Effects of Host Plant Water Stress on Two Insect Pests of Wheat in the Pacific Northwest

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AUTHORIZATION TO SUBMIT THESIS

This thesis of Nathaniel E. Foote, submitted for the degree of Master of Science with a Major in Entomology and titled “**Effects of Host Plant Water Stress on Two Insect Pests of Wheat in the Pacific Northwest**,” 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

Continued or accelerated warming of global atmospheric temperatures are expected to alter rainfall patterns, raise global aridity, and increase the frequency and severity of extreme weather events, including floods, heat waves and droughts. In the United States the frequency and severity of droughts have increased significantly over the past several decades, and global climate model analyses anticipate greater risk of drought on most continents during this century mainly as the result of elevated global mean surface temperatures and increasing aridity.

In cereals, which are the dominant staple crop worldwide, drought continues to be the main abiotic constraint on crop productivity and yield, and in severe cases leads to crop failure. However, impacts from drought can also emerge from its effects on other biotic components of the cropping system. Insect herbivores that are pests of cereals are important in this respect because in addition to damaging crops directly, drought-induced changes to the host plant or its growing conditions can affect the net impact of certain pests. But pest responses to drought can vary in direction and magnitude between species of an herbivore community as well as the level (i.e., severity or duration) of drought stress. For these reasons especially, the effects of drought on cereal pests and herbivores in general remain poorly understood.

Cereal systems in the Pacific Northwest (PNW) region of the United States contribute significantly to the global wheat crop. Economically important pests of cereals in the PNW include the cereal leaf beetle (CLB), *Oulema melanopus* and multiple cereal aphid species, including the bird cherry-oat aphid, *Rhopalosiphum padi* and more recently, the grass aphid *Metopolophium festucae cerealium*. Cereal leaf beetle and both aphid species can cause extensive damage to a variety of cereal crops, but are highly prevalent in wheat grown in the PNW. Research and monitoring efforts have improved our basic biological understanding and control of these pests considerably. However, information detailing responses by these pests to drought stress largely remains limited to mixed findings that can be difficult to interpret or apply within a broader management context.

We tested the effects of plant water stress on the feeding performance of CLB and growth of its host plant, using a study system in wheat (*Triticum aestivum*) and greenhouse experiments. Experimental watering regimes, which provisioned either ample or deficient water supplies at regular intervals, produced healthy and chronically water-stressed potted wheat plants, respectively. We found that water stress may adversely affect CLB performance and significantly impacts the growth and seed production of potted wheat. However, results from these experiments show that there were no significant interactions between water stress and CLB feeding damage on wheat growth or seed production, suggesting that the combined negative effects from these two stressors on the host plant are additive.

We additionally tested the hypothesis that plant water stress mediates interactions between the cereal aphids *M. festucae cerealium* and *R. padi*, again using greenhouse experiments with potted wheat plants grown under experimental watering regimes. We found that experimentally induced water stress resulted in significantly smaller wheat plants, and negatively affected *R. padi* but not *M. festucae cerealium* on host plants colonized by only one or the other species. However, *M. festucae cerealium* was negatively affected on plants simultaneously co-colonized by *R. padi*, and an interaction occurred whereby this effect was significantly greater on the water-stressed plants. Additionally, we found that higher reproduction rates occurred for *R. padi* feeding in close proximity to, or on leaves previously infested by *M. festucae cerealium* on wheat plants that were provided an ample water supply. Our findings suggest that interactions between these two economically important aphid species are ecologically complex, producing asymmetrical competitive outcomes which can depend greatly on the environmental context under which these interactions take place.

This thesis aims to provide better understanding of the biotic constraints on cereal systems in the Inland PNW by investigating the effects of plant water stress on these regionally prevalent pests, using a study system in wheat and greenhouse experiments. Potential implications for pest management in PNW cereal systems under projected climate scenarios will be discussed using a regional context relevant to agriculture globally.

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DEDICATION

This is dedicated to my mother, Marcia Zukosky, who has my deepest gratitude for her unconditional support towards completing this graduate degree. Without her help this would not have been possible, and for this I will always be grateful.

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CHAPTER ONE—Literature review

Introduction

During this century major challenges will face agricultural food production worldwide. As the global demand for food crops increases with a growing human population, uncertainty grows over the future stability of the world food supply (Ericksen et al. 2009, Tilman et al. 2011, Eigenbrode et al. 2013, Challinor et al. 2014). Contributing significantly to this uncertainty are the impacts associated with a globally changing climate and its potential effects on crop production (Tubiello et al. 2007).

Warmer global atmospheric temperatures are expected to alter rainfall patterns (Wang 2005), raise global aridity (Dai 2013), and increase the frequency and severity of extreme meteorological events, including floods (Milly et al. 2002), heat waves (Seneviratne et al. 2006) and droughts (Mishra and Singh 2010). While virtually all forms of agriculture are sensitive to climate variations (Howden et al. 2007), most are exceptionally vulnerable to extreme climate events (Rosenzweig et al. 2001). In the United States drought is considered the most damaging of extreme climate events in terms of economic losses, which currently rank highest in the country compared with other natural disasters (Cook et al. 2007).

The frequency and severity of droughts in the United States have increased significantly over the past several decades (Wilhite and Hayes 1998, Changnon et al. 2000, Mishra and Singh 2010). This has prompted increasing efforts towards assessing drought risk under future climate scenarios (e.g., Dai 2013). Global climate model analyses anticipate greater risk of drought on most continents during this century, mainly as the result of elevated global mean surface temperatures and increasing aridity (Dai 2013). Warmer temperatures, in particular, may intensify the global hydrological cycle and increase atmospheric evaporative demands (Allen and Ingram 2002), which might in turn alter atmospheric circulation patterns and cause further increases in aridity through feedbacks (Dai 2011).

Fundamentally, drought is any event that creates a deficiency of precipitation resulting in water shortage for some activity (e.g., plant growth) or for some group (e.g., farmers) (Wilhite and Glantz 1985). Water shortages pose a serious threat to agriculture, which demands the most water of any human activity; irrigated crops alone consume 75% of total freshwater resources annually while comprising only 17% of total arable land (Tubiello et al. 2007). Dai (2011) specifically refers to agricultural drought as a period with dry soils that results from below average precipitation, intense but less frequent rainfall, or greater than normal evaporation, all of which can impact plant growth leading to reduced crop production.

In cereals, which are the dominant staple crop worldwide (Evans 1998, Araus et al. 2002, Parry et al. 2004), drought is the main abiotic constraint on crop productivity and yield (Boyer 1982, Araus et al. 2002). In severe cases drought leads to plant death and crop failure (Palmer 1968). Cereal grasses can otherwise survive some level of drought-induced water deficits through a variety of physiological adjustments (Hsiao 1973), but at the cost of reduced carbon assimilation and slower growth which can result in lower crop yields (Fleury et al. 2010).

The impacts of drought on cropping systems can potentially also emerge through indirect pathways, including effects on other biotic components of the cropping system (Rosenzweig et al. 2001). Insect pests are important in this regard because they damage crops directly, but also respond to environmental factors in ways that can further impact cropping systems (Cannon 1998). For example, warmer temperatures associated with droughts can affect pest populations by extending the plant growing season, shortening insect generation times, and altering their geographic distributions (Porter et al. 1991).

In cereal production systems, it is not uncommon for multiple economically important herbivore species to cohabitate the same fields and share the same crop plant resources (Schotzko and Bosque-Pérez 2000, Bosque-Pérez et al. 2002, Qureshi and Michaud 2005). However, pest responses to drought can vary in both magnitude and direction between species of an herbivore community complex (Huberty and Denno 2004). Additionally, changes in the abiotic setting can influence or alter the outcomes of important multitrophic interactions that play a role in determining herbivore community structure (Hunter and Price 1992), such as between some biological control agents and their target pest species (see Evans et al. 2012). Pest pressures in cereals under drought might therefore change as the result of individual species responses, but also community level responses and the emergent effects of drought on multi-species interactions.

Drought effects on herbivores and the plant-stress hypothesis

Precisely determining pest responses to drought has remained an ongoing and evolving challenge for researchers. Deriving from the well-documented effects of water stress on plants (e.g., Hsiao 1973), early studies observing herbivore populations during drought events hypothesized plant stress responses as an underlying cause of pest outbreaks. At the molecular level, water stress can disrupt plant protein metabolism and amino acid synthesis (Hsiao 1973), resulting in plant tissues with higher levels of free amino acids (Brodbeck and Strong 1987). Plants may also compensate for low osmotic pressure through the production of nitrogen-containing osmoprotectants (Aspinall and Paleg 1981, Huberty and Denno 2004). Insect herbivores that subsist on otherwise nitrogen-limited plant diets can benefit from these stress-induced changes in plant chemistry (Mattson 1980). This perceived improvement in host plant quality has led to the traditionally accepted plant-stress hypothesis, which suggests that environmental stress in plants improves the performance of herbivorous insects and affects their population and outbreak dynamics (White 1969, Brodbeck and Strong 1987, Mattson and Haack 1987a, 1987b, Huberty and Denno 2004).

While substantial evidence exists in support of the plant-stress hypothesis (see Mattson and Haack 1987a, 1987b), stress-induced changes in the host plant do not always correlate with increased herbivore performance (Scriber 1984, Rodriguez and Rodriguez 1987, Dale 1988, Zou and Cates 1994, English-Loeb et al. 1997). Rather, many experimental studies have demonstrated neutral or negative effects of plant water stress on certain herbivores (e.g., Hale et al. 2003). Reviews combining these mixed results (e.g., Larsson 1989, Huberty and Denno 2004) have emphasized the inherent complexities of assessing insect responses to drought, especially when attempting to compare differential responses by multiple herbivore groups. A synthesis by Huberty and Denno (2004) approached this problem by differentiating insect responses to plant water stress according to herbivore feeding guild.

Insect herbivores are assigned to feeding guilds based on their means of food acquisition from plants. Huberty and Denno (2004) used mouthpart morphology to distinguish two major feeding guilds: ‘chewers’ have mandibulate mouthparts that masticate plant tissues, whereas ‘sap feeders’ tap phloem or xylem sap using stylet-like mouthparts. These distinctions are important for assessing the effects of water stress on herbivores for several reasons. First, different herbivores have different food resource needs of their host plants (e.g., leaf tissue versus phloem sap); second, water stress affects different parts of a plant in unique ways. For example, cuticular resistance can increase as leaf water content decreases (Bengtson et al. 1978), and during prolonged periods of water stress the deposition of epicuticular wax is increased (Skoss 1955, Clark and Levitt 1956, Daly 1964). These water stress-induced changes to the anatomical features of plant tissue can result in physically tougher leaves that deter attack by chewing herbivores, such as leaf beetles (Raupp 1985).

The plant-stress hypothesis was originally formulated based on field observations of the outbreak dynamics of sap feeders, specifically psyllids on *Eucalyptus* trees in the drought-prone regions of Australia (see White 1969). Feeding by sap feeders, however, requires adequate plant cell turgor pressure (Wearing and van Emden 1967, Holtzer et al. 1988, Archer et al. 1995), which is directly related to plant water content (Hsiao 1973, Drake and Gallagher 1984, Taiz and Zeiger 2002). Experimental studies testing sap feeder responses to continuous, rather than intermittent periods of water stress (as observed in field studies) have provided evidence in conflict with the plant-stress hypothesis (Huberty and Denno 2004). The performance of certain pest aphid species, for example, can be adversely affected on water-stressed hosts (Kennedy et al. 1958, Pons and Tatchell 1995, Hale et al. 2003, Huberty and Denno 2004). But the effects of drought stress on aphids is variable and dependent on the exact form of drought stress (Pons and Tatchell 1995, Huberty and Denno 2004), the host plant (Hale et al. 2003) and aphid species involved (Pons and Tatchell 1995).

Drought with climate change: rising temperature effects on herbivores

According to global model projections, warmer temperatures are expected to accompany future occurring drought events (Lehner et al. 2006). While the effects of climate change on insect herbivores are attributed to multiple interacting variables (Eigenbrode et al. 2015), the direct effects of temperature are considered to be a major leading factor (Bale et al. 2002). Understanding pest responses to drought stress therefore also requires addressing the effects of important drought-associated abiotic variables, notably temperature.

As ectotherms, temperature is the dominant abiotic factor affecting development, survival, range and abundance of insect herbivores (Cammell and Knight 1992, Bale et al. 2002). Rising temperatures may benefit numerous pest species in terms of increased overwintering survival and shorter generation times (Olfert and Weiss 2006), but also potentially change the pest status of certain species as a consequence of range expansion and greater abundances (Estay et al. 2009). If this occurs unevenly among closely interacting species, the efficacy of biological control may change as a result of disrupted temporal or spatial synchrony between species (Harrington et al. 1999, Bale et al. 2002, Both et al. 2009, Evans et al. 2012, Eigenbrode et al. 2015).

Additionally, biological control could be undermined by temperature change-driven phenological misalignments between pests and their natural enemies (Eigenbrode et al. 2015). For example, Evans et al. (2012) provide evidence that warmer springs in northern Utah, USA disrupt alignment between the cereal leaf beetle (CLB; see Chapter Two), *Oulema melanopus* L. (Coleoptera: Chrysomelidae), and *Tetrastichus julis* Walker (Hymenoptera: Eulophidae), a specialist parasitoid and the principal natural enemy of invasive CLB populations in North America. Over the course of 10 seasons, the phenological misalignment between these two species during warm springs resulted in severely reduced parasitism of CLB by *T. julis*, and effectively undermined biological control of a major cereal pest (Evans et al. 2012).

Cereal aphids (Hemiptera: Aphididae; see Chapter Three) are another major focus of pest populations in response to rising temperatures, as long-term suction trap networks established in the United States and Europe have detected changes in migrations related to warming (Eigenbrode in preparation). A 17-year time series compiled from one of these networks showed that abundances of the cereal aphid *Diuraphis noxia* (Kurdumov) in the Pacific Northwest USA were negatively correlated with increasing temperatures in the absence of density-dependent effects (Davis et al. 2014a). According to suction trap network data collected in the United Kingdom, however, the cereal aphids *Metopolophium dirhodum* (Walker) and *Sitobion avenae* F. showed little response to warming temperature trends over a 50-year period (Bell et al. 2014). Collectively, these results indicate highly differential responses of aphids to temperature trends, by species as well as region. Furthermore, these findings highlight the effects of weather and climate variables in determining the reliability of pest phenology models and the importance of regional contexts (Eigenbrode in prep.).

Climate projections for Pacific Northwest cereal systems

Climate change is expected to increasingly affect cereal yields globally (Tubiello et al. 2007), but determining system-wide responses of cereal pests to drought requires incorporating regional contexts (Eigenbrode et al. 2013). Cereal systems in the Pacific Northwest (PNW) region of the United States produce over 130 million bushels of wheat (*Triticum aestivum* L.) annually, with an annual total grain production value exceeding $500 million (USDA-NASS 2015). The Inland PNW region (42 - 49 °N, 111 – 121 °W) region has high edaphic and climate heterogeneity, lending to a diversity of cereal production systems ranging from continuous cropping to annual crop-fallow and irrigated (Douglas et al. 1992) with the vast majority of wheat being grown under dryland (rainfed) conditions (Schillinger and Papendick 2008). In fact, dryland cropping in the Inland PNW covers approximately 4.4 million hectares and is the largest contiguous cropping zone in the western United States (Schillinger et al. 2006).

The PNW has experienced a warming trend of approximately 0.1 °C per decade during the past century (Mote et al. 2005), with a mean annual temperature increase of 1.7 to 2.2 °C being predicted for the inland region by middle of this century (Von Walden 2014). By the latter half of the twenty-first century mean annual temperatures are projected to rise 5 °C (Abatzoglou 2015). However, this warming will be highly asymmetric between seasons. According to Abatzoglou et al. (2015), regional climate models project amplified warming rates for overnight low temperatures compared to daytime high temperatures during the winter, but faster warming of daytime high temperatures compared to overnight low temperatures during the summer. Annual precipitation totals are also projected to increase by up to 12.5 % by end-of-century, but predominantly during the winter with likely decreases in rainfall during summer months (Stöckle 2014).

Temperature and precipitation changes projected for the Inland PNW for the latter half of this century are expected to have variable effects on cereal production systems throughout the region, resulting in complex net effects on PNW agriculture in general (Eigenbrode et al. 2013). For example, milder winters may reduce incidence of cold damage to winter cereals while hotter, drier summers will result in increased potential evapotranspiration and moisture stress for (Abatzoglou et al. 2015). In general, future climate changes across the Inland PNW are expected to allow for the establishment of certain novel agricultural systems while also posing risks to those that are not thermally adaptive (Abatzoglou et al. 2015).

Prevalent insect pests of cereals in the PNW include the CLB, and cereal aphids . Both pests cause extensive damage to multiple cereal crops throughout North America, but are particularly prevalent in wheat grown in the PNW. These pests each belong to a different feeding guild and damage the common host plant in very different ways. These insects also respond differently to abiotic stressors such as drought, and therefore pose unique potential threats to PNW cropping systems under projected climate scenarios.

Cereal leaf beetle overview

The cereal leaf beetle is an introduced, widespread pest of small grains causing economic losses to wheat, oats, and barley throughout North America (Haynes and Gage 1981, Olfert et al. 2004). Greatest damage to crop plants occurs during feeding by CLB larvae, which remove long strips of leaf tissue, parallel with leaf veins, resulting in reduced photosynthesis and crop yield (Wellso et al. 1975). The beetle is native to Eurasia and was first identified in southwestern Michigan in 1962 (Castro et al. 1965), but surveys indicate that CLB populations have now become established from Alabama north to Ontario, Canada (NAPIS 2003, Olfert et al. 2004).

Beetle populations have most recently expanded into northwestern North America from Utah northward into British Columbia, Canada (Olfert et al. 2004). Continued cereal leaf beetle range expansion and establishment could be attributed to a warming climate (see Sutherst 2000, Bale et al. 2002). Bio-climatic models that incorporate future climate scenarios have been developed to project the potential distribution and abundance of this invasive pest throughout its expanding range in Canada (Olfert et al. 2004, Olfert and Weiss 2006). But these models do not consider the indirect effects of climate-related stress on the beetle through its predominant host plant, and despite its potential greater pest status under warming climates there are no published studies detailing how CLB is affected by drought stress.

Cereal aphids overview

Aphids are considered among the most important insect pests affecting agriculture globally (Hill 1997, Newman 2005), with the greatest number of species occurring in temperate regions (Dixon 1973). Multiple economically important aphid species occur together in cultivated wheat fields throughout cereal producing regions of the United States (Schotzko and Bosque-Pérez 2000, Bosque-Pérez et al. 2002, Qureshi and Michaud 2005). Two cereal aphid species found infesting the same wheat fields in the Pacific Northwest are the grass aphid, *Metopolophium festucae* (Theobald) subsp. *cerealium* and the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Y. Wu personal communication).

Metopolophium festucae subsp. *cerealium* is an exotic species in the United States, first reported in the state of Oregon in 1994 (Halbert and Sandvol 1995). While little is known about *M. festucae cerealium* ecology in its exotic North American habitat, the aphid is already a well-established pest in Europe (Dent 1983), and has potential to emerge as a major pest of cereal crops in the USA (Davis et al. 2014b). Large populations of the aphid have recently been detected in wheat in the states of Washington, Oregon, and Idaho (Halbert et al. 2013, Davis et al. 2014b) where it appears to have transitioned to invasion dynamics (Sadeghi et al. 2016).

Rhopalosiphum padi is one of the fourteen aphid species considered of most agricultural importance worldwide (Blackman and Eastop 2007), and unlike *M. festucae cerealium,* is a naturalized pest of cereal crops throughout the PNW. It has been studied extensively since the 1950s when it was discovered to transmit crop plant disease-causing yellow dwarf viruses (Plumb 1983, Rochow 1970, Finlay and Luck 2011). In contrast, *M. festucae cerealium* does not appear capable of transmitting BYDV-PAV (Sadeghi et al. 2016), the dominant and most injurious species of yellow dwarf virus encountered in the PNW (Hewings and Eastman 1995).

For aphids the effects of drought stress are variable and dependent on multiple factors. Studies have shown that drought affects *R. padi* either neutrally or negatively depending on the exact form of drought stress (Pons and Tatchell 1995) and host plant species (Hale et al. 2003). However, Davis et al. (2015) demonstrated that *R. padi* feeding on wheat plants infected with *Barley yellow dwarf virus* were less adversely affected by host plant drought stress. Besides one study by Davis et al. (2014b) examining host plant settling behavior of *M. festucae cerealium* on a variety of grass species, very little is known about this aphid in North America and there have been no studies investigating its response to host plant drought stress.

Project context: REACCH PNA

Initiated in 2011, Regional Approaches to Climate Change for Pacific Northwest Agriculture (REACCH PNA) is a five-year transdisciplinary effort to enhance sustainability of Inland PNW cereal production systems under current and projected climate change, while contributing to climate change mitigation. The project pursues specific research objectives; among these is anticipating and developing approaches to climate-related changes in crop protection requirements, which necessitates furthering our understanding of biotic constraints in the region's cereal systems in response to abiotic stress events associated with climate change, such as drought.

Purpose of thesis research

This thesis research aims to provide better understanding of the biotic constraints of cereal systems in the Inland PNW by investigating the effects of experimentally induced plant water stress on two regionally prevalent pests, using a study system in wheat and greenhouse experiments. Wheat plants grown in a greenhouse were ideal for testing the combined impacts of water stress and insect feeding because their water content, growth and seed production could be accurately measured under controlled environment conditions. Using potted wheat plants further allowed for the manipulation of plant water content through experimental water regimes adapted from similar studies (e.g., Davis et al. 2015). The insect species used in this research were selected on the basis of pest status and prevalence in PNW cereal production.

Thesis objectives

The primary objective of this research was to determine the effects of host plant water stress on two insect pests of wheat. Because two distinct groups (i.e., feeding guilds) of herbivores were involved in this research, two studies emerged each with their own specific objectives.

Study 1 (Cereal leaf beetle) specific objectives:

1. Measure changes in feeding performance of the cereal leaf beetle as affected by host plant water stress.
2. Measure changes in the growth and seed production of wheat plants as affected by water stress and CLB feeding damage, separately and in combination.
3. Determine whether the effects of CLB feeding damage on wheat are mediated by water stress.

Study 2 (Cereal aphids, *M. festucae cerealium* and *R. padi*) specific objectives:

1. Measure changes in reproductive performance of *M. festucae cerealium* and *R. padi* as affected by host plant water stress.
2. Measure changes in reproductive performance of *M. festucae cerealium* in the presence of *R. padi*, and vice versa.
3. Determine the ecological outcome of interactions between *M. festucae cerealium* and *R. padi* colonizing the same wheat plants.
4. Determine whether the outcome of interactions between *M. festucae cerealium* and *R. padi* colonizing the same wheat plants is mediated by water stress.

Findings from this research have potential implications for pest management in cereal cropping systems in the PNW and elsewhere. The focused narrative of this thesis involves gathering evidence novel to our currently limited understanding of cereal pest responses to drought. Within the broader context of REACCH PNA, integrating modeling efforts using the data generated from this research and other studies within the Project could potentially improve our capacity for predicting pest pressures in Inland PNW cereal systems under projected climate scenarios. Collectively, these are components of a broader perspective with the goal of improving our understanding of the complex interactions between insect pests, their associated herbivore communities and the environment under projected climate scenarios, where drought may be a more prevalent condition affecting agricultural systems in the PNW and elsewhere in North America.

References

Abatzoglou, J., D. Rupp, and P. Mote. 2015. Asymmetric warming projections for the inland Pacific Northwest, pp. 104-105. *In* Regional Approaches to Climate Change for Pacific Northwest Agriculture Annual Report Year 4.

Allen, M.R., and W.J. Ingram. 2002. Constraints on future changes in climate and the hydrologic cycle. Nature 419: 224-232.

Araus, J.L, G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: what should we breed for? Annals of Botany 89: 925-940

Archer, T.L., E.D. Bynum, Jr., A.B. Onken, and C.W. Wendt. 1995. Influence of water and nitrogen fertilizer on biology of the Russian wheat aphid (Hemiptera: Aphididae) on wheat. Crop Protection 14: 165-169.

Aspinall, D., and L.G. Paleg. 1981. *In* The physiology and biochemistry of drought resistance in plants, pp. 205-241. Academic Press, Sydney, Australia.

Bale, J.S., G.J. Masters, I.D. Hodkinson, C. Awmack, T.M. Bezemer, V.K. Brown, J. Butterfield, A. Buse, J.C. Coulson, J. Farrar, J.E.G. Good, R. Harrington, S. Hartley, T.H. Jones, R.L. Lindroth, M.C. Press, I. Symrnioudis, A.D. Watt, and J.B. Whittaker. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8: 1-16.

Bell, J. R., L. Alderson, D. Izera, T. Kruger, S. Parker, J. Pickup, C. R. Shortall, M. S. Taylor, P. Verrier, and R. Harrington. 2014. Long-term phenological trends, species accumulation rates, aphid traits and climate: five decades of change in migrating aphids. Journal of Animal Ecology 84: 21-34.

Bengston, C., S. Larsson, and C. Liljenberg. 1978. Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. Physiologia Plantarum 44: 319-324.

Blackman, R. L., V.F. Eastop, H.F. van Emden, and R. Harrington. 2007. Aphids as crop pests. CABI, Wallingford, England.

Both, C., M. Van Asch, R.G. Bijlsma, A.B. Burg Van Den, and M.E. Visser. 2009. Climate change and unequal phonological changes across four trophic levels: constraints or adaptations? Journal of Animal Ecolology 78: 73-83.

Boyer, J.S. 1982. Plant productivity and environment. Science 218: 443-448.

Bosque-Pérez, N.A., J.B. Johnson, D.J. Schotzko, and L. Unger. 2002. Species diversity, abundance, and phenology of aphid natural enemies on spring wheats resistant and susceptible to Russian wheat aphid. Biocontrol 47: 667-684.

Brodbeck, B., and D. Strong. 1987. Amino acid nutrition of herbivorous insects and stress to host plants, pp. 347-364. *In* P. Barbosa and J. Schultz (eds.), Insect outbreak: ecological and evolutionary perspectives. Academic Press, New York, NY.

Cammell, M.E., J.D. Knight. 1992. Effects of climatic change on the population dynamics of crop pests. Advances in Ecological Research 22: 117-162.

Cannon, R.J.C. 1998. The implications of predicated climate change for insect pests in the UK, with emphasis on non-indigenous species. Global Change Biology 4: 785-796.

Castro, T.R., R.F. Ruppel, and M.S. Gomulinski. 1965. Natural history of the cereal leaf beetle in Michigan. Michigan Agricultural Experiment Station Quarterly Bulletin 47: 623-653.

Challinor, A. J., J. Watson, D. B. Lobell, S. M. Howden, D. R. Smith, and N. Chhetri. 2014. A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change 4:287-291.

Chaves, M. M., J.S. Pereira, J. Maroco, et al. 2002. How plants cope with water stress in the field? Photosynthesis and growth. Annals of Botany 89: 907-916.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology 30: 239-264.

Changnon, S. A., R.A Pielke Jr, D. Changnon, R.T., Sylves, and R. Pulwarty. 2000. Human Factors Explain the Increased Losses from Weather and Climate Extremes. Bulletin of the American Meteorological Society 81:437-442.

Clark, J.A., and J. Levitt. 1956. The basis of drought resistance in the soyabean plant. Physiologia Plantarum 9: 598-606.

Cook, E. R., R. Seager, M.A. Cane, and D.W. Stahle. 2007. North American drought: reconstructions, causes, and consequences. Earth-Science Reviews 81:93-134.

Dai, A. 2011. Drought under global warming: a review. Wiley Interdisciplinary Reviews: Climate Change 2: 45-65.

Dai, A. 2013. Increasing drought under global warming in observations and models. Nature Climate Change 3: 52-58.

Dale, D. 1988. Plant-mediated effects of soil mineral stresses on insects, pp. 35-110. *In* E.A. Heinrichs (ed.), Plant stress-insect interactions. Wiley, New York, NY.

Daly, G.T. 1964. Leaf-surface wax in *Poa colensoi*. Journal of Experimental Botany 15: 160-165.

Davis, T. S., J. Abatzoglou, N. A. Bosque-Pérez, S. E. Halbert, K. Pike, and S. D. Eigenbrode. 2014a. Differing contributions of density dependence and climate to the population dynamics of three eruptive herbivores. Ecological Entomology 39:566–577.

Davis, T.S., Y. Wu, and S.D. Eigenbrode. 2014b. Host settling behavior, reproductive performance, and effects on plant growth of an exotic cereal aphid, *Metopolophium festucae* subsp. *cerealium* (Hemiptera: Aphididae). Environmental Entomology 43: 682-688.

Davis, T.S., N.A. Bosque-Pérez, N.E. Foote, T.M. Magney, and S.D. Eigenbrode. 2015. Environmentally dependent host-pathogen and vector-pathogen interactions in the barley yellow dwarf virus pathosystem. Journal of Applied Ecology. 52: 1392-1401.

Drake, B.G., and J.L. Gallagher. 1984. Osmotic potential and turgor maintenance in *Spartina alterniflora* Loisel. Oecologia 62: 368-375.

Dixon, A.F.G. 1973. Biology of aphids. Arnold, London, England.

Eigenbrode, S.D., S.M Capalbo, L.L. Houston, J. Johnson-Maynard, C. Kruger, and B. Olen. 2013. Agriculture impacts, adaptation, and mitigation, pp. 149-180. *In* M. Dalton, P. Mote, and A. Snover (eds.), Climate Change in the Northwest Implications for Our Landscapes, Waters, and Communities. Island Press, Washington, DC.

Eigenbrode, S.D., T. S. Davis, and D. W. Crowder. 2015. Climate change and biological control in agricultural systems: Principles and examples from North America, pp. 119-135. *In* C. Bjorkman and P. Niemela (eds.), Climate Change and Insect Pests. CABI, Wallingford, England.

English-Loeb, G., M.J. Stout, and S.S. Duffey. 1997. Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. Oikos 79: 456-468.

Ericksen, P.J., J.S.I. Ingram, D.M., Liverman. 2009. Food security and global environmental change: emerging challenges. Environmental Science and Policy 12: 373-377.

Estay, S.A., M. Lima, and F.A. Labra. 2009. Predicting insect pest status under climat change scenarios: combining experimental data and population dynamics modelling. Journal of Applied Entomology 133: 491-499.

Evans, L.T. 1998. Feeding the ten billion: plants and populations growth. Cambridge University Press, Cambridge, England.

Evans, E. W., N. R. Carlile, M. B. Innes, and N. Pitigala. 2012. Warm springs reduce parasitism of the cereal leaf beetle through phenological mismatch. Journal of Applied Entomology 137: 321-400.

Finlay, K.J., and J.E. Luck. 2011. Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop-vector-virus pathosystem. Agriculture, Ecosystems and Environment 144: 405-421.

Fleury, D., S. Jefferies, H. Kuchel, and P.Langridge. 2010. Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61: 3211-3222.

Gianoli, E. 2000. Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. Environmental Entomology 29: 213-219.

Gonzales, W.L., E. Fuentes-Contreras, and H.M. Niemeyer. 2002. Host plant and natural enemy impact on cereal aphids competition in a seasonal environment. Oikos 96: 481-491.

Halbert, S.E., and L.E. Sandvol. 1995. New or unusual pests in Idaho in 1994, p. 71. *In* Research Reports: 54th Annual Northwest Insect Management Conference, January 1995, Portland, OR. Oregon State University, Corvallis, OR.

Halbert, S.E., Y. Wu, and S.D. Eigenbrode. 2013. *Metopolophium festucae cerealium* (Hemiptera: Aphididae), a new addition to the aphid fauna of North America. Insecta Mundi 301: 295-302.

Hale, B.K., J.S. Bale, J. Pritchard, et al. 2003. Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. Ecological Entomology 28: 666-677.

Harrington, R., I. Woiwood, and T.C. Sparks. 1999. Climate change and trophic interactions. Trends in Ecology and Evolution. 14: 146-150.

Haynes, D.L., and S.H. Gage. 1981. The cereal leaf beetle in North America. Annual Review of Entomology. 26: 259-287.

Hill, D.S. 1997. The Economic Importance of Aphids. Chapman and Hall, London.

Howden, S.M., J. Soussana, F.N. Tubiello, N. Chhetri, M. Dunlop, and H. Meinke. 2007. Adapting agriculture to climate change. Proceedings of the National Academy of Sciences of the United States of America. 104: 19691-19696.

Holtzer, T.O., T.L. Archer, and J.M. Norman. 1988. Host plant suitability in relation to water stress. Pages 111-137 *in* E.A. Heinrichs, editor. Plant stress-insect interactions. John Wiley and Sons, New York, New York, USA.

Hsiao, T.C. 1973. Plant responses to water stress. Annual Review of Plant Physiology. 24: 519-570.

Huberty, A.F., and R.F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85: 1383-1398.

Hunter, M.D., P.W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of botto-up and top-down forces in natural communities. Ecology 73: 724-732.

Inbar, M., A. Eshel, and D. Wool. 1995. Interspecific competition among phloem-feeding insects mediated by induced host-plant sinks. Ecology 76: 1506-1515.

Kennedy, J.S., K.P. Lamb, and C O. Booth. 1958. Responses of *Aphis fabae* Scop. to water shortage in host plants in pots. Entomologia Experimentalis et Applicata 1: 274–291.

Larsson, S. 1989. Stressful times for the plant stress: insect performance hypothesis. Oikos 56: 277-283.

Lehner, B., P. Döll, J. Alcamo, T. Henricks, and F. Kaspar. 2006. Estimating the impact of global change on flood and drought risks in Europe: a continental, integrated analysis. Climatic Change 75: 273-299.

Mattson Jr, W. J. 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics: 119-161.

Mattson, W.J., and R.A. Haack. 1987a. The role of drought in outbreaks of plant-eating insects. BioScience 37: 110-118.

Mattson, W.J., and R.A. Haack. 1987b. The role of drought stress in provoking outbreaks of phytophagous insects, pp. 365-407. *In* P. Barbosa and J. Schultz (eds.), Insect outbreak: ecological and evolutionary perspectives. Academic Press, New York, NY.

Milly, P.C.D., R.T. Wetherald, K.A. Dunne, and T.L. Delworth. 2002. Increasing risk of great floods in a changing climate. Nature 415: 514-517.

Mishra, A. K. and V.P Singh. 2010. A review of drought concepts. Journal of Hydrology 391: 202-216.

Mote, P., E. Salathé, and C. Peacock. 2005. Scenarios of future climate for the Pacific Northwest. Report prepared for King County, WA by the Climate Impacts Group, University of Washington, Seattle, WA.

NAPIS. 2003. First reported occurrence of cereal leaf beetle, *Oulema melanopus*. National Agricultural Pest Information System, Cooperative Agriculture Pest Survey. http://www.ceris.purdue.edu/napis/pests/clb/imap/clbtime.html.

Newman, J.A. 2005. Climate change and the fate of cereal aphids in Southern Britain. Global Change Biology 11: 940-944.

Olfert, O., R.M. Weiss, S. Woods, H. Philip, and L. Dosdall. 2004. Potential distribution and relative abundance of an invasive cerel crop pest, *Oulema melanopus* (Coleoptera: Chrysomelidae), in Canada. Canadian Entomologist. 136: 277-287.

Olfert, O., and R.M. Weiss. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus*, *Meligethes viridescens*, and *Ceutorhynchus obstrictus* in Canada. Agriculture, Ecosystems & Environment. 113 : 295-301.

Palmer, W.C. 1968. Keeping track of crop moisture conditions, nationwide : the new crop moisture index. Weatherwise 21 : 156-161.

Parry, M.L., C. Rosenzweig, A. Iglesias, M. Livermore, and G. Fischer. 2004. Effects of climate change on global food production under SRES emissions and socioeconomic scenarios. Global Environmental Change 14 : 53-67.

Petit, J.R, J. Jouzel, D. Raynaud, et al. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399: 429-436.

Plumb, R. 1983. Barley yellow dwarf virus—a global problem. *In* Plumb, R., Thresh, J. (Eds.), Plant Virus Epidemiology. Blackwell Scientific Publications, Oxford, England, pp. 185-194.

Pons, X. and G.M. Tatchell. 1995. Drought stress and cereal aphid performance. Annals of Applied Biology 126: 19-31.

Porter, J.H, M.L. Parry, T.R. Carter. 1991. The potential effects of climatic-change on agricultural insect pests. Agriculture and Forest Meteorology 57: 221-240.

Qureshi J.A., and J.P. Michaud. 2005. Interactions among three species of cereal aphids simultaneously infesting wheat. Journal of Insect Science 5: 1-8.

Raupp, M.J. 1985. Effects of leaf toughness on manibular wear of the leaf beetle, *Plagiodera versicolora.* Ecological Entomology. 10: 73-79.

Rochow, W., 1970. Barley yellow dwarf virus. Descriptions of Plant Viruses No. 4, Vol. 32. CMI/AAB, Kew, Surrey, United Kingdom.

Rodriguez, J.G., and L.D. Rodriguez. 1987. Nutrtional ecology of phytophagous mites, pp. 177-208. *In* F. Slanksy and J.G. Rodriguez (eds.), Nutritional ecology of insects, mites, spiders, and related invertebrates. Wiley, New York, NY.

Rosenzweig, C., and M.L. Parry. 1994. Potential impact of climate change on world food supply. Nature 367: 133-138.

Rosenzweig, C., A. Iglesius, X.B. Yang, P.R. Epstein, and E. Chivian. 2001. Climate change and extreme weather events—implications for food production, plant diseases, and pests. Global Change and Human Health 2: 90-104.

Sandstrom, J., A. Telang, and N.A. Moran. 2000. Nutrtional enhancement of host plants by aphids—a comparison of three aphid species on grasses. Journal of Insect Physiology 46: 33-40.

Sadeghi, S.E., J. Bjur, L. Ingwell, L. Unger, N.A. Bosque-Pérez, and S.D. Eigenbrode. 2016. Interactions between *Metopolophium festucae cerealium* (Hemiptera: Aphididae) and Barley yellow dwarf virus (BYDV-PAV). Journal of Insect Science 16: 1-6.

Seneviratne, S.I., D. Luthi, M. Litschi, and C. Schar. 2006. Land-atmosphere coupling and climate change in Europe. Nature 443: 205-209.

Skoss, J.D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. Botanical Gazette 117: 55-72.

Schillinger, W.F., R.I. Papendick, S.O. Guy, P.E. Rasmussen, and C. Van Kessel. 2006. Dryland cropping in the western United States, pp. 365-393. *In* G. Peterson et al. (eds.), Dryland Agriculture 2nd ed. Agron. Monogr. 23. ASA, CSSA, and SSSA, Madison, WI.

Schillinger, W.F., and R.I. Papendick. 2008. Then and now: 125 years of dryland wheat farming in the Iland Pacific Northwest. Agronomy Journal 100 Supplement: S-166.

Schotzko, D.J., and N.A. Bosque-Pérez, N. A. 2000. Seasonal dynamics of cereal aphids on Russian wheat aphid (Homoptera: Aphididae) susceptible and resistant wheats. Journal of Economic Entomology 93: 975-981

Scriber, J.M. 1984. Insect/plant interactions-host plant suitability, pp. 159-202. *In* W. Bell and R. Cardé (eds.), The chemical ecology of insects. Chapman and Hall, London, England.

Steudle, E. 2000. Water uptake by roots: effects of water deficit. Journal of Experimental Botany 51: 1531-1542.

Stöckle, 2014. End-of-the-century climate and Pacific Northwest wheat production, pp. 58-59. *In* Regional Approaches to Climate Change for Pacific Northwest Agriculture Annual Report Year 3.

Sutherst, R.W. 2000. Climate change and invasive species: a conceptual framework, pp. 211-240. *In* H.A. Mooney and R.J. Hobbs (eds.), Invasive species in a changing world. Island Press, Washington, D.C.

Taiz, L., and E. Zeiger. 2002. Plant Physiology, 3rd ed. Sinauer, Sunderland, Massachusetts, USA.

Tilman, D., C. Balzer, J. Hill, and B.L. Befort. 2011. Global food production and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences of the United States of America. 108: 20260-20264.

Tubiello, F.N., J. Soussana, and S.M. Howden. 2007. Crop and pasture response to climate change. Proceedings of the National Academy of Sciences of the United States of America. 104: 19686-19690.

(USDA) United States Department of Agriculture-NASS. 2015. Agricultural statistics data base (quick stats). (https://quickstats.nass.usda.gov).

Walden, V. 2014. Weather, climate, and agriculture, pp. 54-55. *In* Regional Approaches to Climate Change for Pacific Northwest Agriculture Annual Report Year 3.

Wang, G. 2005. Agricultural drought in a future climate: results from 15 global climate models participating in the IPCC 4th assessment. Climate Dynamics 25: 739-753.

Wearing, C.H., and H.F. van Emden. 1967. Sutdies on the relations of insect and host plant. I. Effects of water stress in host plants on infestation by *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). Nature 203: 1051-1052.

Wellso, S.G., W.G. Ruesink, and S.H. Gage. 1975. Cereal leaf beetle: relationships between feeding, oviposition, mating, and age. Annals of the Entomological Society of America. 68: 663-668.

White, T.C.R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology 50: 905-909.

Wilhite, D. A., M.H Glantz. 1985. Understanding the drought phenomenon: the role of definitions. Water International 10: 111-120.

Wilhite, D.A., M.J. Hayes. 1998. Drought planning in the Unites States: status and future directions, pp. 33-54. *In* H.J. Bruins and H. Lithwick (eds.), The Arid Frontier. Kluwer, Dordrecht, The Netherlands.

Zou, J., and R.G. Cates. 1994. Role of douglas fir (*Pseudotsuga menziesii*) carbohydrates in resistance to budworm (*Choristoneura occidentalis*). Journal of Chemical Ecology 20: 395-405.

CHAPTER TWO—Impacts of water stress on performance of the cereal leaf beetle (*Oulema melanopus)* and growth of the host plant, wheat (*Triticum aestivum*)

Abstract

The cereal leaf beetle (CLB), *Oulema melanopus* is an introduced, widespread pest of small grains causing economic losses to wheat, oats, and barley throughout North America. Native to Europe and Asia, CLB was first identified in the state of Michigan in 1962. It has since established throughout large portions of the United States including the Pacific Northwest, where it has become a major agricultural pest. Climate models indicate environmental suitability for CLB could increase by mid-century, but they do not consider the indirect effects of abiotic stress on the beetle through its host plant, which could affect its relative performance and pest status. We tested the effects of plant water stress on the feeding performance of CLB and growth of its host plant, using a study system in wheat (*Triticum aestivum*) and greenhouse experiments. We found that water stress may adversely affect CLB performance and significantly impacts the growth and seed production of potted wheat. However, there were no significant interactions between water stress and CLB feeding damage on wheat growth or seed production, indicating that the combined negative effects from these two stressors on the host plant are additive. We conclude that future studies are needed to determine the precise effects of water stress on this economically important insect-plant interaction, especially within the context of projected climate scenarios where drought may be a more prevalent condition affecting agriculture in the Pacific Northwest and elsewhere.

Introduction

The cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae, Criocerinae) is an introduced, widespread pest of small grains causing economic losses to wheat, oats, and barley throughout North America (Haynes and Gage 1981, Olfert et al. 2004). Greatest damage to crop plants occurs during feeding by CLB larvae, which remove long strips of leaf tissue, parallel with leaf veins, resulting in reduced photosynthesis and crop yield (Wellso et al. 1975). The beetle is native to Eurasia and was first identified in southwestern Michigan in 1962 (Castro et al. 1965), but surveys indicate that CLB populations have now become established from Alabama north to Ontario, Canada (NAPIS 2003, Olfert et al. 2004). Beetle populations have more recently expanded into northwestern North America from Utah northward into British Columbia, Canada (Olfert et al. 2004). In past decades, approximately 10-20 percent of wheat fields in northern Alabama and Georgia experienced damaging infestations annually (Bryan et al. 1994, Hudson and Buntin 1997). Winter wheat fields in Virginia and North Carolina have recorded average annual yield losses of 21.6% directly attributed to feeding by CLB larvae (Ihrig et al. 2001). More recently in the northwestern United States, reported yield losses in irrigated spring wheat were as high as 25% (Roberts and Walenta 2012).

Continued cereal leaf beetle range expansion and establishment could be attributed to a warming climate (see Sutherst 2000, Bale et al. 2002). Bio-climatic models that incorporate future climate scenarios have been developed to project the potential distribution and abundance of this invasive pest throughout its expanding range in Canada (Olfert et al. 2004, Olfert and Weiss 2006). However, the indirect effects of climate-related stress on the beetle through its predominant host plant wheat (*Triticum aestivum* L.) and potential resulting changes in CLB feeding damage, remain unknown. Research investigating plant responses to water stress has become increasingly relevant as most climate-change scenarios predict an increase in aridity throughout agricultural systems globally (Petit et al. 1999, Chaves et al. 2003), where drought is already the main abiotic constraint on yield in certain staple crops, including cereals (Araus et al. 2002).

Plant physiological responses to water stress are complex and have varying effects on insect herbivores (Mattson and Haack 1987, Larsson 1989, Bale et al. 2002, Chaves et al. 2003, Huberty and Denno 2004). In many instances, drought-stressed plants have relatively higher nutrient levels that are important to insect herbivores (e.g., amounts of total nitrogen, soluble protein, amino acids, and simple sugars) (Bradford and Hsiao 1982, Morgan 1984, White 1984, Mattson and Haack 1987, Dale 1988, English-Loeb et al. 1997). This perceived improvement in host plant quality has led to the traditionally accepted plant-stress hypothesis, which suggests that environmental stress in plants improves the performance of herbivorous insects and affects their population and outbreak dynamics (White 1969, Brodbeck and Strong 1987, Mattson and Haack 1987a, 1987b, Huberty and Denno 2004). But stress-induced changes in the host plant do not necessarily correlate with increased herbivore performance (Scriber 1984, Rodriguez and Rodriguez 1987, Dale 1988, Zou and Cates 1994, English-Loeb et al. 1997), and more recent investigations have uncovered differential responses from different insect feeding guilds to host plant drought stress (Huberty and Denno 2004). Evidence towards this end is mixed and largely inconclusive, especially for free-living, chewing guilds of insect herbivores (Huberty and Denno 2004).

CLB is an introduced, historically disruptive pest. Despite its high-profile pest status, there are no published studies of the effects of host plant water stress on the insect. As a first step to address this gap, controlled-environment greenhouse studies have two advantages. First, in the greenhouse host plant water status can be manipulated and measured precisely (see Turner 1988); second, CLB reared in laboratory cultures can provide specific stages for experiments (e.g., Clement and Elberson 2010). We used greenhouse experiments to determine (1) whether feeding performance of CLB larvae differs on water-stressed versus water-replete host plants (wheat), and (2) whether the combined stresses of water limitation of the host plant and CLB herbivory interact to impact the water status, growth, and seed production of wheat plants. Knowing the potential effects of a climate-related stress on the beetle as mediated by its predominant crop host plant can improve understanding of this economically important pest in a projected warming climate, and help predict future impacts on cereal grain agriculture throughout North America.

Materials and Methods

EXPERIMENT 1. Effects of water stress on cereal leaf beetle performance and host plant growth and water status

Insect cultures. Overwintered CLB adults were collected using a sweep net from adjacent spring and winter wheat fields near Nine Mile Falls, Washington, USA (47° 49’ 09” N, 117° 38’ 03” W) in May, 2013. Beetles were caged in the greenhouse on potted spring wheat cv. ‘Penawawa’ plants sown roughly three weeks prior to infestation and allowed to feed, mate, and oviposit. Eggs were collected from leaf clippings and placed in covered, unsealed Petri dishes supplied with filter paper moistened with deionized (DI) water. Petri dishes were observed daily for hatched CLB.

Plants. Sixty spring wheat plants, cv. ‘JD’ (Washington State University) were sown individually in 100 cm^3 pots containing 111 grams (oven-dried) horticultural mix (Sunshine mix no. 1; SunGro Horticulture, Agawam, MA, USA) in May, 2013. Plants were grown on a single bench in a greenhouse (30°C mean daytime temperature and 14h:10h, L:D photoperiod) at the University of Idaho Manis Laboratory for Entomological Research in Moscow, Idaho, USA.

Watering regimes. Experimental watering treatments were established from preliminary tests conducted in the greenhouse using the same wheat cultivar ‘JD’. Watering regimes were applied on a gravimetric basis (grams H_2O / grams oven-dried soil) and consisted of two treatments: 0.1 and 0.4 g H_2O per g soil, hereafter referred to as ‘LOW’ and ‘HIGH’ water inputs, respectively. Plants were supplied unlimited water for two weeks after sowing, then placed individually in polystyrene bowls where water was withheld entirely for five days in order to eliminate any excess soil moisture. Thereafter, watering treatments began and persisted throughout the course of the experiment. Exact H_2O volumes were measured using a graduated cylinder and delivered to plants on an individual basis via bottom-watering into polystyrene bowls every 48 h.

Insect measurements. Neonate cereal leaf beetle within 24 h of hatching were placed on the third leaves of four-week old wheat plants (1 CLB / plant) using a fine haired brush, approximately 1 cm from the leaf ligule and oriented randomly. Larvae were observed on plants daily and allowed to feed freely through all instars, after which they dropped to the soil and pupated. Using a transparent mm-squared grid overlaid on damaged leaves under a dissecting microscope, the total leaf area consumed by each CLB larva (per plant) was measured in addition to calculating the area removed per leaf. Finally, the total number of leaves fed upon per plant was counted.

Plant measurements. Five weeks post-sowing (i.e., upon completion of CLB larval feeding and pupation), predawn leaf water potentials of plants were measured using the pressure chamber method (see Turner 1988) on flag leaves excised from the stem within 1 cm of the ligule using a razorblade. Measurements of main stem height and fresh aboveground biomass followed leaf water potential sampling. After oven drying at 70 °C for 48 h, dry aboveground biomass was measured for each plant.

Experimental design and statistical analysis. We used a full factorial design to test the hypotheses that plant water stress negatively affects CLB larval feeding and performance, and that combined water stress and CLB feeding interact to negatively affect the host plant. Sixty plants were arranged on a single greenhouse bench and each potted plant represented an experimental unit (n = 15 for all treatment groups). Treatments included two factors (H_2O and CLB) with two levels each (LOW or HIGH H_2O, and with or without CLB) and these were randomly assigned to pots for a completely randomized factorial design. A fixed-effects model two-way analysis of variance (ANOVA) was used to test for significant main effects of H_2O and CLB factors and their interaction on all plant and insect-related response variables. All statistical tests incorporated a type I error rate of $\alpha = 0.05$ and were performed using Minitab 17 software (Minitab Inc.). Plants which initially received a CLB larva that expired or was missing from its plant before pupation or a plant which expired before predawn leaf water potentials were measured (due to water stress), were considered lost samples and their data not included in the statistical analyses. Initially for all treatment groups, n = 15; for plants with CLB, n = 13; for plants without CLB, n = 29.

EXPERIMENT 2: Effects of combined water stress and cereal leaf beetle feeding on growth and seed production of the host plant

Insect cultures. Overwintered CLB adults were collected using a sweep net from the same wheat fields as in experiment 1 (Nine Mile Falls, WA) in May 2014. Beetles were caged in the greenhouse and allowed to feed, mate, and oviposit on potted spring wheat cv. ‘Alturas’ plants sown roughly 3 weeks prior to use in experiments. Eggs were collected from leaf clippings and placed in covered Petri dishes supplied with filter paper moistened with DI water. Petri dishes were observed daily for hatched CLB. On four-week-old plants, neonate larvae hatched within 24 h were placed on the third leaves of wheat plants (1 CLB / plant) using a fine haired brush approximately 1 cm from the ligule and oriented randomly. Larvae were observed on plants daily and allowed to feed freely through all instars, after which they dropped to the soil and pupated.

Plants. Fifty-four plants of spring wheat cv. ‘Kelse’ (Washington State University) were sown individually in 1,180 cm^3 pots containing 330 grams (oven-dried) horticultural mix (Sunshine mix no. 1; SunGro Horticulture, Agawam, MA, USA) in May 2014. In contrast to Experiment 1, ‘Kelse’ was used in place of ‘JD’ to compliment a parallel field experiment. Also, larger soil capacity pots were used to accommodate for the possibility of plants becoming root-bound during the relatively longer growing time required for seeds to mature. Plants were grown in the same greenhouse (30 °C mean daytime temp. and 14h:10h, L:D photoperiod) at the University of Idaho Manis Laboratory for Entomological Research in Moscow, Idaho, USA.

Watering regime. Except for the greater H_2O volumes delivered due to the relatively larger capacity pots, watering regimes were identical to those used in experiment 1. These were applied on a gravimetric basis (grams H_2O / grams oven-dried soil) and consisted of two treatments: 0.1 and 0.8 g H_2O per g soil, hereafter referred to as 'LOW' and 'HIGH' water inputs, respectively. Compared to experiment 1, 0.8 g (versus 0.4 g) H_2O per g soil was used for the 'HIGH' water input to accommodate for anticipated greater water use by plants being grown to maturity for producing seed. Wheat plants were supplied unlimited water for two weeks post-sowing, then placed individually in polystyrene bowls, at which time watering treatments were initiated and maintained for eight weeks. Exact volumes were measured using a graduated cylinder and supplied to plants every 48 h via bottom-watering into the polystyrene bowls.

Plant measurements. After 10 weeks, water was withheld from all plants to allow for ripening of seeds. Three weeks after watering was withheld and kernels loosened, wheat heads from the main stem and all tillers were clipped. Seeds were hand-threshed from wheat heads, counted and weighed. We used a full factorial design to test the hypothesis that combined water stress and CLB feeding interact to negatively affect host plant performance, measured as aboveground growth and seed production. Fifty-four plants were arranged on a single greenhouse bench where each potted plant represented a single experimental unit. Treatments included two factors (H_2O and CLB) with two levels each (LOW or HIGH H_2O, and with or without CLB) and these were assigned randomly to pots for a completely randomized factorial design. Anticipating (from experiment 1 results) high sample loss for treatment groups with CLB, the number of replicates were doubled for plants with CLB (i.e., n = 18 for plants with CLB and n = 9 for plants without CLB). Plant response variables were analyzed with a fixed-effects model two-way analysis of variance (ANOVA) incorporating a type I error rate of $\alpha = 0.05$ and statistical tests were performed using Minitab 17 software (Minitab Inc.). Plants which initially received a CLB larva that expired or was missing from its plant before pupation or a plant which expired before predawn leaf water potentials were measured (due to water stress), were considered lost samples and their data not included in the statistical analyses. (n = 22 for plants with CLB, n = 18 for plants without CLB).

Results

EXPERIMENT 1

Plant responses. Watering input ‘LOW’ vs. ‘HIGH’ had a significant ($p < 0.05$) effect on all measured plant response variables (Table 2.1). Wheat plants receiving ‘LOW’ H_2O were significantly smaller than plants receiving ‘HIGH’ H_2O: main stem height was reduced by 50.1% while fresh and dried aboveground biomass was reduced by 64.8 and 60.8%, respectively. Predawn leaf water potentials of ‘LOW’ H_2O plants were approximately 0.4 MPa lower (Table 2.1), which would be considered by Hale et al. (2003) a ‘moderate’ level of water stress. Cereal leaf beetle feeding (i.e., from a single CLB larva / plant) did not significantly affect main stem height, dry biomass or leaf water potential, but did reduce fresh AB biomass by 17.5% (Table 2.1). No significant interaction effects resulted from the combination of H_2O and CLB factor treatments for any of the measured plant response variables (Table 2.1).

Table 2.1. ANOVA results for plant response variables as measured under H_2O and CLB factor treatments; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
Main stem height					
H_2O	**1**	**505350**	**505350**	**124.26**	**0.000**
CLB	1	2385	2385	0.59	0.449
H_2O x CLB	1	5	5	0.00	0.972
Error	38	154546	4067		
Total	41	752296			
Fresh aboveground biomass					
H_2O	**1**	**44.3723**	**44.3723**	**231.32**	**0.000**
CLB	**1**	**1.2344**	**1.2344**	**6.44**	**0.015**
H_2O x CLB	1	0.0013	0.0013	0.01	0.934
Error	38	7.2891	0.1918		
Total	41	60.6533			
Dried aboveground biomass					
H_2O	**1**	**3.44870**	**3.44870**	**220.62**	**0.000**
CLB	1	0.02140	0.02140	1.37	0.250
H_2O x CLB	1	0.00003	0.00003	0.00	0.964
Error	36	0.56275	0.01563		
Total	39	4.52458			
Predawn leaf water potential					
H_2O	**1**	**1.43217**	**1.43217**	**15.17**	**0.000**
CLB	1	0.12797	0.12797	1.36	0.252
H_2O x CLB	1	0.00120	0.00120	0.01	0.911
Error	38	3.58708	0.09440		
Total	41	5.37859			

Table 2.2. Comparison of treatment level effects for each factor on plant response variables; significant (p < 0.05) treatment effects are highlighted in bold text.

Variable	Treatment		n	Mean ± SE	% decrease
	Factor	Level			
Main stem height (mm)	Watering input	HIGH	20	476.3 ± 15.2	**50.1**
		LOW	22	237.9 ± 12.2	
Fresh aboveground biomass (g)		HIGH	20	3.44 ± 0.13	**64.8**
		LOW	22	1.21 ± 0.07	
Dried aboveground biomass (g)		HIGH	19	1.02 ± 0.04	**60.8**
		LOW	21	0.40 ± 0.01	
Predawn leaf water potential (MPa)		HIGH	20	-0.26 ± 0.03	**153.8**
		LOW	22	-0.66 ± 0.09	
Main stem height (mm)	Cereal leaf beetle	NO	29	358.0 ± 25.4	5.9
		YES	13	336.7 ± 37.8	
Fresh aboveground biomass (g)		NO	29	2.40 ± 0.22	**17.5**
		YES	13	1.98 ± 0.34	
Dried aboveground biomass (g)		NO	27	0.71 ± 0.07	8.5
		YES	13	0.65 ± 0.10	
Predawn leaf water potential (MPa)		NO	29	-0.43 ± 0.06	30.2
		YES	13	-0.56 ± 0.13	

Insect responses. Watering input did not significantly affect any of the measured insect response variables. These results should be considered, however, with the sample loss that occurred in both 'LOW' and 'HIGH' H_2O treatment groups (initially, n = 15 for all treatment groups; Table 2.4).

Table 2.3. ANOVA results for insect-related response variables response as measured under H_2O factor treatments.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		Leaf area consumed			
H_2O	1	25536	25536	0.19	0.670
Error	11	1474331	134030		
Total	12	1499867			
		Area removed per leaf			
H_2O	1	23088	23088	0.85	0.377
Error	11	299858	27260		
Total	12	322946			
		Number of leaves fed upon			
H_2O	1	1.875	1.875	1.37	0.266
Error	11	15.048	1.368		
Total	12	16.923			

Table 2.4. Comparison of H_2O treatment level effects for each factor on insect-related response variables.

H_2O	n	Mean	± SE	df	F value	p > F	% decrease
			Leaf area consumed				
HIGH	6	820	± 214	11	0.41	0.702	10.9
LOW	7	731	± 49				
			Area removed per leaf				
HIGH	6	310	± 96	11	0.92	0.377	27.4
LOW	7	225	± 22				
			Number of leaves fed upon				
HIGH	6	2.67	± 0.56	11	-1.17	0.266	-28.5
LOW	7	3.43	± 0.37				

EXPERIMENT 2

Watering input (‘LOW’ or ‘HIGH’) had a significant ($p < 0.05$) effect on all measured plant response variables (Table 2.4). Dried aboveground biomass of wheat plants was 67% lower for plants receiving ‘LOW’ H_2O compared to those receiving ‘HIGH’ H_2O (Table 2.5). Seed production (total numbers of seeds per plant) and plant yield (total grams of seed per pot) were reduced by 76 and 71%, respectively, for plants receiving ‘LOW’ H_2O compared to those receiving ‘HIGH’ H_2O (Table 2.5). Cereal leaf beetle feeding (i.e., from a single CLB larva / plant) resulted in a significant ($p = 0.005$) reduction in plant dried AG biomass of 8.4%, but did not significantly affect seed count or plant yield (Table 2.4, 2.5). No significant interaction effects resulted from the combination of H_2O and CLB treatments for any of the measured plant response variables (Table 2.4).

Table 2.4. ANOVA results for plant response variables as measured under H_2O and CLB treatments; significant ($p < 0.05$) treatment effects highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		Dried aboveground biomass			
H_2O	**1**	**235.684**	**235.684**	**1171.86**	**0.000**
CLB	**1**	**1.764**	**1.764**	**8.77**	**0.005**
H_2O x CLB	1	0.141	0.141	0.70	0.408
Error	40	8.045	0.201		
Total	43	251.532			
		Seed production			
H_2O	**1**	**94128**	**94128.2**	**201.24**	**0.000**
CLB	1	1498	1498.0	3.20	0.081
H_2O x CLB	1	890	890.4	1.90	0.176
Error	40	18729	468.2		
Total	43	115051			
		Plant yield			
H_2O	**1**	**52.8490**	**52.8490**	**180.74**	**0.000**
CLB	1	0.8877	0.8877	3.04	0.089
H_2O x CLB	1	0.2717	0.2717	0.93	0.341
Error	40	11.6961	0.2924		
Total	43	66.0955			

Table 2.5. Comparison of treatment level effects for each factor on plant response variables; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Variable	Treatment		n	Mean ± SE	% decrease
	Factor	Level			
Dried aboveground biomass (g)	Watering input	HIGH	22	7.01 ± 0.13	**66.8**
		LOW	22	2.33 ± 0.07	
Seed production (# / plant)		HIGH	22	121 ± 6	**76.0**
		LOW	22	29 ± 2	
Plant yield (g / pot)		HIGH	22	3.07 ± 0.15	**71.0**
		LOW	22	0.87 ± 0.07	
Dried aboveground biomass (g)	Cereal leaf beetle	NO	18	4.91 ± 0.59	**8.4**
		YES	26	4.50 ± 0.47	
Seed production (# / plant)		NO	18	82 ± 14	14.6
		YES	26	70 ± 9	
Plant yield (g / pot)		NO	18	2.14 ± 0.31	13.6
		YES	26	1.85 ± 0.23	

Discussion

We examined interactions between the cereal leaf beetle and wheat to determine the effects of plant water stress on this introduced, historically disruptive pest and its predominant host plant in the Pacific Northwest. We designed factorial experiments to determine the direct effects of water stress on beetle feeding performance, and tested whether water stress interacted with beetle feeding damage to impact the growth and seed production of potted wheat plants. Water stress resulted in significantly smaller plants with lower leaf water potential, in addition to decreased seed production and plant yield. Beetle feeding damage significantly reduced plant aboveground biomass, but did not affect plant seed production or yield. Furthermore, we observed no significant interactions between water stress and CLB feeding damage, suggesting that the effects from both stresses combined are additive. However, high experimental error largely due to sample losses warrants further investigation of CLB responses to drought stress and should consider this challenge in future studies.

While drought-induced water stress in plants has traditionally been thought to benefit most insect pests (Larsson 1989, Huberty and Denno 2004), our findings suggest that host plant water stress has a neutral effect on this economically important herbivore. Furthermore, our results show no interaction between water stress and insect feeding activity on the host plant, indicating that the combined impacts from water stress and CLB herbivory on wheat are additive. However, plant physical characteristics can play a role in determining host plant utilization by insects (Hoffman and Rao 2011), which may change as the result of physiological adjustments to water stress (Chaves et al. 2003, Bowne et al.

2012). In cereals including wheat, water stress initially results in stomatal closure and osmotic adjustment, followed by wilting and reduced cell growth (Steudle 2000). Additionally, cuticular resistance increases as leaf water content decreases (Bengtson et al. 1978), and during prolonged periods of water stress the deposition of epicuticular wax in increased (Skoss 1955, Clark and Levitt 1956, Daly 1964). These water stress-induced changes to the anatomical features of plant tissue can result in physically tougher leaves, which is a primary deterrent of herbivore attack (Raupp 1985). In our study, water stress resulted in smaller plants with lower leaf water content. Despite our finding of a neutral effect of water stress on CLB, the nonsignificant trends in our study (which we consider in light of the high sample losses encountered) indicate a potentially negative effect of water stress on CLB larval feeding performance. The possibility that severe drought conditions in the field might reduce the overall quality of wheat plants for CLB feeding, possibly as the result of increased toughness of foliar tissues on water-stressed plants, should therefore be considered.

A drawback of our study design, which potentially influenced larval feeding behavior, was our transfer of neonate CLB onto predetermined leaves of experimental plants. While this was a methodological requirement for our experiments and has been used in similar studies (e.g., Clement and Elberson 2010), it did not allow for oviposition and egg hatching directly on leaves of experimental plants, and eliminated the opportunity for adult CLB females to choose oviposition sites, a demonstrated behavior which is affected by plant and leaf age, as well as tissue toughness (Hoffman and Rao 2011). Furthermore, CLB adult and egg densities are typically higher in later-planted fields and decline in fields planted earlier in the season (Casagrande et al. 1977, Hoffman and Rao 2010). Due to the time required for our experimental watering regimes to take effect (i.e., producing symptoms of plant water stress), our study used wheat aged four weeks at the time of beetle introductions and were at the stem elongation or booting stage. In this sense, phenological mismatch between the herbivore and its host plant may have been sufficient to influence larval feeding activity.

The use of single potted plants in both experiments also potentially affected our results. Hoffman and Rao (2011) demonstrated using oat plants that adult CLB females prefer older leaves lower on the stem for oviposition, which corresponds with many field observations in other cereals (Gage 1974, Casagrande et al. 1977, Hoffman and Rao 2010). However, late-instar larvae preferentially feed on the upper leaves of wheat, including the flag leaf (Ihrig et al. 2001). Together, these findings indicate that CLB are capable of accessing both lower and upper leaves of their host plant, as both adults and as larvae. In addition, cereals growing in a field can provide larvae access to multiple host plants with interlocking plant crowns. Considering that spatial heterogeneity lends to higher forage resource quality for herbivores (Viswanathan and Thaler 2004), the ability to utilize multiple plants as larvae could have consequences for their performance, especially under conditions of drought-induced water stress. This could be addressed in future studies assessing performance of CLB larvae through the use of garden or greenhouse mesocosm experiments, which would more closely resemble plant growing conditions found in cereal fields.

Findings from this study have potential implications for cereal leaf beetle management in cropping systems in the Pacific Northwest and elsewhere. Our results show that the combined effects of water stress and insect feeding damage on the growth and seed production of potted wheat plants are additive rather than interactive. These findings are important considering that few studies have quantified economic damage from CLB infestations in the Pacific Northwest where CLB is a relatively recently introduced pest, that further incorporate an abiotic stress. The ecological scope of this study could be broadened further through field experiments incorporating other critical factors missing from our greenhouse experiments, from biotic components (e.g., natural enemies) to other climatic stresses associated with drought (e.g., elevated temperature). In addition, continued modeling efforts incorporating field survey and experimental data could advance management of CLB greatly. Further investigations to these ends could help advance our understanding of the complex interactions between this insect pest and the environment under projected climate scenarios, where drought may be a more prevalent condition affecting agricultural systems in the Pacific Northwest and elsewhere in North America.

References

Araus, J.L, G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: what should we breed for? Annals of Botany 89: 925-940.

Bale, J.S., G.J. Masters, I.D. Hodkinson, et al. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8: 1-16.

Bengston, C., S. Larsson, and C. Liljenberg. 1978. Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. Physiologia Plantarum 44: 319-324.

Bowne, J.B., T.A. Erwin, J. Juttner, et al. Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. Molecular Plant 5: 418-429.

Bradford, K.J., and T.C. Hsiao. 1982. Physioloical responses to moderate water stress, pp. 263-324. *In* O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler (eds.), Physiological plant ecology II. Water relations and carbon assimilation. Springer-Verlag, Berlin, Germany.

Brodbeck, B., and D. Strong. 1987. Amino acid nutrition of herbivorous insects and stress to host plants, pp. 347-364. *In* P. Barbosa and J. Schultz (eds.), Insect outbreak: ecological and evolutionary perspectives. Academic Press, New York, NY.

Bryan, M., R.A. Berry, C.J. Copeland, and G.S. Sautter. 1994. Cereal leaf beetle biological control project, FY 1993 Project report, United States Department of Agriculture. APHIS Niles Biological Control Laboratory, Niles, MI.

Castro, T.R., R.F. Ruppel, and M.S. Gomulinski. 1965. Natural history of the cereal leaf beetle in Michigan. Michigan Agricultural Experiment Station Quarterly Bulletin 47: 623-653.

Casagrande, R.A., W.G. Ruesink, and D.L. Haynes. 1977. The behavior and survival of adult cereal leaf beetles. Annals of the Entomological Soceity of America 70: 19-30.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology 30: 239-264.

Clark, J.A., and J. Levitt. 1956. The basis of drought resistance in the soyabean plant. Physiologia Plantarum 9: 598-606.

Clement, S.L., and L.R. Elberson. 2010. Variable effects of grass-*Neotyphodium* associations on cereal leaf beetle (Coleoptera: Chrysomelidae) feeding, development and survival. Journal of Entomological Science 45: 197-203.

Dale, D. 1988. Plant-mediated effects of soil mineral stresses on insects, pp. 35-110. *In* E.A. Heinrichs (ed.), Plant stress-insect interactions. Wiley, New York, NY.

Daly, G.T. 1964. Leaf-surface wax in *Poa colensoi*. Journal of Experimental Botany 15: 160-165.

English-Loeb, G., M.J. Stout, and S.S. Duffey. 1997. Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. Oikos 79: 456-468.

Gage, H.L. 1974. Ecological investigations on the cereal leaf beetle, *Oulema melanopus* (L.), and the principal larval parasite *Tetrastichus julis* (Walker). PhD Dissertation, Michigan State University, East Lansing, MI.

Hale, B.K., J.S. Bale, J. Pritchard, et al. 2003. Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. Ecological Entomology 28: 666-677.

Haynes, D.L., and S.H. Gage. 1981. The cereal leaf beetle in North America. Annual Review of Entomology 26: 259-287.

Hoffman, G.H., and S. Rao. 2010. Cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), attraction to oat plantings of difference ages. Journal of the Entomological Society of British Columbia 107: 57-66.

Hoffman, G.H., and S. Rao. 2011. Oviposition site selection on oat: the effect of plant architecture, plant and leaf age, tissue toughness, and hardness on cereal leaf beetle, *Oulema melanopus*. Entomologia Experimentalis et Applicata 141: 232-244.

Huberty, A.F., and R.F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85: 1383-1398.

Hudson, R.D., and G.D. Buntin. 1997. Small grain insects, p. 37. *In* R.M. McPherson and G.K. Douce (eds.), Summary of losses from insect damage and cost of control in GA, 1996. University of Georgia CAES Special Publication 91, Athens, GA.

Ihrig, R.A., D.A. Herbert, Jr., J.W. Van Duyn, and J.R. Bradley, Jr. 2001. Relationship between cereal leaf beetle (Coleoptera: Chrysomelidae) egg and fourth-instar populations and impact of fourth-instar defoliation of winter wheat yields in North Carolina and Virginia. Journal of Economic Entomology 94: 634-639.

Larsson, S. 1989. Stressful times for the plant stress: insect performance hypothesis. Oikos 56: 277-283.

Mattson, W.J., and R.A. Haack. 1987a. The role of drought in outbreaks of plant-eating insects. BioScience 37: 110-118.

Mattson, W.J., and R.A. Haack. 1987b. The role of drought stress in provoking outbreaks of phytophagous insects, pp. 365-407. *In* P. Barbosa and J. Schultz (eds.), Insect outbreak: ecological and evolutionary perspectives. Academic Press, New York, NY.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology 35: 299-319.

NAPIS. 2003. First reported occurrence of cereal leaf beetle, *Oulema melanopus*. National Agricultural Pest Information System, Cooperative Agriculture Pest Survey. http://www.ceris.purdue.edu/napis/pests/clb/imap/clbtime.html.

Olfert, O., R.M. Weiss, S. Woods, H. Philip, and L. Dosdall. 2004. Potential distribution and relative abundance of an invasive cerel crop pest, *Oulema melanopus* (Coleoptera: Chrysomelidae), in Canada. Canadian Entomologist 136: 277-287.

Olfert, O., and R.M. Weiss. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus*, *Meligethes viridescens*, and *Ceutorhynchus obstrictus* in Canada. Agriculture, Ecosystems & Environment 113 : 295-301.

Petit, J.R., J. Jouzel, D. Raynaud, et al. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399: 429-436.

Raupp, M.J. 1985. Effects of leaf toughness on manibular wear of the leaf beetle, *Plagiodera versicolora.* Ecological Entomology 10: 73-79.

Roberts, D., and D.L. Walenta. 2012. Integrated pest management (IPM) for the cereal leaf beetle in Washing State. Washington State University Extension.

Rodriguez, J.G., and L.D. Rodriguez. 1987. Nutrtional ecology of phytophagous mites, pp. 177-208. *In* F. Slanksy and J.G. Rodriguez (eds.), Nutritional ecology of insects, mites, spiders, and related invertebrates. Wiley, New York, NY.

Scriber, J.M. 1984. Insect/plant interactions-host plant suitability, pp. 159-202. *In* W. Bell and R. Cardé (eds.), The chemical ecology of insects. Chapman and Hall, London, England.

Skoss, J.D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. Botanical Gazette 117: 55-72.

Steudle, E. 2000. Water uptake by roots: effects of water deficit. Journal of Experimental Botany 51: 1531-1542.

Sutherst, R.W. 2000. Climate change and invasive species: a conceptual framework, pp. 211-240. *In* H.A. Mooney and R.J. Hobbs (eds.), Invasive species in a changing world. Island Press, Washington, D.C.

Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. Irrigation Science 9: 289-308.

Viswanathan, D.V., and J.S. Thaler. 2004. Plant vascular architecture and within-plant spatial patterns in resource quality following herbivory. Journal of Chemical Ecology30: 531-543.

Wellso, S.G., W.G. Ruesink, and S.H. Gage. 1975. Cereal leaf beetle: relationships between feeding, oviposition, mating, and age. Annals of the Entomological Society of America 68: 663-668.

White, T.C.R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology 50: 905-909.

White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90-105.

Zou, J., and R.G. Cates. 1994. Role of douglas fir (*Pseudotsuga menziesii*) carbohydrates in resistance to budworm (*Choristoneura occidentalis*). Journal of Chemical Ecology20: 395-405.

CHAPTER THREE—Interspecific interactions between the aphids *Metopolophium festucae cerealium* and *Rhopalosiphum padi* mediated by host plant water stress

Abstract

The aphid *Metopolophium festucae* subsp. *cerealium* is a recently established species in the Pacific Northwestern USA, having the potential to emerge there as a major pest of cereal crops. Also present in the region is the naturalized bird cherry-oat aphid (*Rhopalosiphum padi*), which shares the same graminaceous host plants. Nothing is known of how interactions between these two species may affect their relative performance and pest status, especially in combination with certain climate-related factors that impact the insect herbivore community as a whole. We tested the hypothesis that plant water stress mediates interactions between *M. festucae cerealium* and *R. padi*, using a study system in wheat (*Triticum aestivum*) and greenhouse experiments. Experimental watering regimes, which provisioned either ample or deficient water supplies at regular intervals, produced healthy and chronically water-stressed potted wheat plants, respectively. An approximate -0.7 MPa water stress resulted in significantly smaller wheat plants, and negatively affected *R. padi* but not *M. festucae cerealium* on host plants colonized by only one or the other species. However, *M. festucae cerealium* was negatively affected on plants simultaneously co-colonized by *R. padi*, and an interaction occurred whereby this effect was significantly greater on the water-stressed plants. Additionally, we found that higher reproduction rates occurred for *R. padi* feeding in close proximity to, or on leaves previously infested by *M. festucae cerealium* on wheat plants that were provided an ample water supply. Our findings suggest that interactions between these two economically important aphid species

are ecologically complex, producing asymmetrical competitive outcomes which can depend greatly on the environmental context under which these interactions take place. We discuss implications for pest management in cereal production systems under projected climate scenarios, where drought stress may be a more prevalent condition affecting agriculture in the Pacific Northwest and elsewhere.

Introduction

Climate change is expected to produce future environments with greater frequencies of extreme events, including drought (Chaves et al. 2003, Dai 2013). This potentially threatens productivity of agricultural systems globally where drought is already the main abiotic constraint on yield in certain staple crops, including cereals (Araus et al. 2002). The impacts of drought on the cropping system result from direct effects on the plant and indirect effects on other trophic levels, including insect pests. While the direct effects of drought stress on crop plants have been documented extensively (e.g., Hsiao 1973), the indirect effects on insect herbivores remain less well understood (Hale et al. 2003, Huberty and Denno 2004).

Aphids (Hemiptera: Aphididae) are considered among the most important insect pests affecting agriculture globally (Hill 1997, Newman 2005), with the greatest number of species occurring in temperate regions (Dixon 1973). Aphids feed exclusively on phloem sap, which they extract from plant tissues using specialized stylet-like mouthparts (Dixon 1973). Successful feeding and nutrient uptake by aphids require adequate plant cell turgor pressure (Wearing and van Emden 1967, Holtzer et al. 1988, Archer et al. 1995), which is directly related to plant water content (Hsiao 1973, Drake and Gallagher 1984, Taiz and Zeiger 2002). Consequently, drought-stressed plants generally are poor hosts for aphids by adversely affecting their performance (Kennedy et al. 1958, Huberty and Denno 2004). But the effects of drought stress on aphids is variable and dependent on the exact form of drought stress (Pons and Tatchell 1995, Huberty and Denno 2004), the host plant (Hale et al. 2003) and aphid species involved (Pons and Tatchell 1995). Indirect effects of drought stress on aphids can be further complicated when multiple aphid species co-occur on the same host, each responding uniquely to drought stress in the host (Pons and Tatchell 1995, Hale et al. 2003) and to the presence of heterospecifics (Inbar et al. 1995, Gianoli 2000, Sandstrom et al. 2000, Gonzales et al. 2002).

The net effects of interactions on individuals and populations of different aphid species resulting from drought stress can be negative, neutral or positive (Mitchell and Arthur 1996). These effects may also range in magnitude (Chamberlain et al. 2014) and have varying degrees of symmetry (e.g., mutualism versus commensalism; Denno et al. 1995). Additionally, changes in the abiotic setting may have differential impacts on each species, thereby changing the relative importance of host plant quality, which is directly influenced by environmental factors (Gonzales et al. 2002). Consequently, these outcomes may be environmentally conditioned or context dependent, changing as a function of the abiotic conditions under which these interactions occur (Chamberlain et al. 2014, Davis et al. 2015). Such interactions might therefore produce emergent effects of climatic stresses on the response of insect herbivore communities, with implications for ecology and pest management.

Multiple economically important aphid species occur sympatrically in cultivated wheat (*Triticum aestivum* L.) fields throughout cereal producing regions of the United States (Schotzko and Bosque-Pérez 2000, Bosque-Pérez et al. 2002, Qureshi and Michaud 2005). Two cereal aphid species found infesting the same wheat fields in the Pacific Northwest USA are the grass aphid, *Metopolophium festucae* (Theobald) subspecies *cerealium* , and the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Y. Wu, personal communication). *Metopolophium festucae cerealium* is an exotic species in the United States, first reported in the state of Oregon in 1994 (Halbert and Sandvol 1995). While little is known about *M. festucae cerealium* ecology in its exotic North American habitat, the aphid is already a well-established pest in Europe (Dent 1983), and has potential to emerge as a major pest of cereal crops in the USA, where large populations of the aphid have recently been detected in wheat in the states of Washington, Oregon, and Idaho (Halbert et al. 2013, Davis et al. 2014). *Rhopalosiphum padi* is one of the fourteen aphid species considered of most agricultural importance worldwide (Blackman and Eastop 2007), and unlike *M. festucae cerealium,* is a naturalized pest of cereal crops throughout the Pacific Northwest. It has been studied extensively since the 1950s when it was discovered to transmit crop plant disease-causing yellow dwarf viruses (Plumb 1983, Rochow 1970, Finlay and Luck 2011). In contrast, *M. festucae cerealium* does not appear capable of transmitting *Barley yellow dwarf virus* (Sadeghi et al. 2016).

Interactions between sympatrically occurring cereal aphids colonizing the same plants have rarely been studied (Qureshi and Michaud 2005), and nothing is known of how interactions between *M. festucae cerealium* and *R. padi* may affect the performance and pest status for each of these species, especially under changing environmental conditions that can impact agricultural systems and their insect pest communities as a whole. We tested the hypothesis that an abiotic stress imposed via host plant water limitation mediates interactions between these two aphid species using a study system in wheat. In this study, we used greenhouse experiments to examine the interactions between *M. festucae cerealium* and *R. padi* colonizing the same wheat plants to test whether water stress mediated their interactions to produce alternate outcomes for either aphid species. We also tested whether these outcomes were dependent on temporal co-occurrence versus precedence of each species, and whether changes occurred under different spatial scales. Our broader goal is to understand how these interactions can affect co-occurring insect pests of cereal grains under projected climate scenarios, where drought-induced water stress may be a more prevalent condition affecting agriculture in the Pacific Northwest and elsewhere in North America.

Materials and Methods

Insect cultures. Stock cultures of *M. festucae cerealium* originated from females collected within the vicinity of Moscow, ID, USA (46.7317° N, 116.9972° W) during May 2013. Stock cultures of *R. padi* originated from females collected within the vicinity of Moscow, ID in 2001. In the summer of 2014, both aphid species were maintained as nonviruliferous cultures by transferring first-instar nymphs produced off-plant to potted winter barley cv. ‘Eight-twelve’ (sown 2-3 weeks prior). These were kept in environmental growth chambers (Percival Scientific, Perry, IA, USA) at 25 ± 1° C under a 16L: 8D photoperiod at the University of Idaho Manis Entomological Laboratory.

EXPERIMENT 1. Effects of concurrent colonization on reproduction of *M. festucae cerealium* and *R. padi* across differing water inputs of the shared host plant

Plants. Ninety-six spring wheat plants cv. ‘Kelse’ (Kidwell et al. 2009) were sown individually in 100 cm^3 pots containing 111 grams (oven-dried) horticultural mix (Sunshine mix no. 1; SunGro Horticulture, Agawam, MA, USA) in October 2014. Plants were grown on a single bench in a greenhouse (27°C mean daytime temperature and 16h: 8h, L: D photoperiod) at the University of Idaho Manis Entomological Laboratory.

Watering regimes. Experimental watering treatments were established from preliminary tests conducted in the greenhouse using cultivar Kelse. Watering regimes were applied on a gravimetric basis (grams H_2O / grams oven-dried soil) and consisted of two treatments: 0.1 and 0.8 g H_2O per g soil, hereafter referred to as ‘LOW’ and ‘HIGH’ water inputs, respectively. Watering regimes were initiated one week post-sowing and maintained throughout the course of the experiment. Exact H_2O volumes were measured using a graduated cylinder and delivered to plants on an individual basis via bottom-watering into polystyrene bowls every 72 h.

Plant measurements. Seventeen days post-sowing (i.e., ten days following initiation of the watering regimes and correlating with onset of reproduction by *M. festucae cerealium*), predawn leaf water potentials of aphid-free control plants were measured using the pressure chamber method (see Turner 1988) on second-emerged leaves of wheat plants, excised from the stem within 1 cm of the ligule using a razorblade. After oven drying at 70 °C for 48 h, dry aboveground biomass was measured for all ninety-six plants immediately following termination of the experiment.

Insect measurements. Wheat plants were populated with aphids using materials similar to those used by Jiménez-Martínez and Bosque-Pérez (2004). Two weeks after sowing, wheat plants (subjected to watering regimes for 7 d) were fit with transparent cages constructed from dialysis tubing (3 x 1 cm length x diam., Spectrum Lab, Inc., Rancho Domingues, CA, USA). Cages were placed on the second-emerged leaves of plants at their mid-lengths and stoppered with foam at both ends to prevent aphid escape, and positioned such that both adaxial and abaxial surfaces of the caged leaf material were exposed to the aphids. Two thirds of these plants received either *M. festucae cerealium* or *R. padi* alone, and one third received both aphid species. To control for potential aphid density-dependent effects, the total number of aphids populating cages was the same whether one or both species were present. Using methods adapted from Wu et al. (2014), a total of six mature, apterous females from aphid stock cultures were caged and allowed to feed and reproduce on plants for 24 h, after which time all except four nymphs (i.e., foundresses) were removed. These consisted of either four *M. festucae cerealium*, four *R. padi*, or two *M. festucae cerealium* plus two *R. padi* foundresses to yield a starting density of four aphids per plant. Foundresses were checked daily thereafter, and the numbers of offspring (per foundress by species) were recorded every 24 hours for 12 days to determine cumulative reproduction per day.

Experimental design and statistical analyses. Ninety-six plants were divided among eight treatment groups consisting of two factors (water regime and aphid species mixture) with two levels each (‘LOW’ or ‘HIGH’ water, and either conspecific or heterospecific aphid species mixtures), in addition to an aphid-free control group (see Table 3.1). Accounting for potential light and temperature variability within the greenhouse, treatment replicates (n = 12) were divided evenly among adjoining sections of a single greenhouse bench for a balanced, randomized complete block design, where sections were treated as blocks (b = 3). Means of ‘LOW’ and ‘HIGH’ leaf water potentials were compared using one-way ANOVA with block as a random effect and watering input as a fixed effect. Daily cumulative reproduction for each aphid species was compared using two-way repeated-measures ANOVA where each plant was considered an experimental unit with block as a random effect, watering input and aphid species as fixed effects and each day as a random repeated-measures effect. Statistical tests incorporated a type I error rate of $\alpha = 0.05$ and were performed using Minitab 17 software (Minitab Inc.).

Table 3.1. Experiment 1 design showing division of ninety-six plants across treatment groups.

		Starting densities, *M. festucae cerealium*: *R. padi*			
		4: 0	2: 2	0: 4	0: 0 (control)
Water input	‘LOW’ 0.1 g H_2O per g soil · 72 h^{-1}	n = 12	n = 12	n = 12	n = 12
	‘HIGH’ 0.8 g H_2O per g soil · 72 h^{-1}	n = 12	n = 12	n = 12	n = 12

EXPERIMENT 2. Plant-mediated effects of previous colonization by *M. festucae cerealium* on life history traits of *R. padi*

Plants. Twenty-four wheat cv. Kelse plants were sown individually in 100 cm^3 pots containing 111 grams (oven-dried) horticultural mix (Sunshine mix no. 1; SunGro Horticulture, Agawam, MA, USA) in December 2014. Plants were grown in the same greenhouse (27°C mean daytime temperature and 16h: 8h, L: D photoperiod) and administered the same 'HIGH' watering input regime used in experiment 1.

Insect measurements. Wheat plants were populated with aphids using materials similar to those used by Jiménez-Martínez and Bosque-Pérez (2004). Two weeks after sowing, wheat plants were fit with transparent cages constructed from dialysis tubing (3 x 1 cm length x diam., Spectrum Lab, Inc., Rancho Domingues, CA, USA). Cages were placed on the second-emerged leaves of plants at their mid-lengths and stoppered with foam at both ends to prevent aphid escape, and positioned in such a way as to expose both adaxial and abaxial surfaces of the caged leaf material to the aphids. Plants received either four mature, apterous *M. festucae cerealium* or *R. padi* females from aphid stock cultures, and females were allowed to feed and reproduce on caged leaf material for seven days. After this period, these aphids and any offspring were removed and replaced with two mature *R. padi* apterae on the same caged leaf material. After 24 h, all except one *R. padi* nymph (i.e., foundress) were removed to yield a starting density of one aphid per plant. Foundresses were checked daily thereafter, and new nymphs were recorded and removed every 48 hours until reproduction ceased. These data were parameterized to construct life tables using equations from Birch (1948) and Wyatt and White (1977), shown in Table 3.8.

Experimental design and statistical analyses. Twenty-four plants were divided among two treatment groups on the basis of initial colonizing aphid species (*M. festucae cerealium* or *R. padi*). Accounting for potential light and temperature variability within the greenhouse, treatment replicates (n = 12) were divided evenly among adjoining sections of a single greenhouse bench for a balanced, randomized complete block design, where sections were treated as blocks (b = 2). Differences in age-specific fecundity of *R. padi* were tested using one-way repeated-measures ANOVA, where each plant was considered an experimental unit, with block as a random effect, aphid species as a fixed effect and each day as a random repeated-measures effect. All other life table parameters were compared using one-way ANOVA where each plant was considered an experimental unit with block and aphid species as fixed effects. Statistical tests incorporated a type I error rate of $\alpha = 0.05$ and were performed using Minitab 17 software (Minitab Inc.). In the event an *R. padi* foundress exhibited unusually delayed reproduction (i.e., by more than two days) or early mortality (i.e., within two days following reproduction), the sample was considered an outlier and excluded from statistical analyses. For plants initially colonized by *M. festucae cerealium*, n = 11; by *R. padi*, n = 7.

EXPERIMENT 3. Effects of concurrent colonization on abundances of *M. festucae cerealium* and *R. padi* across differing water inputs of the shared host plant

Plants. Ninety-six wheat plants (cv. Kelse) were sown individually in 1,180 cm^3 pots containing 330 grams (oven-dried) horticultural mix (Sunshine mix no. 1; SunGro Horticulture, Agawam, MA, USA) in February 2015. In contrast to the previous two experiments, larger soil capacity pots were used to accommodate for the possibility of plants becoming root-bound during the relatively longer duration required for this experiment. Plants were grown in the same greenhouse (27°C mean daytime temperature and 16h: 8h, L: D photoperiod) used in experiments 1 and 2.

Watering regimes. Excepting for H_2O volumes delivered due to the relatively larger soil capacity pots, watering regimes were identical to those used in experiment 1. These were applied on a gravimetric basis (grams H_2O / grams oven-dried soil) and consisted of two treatments: 0.1 and 0.8 g H_2O per g soil, hereafter referred to as 'LOW' and 'HIGH' water inputs, respectively. Watering regimes were initiated one week post-sowing and maintained throughout the course of the experiment. Exact H_2O volumes were measured using a graduated cylinder and delivered to plants on an individual basis via bottom-watering into polystyrene bowls every 72 h.

Plant measurements. Four weeks post-sowing (i.e., three weeks following initiation of watering regimes and correlating with the same day plants were populated with aphids), predawn leaf water potentials of aphid-free control plants were measured using the pressure chamber method (see Turner 1988) from third leaves (top down, referring to the most recently emerged leaf as first) on the main stem of wheat plants, excised from the stem within 1 cm of the ligule using a razorblade. Fresh biomass was measured for all ninety-six plants immediately following termination of the experiment. After oven drying at 70 °C for 48 h, dry aboveground biomass was additionally measured.

Insect measurements. Four weeks post-sowing, wheat plants were fit with 10 mm diameter clip cages placed on the third leaves (top down, referring to the most recently emerged leaf as first) approximately 3 cm from the main stem. Cages on two thirds of these plants received either *M. festucae cerealium* or *R. padi* alone, and one third received both aphid species. Using methods adapted from Wu et al. (2014), a total of six mature, apterous females per species from aphid stock cultures were caged and allowed to feed and reproduce on plants for 24 h, after which time all except five nymphs (i.e., foundresses) per species were removed in addition to the clip cages, and whole plants were enclosed individually using 1 x 1 mm mesh screen. Therefore, two thirds of plants receiving only one species had a starting density of five aphids, and one third of plants receiving both species had a starting density of ten aphids. Foundresses were left to populate plants for three weeks thereafter, and ending population sizes for each aphid species were recorded at which point the experiment was terminated. In contrast to experiment 1 which kept the starting density of aphids equal whether one or both species were present, the design of this experiment allowed for the direct comparison of ending population sizes between aphid species. In the event that a plant died (i.e., due to water limitation) and was no longer able to support aphids, the sample was removed from the data set.

Experimental design and statistical analyses. Ninety-six plants were divided among eight treatment groups consisting of two factors (water regime and aphid species mixture) with two levels each (‘HIGH’ or ‘LOW’ water, and either conspecific or heterospecific aphid species mixtures), in addition to an aphid-free control group (see Table 3.3). Accounting for potential light and temperature variability within the greenhouse, treatment replicates ($n = 12$) were divided evenly among adjoining sections of a single greenhouse bench for a balanced, randomized complete block design, where sections were treated as blocks ($b = 3$). Means of ‘LOW’ and ‘HIGH’ leaf water potentials were compared using one-way ANOVA with block as a random effect and watering input as a fixed effect. Data were normalized using a square-root transformation ($\lambda = 0.5$) and ending population sizes for each aphid species were compared using two-way ANOVA where each plant was considered an experimental unit with block, watering input, and aphid species as fixed effects. Statistical tests incorporated a type I error rate of $\alpha = 0.05$ and were performed using Minitab 17 software (Minitab Inc.).

Table 3.2. Experiment 3 design showing division of ninety-six plants across treatment groups (in contrast to experiment 1; see Table 3.1)

		Starting densities, *M. festucae cerealium*: *R. padi*			
		5: 0	5: 5	0: 5	0: 0 (control)
Water input	‘LOW’ 0.1 g H_2O per g soil · 72 h^{-1}	n = 12	n = 12	n = 12	n = 12
	‘HIGH’ 0.8 g H_2O per g soil · 72 h^{-1}	n = 12	n = 12	n = 12	n = 12

Results

EXPERIMENT 1

Plant responses. Watering input had a significant ($p < 0.005$) effect on measured plant response variables (Table 3.3). Wheat plants receiving 'LOW' water were significantly smaller than plants receiving 'HIGH' water (Figure 3.1), whereby dried aboveground biomass was reduced by 62 % (Table 3.4). Predawn leaf water potentials for plants receiving 'LOW' water were approximately 0.4 MPa lower than plants receiving 'HIGH' water (Figure 3.2), translating to a 181.8% reduction (Table 3.4).

Table 3.3. ANOVA results for plant response variables as measured under water treatments (predawn leaf water potential results from aphid-free control plants only); significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
Dried aboveground biomass					
Block	**2**	**0.2158**	**0.10792**	**9.86**	**0.000**
H_2O	**1**	**5.7526**	**5.75260**	**525.84**	**0.000**
Error	92	1.0065	0.01094		
Total	95				
Predawn leaf water potential					
Block	**2**	**0.6748**	**0.33740**	**3.83**	**0.039**
H_2O	**1**	**0.9600**	**0.96000**	**10.89**	**0.004**
Error	20	1.7630	0.08815		
Total	23				

Table 3.4. Comparison of water treatment effects on plant response variables; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Variable	H_2O	n	Mean ± SE	% decrease
Dried aboveground biomass (g)	HIGH	48	0.79 ± 0.02	**62.0**
	LOW	48	0.30 ± 0.01	
Predawn leaf water potential (MPa)	HIGH	12	-0.22 ± 0.01	**181.8**
	LOW	12	-0.62 ± 0.14	

Insect responses. Watering input did not significantly affect daily cumulative reproduction for either *M. festucae cerealium* or *R. padi* (Table 3.5, Figures 3.3 and 3.5). Aphid species mixture did not significantly affect reproduction of *M. festucae cerealium*. Aphid species mixture did, however, significantly affect reproduction of *R. padi*: the presence of *M. festucae cerealium* resulted in significantly ($p < 0.05$) increased total nymph reproduction of *R. padi* foundresses measured on days eleven and twelve compared to *R. padi* alone (Table 3.5, Figure 3.4).

Table 3.5. Repeated-measures ANOVA results showing effects of water and aphid species mixture treatments (nested within day) on aphid foundress daily cumulative reproduction per tube cage by species; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		M. festucae cerealium			
Block	2	18.83	9.415	2.13	0.123
Day	**2**	**787.90**	**393.948**	**80.40**	**0.003**
H_2O(Day)	3	5.29	1.762	1.36	0.403
Aphid sp. mix. (Day)	3	13.29	4.430	3.43	0.169
H_2O x Aphid sp. mix. (Day)	3	3.88	1.293	0.29	0.830
Error	121	533.87	4.412		
Total	134	1389.86			
		R. padi			
Block	2	10.6	5.29	0.29	0.749
Day	**5**	**12681.3**	**2536.26**	**23.40**	**0.000**
H_2O(Day)	6	53.5	8.91	2.65	0.131
Aphid sp. mix. (Day)	**6**	**616.9**	**102.81**	**30.63**	**0.000**
H_2O x Aphid sp. mix. (Day)	6	20.2	3.37	0.18	0.981
Error	231	4229.4	18.31		
Total	256	17747.4			

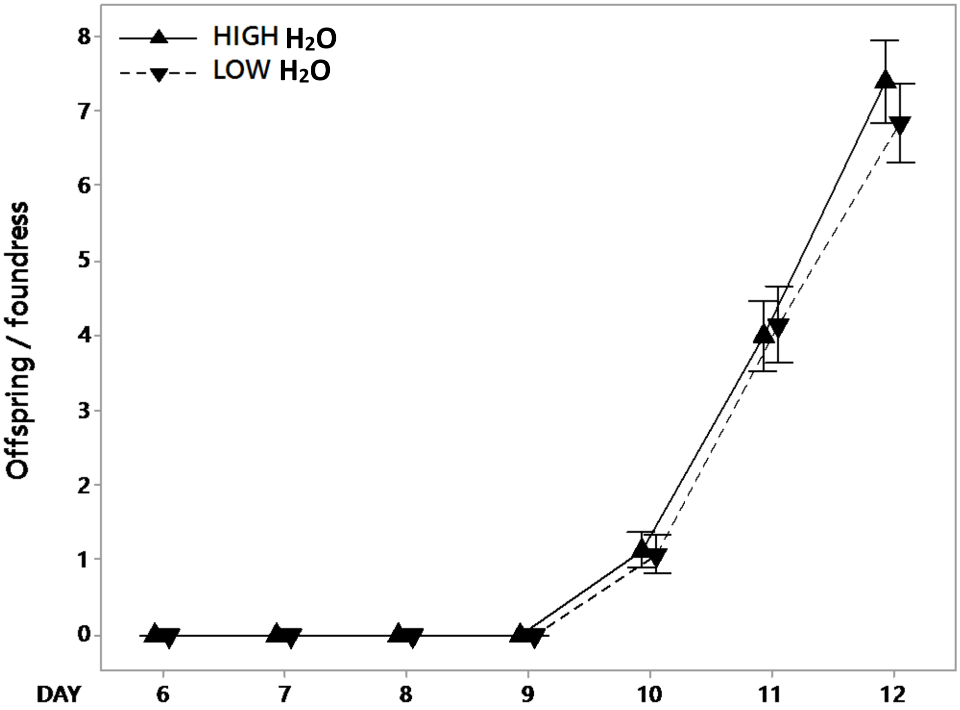

Figure 3.1. Cumulative total offspring produced per day by *M. festucae cerealium* foundresses on plants receiving 'HIGH' or 'LOW' water inputs; symbols represent group means and error bars show standard error estimates.

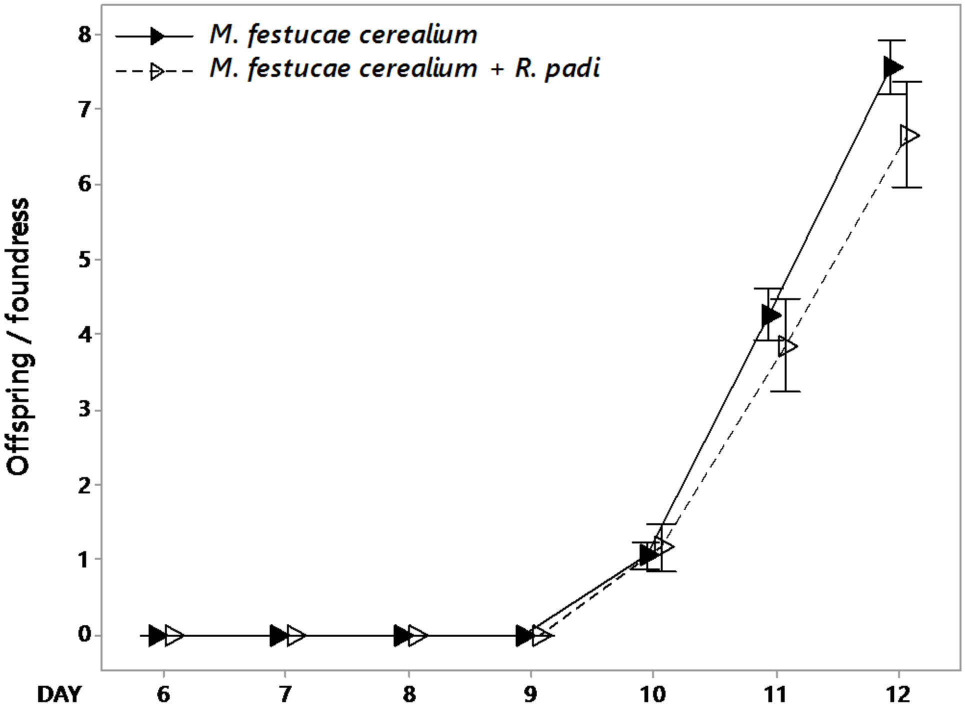

Figure 3.2. Cumulative total offspring produced per day by *M. festucae cerealium* foundresses in cages populated with *M. festucae cerealium* alone or *M. festucae cerealium* with *R. padi*; symbols represent group means and error bars show standard error estimates.

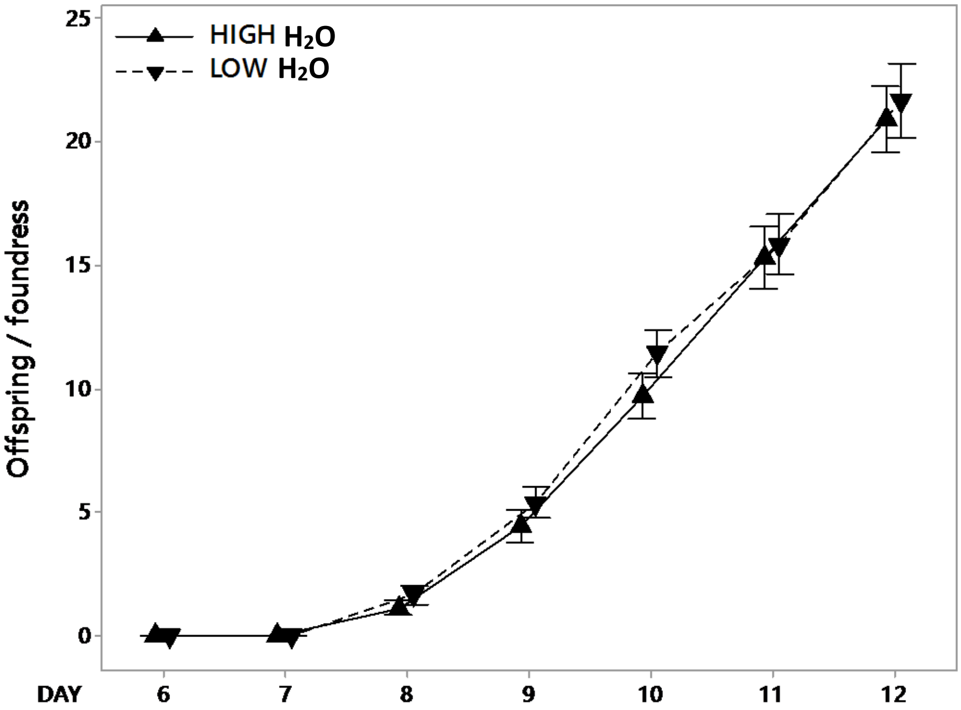

Figure 3.3. Cumulative total offspring produced per day by *R. padi* foundresses on plants receiving 'HIGH' or 'LOW' H_2O inputs; symbols represent group means and error bars show standard error estimates.

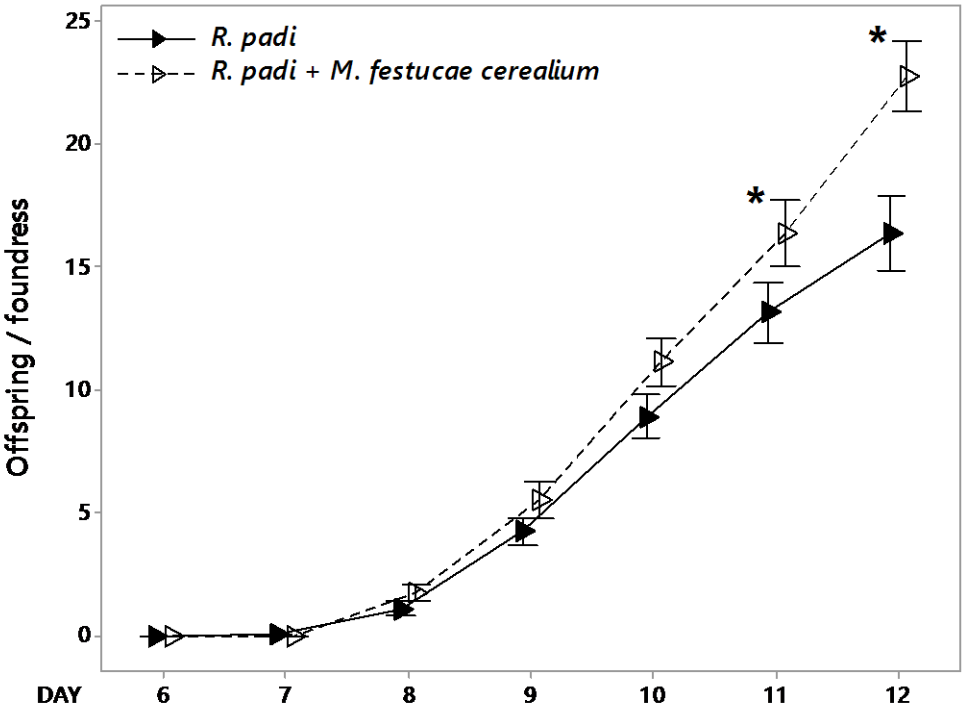

Figure 3.4. Cumulative total offspring produced per day by *R. padi* foundresses in cages populated with *R. padi* alone or *R. padi* with *M. festucae cerealium*; symbols represent group means and error bars show standard error estimates. Asterisks indicate significant (α = 0.05) differences.

EXPERIMENT 2

Previous colonizing aphid species significantly affected age-specific fecundity ($p < 0.0001$), basic reproductive rate ($p < 0.0001$), and intrinsic rate of increase ($p = 0.013$), but not mean generation time or lifetime fecundity of *R. padi* (Tables 3.6 and 3.7, Figure 3.7). Basic reproductive rate and intrinsic growth rate of *R. padi* foundresses increased by 58.3 and 25.3%, respectively, as a result of previous colonization of the same leaf tissue by *M. festucae cerealium* as opposed to *R. padi*. While not statistically significant, *R. padi* foundresses on average were 40.6% more fecund and experienced 10% shorter mean generation times when reproducing on leaf tissue previously colonized by *M. festucae cerealium* as compared to its own species (Table 3.8).

Table 3.6. Repeated-measures ANOVA results showing effects of previous colonizing aphid species (nested within day) on *R. padi* foundress age-specific fecundity; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		Age-specific fecundity			
Block	**1**	**0.44**	**0.44**	**7.69**	**0.007**
Day	**10**	**1163.43**	**116.343**	**4.90**	**0.007**
Aphid sp. (Day)	**11**	**271.44**	**24.676**	**434.62**	**0.000**
Error	104	5.90	0.057		
Total	126	1777.21			

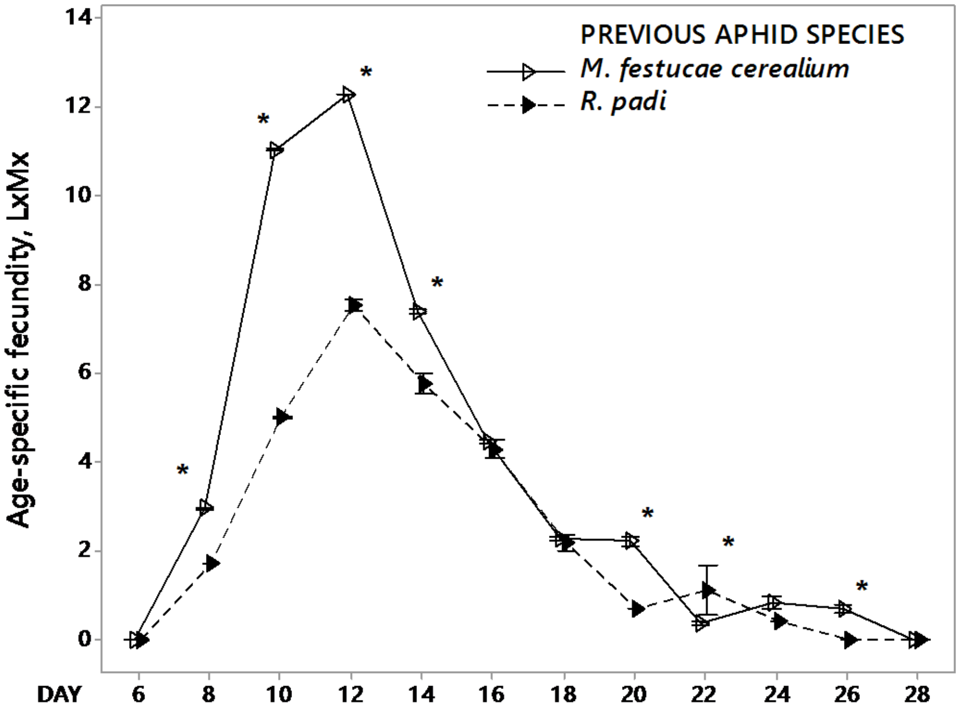

Figure 3.7. Age-specific fecundity of *R. padi* foundresses reproducing on plant leaf tissue previously colonized by either *M. festucae cerealium* or *R. padi*; symbols represent group means and error bars show standard error estimates. Asterisks indicate significant (α = 0.05) differences.

Table 3.7. ANOVA results showing effects of previous colonizing aphid species on *R. padi* foundress life history traits; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		Basic reproductive rate, R_0			
Block	**1**	**87.24**	**87.24**	**9.17**	**0.008**
Aphid sp.	**1**	**908.80**	**908.80**	**95.52**	**0.000**
Error	15	142.71	9.514		
Total	17	1216.89			
		Generation time, T			
Block	**1**	**31.989**	**31.989**	**8.18**	**0.012**
Aphid sp.	1	6.255	6.255	1.60	0.225
Error	15	58.649	3.910		
Total	17	100.664			
		Intrinsic growth rate, r_m			
Block	**1**	**0.012675**	**0.012675**	**10.12**	**0.006**
Aphid sp.	**1**	**0.009862**	**0.009862**	**7.87**	**0.013**
Error	15	0.018794	0.001253		
Total	17	0.044208	0.001342		
		Lifetime fecundity			
Block	1	1035.1	1035.1	4.27	0.056
Aphid sp.	1	757.5	757.5	3.13	0.097
Error	15	3634.2	242.3		
Total	17	5246.0			

Table 3.8. Summary of *R. padi* foundress life history traits showing effects of previous colonizing aphid species; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Basic reproductive rate, R_0[*]			
Previous aphid sp.	n	Mean ± SE	% increase
R. padi	7	26.04 ± 1.60	**58.3**
M. festucae cerealium	11	41.22 ± 1.06	
Generation time, T[†]			
Previous aphid sp.	n	Mean ± SE	% increase
R. padi	7	15.49 ± 1.14	-9.9
M. festucae cerealium	11	13.96 ± 0.57	
Intrinsic growth rate, r_m[‡]			
Previous aphid sp.	n	Mean ± SE	% increase
R. padi	7	0.217 ± 0.017	**25.3**
M. festucae cerealium	11	0.272 ± 0.013	
Lifetime fecundity			
Previous aphid sp.	n	Mean ± SE	% increase
R. padi	7	28.57 ± 3.18	40.6
M. festucae cerealium	11	40.18 ± 6.21	

[*] Basic reproductive rate, R_0, refers to the average number of offspring produced per individual foundress.

[†] Generation time, *T*, represents average time (in days) between two consecutive generations.

[‡] Intrinsic growth rate, r_m, represents the theoretical maximum population growth rate in the absence of density-dependent effects, where $(dN/dt)(1/N) = r$.

EXPERIMENT 3

Plant responses. Watering input significantly affected all measured plant response variables ($p < 0.0001$; Table 3.9). Wheat plants receiving 'LOW' water were significantly smaller than plants receiving 'HIGH' water: fresh and dried aboveground biomass was reduced by 90.7 and 85.7%, respectively (Table 3.10). Predawn leaf water potentials of plants receiving 'LOW' water were approximately 0.7 MPa lower than plants receiving 'HIGH' water, translating to a 400% reduction (Table 3.10).

Table 3.9. ANOVA results for plant response variables as measured under water and aphid treatments (predawn leaf water potential results from aphid-free control plants only); significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
Fresh aboveground biomass					
Block	2	0.5	0.3	0.00	0.997
H_2O	**1**	**36945.0**	**36945.0**	**453.00**	**0.000**
Aphid	3	126.3	42.1	0.52	0.672
H_2O x Aphid	3	162.3	54.1	0.66	0.577
Error	78	6361.4	81.6		
Total	87	44622.0			
Dried aboveground biomass					
Block	2	1.63	0.81	1.15	0.321
H_2O	**1**	**1689.38**	**1689.38**	**2394.17**	**0.000**
Aphid	3	0.37	0.12	0.18	0.912
H_2O x Aphid	3	0.57	0.19	0.27	0.849
Error	78	55.04	0.71		
Total	87	1800.14			
Predawn leaf water potential					
Block	2	15.94	7.972	1.09	0.356
H_2O	**1**	**276.08**	**276.08**	**37.66**	**0.000**
Error	20	146.61	7.331		
Total	23	438.64			

Table 3.10. Comparison of water treatment effects on plant response variables; significant (p < 0.05) treatment effects are highlighted in bold text.

Variable	Water	n	Mean ± SE	% decrease
Fresh aboveground biomass (g)	HIGH	48	46.0 ± 1.7	**90.7**
	LOW	40	4.3 ± 0.2	
Dried aboveground biomass (g)	HIGH	48	10.5 ± 0.2	**85.7**
	LOW	40	1.5 ± 0.1	
Predawn leaf water potential (MPa)	HIGH	12	-0.17 ± 0.02	**400.0**
	LOW	12	-0.85 ± 0.11	

Insect responses. Watering input significantly affected the final per-plant abundance of *R. padi* (p < 0.0001) but not *M. festucae cerealium* (Tables 3.12 and 3.13, Figures 3.10 and 3.11). On average, *R. padi* abundance was 64.8% lower on plants receiving 'LOW' water compared to plants receiving 'HIGH' water (Table 3.14). Aphid species mixture significantly affected the final per-plant abundance of *M. festucae cerealium* (p < 0.0001) but not *R. padi* (Tables 3.12 and 3.13). On average, *M. festucae cerealium* abundance on plants also populated with *R. padi* was reduced by 48.7% when compared to plants populated only with its own species (Table 3.14). While not statistically significant, the abundance of *R. padi* on plants also populated with *M. festucae cerealium* increased by 11.3% on average (Table 3.14). A significant water by aphid species mixture treatment interaction occurred for *M. festucae cerealium* (p = 0.039), but not *R. padi* (Tables 3.12 and 3.13, see Figure 3.5). The presence of *R. padi* resulted in a 28.0% decrease in *M. festucae cerealium* abundance on plants receiving 'HIGH' water and a 71.3% decrease on plants receiving 'LOW' water (Table 3.15).

Table 3.11. Square-root transformed ANOVA results showing effects of water and aphid species mixture treatments on aphid abundance per plant by species; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
		M. festucae cerealium			
Block	2	1.092	0.546	0.05	0.955
Water	1	34.642	34.642	2.92	0.096
Aphid sp. mixture	**1**	**204.840**	**204.840**	**17.25**	**0.000**
Water x Aphid sp. mixture.	**1**	**54.212**	**54.212**	**4.57**	**0.039**
Error	37	439.352	11.874		
Total	42	722.084			
		R. padi			
Block	2	**697.02**	**348.51**	**3.68**	**0.035**
Water	**1**	**1188.57**	**1188.57**	**12.54**	**0.001**
Aphid sp. mixture	1	3.66	3.66	0.04	0.845
Water x Aphid sp. mixture	1	2.06	2.06	0.02	0.884
Error	37	3506.01	94.76		
Total	42	5202.3			

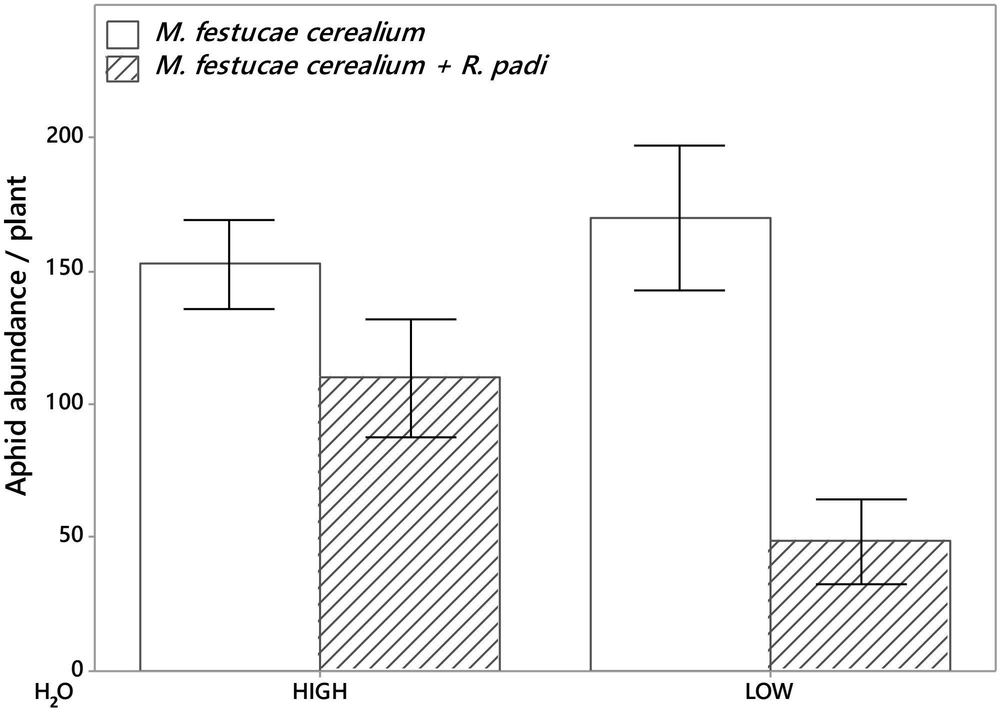

Figure 3.5. Abundance of *M. festucae cerealium* per plant for all treatment combinations after 3 weeks; bars represent group means and error bars show standard error estimates.

Table 3.12. Square-root transformed ANOVA results showing effects of water and aphid species mixture treatments on *M. festucae cerealium* abundance per plant by species; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
Block	2	1.092	0.546	0.05	0.955
Water	1	34.642	34.642	2.92	0.096
Aphid sp. mixture	**1**	**204.840**	**204.840**	**17.25**	**0.000**
Water x Aphid sp. mixture	**1**	**54.212**	**54.212**	**4.57**	**0.039**
Error	37	439.352	11.874		
Total	42	722.084			

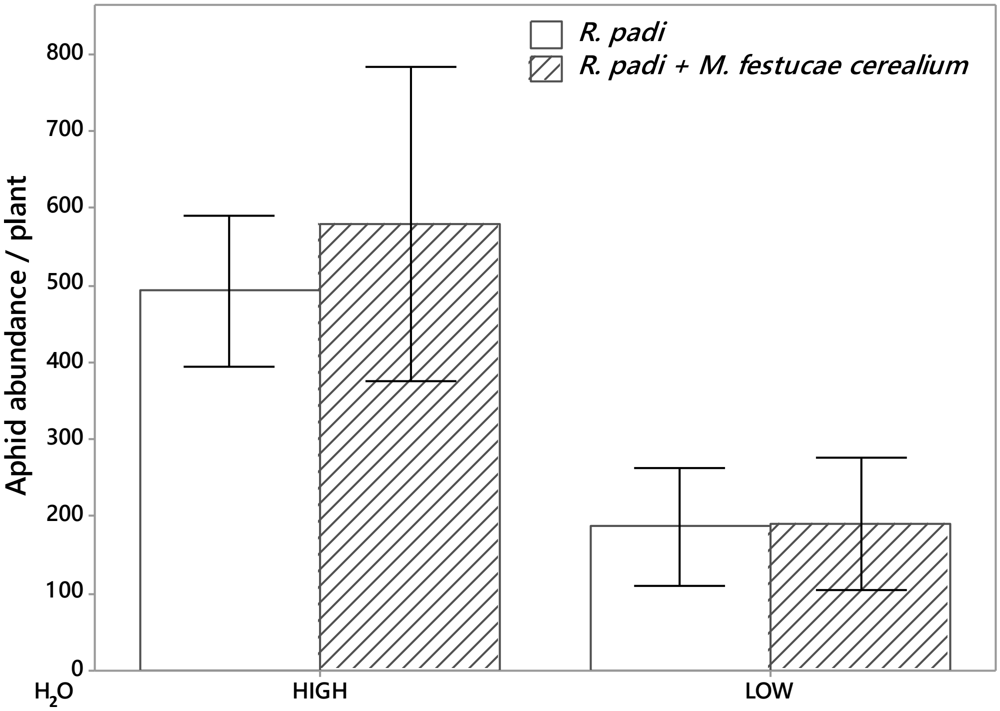

Figure 3.6. Abundance of *R. padi* per plant for all treatment combinations after 3 weeks; bars represent group means and error bars show standard error estimates.

Table 3.13. Square-root transformed ANOVA results showing effects of water and aphid species mixture treatments on *R. padi* abundance per plant by species; significant ($p < 0.05$) treatment effects are highlighted in bold text.

Source	df	Adjusted SS	Adjusted MS	F value	p > F
Block	2	**697.02**	**348.51**	**3.68**	**0.035**
Water	**1**	**1188.57**	**1188.57**	**12.54**	**0.001**
Aphid sp. mixture	1	3.66	3.66	0.04	0.845
Water x Aphid sp. mixture	1	2.06	2.06	0.02	0.884
Error	37	3506.01	94.76		
Total	42	5202.3			

Table 3.14. Comparison of water and aphid species mixture treatment main effects on aphid abundance per plant; significant ($p < 0.05$) treatment effects are highlighted in bold text.

M. festucae cerealium (aphids / plant)

Treatment		n	Mean ± SE	% decrease
Factor	Level			
Water	HIGH	24	131.4 ± 14.2	19.1
	LOW	19	106.3 ± 20.7	
R. padi	**Absent**	**21**	**160.2 ± 14.8**	**48.7**
	Present	**22**	**82.2 ± 15.2**	

R. padi (aphids / plant)

Treatment		n	Mean ± SE	% decrease
Factor	Level			
Water	**HIGH**	**24**	**536.0 ± 111.0**	**64.8**
	LOW	**19**	**188.9 ±57.0**	
M. festucae cerealium	Absent	21	362.0 ± 71.6	-11.3
	Present	22	403.0 ±123.0	

Table 3.15. Comparison of water and aphid species mixture treatment simple main effects on aphid abundance per plant; significant ($p < 0.05$) treatment effects are highlighted in bold text.

M. festucae cerealium (aphids / plant)

Water	*R. padi*	n	Mean ± SE	% decrease
HIGH	Absent	12	152.8 ± 16.6	28.0
	Present	12	110.0 ± 22.0	
LOW	**Absent**	**9**	**170.1 ± 27.4**	**71.3**
	Present	**10**	**48.8 ±15.9**	

R. padi (aphids / plant)

Water	*M. festucae cerealium*	n	Mean ± SE	% decrease
HIGH	Absent	12	493.3 ± 97.1	-17.6
	Present	12	580.0 ± 204.0	
LOW	Absent	9	186.9 ± 77.0	-2.0
	Present	10	190.7 ± 87.2	

Discussion

We examined interspecific interactions between two sympatrically occurring cereal aphid pests colonizing the same wheat plants in order to determine whether an abiotic stress, imposed via host plant water limitation, mediated these interactions to produce emergent effects for either aphid species. Evidence from our experiments indicates that the relative performance of each species can change when the other species is present. The outcomes for each species, however, were asymmetrical and mediated by host plant water stress. When caged on the same leaf, we observed enhanced daily reproductive output by *R. padi* when *M. festucae cerealium* was also present (i.e., concurrently feeding and reproducing on the same leaf tissue). Reproduction of *M. festucae cerealium*, however, was unaffected by the presence of *R. padi*. The responses from neither aphid species changed when we imposed an approximate -0.4 MPa water stress. This water stress did, however, affect plant aboveground biomass and predawn leaf water potential, and would be considered by Hale et al. (2003) a 'moderate' level of water stress (see Hale et al. 2003; Figures 3.1 and 3.2, Table 3.4). We further demonstrated that previous activity by *M. festucae cerealium* benefitted *R. padi* when feeding on the same leaf tissue. Age-specific fecundity (Figure 3.7) in addition to multiple life history traits (Table 3.8) were significantly enhanced for *R. padi* feeding on leaf tissue previously colonized by *M. festucae cerealium* compared to its own species.

Different outcomes were observed during a whole-plant assay where both aphid species simultaneously populated individual caged plants. At the time of aphid introductions, plants were relatively older, larger, and had received administered watering regimes for a longer period. This resulted in a more pronounced effect of water input on plant aboveground biomass and predawn leaf water potential (Table 3.10). Here, reduced water input affected *R. padi* negatively but *M. festucae cerealium* remained unaffected. However, a water regime x aphid species interaction occurred for *M. festucae cerealium*: the presence of *R. padi* reduced *M. festucae cerealium* abundance on plants receiving both water inputs, but the effect was significantly greater (i.e., 71% versus 28% fewer aphids) on water-stressed plants compared to plants receiving an ample water supply (Figure 3.5, Table 3.15).

Our results were consistent with those from previous studies demonstrating either neutral (Pons and Tatchell 1995, Bethke et al. 1998), negative (Kennedy et al. 1958, Sumner et al. 1983), or both (Hale et al. 2003) effects of host plant water stress on aphid performance. Also, evidence from these experiments supports our hypothesis of a context dependent outcome from interactions between these two herbivore species: we observed an amensal interaction between *M. festucae cerealium* and *R. padi* in a whole-plant assay, but the relative magnitude of the negative effect on *M. festucae cerealium* increased on water-stressed plants, while it remained consistent across water-stress treatments for *R. padi*. The asymmetrical outcome resulting from interactions between these two aphid species corroborates findings from studies involving other insect herbivore assemblages. For example, the cereal aphids *Sipha flava* and *Rhopalosiphum maidis* exhibit asymmetrical competitive interactions on the shared host plant, *Sorghum halepense*, and the outcome for each species is dependent on season (i.e., spring versus fall) in addition to the presence or absence of natural enemies (Gonzales et al. 2002). Contrasting results by Gianoli (2000) found symmetrical competition between *R. padi* and the sympatric English grain aphid, *Sitobion avenae*, on seedling and tillering wheat plants, whereby the reproductive rate of each species was negatively impacted by presence of the other. However, no abiotic stress was imposed on plants in these greenhouse experiments. Using a cereal-aphid-virus association that incorporated an abiotic stressor, Davis et al. (2015) demonstrated that chronic water limitation imposed over the lifetime of host plants shifted the sign of interactions between wheat and the *R. padi*-transmitted *Barley yellow dwarf virus* (BYDV-PAV) from exploitative to commensal (i.e., negative to positive net effect on wheat). Additionally, *R. padi* exhibited improved performance on water-stressed wheat infected with BYDV compared to *R. padi* on water-stressed virus-free plants.

Interactions between insect herbivores are commonly mediated by a shared host plant (Fritz et al. 1986, Mopper et al. 1990, Moran and Whitham 1990, Fritz 1992, Denno et al. 1995, Inbar et al. 1995, Gonzales et al. 2002). In many instances, insect herbivory can alter host plant quality to the benefit or detriment of the inducing insect as well as later-colonizing species (Hanhimaki 1989, Karban 1993, Karban and Baldwin 1997, Denno et al. 2000, Gonzales et al. 2002). Considering the generally poor nutritional quality of phloem sap (Slanksy and Scriber 1985), it has been suggested that some aphids are capable of manipulating phloem nutritional quality to their benefit, presumably through ultrastructural changes to plant cells induced by feeding on susceptible hosts (Al-Mousawi 1983). Sandstrom et al. (2000) demonstrated that the aphids *Schizaphis graminum* and *Diuraphis noxia* ingested phloem sap with a substantially higher concentration of amino acids than *R. padi* when feeding on both wheat and barley. Furthermore, *S. graminum* and

D. noxia induced macroscopic chlorotic lesions to infested leaf tissues, whereas *R. padi* did not. It should be noted that these lesions are symptoms of feeding activity and do not directly indicate nutritional manipulation by the herbivore; however, other reports exist linking aphid-induced chlorotic lesions with the enhanced performance of aphids. For example, Dorschner et al. (1987) found that an *S. graminum* clone incapable of inducing chlorotic lesions on a particular wheat cultivar experienced enhanced performance on plants previously infested by another *S. graminum* clone which had the ability to induce lesions. We likewise observed distinctive chlorotic lesions on leaf tissues exposed to *M. festucae cerealium* feeding, compared with *R. padi* which produced no visual changes to plant tissues in our experiments. This inability by *R. padi* to induce such lesions has been reported in other studies (e.g., Sandstrom et al. 2000, Ni and Quisenberry 2006). This is one indication that *M. festucae cerealium* might be able to alter host plant nutritional quality, potentially to its benefit, as well as heterospecifics including *R. padi* when occupying the same leaf tissues. While analyses of phloem sap are needed to directly support a hypothesis of feeding-induced nutritional enhancement (see Sandstrom et al. 2000), the significant changes to *R. padi* life history traits on leaf tissue previously infested by *M. festucae cerealium* lead us to conclude that the mechanism(s) responsible were plant mediated. However, because *R. padi* was placed on the same previously infested section of leaf tissue immediately after the removal of *M. festucae cerealium*, the possibility of non-localized and temporally sensitive residual effects following *M. festucae cerealium* infestation cannot presently be determined.

In our caged leaf experiments, we observed enhanced performance of *R. padi* when feeding on leaf tissue simultaneously and previously infested by *M. festucae cerealium*. However, these experiments controlled for the placement of both aphid species on host plants and further confined their distribution to a single leaf. Although similar methods have been used in other studies (e.g., Gianoli 2000, Davis et al. 2015), selection of feeding sites and movement of *M. festucae cerealium* and *R. padi* that would otherwise occur in the field were restricted in this study. While this was required for our experimental design, the distinct temporal and spatial patterns of resource use by cereal aphids must be considered, especially when examining interactions among sympatric species (Gianoli 2000).

Basic biological information is limited for *M. festucae cerealium* in the Pacific Northwest and in general (Davis et al. 2014). However, surveys for *M. festucae cerealium* show seasonal overlap with *R. padi* elsewhere; outbreaks have been reported during April in Europe (Massee 1935, Janson 1959) as well as generally high abundances on grasses in the spring (Hand 1989), and the aphid was detected in sweep net samples throughout the states of Idaho, Oregon and Washington during May and June of 2011 and 2012 (Halbert et al. 2013, Davis et al. 2014). Furthermore, Dent and Wratten (1986) observed evenly distributed *M. festucae cerealium* apterae on wheat plants in both laboratory and field settings. While spatial data were not recorded during our study, we observed generally even distributions for both aphid species when allowed to simultaneously colonize entire plants, showing no indications of avoidance or deterrence. Collectively, these findings suggest first, that *M. festucae cerealium* and *R. padi* both have the potential to infest wheat fields relatively early in the growing season and experience a temporal overlap; and second, that the distributions of both species co-colonizing the same wheat plant are not spatially separated, leading to potential colonization of the same (or closely-neighboring) feeding sites.

The likelihood of spatio-temporal overlap and potential for competitive interactions between *M. festucae cerealium* and *R. padi* in the Pacific Northwest must be considered together with our findings of differential impacts of host plant water stress on each aphid species. While a significant negative effect occurred for *R. padi*, the same -0.7 MPa water stress in potted wheat plants had no effect on *M. festucae* unless *R. padi* was present. The resilience of *M. festucae cerealium* to water-stressed wheat compared to *R. padi* may be partially due to its different life history traits. According to Dent and Wratten (1986), the highest reproductive rate achieved by *M. festucae cerealium* on cereals was twenty-eight nymphs after ten days, whereas *R. padi* can produce as many as forty nymphs after 6 – 7.5 days (Leather and Dixon 1981, Jiménez-Martínez et al. 2004). In all our experiments, *M. festucae cerealium* reproduction consistently occurred three days following that of *R. padi* (i.e., pre-reproductive periods of ten and seven days, respectively). In the whole-plant assay, average per-plant abundance of *M. festucae cerealium* compared to *R. padi* was nearly seventy percent lower on plants receiving an ample water supply. On water-stressed plants, however, abundance of *M. festucae cerealium* compared to *R. padi* was only nine percent lower. Thus, in the absence of heterospecifics, the relatively slower reproductive rate of *M. festucae cerealium* may be advantageous over faster-reproducing cereal aphid species like *R. padi*, which may encounter intraspecific competition or exhaust plant resources sooner on water-stressed hosts. But co-colonization by *R. padi* on water-stressed plants resulted in significantly reduced abundance of *M. festucae cerealium*. While further testing is needed to determine the underlying mechanisms responsible for this outcome, the relative higher reproductive rate of *R. padi* combined with the increased reproductive output on leaf tissues colonized by *M. festucae cerealium* may interact to produce numbers of *R. padi* great enough to result in competitive exclusion of *M. festucae cerealium* on water-stressed wheat plants. Furthermore, the statistically non-significant effect of *M. festucae cerealium* presence on *R. padi* in the whole-plant assay may have resulted from experimental error due to escaped or otherwise uncounted *R. padi*.

Findings from this study have potential implications for pest management in cereal cropping systems in the Pacific Northwest and elsewhere. We show that interspecific interactions between two economically important aphid species are complex, producing unique outcomes for each species which can depend greatly on the environmental context under which these interactions take place. This may in turn influence the future prevalence and pest status of both species and result in emergent effects on the insect herbivore community as a whole. Continued monitoring for *M. festucae cerealium* and *R. padi* in the Pacific Northwest has potential to reveal future ecological trends for each species at the population level. In the event that both species continue to have sympatric distributions and co-colonize fields, competitive pressure by *R. padi* may displace *M. festucae* populations in severe drought years. Alternately, *M. festucae cerealium* infesting wheat fields where *R. padi* and other potential competitors are generally absent may remain relatively unaffected by adverse environmental conditions that could typically disrupt a cereal aphid population. A component our study did not address was economic damage to wheat and other cereals (e.g., barley) resulting from infestation by both aphid species, and whether yield effects are additive or interactive when both species are present. Additionally, the ecological scope of this study could be broadened significantly through field experiments incorporating other critical factors missing from our greenhouse experiments, from biotic components (e.g., natural enemies) to other climatic stresses associated with drought (e.g., elevated temperature). Further investigations to these ends could help advance our understanding of the complex interactions between insect communities and the environment under projected climate scenarios, where drought may be a more prevalent condition affecting agricultural systems in the Pacific Northwest and elsewhere in North America.

References

Al-Mousawi, A.H., P.E. Richardson, and R.L. Burton. 1983. Ultrastructural studies of greenbug (Homoptera: Aphididae) feeding damage to susceptible and resistant wheat cultivars. Annals of the Entomological Society of America76: 964–971.

Araus, J.L, G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C_3 cereals: what should we breed for? Annals of Botany 89: 925-940.

Archer, T.L., E.D. Bynum, Jr., A.B. Onken, and C.W. Wendt. 1995. Influence of water and nitrogen fertilizer on biology of the Russian wheat aphid (Hemiptera: Aphididae) on wheat. Crop Protection 14: 165-169.

Bethke, J.A., R.A. Redak, and U.K. Schuck. 1998. Melon aphid performance on chrysanthemum as mediated by cultivar, and deferential levels of fertilization and irrigation. Entomologia Experimentalis et Applicata 88: 41-47.

Birch, L.C. 1948. The intrinsic rate of natural increase of an insect population. The Journal of Animal Ecology: 15-26.

Blackman, R.L., and V.F. Eastop. 2000. Aphids on the world's herbaceous plants and shrubs. Wiley with the Natural History Museum of London, England.

Bronstein, J.L. 1994. Conditional outocmes in mutualistic interactions. Trends in Ecology and Evolution 9: 214-217.

Bosque-Pérez, N.A., J.B. Johnson, D.J. Schotzko, and L. Unger. 2002. Species diversity, abundance, and phenology of aphid natural enemies on spring wheats resistant and susceptible to Russian wheat aphid. Biocontrol 47: 667-684.

Chamberlain, S.A., J.L. Bronstein, and J.A. Rudgers. 2014. How context dependent are species interactions? Ecology Letters 17: 881-890.

Chaves, M.M, J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology 30: 239-264.

Dai, A. 2013. Increasing drought under global warming in observations and models. Nature Climate Change 3: 52-58.

Davis, T.S., Y. Wu, and S.D. Eigenbrode. 2014. Host settling behavior, reproductive performance, and effects on plant growth of an exotic cereal aphid, *Metopolophium festucae* subsp. *cerealium* (Hemiptera: Aphididae). Environmental Entomology 43: 682-688.

Davis, T.S., N.A. Bosque-Pérez, N.E. Foote, T.M. Magney, and S.D. Eigenbrode. 2015. Environmentally dependent host-pathogen and vector-pathogen interactions in the barley yellow dwarf virus pathosystem. Journal of Applied Ecology 52: 1392-1401.

Dent, D.R., 1983. The biology of host plant relationships of the grass aphid *Metopolophium festucae* (Theobald) subspecies *cerealium*. PhD thesis, University of Southampton, Southampton, England.

Dent, D.R., and S.D. Wratten. 1986. The host-plant relationships of apterous virginoparae of the grass aphid *Metopolophium festucae cerealium*. Annals of Applied Biology 108: 567-576.

Denno, R.F., M.S. McClure, and J.R. Ott. 1995. Interspecific interactions in phytophagous insections: competition reexamined and resurrected. Annual Review of Entomology 40: 297-331.

Denno, R.F., M.A. Peterson, C. Gratton, et al. 2000. Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. Ecology 81: 1814–1827.

Dixon, A.F.G. 1973. Biology of aphids. Arnold, London, England.

Dorschner, K.W., J.D. Ryan, R.C. Johnson, and R.D. Eikenbary. 1987. Modification of host nitrogen levels by the greenbug (Homoptera: Aphididae): Its role in resistance of winter wheat to aphids. Environmental Entomology 16: 1007–1011.

Drake, B.G., and J.L. Gallagher. 1984. Osmotic potential and turgor maintenance in *Spartina alterniflora* Loisel. Oecologia 62: 368-375.

Finlay, K.J., and J.E. Luck. 2011. Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop-vector-virus pathosystem. Agriculture, Ecosystems & Environment 144: 405-421.

Fritz, R.S. 1992. Community structure and species interactions of phytophagous insects on resistant and susceptibles host plants, pp. 240-277. *In* R.S. Fritz and E.L. Simms (eds.), Plant resistance to herbivores and pathogens. Univ. of Chicago Press, Chicago.

Fritz, R.S., C.F. Sacchi, and P.W.Price. 1986. Competition versus plant phenotypes in species composition: willow sawflies. Ecology 67: 1608–1618.

Gianoli, E. 2000. Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. Environmental Entomology 29: 213-219.

Gonzales, W.L., E. Fuentes-Contreras, and H.M. Niemeyer. 2002. Host plant and natural enemy impact on cereal aphids competition in a seasonal environment. Oikos 96: 481-491.

Halbert, S.E., and L.E. Sandvol. 1995. New or unusual pests in Idaho in 1994, p. 71. *In* Research Reports: 54th Annual Northwest Insect Management Conference, January 1995, Portland, OR. Oregon State University, Corvallis, OR.

Halbert, S.E., Y. Wu, and S.D. Eigenbrode. 2013. *Metopolophium festucae cerealium* (Hemiptera: Aphididae), a new addition to the aphid fauna of North America. Insecta Mundi 301: 295-302.

Hale, B.K., J.S. Bale, J. Pritchard, G.J. Masters, and V.K. Brown. 2003. Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. Ecological Entomology 28: 666-677.

Hand, S.C. 1989. The overwintering of cereal aphids on Graminae in southern England, 1977-1980. Annals of Applied Biology 115: 17-29.

Hanhimaki, S. 1989. Induced resistance in mountain birch: defense against leaf-chewing insect guild and herbivore competition. Oecologia 81: 242–248.

Hill, D.S. 1997. The Economic Importance of Aphids. Chapman and Hall, London, England.

Hodge, S., and W. Arthur. 1996. Contramensal interactions between species. Oikos 77: 371-375.

Holtzer, T.O., T.L. Archer, and J.M. Norman. 1988. Host plant suitability in relation to water stress. Pages 111-137 *in* E.A. Heinrichs, editor. Plant stress-insect interactions. John Wiley and Sons, New York, NY.

Hsiao, T.C. 1973. Plant responses to water stress. Annual Review of Plant Physiology 24: 519-570.

Huberty, A.F., and R.F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85: 1383-1398.

Hunter, M.D., and P.W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology 73: 724-732.

Inbar, M., A. Eshel, and D. Wool. 1995. Interspecific competition among phloem-feeding insects mediated by induced host-plant sinks. Ecology 76: 1506-1515.

Janson, H.W. 1959. Aphids on cereals and grasses in 1957. Plant Pathology 8: 38.

Jiménez-Martínez, E.S., and Bosque-Pérez. 2004. Variation in barley yellow dwarf virus transmission efficiency by *Rhopalosiphum padi* after acquisition on transgenic and nontransformed wheat genotypes. Journal of Economic Entomology 97: 1790-1796.

Jiménez-Martínez, E.S., N.A. Bosque-Pérez, P.H. Berger, and R.S. Zemetra. 2004. Life history of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae) on transgenic and untransformed wheat challenged with *Barley yellow dwarf virus.* Journal of Economic Entomology 97: 203-212.

Karban, R. 1993. Induced resistance and plant density of a native shrub, Gossypium thurberi, affect its herbivores. Ecology 74: 1–8.

Karban, R., and I.T. Baldwin. 1997. Induced responses to herbivory. Univ. of Chicago Press, Chicago.

Kennedy, J.S., K.P. Lamb, and C O. Booth. 1958. Responses of *Aphis fabae* Scop. to water shortage in host plants in pots. Entomologia Experimentalis et Applicata 1: 274–291.

Kidwell, K.K., G.B. Shelton, V.L. DeMacon, X.M. Chen, J.S. Kuehner, B. Baik, D.A. Engle, A.H. Carter, and N.A. Bosque-Pérez. 2009. Registration of 'Kelse' wheat. Journal of Plant Registrations 3: 269-272.

Leather, S.R., and A.F.G. Dixon. 1981. The effect of cereal growth stage and feeding site on the reproductive activity of the bird-cherry aphid, *Rhopalosiphum padi*. Annals of Applied Biology 97: 135-141.

Massee, A.M. 1935. Notes on some interesting mites and insects observed on hops and fruit trees in 1983. Report of East Malling Research Station for 1935, pp. 164-170

Mopper, S., T.G. Whitham, and P.W. Price. 1990. Plant phenotype and interspecific competition between insects determine sawfly performance and density. Ecology 71: 2135–2144.

Moran, N.A. and T.G. Whitham. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. Ecology 71: 1050–1058.

Newman, J.A. 2005. Climate change and the fate of cereal aphids in Southern Britain. Global Change Biology 11: 940-944.

Ni, X., and S.S. Quisenberry. 2006. *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae) interactions and their injury on resistant and susceptible cereal seedlings. Journal of Economic Entomology 99: 551-558.

Plumb, R. 1983. Barley yellow dwarf virus—a global problem, pp 185-194. *In* R. Plumb, R. and J. Thresh (eds.), Plant Virus Epidemiology. Blackwell Scientific Publications, Oxford, England.

Pons, X, and G.M. Tatchell. 1995. Drought stress and cereal aphid performance. Annals of Applied Biology 126: 19-31.

Qureshi J.A., and J.P. Michaud. 2005. Interactions among three species of cereal aphids simultaneously infesting wheat. Journal of Insect Science 5: 1-8.

Rochow, W., 1970. Barley yellow dwarf virus. Descriptions of Plant Viruses, vol. 32. CMI/AAB, Kew, Surrey, England.

Sadeghi, S.E., J. Bjur, L. Ingwell, L. Unger, N.A. Bosque-Pérez, and S.D. Eigenbrode. 2016. Interactions between *Metopolophium festucae cerealium* (Hemiptera: Aphididae) and Barley yellow dwarf virus (BYDV-PAV). Journal of Insect Science 16: 1-6.

Sandstrom, J., A. Telang, and N.A. Moran. 2000. Nutrtional enhancement of host plants by aphids—a comparison of three aphid species on grasses. J. Insect Physiol. 46: 33-40.

Schotzko, D.J., and N.A. Bosque-Pérez, N. A. 2000. Seasonal dynamics of cereal aphids on Russian wheat aphid (Homoptera: Aphididae) susceptible and resistant wheats. Journal of Economic Entomology 93: 975-981.

Slansky, F., and J.M. Scribner. 1985. Food consumption and utilization, pp. 87-163. *In*: G.A. Kerkut and L.I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 4. Pergamon Press, Oxford, England.

Sumner, L.C., J.T. Need, R.W. McNew, K.W. Dorschner, R.D. Eikenbary, and R.C. Johnson. 1983. Response of *Schizaphis graminum* (Homoptera: Aphididae) to drought-stressed wheat, using polyethylene glycol as a matricum. Environmental Entomology 12: 919-922.

Taiz, L., and E. Zeiger. 2002. Plant physiology, 3rd ed. Sinauer, Sunderland, Massachusetts, MA.

Wearing, C.H., and H.F. van Emden. 1967. Studies on the relations of insect and host plant. I. Effects of water stress in host plants on infestation by *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). Nature 203: 1051-1052.

Wu, Y., T.S. Davis, and S.D. Eigenbrode. 2014. Aphid behavioral responses to virus-infected plants are similar despite divergent fitness effects. Entomologia Experimentalis et Applicata 153: 246-255.

CHAPTER 4—Summary

This thesis research investigated the effects of insect herbivore responses to host plant water stress, using a study system in wheat (*Triticum aestivum* L.). Greenhouse experiments were used to examine the effects of host plant drought stress on two prevalent insect pests of wheat in the Pacific Northwest USA: the cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae, Criocerinae) and two sympatric species of cereal aphid (Hemiptera: Aphididae), *Metopolophium festucae* (Theobald) subsp. *cerealium* and *Rhopalosiphum padi* L.

In experiments with cereal leaf beetle (Chapter Two), water stress resulted in significantly smaller plants with lower leaf water potential, in addition to decreased seed production and plant yield. Beetle feeding damage significantly reduced plant aboveground biomass, and there were non-significant trends of reduced seed production and plant yield due to CLB. However, we observed no significant interactions between water stress and CLB feeding damage, suggesting that the effects from both stresses combined are additive.

In experiments with the cereal aphids *Metopolophium festucae cerealium* and *Rhopalosiphum padi* (Chapter Three), we found that the relative performance of each species could change when the other species was present. The outcomes for each species, however, were asymmetrical and mediated by host plant water stress. When caged on the same leaf, we observed enhanced daily reproductive output by *R. padi* when *M. festucae cerealium* was also present (i.e., concurrently feeding and reproducing on the same leaf tissue). Reproduction of *M. festucae cerealium*, however, was unaffected by the presence of *R. padi*. The responses from neither aphid species changed when we imposed an approximate -0.4 MPa water stress. We further demonstrated that previous activity by *M. festucae cerealium* benefitted *R. padi* when feeding on the same leaf tissue. Age-specific fecundity in addition to multiple life history traits were significantly enhanced for *R. padi* feeding on leaf tissue previously colonized by *M. festucae cerealium* compared to its own species.

Different outcomes were observed during a whole-plant assay where both aphid species simultaneously populated individual caged plants. At the time of aphid introductions, plants were relatively older, larger, and administered watering regimes for a longer duration. This resulted in a more pronounced effect of water input on plant aboveground biomass and predawn leaf water potential. Here, water input affected *R. padi* negatively but *M. festucae cerealium* remained unaffected. However, a water regime by aphid species interaction occurred for *M. festucae cerealium*: the presence of *R. padi* reduced *M. festucae cerealium* abundance on plants receiving both water inputs, but the effect was significantly greater (i.e., 71% versus 28% fewer aphids) on water-stressed plants compared to plants receiving an ample water supply.

Future research needs and conclusion

Findings from this thesis research have potential implications for pest management in cereal cropping systems in the Pacific Northwest and elsewhere. The two greenhouse studies in this thesis (Chapters Two and Three) allowed for the experimental manipulation and precise measurement of host plant water content of potted wheat plants in order to test specific hypotheses regarding the effects of water stress on two problematic insect pests of wheat in the PNW. However, these controlled-environment greenhouse experiments imposed certain limitations that could be avoided through the incorporation of field experiments, but also potentially yield results different from what we observed in the greenhouse. For example, our results indicate a neutral effect of plant water stress on CLB larval feeding activity, but much different findings were presented in a study investigating drought stress responses by another herbivore within the same feeding guild as CLB. Franzke and Reinhold (2011) found that grasshoppers feeding on grasses supplied sixty percent less precipitation than the summer seasonal average in Europe showed enhanced reproductive performance (i.e., shorter development time and higher fecundity) compared to grasshoppers fed control plants.

By adapting the designs of these greenhouse experiments for implementation in a field setting, the scope of this research could be broadened by incorporating additional biotic factors that more accurately represent the ecological complexity of this and similar agrosystems. Barton and Ives (2014) demonstrated the ecological complexity between two aphid species and a generalist predator in alfalfa using a field experiment that experimentally simulated drought through rain exclusion. Drought treatments that reduced water content in alfalfa had no direct effect on the spotted aphid, *Therioaphis maculata*, but reduced numbers of the pea aphid, *Acyrthosiphon pisum*, which in high densities attract the Asian ladybeetle, *Harmonia axyridis*. As a result, ladybeetle densities became lower in drought treatments, thereby releasing spotted aphid densities through apparent competition (Barton and Ives 2014).

In addition to incorporating a field component, complementary greenhouse experiments could be used to include drought stress effects on these pests testing other important crop plant species grown in the PNW. Barley (*Hordeum vulgare*) and oats (*Avena sativa*), for example, are generally less resistant to CLB than wheat (Haynes and Gage 1981). Similarly, the aphids *M. festucae cerealium* and *R. padi* are oligophagous herbivores on multiple grass species. Davis et al. (2014) found that *M festucae cerealium* preferentially settled on wheat but did not discriminate between barley, oats and native grasses and exhibited highest fecundities on both wheat and barley. Using greenhouse experiments, Hale et al. (2003) tested the effects of plant water stress on *R. padi* performance using four closely related grass species, but found that drought stress treatments did not affect *R. padi* consistently, further demonstrating the need for testing multiple crop plant species.

Additional greenhouse experiments could also test for the effects of plant water stress on other regionally problematic herbivore species. The English grain aphid (*Sitobion avenae*), for example, demonstrates a sympatric distribution with both *M. festucae cerealium* and *R. padi* populations in the PNW. In a greenhouse study Gianoli (2000) found symmetrical competition between *R. padi* and *S. avenae* on seedling and tillering wheat plants, whereby the reproductive rate of each species was negatively impacted by presence of the other. However, no abiotic stress was imposed on plants in these experiments, warranting further investigations on multi-species responses to drought stress.

Continued modeling efforts incorporating field survey and experimental data could advance management of these pest herbivores greatly. Additionally, integrating these experimental data with pest phenology and climate models (e.g., Olfert et al. 2006 and Dai 2013, respectively) could assist with adapting findings from these and potential future studies for use by cereal growers in the PNW and elsewhere, incorporating both current and projected climate scenarios. Further investigations to these ends could help advance our understanding of the complex interactions between these insect pests and the environment under projected climate scenarios, where drought may be a more prevalent condition affecting agricultural systems in the Pacific Northwest and elsewhere in North America.

References

Barton, B.T., and A.R. Ives. 2014. Species interactions and a chain of indirect effects drived by reduced precipitation. Ecology 95: 486-494.

Dai, A. 2013. Increasing drought under global warming in observations and models. Nature Climate Change 3: 52-58.

Davis, T.S., Y. Wu, and S.D. Eigenbrode. 2014. Host settling behavior, reproductive performance, and effects on plant growth of an exotic cereal aphid, *Metopolophium festucae* subsp. *cerealium* (Hemiptera: Aphididae). Environmental Entomology 43: 682-688.

Franzke, A., and K. Reinhold. 2011. Stressing food plants by altering water availability affects grasshopper performance. Ecosphere 2: 1-13.

Gianoli, E. 2000. Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. Environmental Entomology 29: 213-219.

Hale, B.K., J.S. Bale, J. Pritchard, et al. 2003. Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. Ecological Entomology 28: 666-677.

Haynes, D.L., and S.H. Gage. 1981. The cereal leaf beetle in North America. Annual Review of Entomology 26: 259-287.

Hoffman, G.H., and S. Rao. 2011. Oviposition site selection on oat: the effect of plant architecture, plant and leaf age, tissue toughness, and hardness on cereal leaf beetle, *Oulema melanopus*. Entomologia Experimentalis et Applicata 141: 232-244.

Olfert, O., and R.M. Weiss. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus*, *Meligethes viridescens*, and *Ceutorhynchus obstrictus* in Canada. Agriculture, Ecosystems & Environment 113: 295-301.