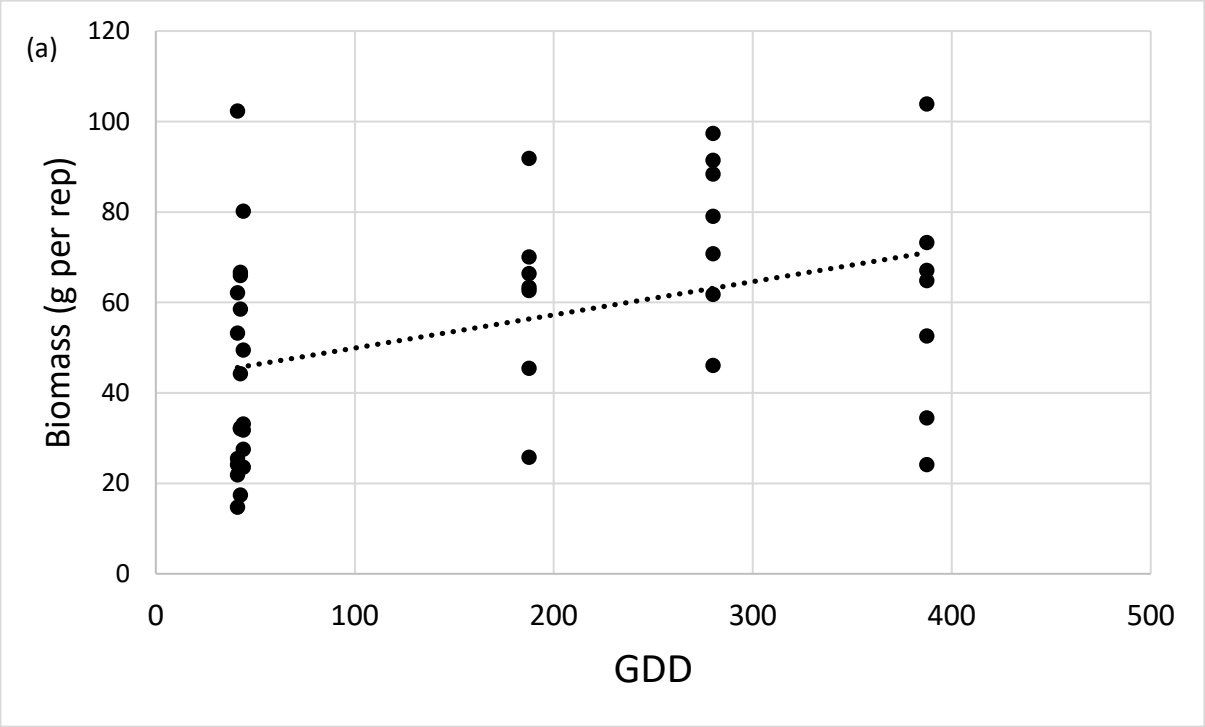
Pea aphid monitoring described in Chapter 2 indicated that pea aphid fall migrations are rare, and suction trap records also suggest they occur, but are less abundant than spring and summer movements by this aphid. Still, 2 years is a small sample, and a virus outbreak could occur in the longer run in fall-sown pea. Husebye et al. (2013) created a meteorological model to predict levels of incoming viruliferous aphids in the Palouse region. This model was built using a historical record of aphid abundance in the spring and was parameterized with winter and spring meteorological data. A similar model for fall infestations could be built eventually, but it would need a long-term record on which to base it. The Legume Virus project (https://www.legumevirusproject.org) uses tools to track aphid arrival and virus prevalence in spring-sown pea throughout the Palouse region. Recently, fall-sown pea has been added to the project. The information provided by the Legume Virus Project regarding fall-sown pea and the results of this study can help producers determine if control actions on virus vectors are necessary in fall-sown pea. It seems unlikely based on this study, but with projected climate change (Stöckle et al. 2018) in certain years or locations in the future, treating aphids in the fall to manage virus in fall-sown pea may be justified.

Table 3.1 – Inoculation dates and growing degree days (GDD) experienced at the date of inoculation from all three experiments. (“SP” denotes spring-sown pea)

Location	Treatment	Date Seeded	Date of Emergence	Inoculation date	GDD experienced at inoculation	Number of plants in treatment
Kambitsch	1	10-2-17	10-24-17	10-31-17	41	70
Kambitsch	2	10-2-17	10-24-17	11-7-17	42.5	70
Kambitsch	3	10-2-17	10-24-17	11-14-17	44	67
Kambitsch	4	10-2-17	10-24-17	4-24-18	187.5	60
Kambitsch	5	10-2-17	10-24-17	5-1-18	280	65
Kambitsch	6	10-2-17	10-24-17	5-8-18	387.5	60
Kambitsch	Control	10-2-17	10-24-17	N/A	N/A	60
Parker	1	10-6-17	10-26-17	11-1-17	33.5	70
Parker	2	10-6-17	10-26-17	11-8-17	35	70
Parker	3	10-6-17	10-26-17	11-15-17	36.5	70
Parker	4	10-6-17	10-26-17	4-25-18	188.5	70
Parker	5	10-6-17	10-26-17	5-2-18	280	70
Parker	6	10-6-17	10-26-17	5-9-18	394.5	70
Parker	Control	10-6-17	10-26-17	N/A	N/A	70
Greenhouse	1	5-7-18	5-15-17	5-18-18	315	12
Greenhouse	2	5-7-18	5-15-17	5-25-18	535.5	12
Greenhouse	3	5-7-18	5-15-17	6-1-18	756	12
Greenhouse	4	5-7-18	5-15-17	7-24-18	1102.5	12
Greenhouse	5	5-7-18	5-15-17	7-31-18	1323	12
Greenhouse	6	5-7-18	5-15-17	8-7-18	1543.5	12
Greenhouse	Control	5-7-18	5-15-17	N/A	N/A	7
Kambitsch-SP	1	4-27-18	5-10-17	5-14-18	56.5	70
Kambitsch-SP	2	4-27-18	5-10-17	5-21-18	198.5	70
Kambitsch-SP	3	4-27-18	5-10-17	5-25-18	288.5	70
Kambitsch-SP	Control	4-27-18	5-10-17	N/A	N/A	70

Table 3.2 - ANOVA table containing the degrees of freedom (df), mean squares (MS), F-value (F), and P-value of the total biomass, mean U.S. #1 grade weight per plant and 100-seed weight of the fall-sown pea at the Kambtisch Farm.

ANOVA				
Kambitsch Total Biomass	df	MS	F	P-value
Regression	1	4003.103	7.291	0.0102
Residual	39	549.072		
Total	40			
Kambitsch Mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	13.768	17.769	0.0001
Residual	39	0.775		
Total	40			
Kambitsch 100-seed weight	df	MS	F	P-value
Regression	1	3.021	0.840	0.3651
Residual	39	3.597		
Total	40			



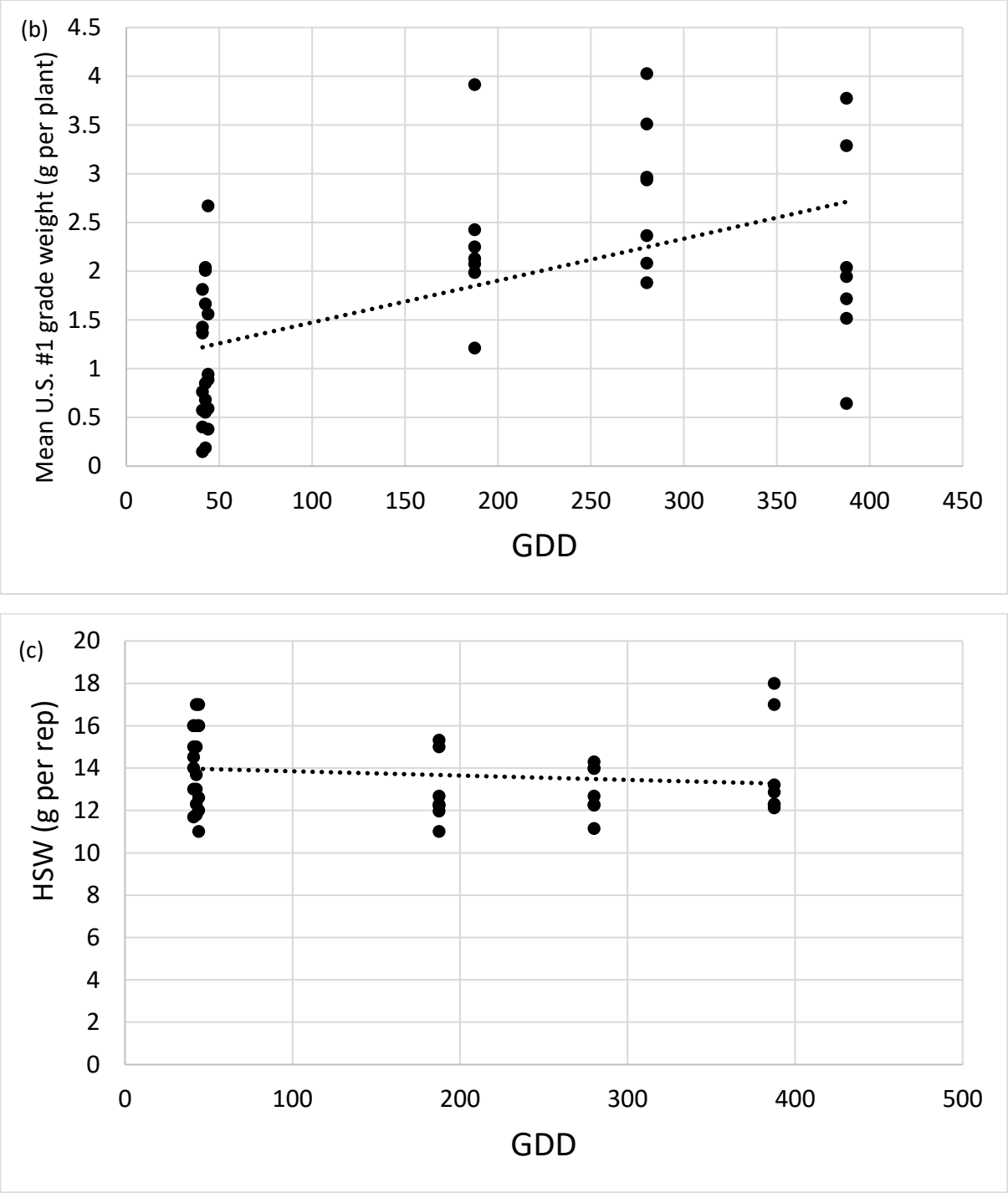
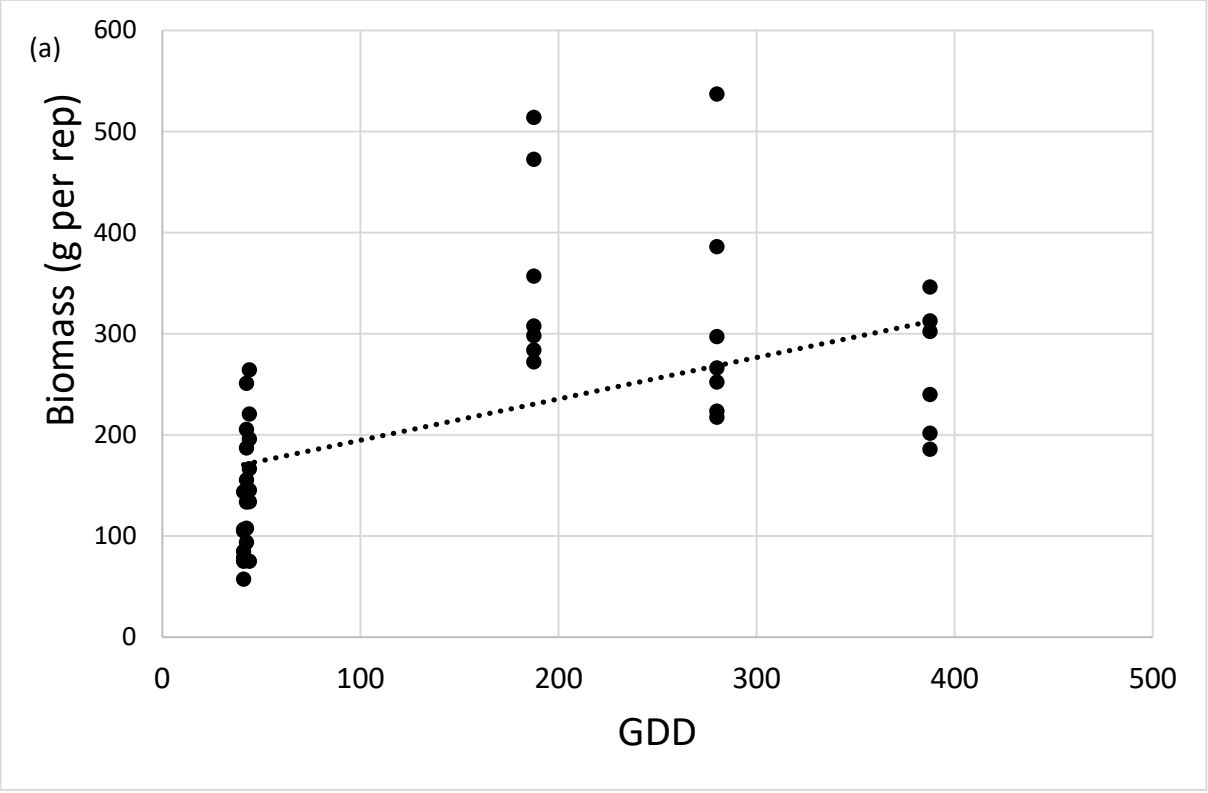


Figure 3.1 - Linear regression models for the relationship between the total plant biomass (a) (y = 42.58 + 0.07x), mean USWPP (b) (y = 1.04 + 0.004x) and HSW (c) (y = 14.05 + -0.002x) with the inoculation timing with PEMV aphids at the University of Idaho Kambitsch Farm.

Table 3.3 - ANOVA table containing the degrees of freedom (df), mean squares (MS), F-value (F), and P-value of the total biomass, mean U.S. #1 grade weight per plant and HSW of the fall-sown pea at the Parker Farm.

ANOVA				
Parker Total Biomass	df	MS	F	P-value
Regression	1	126575.214	10.621	0.0023
Residual	40	11917.700		
Total	41			
Parker Mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	219.280	13.270	0.0008
Residual	39	16.524		
Total	40			
Parker HSW	df	MS	F	P-value
Regression	1	1.103	0.729	0.3982
Residual	39	1.513		
Total	40			



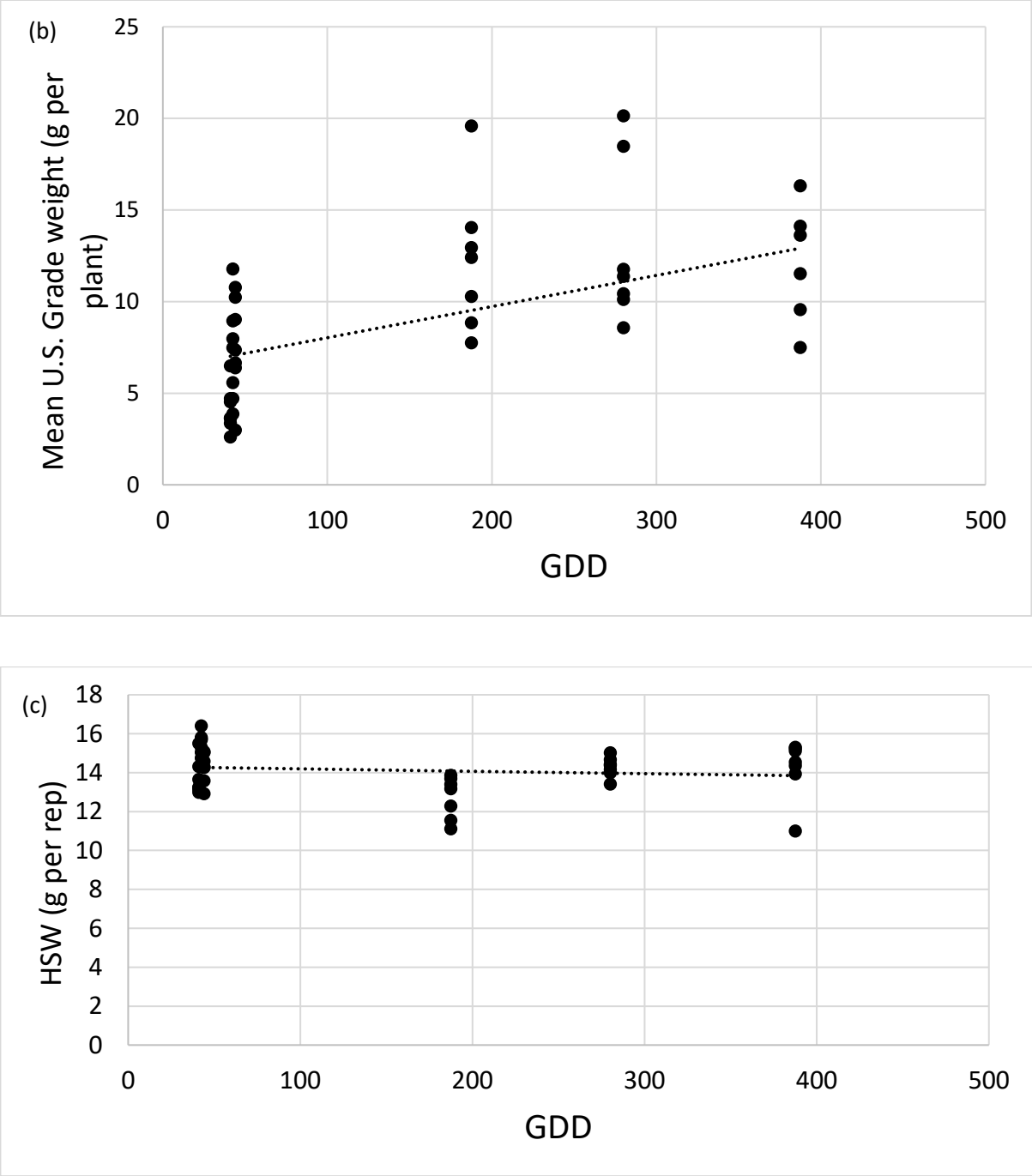
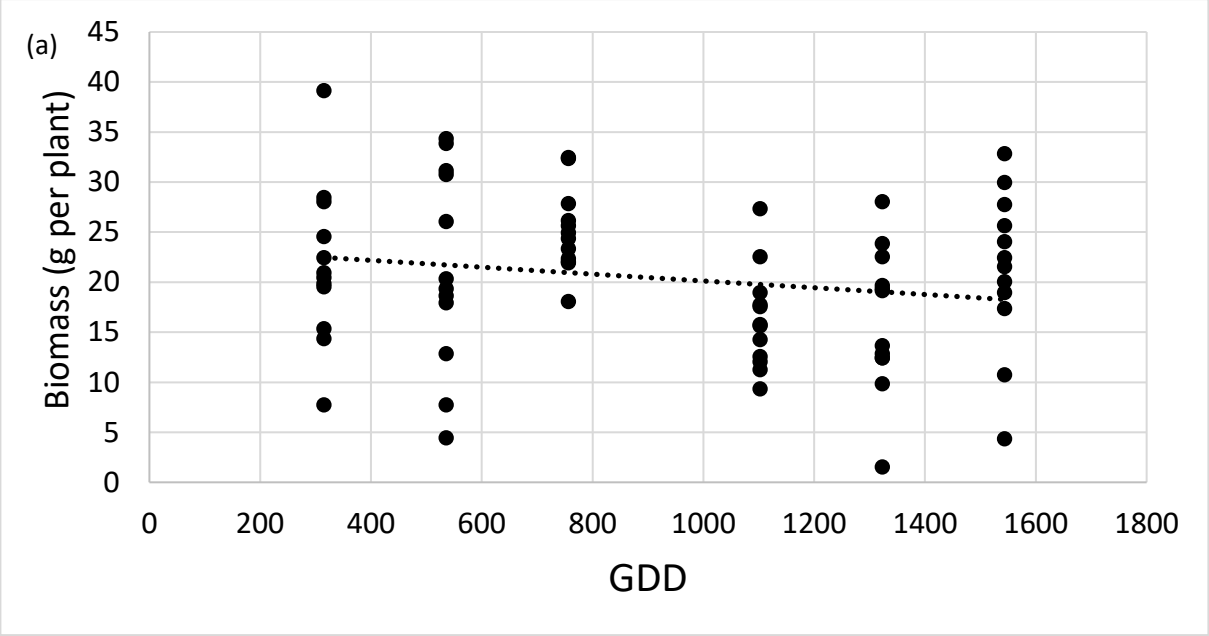
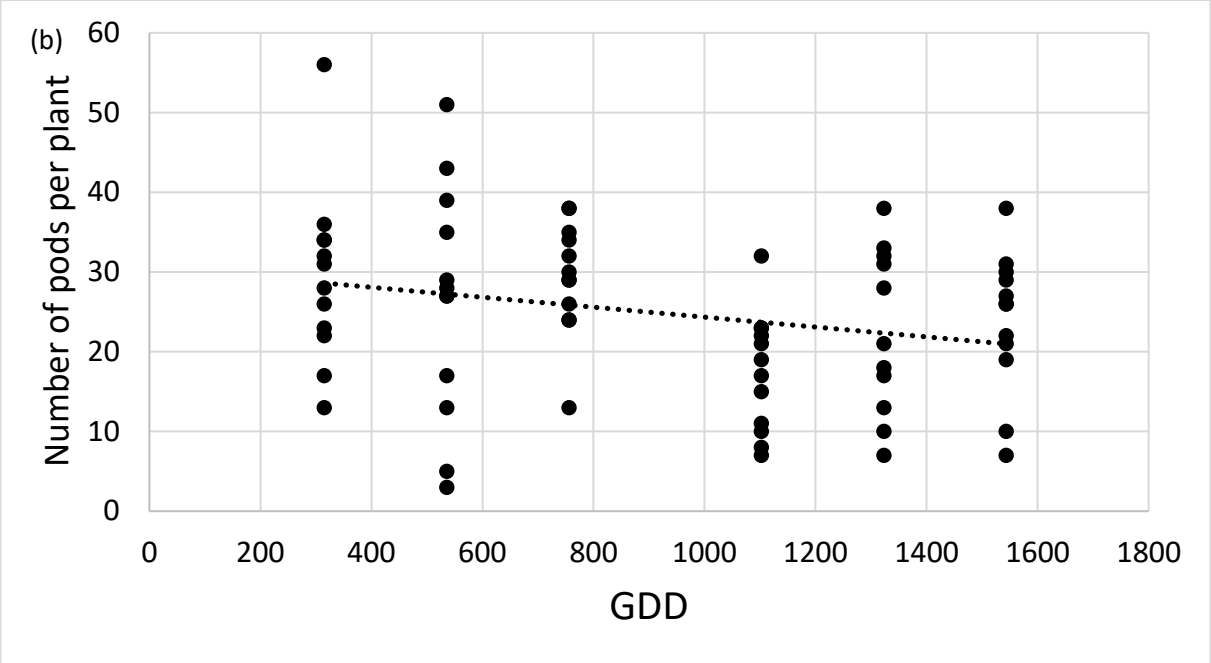


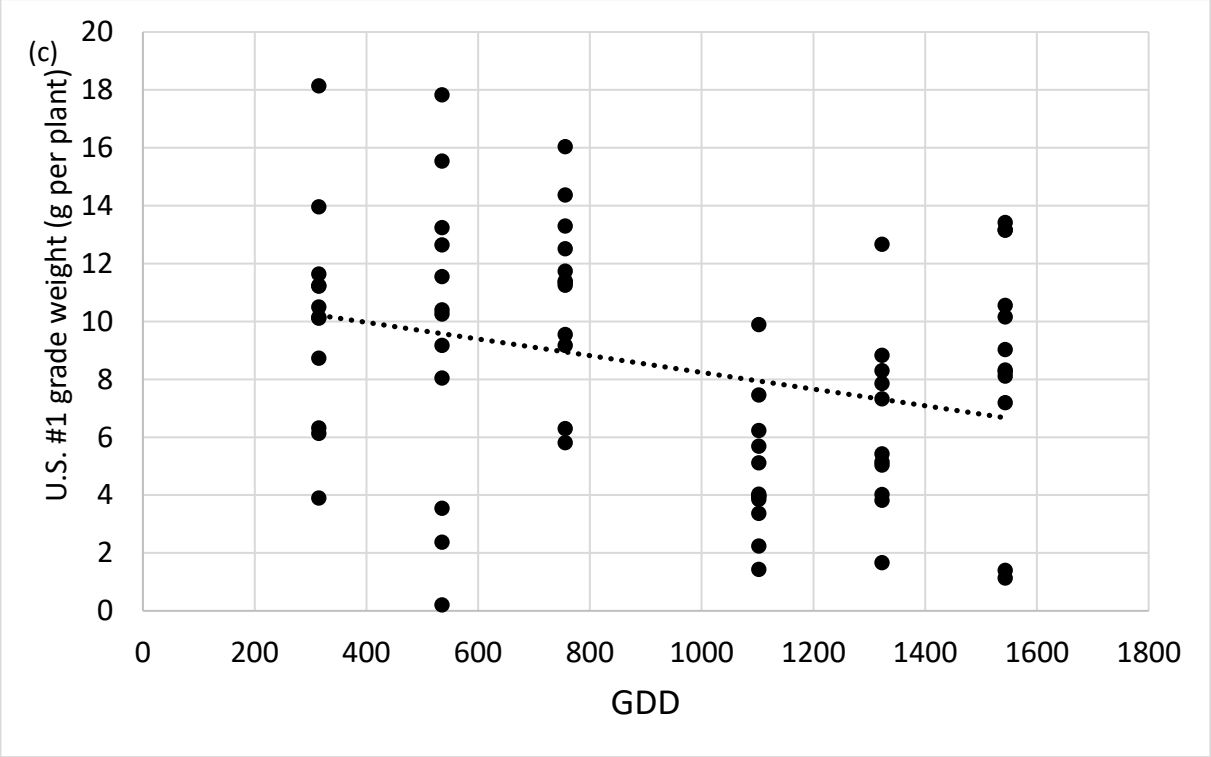
Figure 3.2 - Linear regression models for the relationship between the total plant biomass (a) (y = 153.79 + 0.41x), mean U.S. #1 seed weight per plant (b) (y = 6.32 + 0.02x) and HSW (c) (y = 14.31 + -0.001x) with the inoculation timing with PEMV aphids at the University of Idaho Parker Farm.

Table 3.4 - ANOVA table containing the degrees of freedom (df), mean squares (MS), F-value (F), and P-value of the total biomass, pod-count per plant, mean U.S. #1 grade weight per plant and HSW of the fall-sown pea at the Manis Laboratory.

ANOVA				
Greenhouse total biomass	df	MS	F	P-value
Regression	1	157.356	2.680	0.1061
Residual	70	58.710		
Total	71			
Greenhouse pod-count per plant	df	MS	F	P-value
Regression	1	514.664	4.695	0.0337
Residual	69	109.613		
Total	70			
Greenhouse mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	111.018	6.795	8.68E-15
Residual	69	16.338		
Total	70			
Greenhouse HSW	df	MS	F	P-value
Regression	1	1.219	0.129	0.7204
Residual	69	9.435		
Total	70			




(b)
Number of pods per plant
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
1600
1800
GDD


(c)
U.S. #1 grade weight (g per plant)
20
18
16
14
12
10
8
6
4
2
0
0
200
400
600
800
1000
1200
1400
1600
1800
GDD

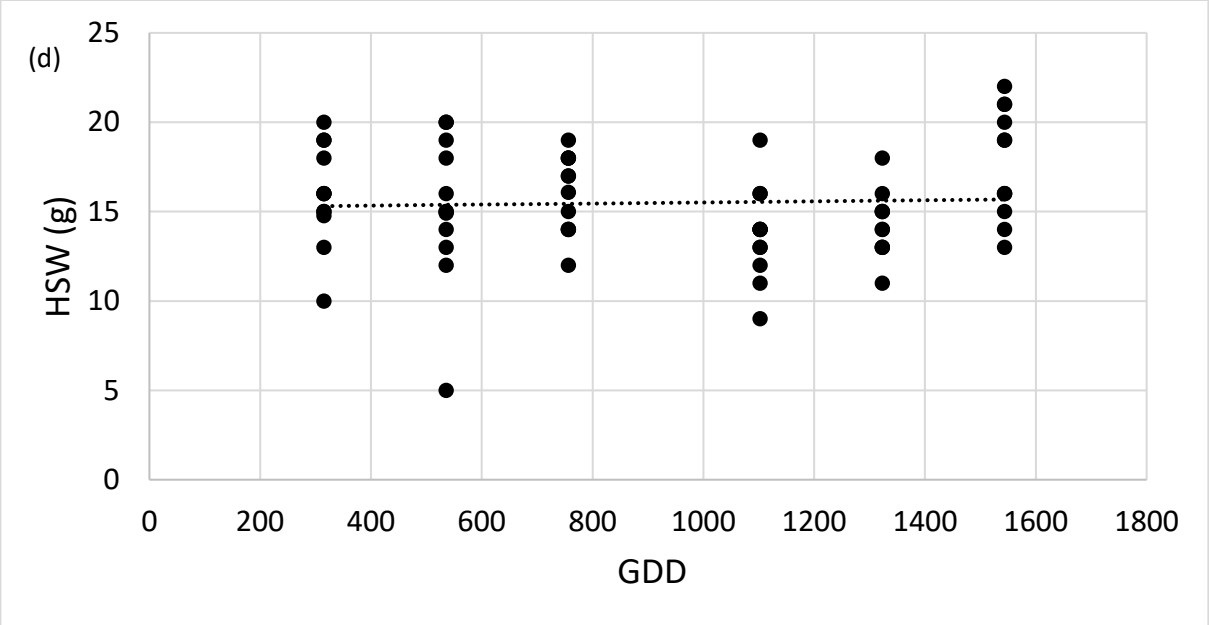
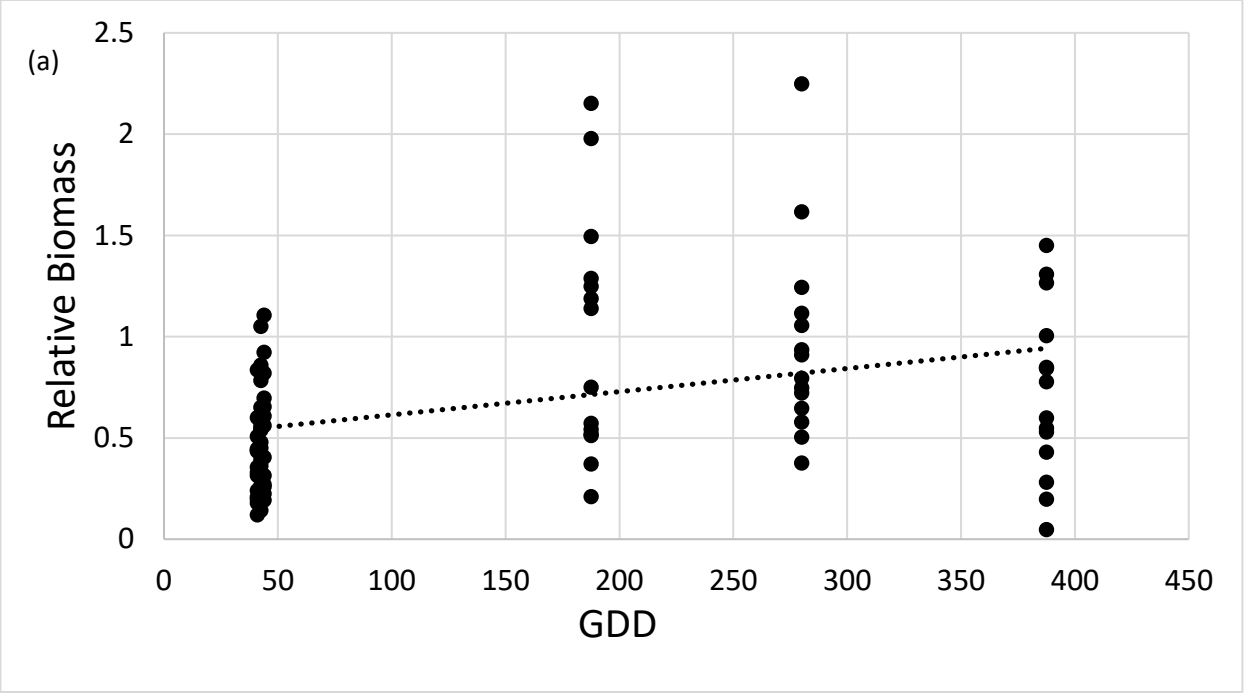
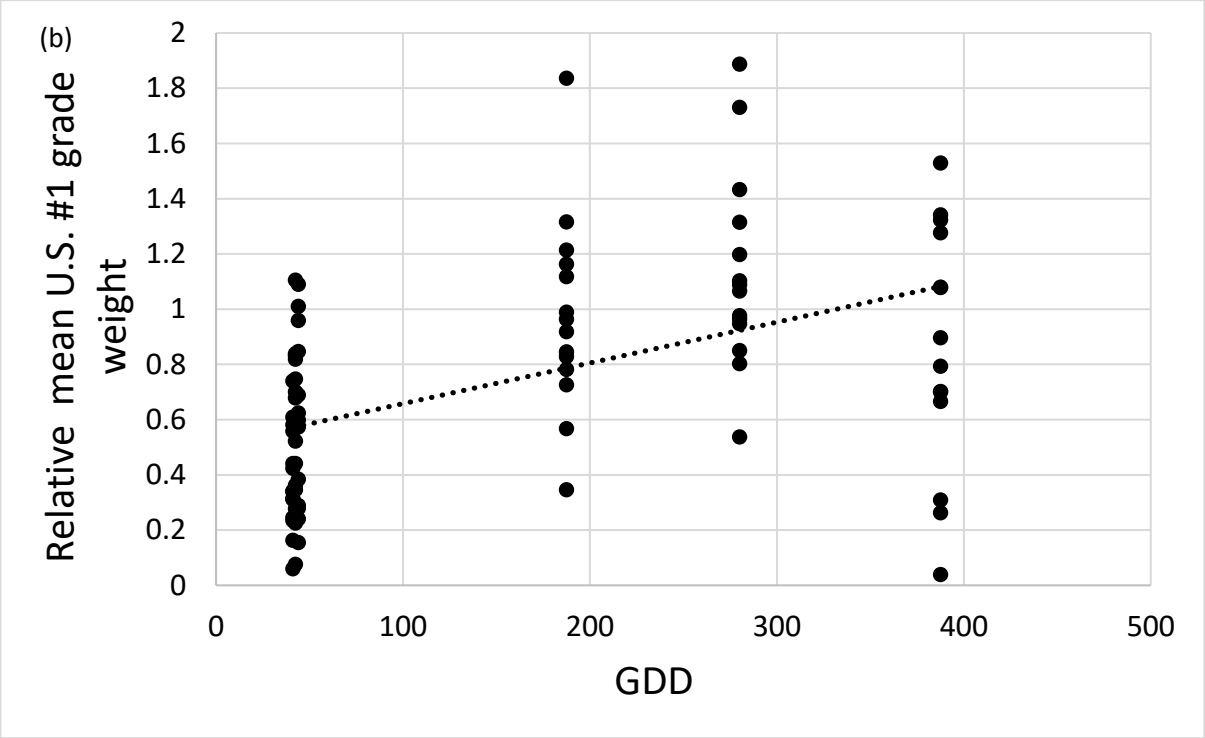


Figure 3.3 - Linear regression models for the relationship between the total plant biomass (a) (y = 23.54 + -0.003x), pod-count per plant (b) (y = 30.57 + -0.006x), U.S. #1 seed weight per plant (c) (y = 11.12 + -0.003x) and HSW (d) (y = 15.21 + 0.0003x) with the inoculation timing with PEMV aphids at the University of Idaho Manis Laboratory.

Table 3.5 - ANOVA table containing the degrees of freedom (df), mean squares (MS), F-value (F), and P-value of the combined relative biomass, combined relative mean U.S. #1 grade weight per plant and combined relative HSW of the fall-sown pea at the Kambitsch Farm and Parker Farm.

ANOVA				
Combined relative biomass	df	MS	F	P-value
Regression	1	1.956	10.544	0.0017
Residual	81	0.185		
Total	82			
Combined relative mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	3.266	23.995	4.86E-06
Residual	81	0.136		
Total	82			
Combined relative HSW	df	MS	F	P-value
Regression	1	0.018	1.355	0.2478
Residual	81	0.013		
Total	82			


(a)
Relative Biomass
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
300
350
400
450
GDD


(b)
Relative mean U.S. #1 grade weight
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
GDD

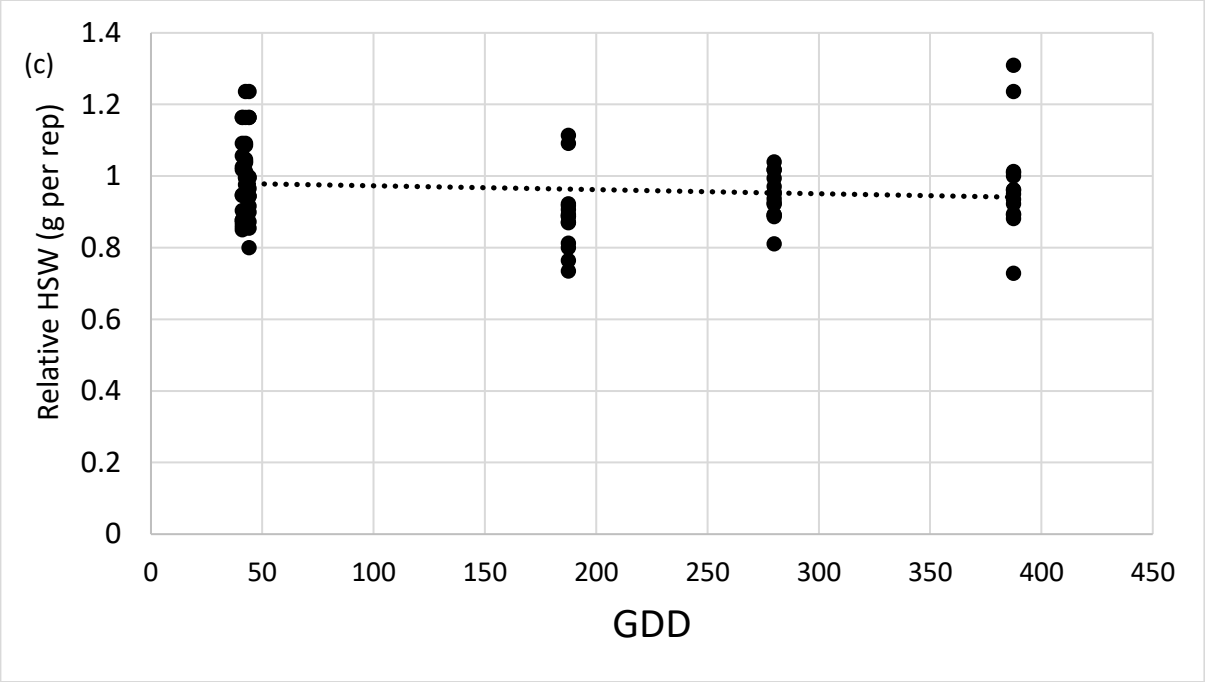


Figure 3.4 - Linear regression models for the relationship between the combined data from the Kambitsch Farm and Parker Farm expressed at relative total plant biomass (a) (y = 0.50 + 0.001x), relative mean U.S. #1 seed weight per plant (b) (y = 0.51 + 0.001x) and relative HSW (c) (y = 0.98 + -0.0001x) with the inoculation timing with PEMV aphids.

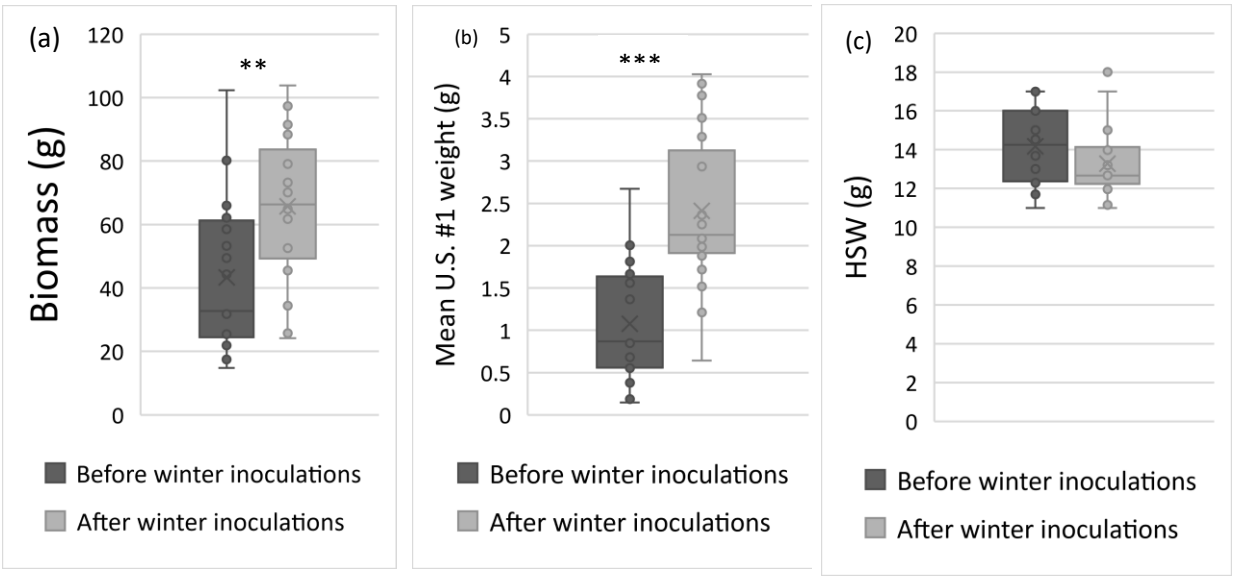


Figure 3.5 - Boxplots of the total biomass (a), mean U.S. #1 grade weight per plant (b) and HSW (c) of the pooled "before winter" inoculations and the pooled "after winter" inoculations at the Kambitsch Farm (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

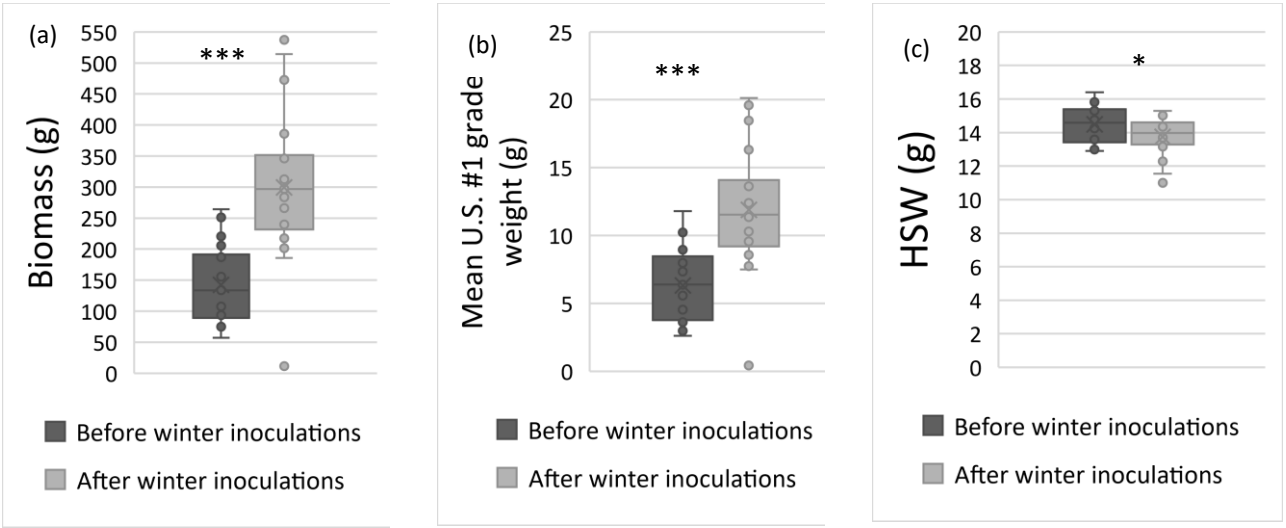


Figure 3.6 - Boxplots of the total biomass (a), mean U.S. #1 grade weight per plant (b) and HSW (c) of the pooled "before winter" inoculations and the pooled "after winter" inoculations at the Parker Farm (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

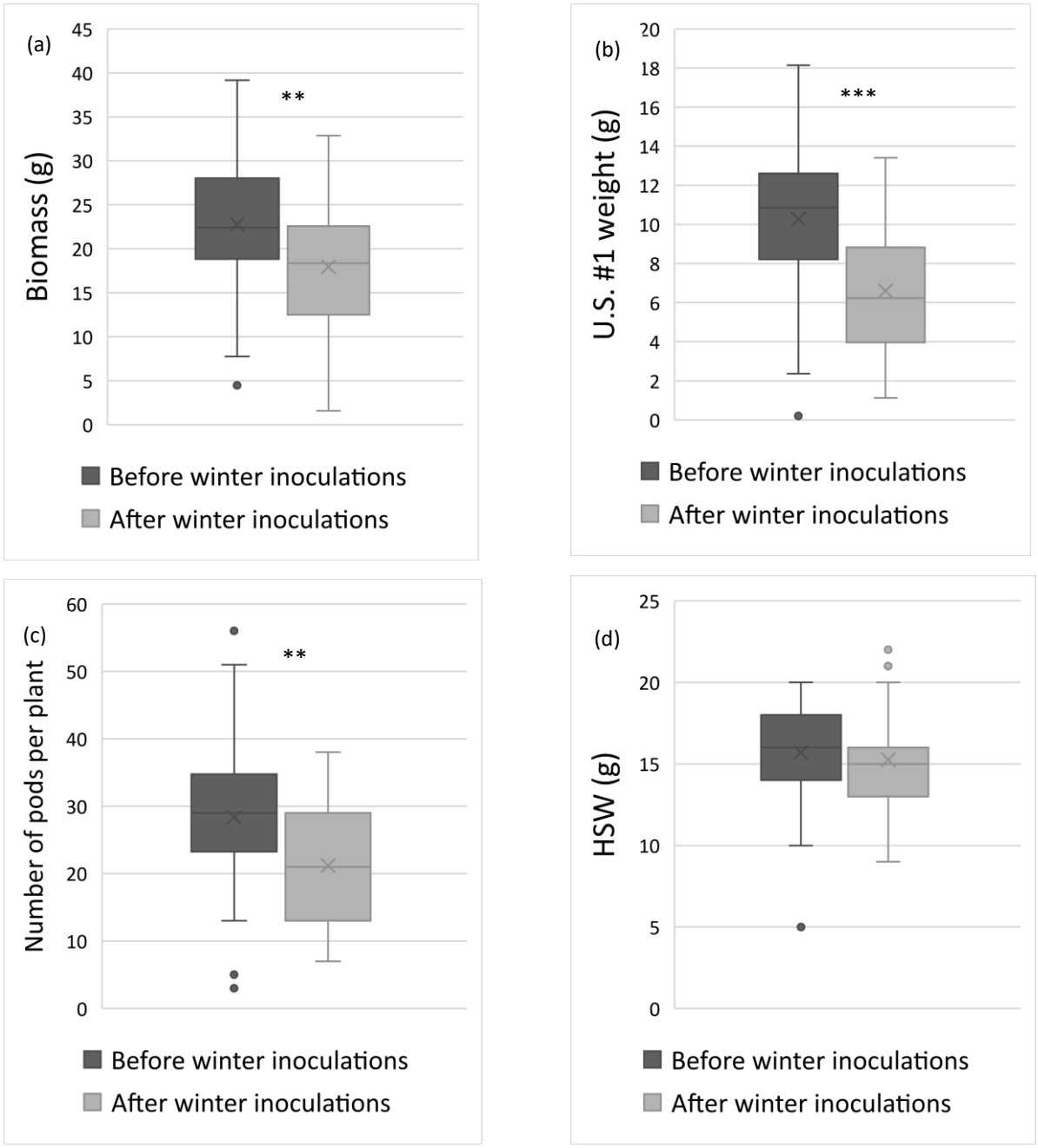


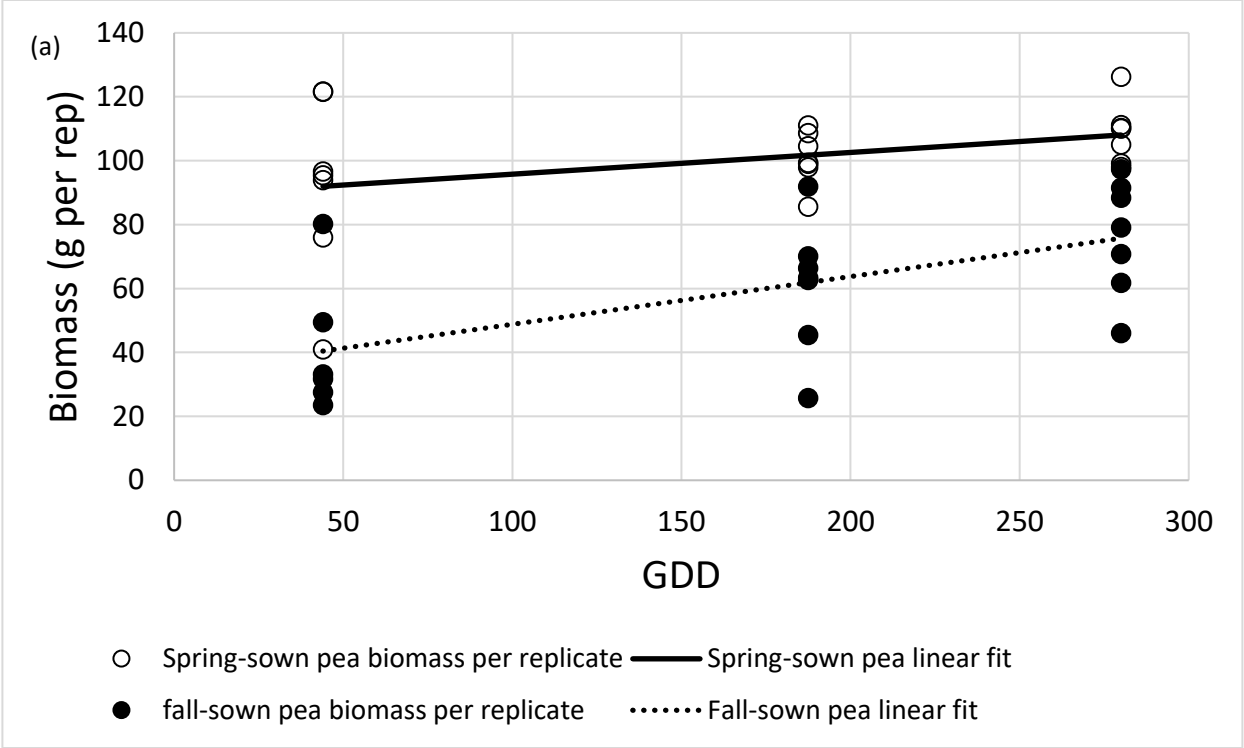
Figure 3.7 - Boxplots of the total biomass (a), U.S. #1 grade weight per plant (b), pod-count per plant (c) and HSW (d) of the pooled “before winter” inoculations and the pooled “after winter” inoculations at the Manis Laboratory (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

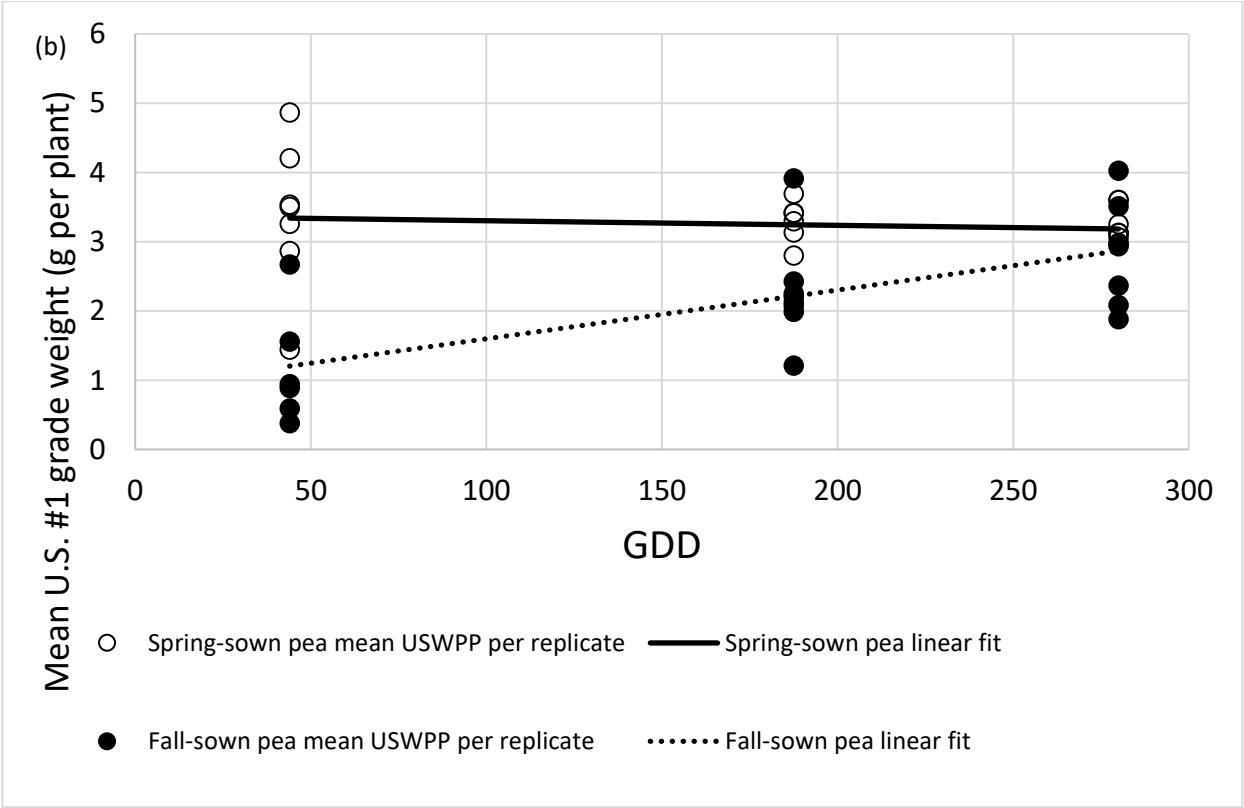
Table 3.6 - ANOVA table containing the degrees of freedom (df), mean squares (MS), F-value (F), and P-value of the biomass, mean U.S. #1 grade weight per plant and HSW of the spring-sown pea and fall-sown pea that experienced inoculations at similar GDD at the Kambitsch Farm.

ANOVA				
Spring-sown pea total biomass	df	MS	F	P-value
Regression	1	917.419	3.101	0.094
Residual	19	295.883		
Total	20			
Fall-sown pea total biomass	df	MS	F	P-value
Regression	1	4051.562	10.745	0.0042
Residual	18	377.078		
Total	19			
Spring-sown pea mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	0.086	0.183	0.6737
Residual	19	0.472		
Total	20			
Fall-sown pea mean U.S. #1 grade weight per plant	df	MS	F	P-value
Regression	1	8.990	14.533	0.0013
Residual	18	0.619		
Total	19			
Spring-sown pea HSW	df	MS	F	P-value
Regression	1	7.860	17.413	0.0005
Residual	19	0.451		
Total	20			
Fall-sown pea HSW	df	MS	F	P-value
Regression	1	4.700	1.491	0.2379
Residual	18	3.153		
Total	19			

Table 3.7 - The p-values produced by ANCOVA comparing the total biomass, mean USWPP and HSW of the spring-sown pea and fall-sown pea at Kambitsch Farm.

Spring-sown pea vs. fall-sown pea	P-value
Biomass	0.1795
Mean U.S. #1 grade weight per plant	0.0027
HSW	0.7814


(a)
Biomass (g per rep)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
GDD
Spring-sown pea biomass per replicate
Spring-sown pea linear fit
fall-sown pea biomass per replicate
Fall-sown pea linear fit


(b)
Mean U.S. #1 grade weight (g per plant)
6
5
4
3
2
1
0
0
50
100
150
200
250
300
GDD
Spring-sown pea mean USWPP per replicate
Spring-sown pea linear fit
Fall-sown pea mean USWPP per replicate
Fall-sown pea linear fit

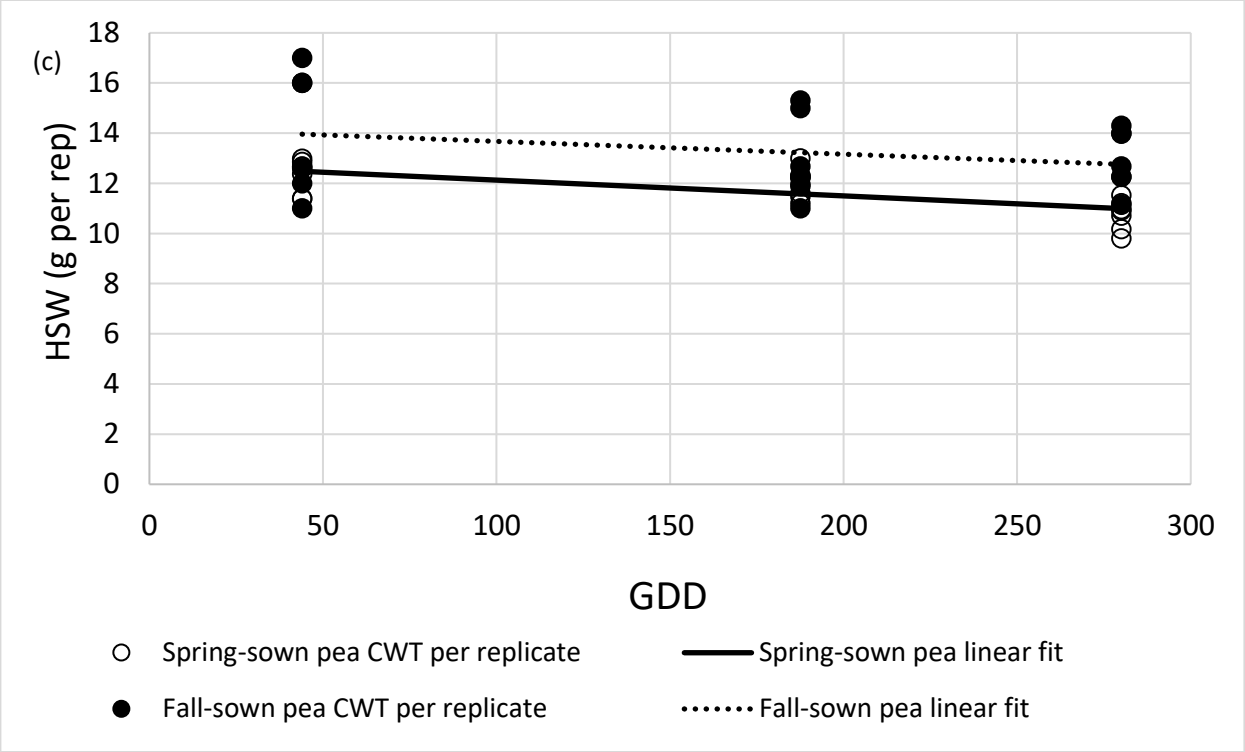


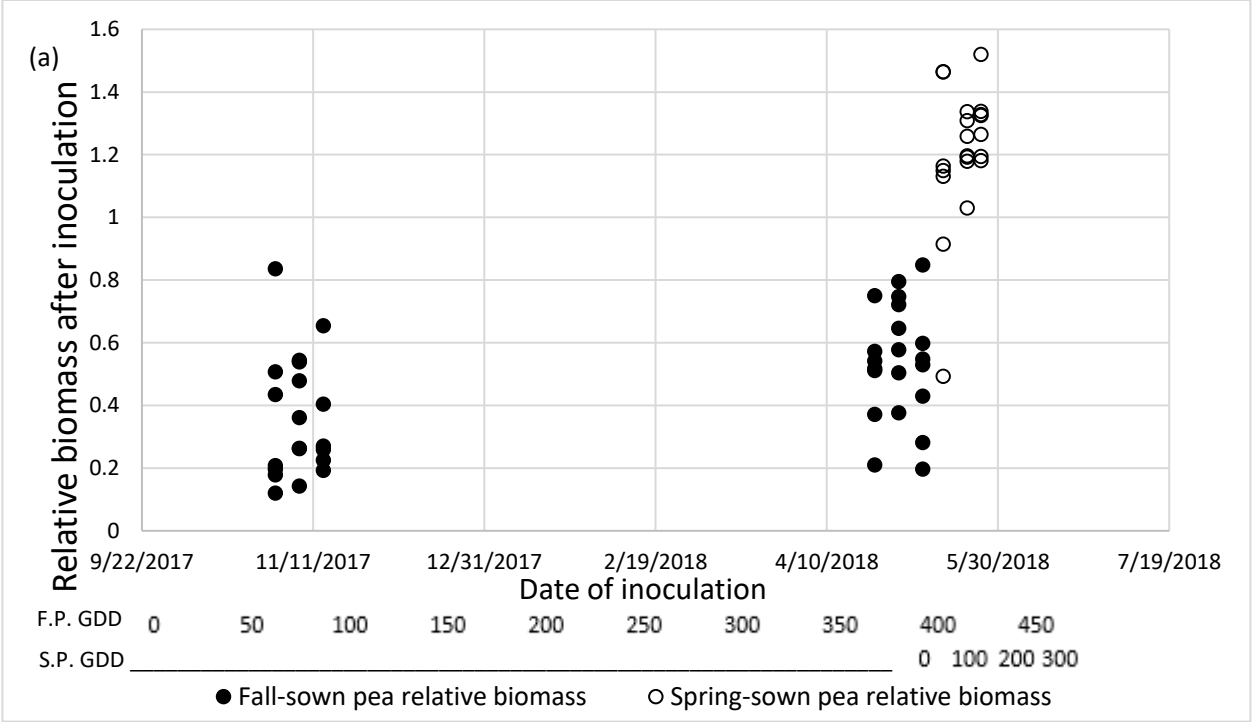
Figure 3.8 - Linear regression models for the relationship between the total plant biomass (a) (y = 153.79 + 0.41x), mean U.S. #1 seed weight per plant (b) (y = 6.32 + 0.02x) and HSW (c) (y = 14.31 + -0.001x) with the inoculation timing with PEMV aphids of the spring-sown pea and fall-sown pea that experienced inoculations at similar GDD at the Kambitsch Farm.

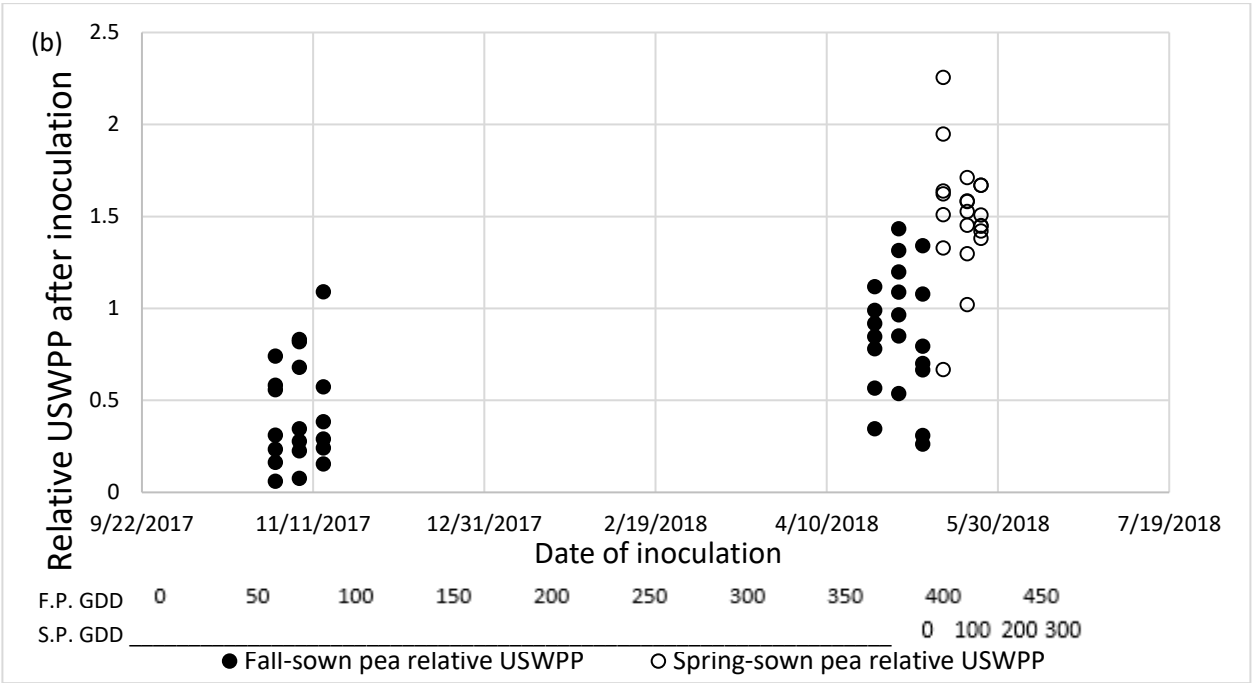
Table 3.8 - The p-values produced by a Welch's two-sample *t*-test comparing the relative total biomass, mean USWPP and HSW of the spring-sown pea and fall-sown pea inoculated at similar GDD at Kambitsch Farm.

Spring-sown pea vs. fall-sown pea biomass	P-value
Plants inoculated at 44 GDD	0.002
Plants inoculated at 187.5 GDD	2.47e-6
Plants inoculated at 280 GDD	8.82e-7

Spring-sown pea vs. fall-sown pea USWPP	P-value
Plants inoculated at 44 GDD	0.0007
Plants inoculated at 187.5 GDD	0.0003
Plants inoculated at 280 GDD	0.0057

Spring-sown pea vs. fall-sown pea HSW	P-value
Plants inoculated at 44 GDD	0.7002
Plants inoculated at 187.5 GDD	0.1169
Plants inoculated at 280 GDD	0.6263


(a)
Relative biomass after inoculation
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
9/22/2017
11/11/2017
12/31/2017
2/19/2018
4/10/2018
5/30/2018
7/19/2018
Date of inoculation
F.P. GDD 0 50 100 150 200 250 300 350 400 450
S.P. GDD 0 100 200 300
Fall-sown pea relative biomass
Spring-sown pea relative biomass


(b)
Relative USWPP after inoculation
2.5
2
1.5
1
0.5
0
9/22/2017
11/11/2017
12/31/2017
2/19/2018
4/10/2018
5/30/2018
7/19/2018
Date of inoculation
F.P. GDD 0 50 100 150 200 250 300 350 400 450
S.P. GDD 0 100 200 300
Fall-sown pea relative USWPP
Spring-sown pea relative USWPP

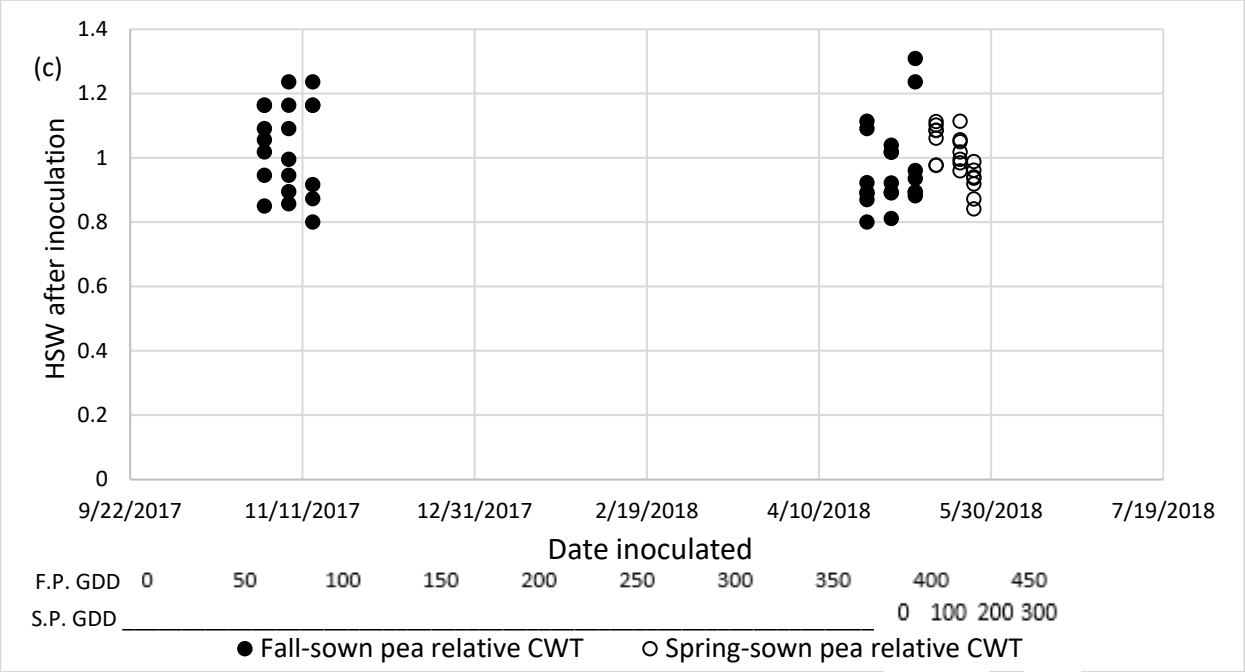


Figure 3.9 - The relative biomass (a), USWPP (b), and HSW (c) of spring-sown pea and fall-sown pea that experienced inoculations at similar GDD at the Kambitsch Farm. The primary x-axis represents the date plants were inoculated. The secondary x-axis represents the GDD experienced by fall-sown pea at inoculation. The tertiary x-axis represents the GDD experienced by spring-sown pea at inoculation.

LITERATURE CITED

Andret-Link, P., & Fuchs, M. (2005). Transmission Specificity of Plant Viruses by Vectors. Journal of Plant Pathology, 153-165.

Clement, S. L. (2006). Main Content Area Pea Aphid Outbreaks and Virus Epidemics on Peas in the US Pacific Northwest: Histories, Mysteries, and Challenges. Plant Health Progress, 1099-1108.

de Zoeten, Gustaaf A., and Skaf, Jihad S. (2001). Pea Enation Mosaic and the Vagaries of a Plant Virus. Advances in Virus Research. 57, 323-350.

DiFonzo, C. D., Ragsdale, D. W., Radcliffe, E. B., & Banttari, E. E. (1994). Susceptibility of Potato Leafroll Virus in Potato: Effects of Cultivar, Plant Age at Inoculation, and Inoculation Pressure on Tuber Infection. Plant Disease *(USA)*.

Elbakidze, L., Lu, L., & Eigenbrode, S. (2011). Evaluating Vector-Virus-Yield Interactions for Peas and Lentils under Climatic Variability: A Limited Dependent Variable Analysis. Journal of Agricultural and Resource Economics, 504-520.

Fabre, F., Dedryver, C. A., Leterrier, J. L., & Plantegenest, M. (2003). Aphid Abundance on Cereals in Autumn Predicts Yield Losses Caused by Barley Yellow Dwarf Virus. Phytopathology, 93(10), 1217-1222.

Grünwald, N. J., Chen, W., and Larsen, R. C. (2004). Pea Diseases and Their Management. Diseases of Fruits and Vegetables, 301-331.

Guy, & Cox. (2002). Reduced Tillage Increases Residue Groundcover in Subsequent Dry Pea and Winter Wheat Crops in the Palouse Region of Idaho. Soil & Tillage Research,66(1), 69-77.

Hull, R. (2002). Matthews' Plant Virology 4th Ed. Academic Press, San Diego, California, USA.

ICTVdB Management (2006). 00.078.0.01.007. Pea enation mosaic virus-2. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

Kennedy, G. G. (1976). Host Plant Resistance and the Spread of Plant Viruses. Environmental Entomology, 5(5), 827-832.

Lindblad, M., & Sigvald, R. (2004). Temporal Spread of Wheat Dwarf Virus and Mature Plant Resistance in Winter Wheat. Crop Protection, 23(3), 229-234.

Liu, Sijun., Sivakumar, S., Sparks, Wendy O., Miller, Allen W., and Bonning, Bryony C. (2010). A Peptide that Binds the Pea Aphid Gut Impedes Entry of Pea Enation Mosaic Virus into the Aphid Hemocoel. Virology 401, 107-116.

McGee, R. J., Eigenbrode, S., Nelson, H., & Schillinger, W. (2017). Re-Inventing Austrian Winter Pea Towards Developing Food Quality Winter Peas. Crops and Soils, 50(4), 4-46.

Nelson, Howard (2017). Fall Planted Pea Production Guidelines. Central Washington Grain Growers inc. Pg. 1-12.

Panter, S. N., & Jones, D. A. (2002). Age-related resistance to plant pathogens.

Paudel, S., Bechinski, E. J., Stokes, B. S., Pappu, H. R., & Eigenbrode, S. D. (2018). Deriving Economic Models for Pea Aphid (Hemiptera: Aphididae) as a Direct-Pest and a Virus-Vector on Commercial Lentils. Journal of Eeconomic Entomology, 111(5), 2225-2232.

Riedell, W. E., Kieckhefer, R. W., Haley, S. D., Langham, M. A., & Evenson, P. D. (1999). Winter Wheat Responses to Bird Cherry-Oat Aphids and Barley Yellow Dwarf Virus Infection. Crop Science, 39(1), 158-163.

Schillinger, W. F. (2017). Winter Pea: Promising New Crop for Washington's Dryland Wheat-Fallow Region. Frontiers in Ecology and Evolution, *5*, 43.

Smith, H. C. (1963). Control of Barley Yellow Dwarf Virus in Cereals. New Zealand Journal of Agricultural Research, 6(3-4), 229-244.

Stöckle, C. O., Higgins, S., Nelson, R., Abatzoglou, J., Huggins, D., Pan, W., ... & Brooks, E. (2018). Evaluating Opportunities for an Increased Role of Winter Crops as Adaptation to Climate Change in Dryland Cropping Systems of the US Inland Pacific Northwest. Climatic Change, 146(1-2), 247-261.

Stokes, B.S. (2012). Bioeconomics of Pea Aphids*, Acyrthosiphon pisum* (Harris), on Commercial Dry Peas, *Pisum sativum* 'Aragorn'. M.S. thesis, University of Idaho.

Strydhorst, S., Olson, M. A., Vasanthan, T., McPhee, K. E., McKenzie, R. H., Henriquez, B., ... & Coles, K. (2015). Adaptability and Quality of Winter Pea and Lentil in Alberta. Agronomy Journal, 107(6), 2431-2448.

Vemulapati, B., K.L. Druffel, S.D. Eigenbrode, A. Karasev, and H.R. Pappu.

(2010). Molecular Characterization of Pea Enation Mosaic Virus and Bean

Leafroll Virus from the Pacific Northwest, USA. Arch. Virol. 155, 1713-1715.

Waigmann, E., Ueki, S., Trutnyeva, K., & Citovsky, V. (2004). The Ins and Outs of Nondestructive Cell-to-Cell and Systemic Movement of Plant Viruses. Critical Reviews in Plant Sciences, 23(3), 195-250.

Chapter 4 Summary and Conclusions

The primary goal of this research was to assess the relationship between sowing date of dry pea, *Pisum sativum*, and viral damage stemming the biology and ecology of the pea aphid, *Acyrthosiphon pisum* (Harris). Additionally, the effects of timing of inoculation with *Pea enation mosaic virus* (PEMV) on yield of fall-sown dry pea were evaluated. A two-year survey monitoring pea aphid arrival, abundance, and virus status was performed at multiple locations across the Palouse region of eastern Washington and northern Idaho. Virus prevalence among plants within the field was also evaluated. Two field experiments and one greenhouse experiment were also conducted to determine the effect of timing of PEMV inoculation on yield.

The questions posed in this research were:

I. Is there differential abundance of pea aphid during the spring in fall-sown pea fields compared to spring-sown pea fields?
II. Does virus status of pea aphids captured at fall-sown pea fields differ from the virus status of pea aphids captured at spring-sown pea fields?
III. Does virus prevalence among plants within the field differ between fall-sown and spring-sown pea locations?
IV. Are pea aphids present and carrying virus in the fall after fall-sown pea has been planted?
V. Does timing of PEMV inoculation have an effect on yield?

Field surveys indicated that end-of-season pea aphid abundance did not differ between fall-sown and spring-sown pea despite the fact that pea aphids were collected at fall-sown pea locations before traps were placed at spring-sown pea locations. Each spring, virus was detected in aphids in fall-sown crops before traps had been placed at spring-sown pea fields, but by the end of the growing season, virus status of aphids trapped at fall-sown or spring-sown pea locations did not differ. Plant tissue samples from both years revealed that end-of-season virus prevalence was the same between fall-sown and spring-sown pea.

In the fall of both years, pea aphids were captured at fall-sown pea locations. Only three aphids were captured in the fall each year, and none of the aphids tested positive for virus. Both PEMV and BLRV were detected in plant samples from fall sown pea locations during both years, but the overall proportion of plant samples that tested positive for virus in the was low both years (18% and 3% respectively). This confirmed that pea aphids are present after fall-sown pea has been planted, and that plants are susceptible to virus inoculation in the fall.

To research question V, fall-sown pea plants were periodically inoculated with PEMV using viruliferous aphids from a colony. Experiments took place at two University of Idaho Experimental Research Farms and at the University of Idaho Manis Entomological Laboratory. Previous studies indicated that plants inoculated earlier in their development are more prone to a higher yield loss than plants inoculated at later development stages. The field experiments in this research supported the hypothesis that plants inoculated at early growth stages exhibit greater yield losses than plants inoculated later. Additionally, spring-sown pea plants were

inoculated at similar growth stages as fall-sown pea plants that were inoculated. This comparison revealed that fall-sown pea exhibited greater yield loss than spring-sown pea when both phenotypes were inoculated at similar growth stages.

This experiment was conducted using PEMV only, but BLRV is an important virus on the Palouse as well. Previous work has shown that spring-sown pea and spring-sown lentil exhibit the same age-related tolerance to BLRV as they do to PEMV, the effects of fall inoculation by BLRV may have different effects on fall-sown pea than does PEMV, and therefore warrants future study.

Although fall-sown pea was susceptible to virus inoculation in the fall and suffered greater yield loss than fall-sown pea plants infected in the spring, pea aphid density in pan traps at fall-sown pea fields in the fall was very low, as was virus infection of plants. Research suggests that fall-sown pea does not require additional steps to manage virus risk in the fall.

Pea aphid monitoring in this study was conducted over just two years. The field inoculation experiment documented the effects of virus after just one unique winter season. Longer, shorter, colder, warmer seasons occur in other years that could influence these overwintering effects. This experiment only showed that plants infected in the fall are more strongly affected, but not why they are more strongly affected. Perhaps the virus multiplies in the dormant plant tissue during the winter to create a higher virus titer within the plant when spring arrives. If this is the case, do the plants that are infected in the fall become strong nodes for secondary spread of the virus in the spring? Questions such as these warrant further research. Additionally, the greenhouse experiment was not successful, but future controlled

studies could test the effects of varying winter lengths and other possible conditions. Further studies involving more GDD overlapping for spring and fall-sown pea could strengthen inferences about fall-sown pea vulnerability to infection before winter.

This project generated many important questions to be answered by subsequent research. It remains possible that virus injury in fall-sown pea could be substantial in the fall on the Palouse or in the more arid production zones of eastern Washington in the future, justifying management action in the fall. It is important to maintain pea-aphid monitoring in the coming years to help producers best prepare for a possible virus outbreak.