FACTORS INFLUENCING BIRD CHERRY-OAT APHID (*RHOPALOSIPHUM PADI* L.) FEEDING BEHAVIOR AND FITNESS

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Abstract: The first objective of this dissertation was to determine if increasingly complex host plant habitats resulted in the most fit *Rhopalosiphum padi*. I hypothesized that the addition of suitable host plant species to *R. padi* rearing cages, in an effort to mimic heterogeneous agroecosystems, would increase aphid fitness. By comparing the weight and number of *R. padi* produced between treatments, the primary hypothesis that the availability/utilization of additional suitable host plants to aphid rearing cages would increase fitness was rejected. Conversely, the data revealed a potentially antagonistic relationship between host plants when grown in close proximity and resulted in a negative effect on aphid fitness. Factors including natal experience effects, plant-plant interactions, lack of host plant conditioning, and/or host plant composition effects may have impacted *R. padi* fitness in this study. The second objective of this study was to quantify host-plant feeding behaviors for *R. padi* reared under different conditions. Results from the first objective indicated that experimental *R. padi* reared uncrowded on wheat, under ideal environmental conditions, were larger. In comparison, *R. padi* from the source colony were substantially smaller and would be predicted to be less fit, and thus cause less plant injury. I hypothesized that differences in rearing conditions had the potential to influence *R. padi* feeding behavior. Behaviors were quantified using salivary sheath staining and electropenetrography techniques. Results supported the hypothesis that differences in rearing conditions of *R. padi* impact feeding behaviors, as significant differences were revealed for typical feeding behaviors between the two aphid rearing conditions. Results indicated that the most "fit" experimental colony aphids (i.e. larger and with higher fitness) may not be the best for plant injury evaluations, but because of their propensity to initiate more feeding attempts, may be highly beneficial in barley yellow dwarf virus (BYDV) transmission study evaluations. Alternatively, the "stressed" source colony, reared under crowded, less optimal environmental conditions, appear more likely to feed for extended periods of time and induce plant injury. These differences should be considered when evaluating the impact of aphid feeding on host plants during screening of resistant plant sources.

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CHAPTER I

INTRODUCTION

The United States is the third largest producer of wheat in the world and is projected to export 26.5 million tonnes of wheat during the 2017-18 production season (IGC 2017). The Southern Great Plains of the U.S. accounts for nearly 30% of the country's total wheat production (USDA-NASS 2017), and winter wheat, *Triticum aestivum* L., is most commonly grown in Oklahoma, with upwards of 6 million acres of Oklahoma land sown to hard red winter wheat annually (Luper et al. 2005).

Wheat is susceptible to barley yellow dwarf (BYD), a disease of cereal crops comprised of two viruses: barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CYDV). BYDV is a *Luteovirus* composed of several different strains (Flanders et al. 2006) that can only be transmitted by aphids which acquire the virus by feeding on the phloem sap of infected plants, often grasses or other crops (Gray et al. 1991; Power et al. 1991; Power and Gray 1995). There are over 25 species of aphid capable of transmitting BYD viruses (Halbert and Voegtlin 1995), however, the highly polyphagous *Rhopalosiphum padi* (L.) (bird cherry-oat aphid) (Blackman and Eastop 2006) is regarded as one of the most economically important, as this species possess the ability to efficiently transmit strains of both BYDV and CYDV infections (Gourmet et al. 1994; Chouhury et al. 2017) and cause direct feeding damage (Stern 1967; Jiménez-Martínez

et al. 2004). Mechanical damage to plants by aphids is caused by 1) the physical puncturing of cells by the stylets and 2) by the deposition of saliva during the feeding process (Miles 1999), however the level of damage inflicted on the plant varies by aphid species (Saheed et al. 2007). *Rhopalosiphum padi* is a heteroecious aphid species, as it typically alternates hosts throughout the year (Dixon 1971). However, it has been speculated that some populations of this aphid species located in the Central Plains of the U.S., specifically southern areas of Kansas and northern Oklahoma, no longer utilize host alternation as a means of survival. Instead, this species may have become monoecious and only reproduce parthenogenetically (Michaud 2008). Elliot and Kieckhefer (1989) suggest that this aphid species is able to survive colder temperatures in the northern reaches of the Central Plains by migrating down to the base of wheat plants or to just below the soil surface. It is plausible that *R. padi* populations commonly found in wheat in the Southern Plains are capable of utilizing this same survival strategy, however the biology and ecology of this aphid species specific to this region remains largely unexplored.

Current management efforts for *R. padi* are heavily focused on preventative and curative insecticide use (Royer 2016; Royer and Giles 2017). The development of *R. padi* resistant wheat cultivars, however, has the potential to protect yield while reducing management inputs. There have been considerable efforts aimed at identifying resistant germplasm sources and developing regionally adapted cultivars (Aradottir et al. 2017; Khan et al. 2017; Girvin et al. 2017). Most recently, hard red and soft white wheat cultivars from Kansas expressed resistance capable of suppressing *R. padi* populations or tolerance of its feeding Girvin et al. 2017). Such germplasm resistance screening assays are typically artificially infested with laboratory reared aphids, but *R. padi* are reared in a variety of conditions and on a variety of hosts prior to resistance screening efforts (Rochow 1969; Gray et al. 1998; Hesler and Tharp 2005; Razmjou et al. 2012; Aradottier et al. 2017). Variation among rearing protocols has the potential to undermine aphid fitness (Dixon 1985). Indeed, *R. padi* currently used for wheat germplasm screenings at Oklahoma State

University are significantly smaller than wild aphids that invade winter wheat fields in the fall (personal observation). It has been demonstrated that aphid body size is strongly affected by temperature and food quality (Dixon 1985) and small aphids been demonstrated to have reduced feeding capacity, reproduction, and ability to cause plant injury (Michaud 2012). Thus as a potential result of rearing condition variability, *R. padi* used in germplasm resistance evaluations may not be representative of the effects of wild populations (i.e. behavior and fitness).

Based on the typical heteroecious nature of *R. padi*, multiple host plants within a rearing environment may be essential for this species to maximize fitness. By mimicking a more naturally diverse environment, appropriate host plant complexity may allow for the production of aphids that are more representative of aphids that regularly colonize wheat fields. I hypothesized that increased host plant complexity within heteroecious aphid habitats has the potential to alter aphid fitness and influence feeding behavior. The objectives of this dissertation were:

I. Determine the effect of increased host plant complexity on aphid fitness. It is hypothesized that the addition of suitable host plant species to *Rhopalosiphum padi* rearing cages, in an effort to mimic the heterogeneous complexity of agroecosystems, will increase aphid fitness.

II. Quantify host-plant feeding behaviors for *Rhopalosiphum padi* reared under different conditions. It is hypothesized that differences in *R. padi* rearing conditions will influence aphid feeding behaviors.

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CHAPTER II

REVIEW OF LITERATURE

Wheat Production in the United States and Southern Great Plains

Cereal crops are a valuable resource for human food and a component of livestock feed the world over, with over 2,000 million tons of seed projected to be produced in the 2017-18 growing season (IGC 2017). According to the International Grain Council, global wheat production is projected to be upwards of 732 million tons for the 2017-18 growing season. Approximately 69% of that seed will be used as food products, while another 19% will be utilized in livestock feed. The United States is a significant producer of wheat, with production estimates for the current growing season at approximately 47 million tons, with approximately half of all wheat produced in the U.S. being exported (IGC 2017). In terms of planted acreage and production, wheat is the third largest field crop in the United States, ranking only behind corn and soybeans (IGC 2017; USDA-ERS 2017).

The Southern Plains of the United States, specifically the states of Oklahoma and Texas, are significant producers of wheat. Combined, these two states harvested a total of 6.3 million acres, resulting in over 226 million bushels of wheat in 2016 (USDA-NASS 2016). Winter wheat, *Triticum aestivum* L., is most commonly grown in Oklahoma; its uses range from human food products to livestock feed and forage, with upwards of 6 million acres of land sown to hard red winter wheat annually (Luper et al. 2005). Wheat production is vital to the cattle industry,

which utilizes 30-50% of the wheat acreage in Oklahoma for grazing during the winter season, helping to offset the cost of feed (Edwards 2015). Depending upon production goals and environmental conditions, winter wheat is typically planted from early September through mid-November. However, wheat that will be used as forage only on dual purpose (i.e. grazing and grain yield) is planted even earlier, in late August into early September. Early planting allows wheat plants to grow tall enough to allow cattle grazing starting in November. Typically, for systems with grain, harvest begins in late May, but can run through late June when plants are mature late or if rainfall prevents early harvest (USDA-NASS 1997).

Barley Production in the United States and Southern Great Plains

Globally, barley (*Hordeum vulgare* L.) is produced at much lower volume amounts than wheat, with only 138 million tons projected to be produced during the 2017-18 growing season. In 2016, approximately 2.5 million acres of barley were harvested in the U.S., producing 199 million bushels. The majority of U.S. barley production is utilized in livestock feed (USDA-NASS 2017). Barley parallels wheat as it has similar planting and harvest dates and has winter and spring varieties (USDA-NASS 1997). High protein, or "feed barleys," are often used for animal feed "fatteners" and can be fed to animals whole, ground, flaked, or in pelleted forms (Garvin et al. 2003). Pasturing on barley can also serve to supplement cattle before the "fattening" period on the grain feed (Pope et al. 1963).

Important Pathogens and Arthropod Pests on Cereal Crops

As small grains crops, both wheat and barley are susceptible to barley yellow dwarf (BYD). In 1951, Oswald and Houston (1953) first described BYD in California barley; now barley yellow dwarf (BYD) is considered the most highly detrimental viral disease of small grains worldwide (Flanders et al. 2006). Estimated yield losses of 13-25 kg/ha have been reported in wheat due to BYD infection (McKirdy et al. 2002). BYD is caused by a suite of viruses in two

main genera in the *Luteoviridae* family: *Luteovirus* and *Polerovirus* (Liu et al. 2007). Barley yellow dwarf virus (BYDV) is a *Luteovirus*, composed of strains MAV, PAV, and PAS, while cereal yellow dwarf virus (CYDV) is composed of strains RPV and RPS (Flanders et al. 2006). Strains SGV, GPV, and RMV have been identified, but have not yet been categorized as either a *Luteovirus* or a *Polerovirus* but are labeled in the literature as BYDV strains (Van Regenmortel et al. 2000; Wu et al. 2011; Chouhury et al. 2017). The strains of BYDV and CYDV are named after their specific species vector (Rochow 1969; Rochow and Muller 1971).

While over 25 species of aphid are capable of transmitting BYD viruses (Halbert and Voegtlin 1995), *Rhopalosiphum padi* (L.), the bird cherry-oat aphid, is regarded as the most detrimental aphid vector because of its ability to transmit both BYDV and CYDV infections (Gourmet et al. 1994; Chouhury et al. 2017). Some of the other key aphid species include *Schizaphis graminum* (green bug), *Sitobion avenae* (English grain aphid), and *Rhopalosiphum maidis* (corn leaf aphid); each of these aphid species transmits specific strains of BYD. *Sitobion avenae*, *Rhopalosiphum padi*, *Rhopalosiphum maidis*, and *Schizaphis graminum* are the most effective vectors of BYDV-MAV, -RMV, -SGV, and CYDV-RPV. While BYDV-PAV is most efficiently transmitted by *R. padi* and *S. avenae* (Chouhury et al. 2017). *R. padi* is not limited to transmitting only BYD viruses, but is also a known vector for maize dwarf mosaic virus (MDMV), a viral disease of many perennial grasses and annual crop species (including small grains) within the Gramineae family (Thongmeearkom et al. 1976).

In general, BYD symptoms on cereal crops may include: leaf discoloration, curling or chlorosis of the leaves, stunted or dwarfed plants (if infection occurs early in development), tiller reduction, negative impact on kernel weight and number per spike, and impaired root growth (Riedell et al. 2003). Reduced grain yield is a potential result of infection, as is plant death if the infection is severe enough (Flanders et al. 2006). In barley, BYD infection typically causes bright

yellow leaf discoloration, while infections in wheat cause discoloration ranging from yellow to reddish-purple (Chouhury et al. 2017).

Visual diagnosis of BYD can be difficult, as symptoms can mimic a range of other crop ailments. Symptoms may mimic drought, injury from cold temperatures, nutrient deficiency, herbicide damage, or other viral infections (i.e. wheat streak mosaic virus or wheat spindle streak virus). Further diagnosis complications arise from the highly variable nature of BYD disease symptoms. Symptom expression is remarkably inconsistent and depend on a variety of factors, such as the strain of virus, crop species and variety, weather and soil conditions, and growth stage of the crop at the time of infection (Bruehl 1961; Rochow 1961; Burnett 1984; D'Arcy 1995). For example, early infections in wheat result in reddish-purple to yellow flag leaves and stunted plants in the spring. Whereas infections occurring in spring result in leaf discoloration (typically yellow) and an absence of plant stunting (Flanders et al. 2006).

Transmission of BYD to healthy plants can only occur when plants are fed upon by aphids carrying the virus (known as viruliferous aphids). The complex of BYD viruses cannot be spread mechanically or through propagation (Miller et al. 2002a). Aphids are only able to obtain the virus by feeding on the sap of infected plants, often acquiring viruses from grasses or other crops. Virus acquisition time varies with the virus strain-aphid vector combination, but can range from minutes to hours (Gray et al. 1991; Power et al. 1991; Power and Gray 1995). BYD is a circulative virus and viruliferous aphids can be, but are not always, infective for the rest of their lives (approximately 21 d) (Nault 1997). Viruses acquired by adult aphids cannot be directly passed to their offspring (Flanders et al. 2006).

In order for a virus to be transmitted by an aphid vector, virions must be innoculated from an infected plant into appropriate tissues of a healthy one. Virions are particles of a virus that contain an RNA or DNA core surrounded by a protein coat (Katis et al. 2007). The transmission

cycle is a series of a up to four events: 1) Acquisition, when the vector obtains the virus particles from an infected plant, 2) Retention, when the vector carries the virions internally or externally at specific carrier sites, 3) Latent period, transmission is delayed and cannot occur immediately after acquisition, 4) Inoculation, when virions are passed into a susceptible plant in a manner that results in infection. Regarding aphid vectors, there are three modes of transmission: non-persistent, semi-persistent, and persistent. These categories are components of the virus-vector relationship and are based upon the retention period of the virions by the aphid. These categories are often used to describe a virus (i.e. BYDV is a persistently transmitted virus) (Katis et al. 2007.)

Non-persistent transmission, often referred to as "stylet-borne" transmission because of the retention of the virions within the aphid (Pirone and Perry 2002) requires only a brief period (less than one minute) of stylet penetration for virion acquisition and inoculation. There is no latent period, allowing complete transmission to occur in a matter of minutes. Aphids that acquire these types of viruses quickly lose the ability to inoculate other plants (Katis et al. 2007). Semi-persistent transmission also lacks a latent period, however acquisition and inoculation require more time; aphids must have access to an infected plant for at least 15 minutes in order to acquire the virions (Palacios et al. 2002). Aphids acquiring virions in this category are able to inoculate for longer periods, in some cases up to two days after obtaining the virions. Semi-persistently transmitted viruses remain attached to the cuticular receptors longer. Viruses in these two transmission categories are considered to be non-circulative because of where the virions reside inside of their aphid vector (i.e. on/in the foregut or stylets) (Katis et al. 2007). In either case, decreased inoculation period can be attributed to 1) the release of virions from the foregut receptors and 2) molting of the stylets and foregut lining (Fereres and Collar 2001; Katis et al. 2007).

Confined to phloem elements within the plant (i.e. sieve tube and accompanying cells), persistent transmission requires long periods of contact with an infected plant, with a latent period occurring between the acquisition and inoculation phases (Katis et al. 2007). Aphid vectors of persistent viruses retain the ability to transmit the virions for several days after acquisition, and in some cases, even for the duration of their lives (Gray and Gildow 2003; Katis et al. 2007). Viruses in this category are considered to be circulative or propagative in nature. Circulative viruses are non-propagative and must cycle through the insect gut, into the hemolymph, then into the accessory salivary glands before inoculation can occur (Figure 2.1). Persistently transmitted propagative viruses follow the same requirement of passing through the aphid gut, hemolymph, and salivary glands, but are capable of repliating within their aphid vector (Nault 1997).

Aphid Biology and Ecology

Aphids (Hemiptera, Sternorrhyncha: Aphididae) are small, pear-shaped, soft-bodied insects that are typically found feeding on stems and leaves of plants, drawing sap through their needle-like mouthparts, known as "piercing-sucking" mouthparts (Figure 2.2). Aphids tend to amass in large numbers, with the population consisting of individuals in all stages of development. Along with their characteristic pear-shape, these insects have long, slender antennae (often longer than their bodies) and a pair of cornicles located at the posterior end of their abdomen. The cornicles are used to excrete defensive secretions. Winged morphs, known as alates, typically hold their wings vertically over their bodies and can be identified by the proportions of the front and hind wing and their venation (Triplehorn and Johnson 2005).

Aphid life cycles can be extremely complex with different stages, each with their own distinct aphid morph, or specialist aphid life stage. These morphs harbor specific functions that are required for the completion of that life stage. There are a variety of morphs, such as

reproductive and dispersal specialists, and those that are capable of surviving undesirable climatic or nutritional conditions (Williams and Dixon 2007).

The two most common types of aphid life cycles are heteroecious, or host-alternating, and monoecious, or non-host alternating. These life cycles have been described based on how the aphids utilize their host plants. Heteroecious aphids feed on different species of plants, depending on the season and geographic location. In general, the primary host is favored during colder, more stressful conditions, while the secondary host is preferred during favorable environmental conditions (Figure 2.3). Distinction among aphid populations in Europe, the Northern Great Plains, and the Southern Great Plains regions of the U.S. will be expanded upon during discussion of bird cherry-oat aphid biology later in this chapter. Monoecious aphids only feed on one species of plant and do not migrate to another species (Figure 2.4). When migration does occur, it is confined to similar species of their host plant (Williams and Dixon 2007).

Heteroecious aphids have been well studied due to their propensity for utilizing several crop plant species as their secondary hosts, despite the fact that only approximately 10% of all aphid species display this behavior (Blackman and Eastop 2007). Heteroecious aphids mate in autumn on their primary host plant; often the primary host plant is a woody plant species. Eggs are deposited on the primary host and remain for the winter. Eggs hatch in spring and produce two distinct morphs: fundatrices, also referred to as stem mothers, and fundatrigenia. The fundatrix is generally apterous (wingless), although winged forms or vestigial wings are found in some species (Williams and Dixon 2007). The fundatrix bears reduced sensory organs and dispersal capabilities, but harbors a larger number of ovarioles (Dixon 1975; Wellings et al. 1980; Williams and Dixon 2007). The increased number of ovarioles allows for higher offspring production (Wellings et al. 1980; Leather and Wellings 1981; Williams and Dixon 2007). The fundatrigenia are similar in both morphology and function to the fundatrices. The main purpose of these morphs is to produce large numbers of the parthenogenetic generations that will occupy

the secondary hosts during the spring and summer (Williams and Dixon 2007). Taylor (1977) estimated that only 0.2-1% of these offspring will successfully locate the secondary host, which explains the strategy of producting of so many offspring and the need for two reproductive morphs.

Successful spring migrants will colonize the secondary host and will reproduce parthenogenetically throughout the summer (Williams and Dixon 2007). It is during this season that heteroecous aphids tend to become pests in crops due to their rapidly expanding populations. Near the end of the summer season, alate (winged) males and gynoparae morphs are produced; both will migrate back to the primary host. Gynoparae give rise to oviparae (sexual females) morphs. The oviparae mate with the males and produce eggs that will overwinter on the primary host to perpetuate the cycle (Williams and Dixon 2007). The term 'holocyclic' is given to aphids that interrupt parthenogenetic reproduction with sexual reproduction (Williams and Dixon 2007).

Monoecious, or non-host-alternating aphids, tend to remain on a single plant species or only migrate among plants of closely related species. If the aphid species produces eggs, eggs are laid on the same host plants that are utilized by all parthenogenetically produced offspring. However, not all aphid species produce eggs. These species are termed 'anholocyclic' and reproduce parthenogenetically. Further complexity arises in aphid biology with some species displaying both holocyclic and anholocyclic reproduction (Williams and Dixon 2007).

Most monoecious aphids are typically not known as agriculturally significant pests due to their habit of infesting woody plant species (typically trees). However, there are some species that inhabit crop plant species and can be found in these areas throughout the year; examples include *Acrythosiphon pisum* (pea aphid) or *Sitobion avenae* (English grain aphid). It is believed that these species evolved from a heteroecious aphid species and simply no longer utilize the primary host (Williams and Dixon 2007). The life cycle of monoecious aphids closely mirrors that of

heteroecious aphids, with only a few slight differences in morph types. Unlike the fundatrix morph found in heteroecious aphid species, monoecious fundatrices are more similar in morphology to the other morphs found in their life cycle. These aphids reproduce parthenogenetically throughout the summer and produce sexuparae morphs in autumn, which produce males and oviparae. Males and oviparae mate and produce eggs that will overwinter (Williams and Dixon 2007).

Rhopalosiphum padi Biology and Ecology

Rhopalosiphum padi (L.) (bird cherry-oat aphid) is a arguably the most economically important aphid species in wheat systems because of its propensity for transmitting BYDV and causing direct damage by feeding (Jiménez-Martínez et al. 2004, Stern 1967). Of the several cereal aphid species known to infest wheat in the U.S., green bug, (*Schizaphis graminum*) and bird cherry-oat aphid (BCOA) are regularly the most damaging (Elliott, Kieckhefer, and Walgenbach 1990). Bird cherry-oat aphids are a large species of aphid, approximately 1/16 inch long, commonly found in winter and early spring wheat fields. These aphids are characterized by a pear-shaped body, flanked by black-tipped legs and antennae, dark olive green body color, with a distinguishing rusty orange to brown colored area around the base of the cornicles (Whitworth and Ahmad 2008). This species of aphid is heteroecious (Moran 1988). These aphids divide their time between their primary hosts (a wide variety of plant species), and a secondary host, typically grasses including seasonal crop plants such as wheat or barley (Taheri, Razmjou and Rastegari 2010).

In Europe, the bird cherry-oat aphid life cycle follows the general life cycle pattern previously described for heteroecious aphid species. In North America, overwintering eggs are commonly laid on *Prunus padus*, commonly known as bird cherry, which serves as the primary host. Surrounding grasses and cereal crops serve as their secondary hosts (Dixon 1971) (Figure

2.5). Bird cherry-oat aphids can display both holocyclic and anholocyclic life cycles. Often, anholocyclic life cycles are initiated when the habitat range of the aphid species is greater than that of its primary host (Williams and Dixon 2007). In the United Kingdom, anholocyclic populations of the bird cherry-oat aphid have become more common as the primary host becomes more scarce geographically (Williams et al. 2000; Williams and Dixon 2007).

While the aforementioned heteroecious life cycle is typical of European BCOA populations, very little is known about the annual life cycle of the bird cherry-oat aphid in the Southern Great Plains of the United States. It has been suggested that anholocyclic populations of BCOA occur in Oklahoma and southern Kansas (Michaud 2008). In the northern Great Plains, the typical heteroecious life cycle of this aphid species is predominately suppressed, with aphids migrating from the south to recolonize cereals each spring (Elliott and Kieckhefer 1989). In the Southern Great Plains, BCOA has been observed to infest winter wheat throughout the entire growing season. In early fall, BCOA will position themselves at the crown of the plant, often below the soil level, to escape cold temperatures and they can survive until the soil freezes (Elliott and Kieckhefer 1989). Bird cherry-oat aphids are able to survive mild winters (Carter et al. 1980), thus it may be inferred that the combination of adaptation to mild winter temperatures and subsequent subterranean survival is allowing BCOA to persist in the southern plains during most of the winter wheat growing season.

In North America, the bird cherry-oat aphid typically colonizes bird cherry as its primary host in northern climates. However, this aphid is highly polyphagous and can utilize a variety of plants as its primary host. In Europe, this aphid species has been found colonizing weed species such as *Capsella bursa-pastoris*, commonly called Shepherd's purse, and *Stellaria media,* or common chickweed (Blackman and Eastop 2007). Both of these weed species are readily found in the United States and in Oklahoma. *Stellaria media* is considered an annual, however it has the ability to tolerate colder temperatures, allowing it to persist during winter months in milder

climates. Because it can readily establish in disturbed soils, *Stellaria media* can become a serious problem in pastures and overwintering cereal crops (Wertz 2015). *Capsella bursa-pastoris* is considered a winter annual, but can survive all year in milder climates. Much like *Stellaria media*, this weed species prefers disturbed soils and is readily found inhabiting agricultural fields (Azulai et al. 2014).

Host Plant Selection and Aphid Feeding Behavior

Aphids must be able to efficiently locate and utilize their host plants. This ability becomes even more important in regard to heteroecious aphids that must are to distinguish their secondary host plants from their primary host plants. Approximately 10% of all aphid species demonstrate seasonal host alternation between two completely different host plant species (Eastop 1986; Dixon 1990), with only 0.5% of those species posing a direct threat to agricultural crops (Irwin et al. 2007). It is believed that several factors contribute to host plant alternation; including factors such as: host plant nutritional quality, presence and activity of natural enemies, or a combination of such factors (Davidson 1927; Mordvilko 1928; Kennedy and Booth 1951; Dixon 1971, 1985, 1990). Moran (1983) further suggested that the summer host-plant species are nutritionally superior to the overwintering hosts, while the overwintering host plant provides superior ovipositioning sites. It is thought that aphids must be able to efficiently distinguish between their host plants and are therefore reliant on plant stimuli for locating hosts.

Successful host finding is dependent upon the aphid's ability to distinguish between a suitable host and a poor and/or non-host. Aphids employ a step-wise behavioral process for finding a host: orientation to the host plant, locating the host plant, host selection, and finally acceptance of the host plant (Pettersson et al. 2007). Orientation to the host plant is accomplished through visual and olfactory cues. It has been well established that the majority of aphid species tend to favor flying during daylight hours (Taylor 1958; Johnson 1969; Dixon 1988; Isard and

Irwin 1993; Isard and Gage 2001; Irwin et al. 2007) and favor yellow-colored surfaces for landing (Moericke 1955; Prokopy and Owens 1983; Robert 1987; Petterson et al. 2007). The bird cherry-oat aphid, however, differs in this aspect by tending to favor green-colored landing surfaces instead (Hardie 1989; Nottingham et al. 1991; Pettersson et al. 2007). It is believed that aphid landing is not a passive event, but rather an insect-guided one (Irwin et al. 2007). Although aphids are known for being weak flyers, migrating alate morphs of the bird cherry-oat aphid have been observed in sustained flight for 2-3 hours before responding to host plant stimuli (David and Hardie 1988; Nottingham and Hardie 1989; Nottingham et al. 1991a; Pettersson et al. 2007).

Historically, it was believed that plant volatiles did not play a significant role in host plant location by alate aphids (Kennedy et al. 1963a, b). However, studies by Kennedy (1986) changed this opinion and plant volatiles are now regarded to be important not only for locating host plants, but also discerning hosts from non-hosts; although the mechanisms by which these events are accomplished are not understood (Pettersson et al. 2007). Extensive electrophysiological studies have been performed that demonstrate the possession and reaction of olfactory organs on aphid antennae to an assortment of plant volatiles but correlation with behavior have been difficult to demonstrate (See Anderson and Bromley 1987; Pickett et al. 1992; Visser and Piron 1997; Park and Hardie 1998; Pickett and Glinwood 2007).

The primary rhinaria are the major olfactory organs found on each of the two terminal segments of the antennae of all aphid morphs (adults and immature stages). These organs have been found to contain receptors for common leaf volatiles (van Giessen et al. 1994: Park and Hardie 2002, 2004; Pettersson et al. 2007). Alate adults also possess a secondary set of rhinaria, found on the third antennal segment. These organs are often not as well developed or are entirely absent on apterous adults. This is to be expected, however, as the role of alate adults is to find new hosts, while apterous adults are responsible for producing offspring and do not wander from the host. In electroantennagram (EAG) studies, van Giessen et al. (1994) and Park and Hardie

(2002) were unable to determine the function of the secondary rhinaria and concluded they contribute little to the comprehensive EAG response of aphids to plant volatiles.

In EAG studies with bird cherry-oat aphid, differences in EAG responses were significant between alate virginoparae and gynoparae. Virginoparae morphs were more sensitive to benzaldehyde, a significant component of volatiles emitted by *P. padus* (bird cherry) the winter host of bird cherry-oat aphid. However, only the gynoparae produced a behavioral response to benzaldehyde in an olfactometer (Pettersson 1970; Park et al. 2000; Pettersson et al. 2007). Even with these findings, no general conclusions can be made due to small sample sizes of aphid species being testing for olfactory responses to plant volatiles, whether from host or non-host plants (Pettersson et al. 2007).

Landing behavior of flying aphids appears to be affected by both the chemistry and the morphology of the plant, with aphids typically preferring to settle on a portion of the plant that provides the best quality and quantity of food. These areas of the plant, generally lower leaf surfaces (Müller 1984), tend to also provide shelter from adverse weather and protection from natural enemies (Pettersson et al. 2007). The influence of plant structures (such as waxes and trichomes) on aphid behavior have been extensively studied (Kilngauf et al. 1978, Lapointe and Tingey 1986, Åhman 1990, Tingey 1991, Powell et al. 1999). Some species of aphids, such as the bird cherry-oat aphid, tend to show seasonal preference in feeding sites on their host plants. Wiktelius et al. (1990) discovered colonies of bird cherry-oat aphid in cereal crops prefer feeding at the base of the stem or just below the soil surface of the plant in early summer, with colonies then shifting to the upper surfaces of the plant by late summer.

Aphids are exceptionally sensitive to changes in their environment (van Emden 1971), with any change or variation impacting their olfactory and gustatory responses when initiating feeding (Pollard 1973). Aphid feeding employs a specific series of behavioral events, with the

completion of one triggering the beginning of another. These behaviors follow a precise pattern: 1) host plant orientation, 2) host acceptance through examination and probing, 3) feeding site location 4) ingestion 5) stylet withdrawal, 6) reactions to adverse materials (toxins, viruses, etc.) and cleaning of the mouth parts. Each action in the sequence has been characterized by specific behaviors and body movements (Pollard 1973).

Much like the entire feeding process, probing itself is composed of a series of events (host plant orientation and acceptance have been previously discussed). This basis of aphid feeding behavior was observed by Ibbotson and Kennedy (1959) with apterous adult *A. fabae*. During this study, antennae were noted to extend forward and vibrate, the rostrum (Figure 2.1 B) was protracted and prodded along the leaf surface, and the antennae settle back over the body when an adequate feeding site is determined. If the leaf surface was uneven, aphids were observed moving sideways, while dragging the tip of the rostrum across the surface before continuing to prod. Purposeful side to side probing behavior was first described by Zweigelt (1915), and it was suggested that taste organs located on the stylets tips were responsible for guiding the stylets. However, Forbes (1966) and Parrish (1967) discovered a pair of nerve extensions (dendrites) within each mandibular stylet. These nerves are believed to serve as proprioreceptors, aiding the insect in guiding stylet position and movement (Wensler 1977). Initial probing of the stylets are often short and are termed “test probes.” It is believed that chemicals from the plant are sampled and “tasted” during test probes to determine if the plant is a suitable host (Pettersson et al. 2007). Test probes occur before any sustained feeding, wherein the aphid will more deeply penetrate the plant tissues and excrete saliva (Tjallingii 2006).

Electropenetrography

The role of saliva and salivary sheaths in feeding and virus transmission by insects with piercing-sucking mouthparts has been well documented (Prillieux 1878; Büsgen 1891; Zweigelt

1915; Smith 1926, 1933; Withycombe 1926; Weber 1928; Bennett 1934; Storey 1939; Braun 1951; Day and Irzykiewicz 1954; Martini 1958; Miles 1958, 1959, 1965, 1972; Kloft 1960; Ehrhardt 1961; Esau 1961; Kloft and Ehrhardt 1962; Saxena 1963; McLean and Kinsey 1965, 1967; Hennig 1966; Nault 1966; Kinsey and McLean 1967; Naito and Masaki 1967; Pollard 1973). The majority of piercing-sucking insects produce salivary sheaths when feeding (Miles 1972). This sheath is formed from viscous fluid composed of proteins and lipids that forms a protective, lubricating gel around the stylet bundle that hardens upon exudation (Figure 2.1 C, D) as it penetrates the plant. As the stylets are withdrawn at the termination of feeding, this sheath is left behind within in the plant (Backus et al. 1988).

Several different histological studies were developed to study stylet sheath structure (Houston et al. 1947; McLean and Kinsey 1967; Pollard 1971; Kimmins and Tjallingii 1985). Barlow and McCully (1972) used histological studies to determine aohid stylet location within a plant by cutting the stylets during feeding and collecting the fluid exudates. More modern studies by Will et al. (2012) employed the used of confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) in order to study salivary sheath structure. And while these studies merited helpful information regarding the structure of sheaths, they could not be performed in a time-efficient manner (Backus et al. 1988).

Once the stylets are withdrawn, a flange is typically left on the surface of the plant leaf. In early studies (Bowling 1979; Viator et al. 1983; Marion-Poll et al. 1987), this flange was stained and used as an indication of an underlying sheath. However, in some insects, the presence of a surface flange does not always indicate an underlying sheath (Cobben 1978). Backus et al. (1988) developed a staining technique for leafhopper salivary sheaths that results in staining of the entire sheath within the plant. This technique, modified from McBride (1936), can be utilized with any insect that produces a salivary sheath. By staining and counting salivary sheaths, it is possible to quantify feeding attempts by piercing-sucking insects.

In contrast, histological and virus transmission studies cannot completely reveal what the insect is doing during active feeding. Electropenetrography (EPG) is a technique that has been evolving since the late 1950s when McLean and Kinsey (1964) first developed an electric monitoring system for studying insect feeding behavior, and has allowed for major breakthroughs describing piercing-sucking insect behavior. EPG monitors are comprised of two components that are electrically connected to one another: a voltage source and an input resistor. These components are housed in a box that has external receptacles for each: an output receptacle (connected to the voltage source) and an input receptacle (connected to the input resistor). By connecting the output receptacle to the input receptacle (via a wire) it creates a simple electrical circuit by allowing electricity to flow from the voltage source, through the input resistor, and back to the voltage source (Walker 2000). In order to study the feeding behavior of piercing-sucking insects, the insect and plant are incorporated into the circuit (Walker 2000, Pettersson et al. 2007) (Figure 2.6). Typically, a fine gold wire (2.5-25µm) is attached to the dorsal surface of the insect with conductive paint or glue, the wire is attached to a conductive stub that is inserted into the input of a head stage amplifier (head amp), and the head amp is attached to the input receptacle by a wire. A plant electrode, typically a stiff copper wire, is inserted into the soil at the base of the plant (near the roots) and is attached to the output receptacle on the EPG monitor by a wire (Walker 2000; Backus et al. 2016). The electrical current passes from the voltage source, through the plant, through the insect, through the input resistor, and back to the voltage source when the insect inserts its stylets into the plant, and thus, completing the circuit (Walker 2000).

Fluids within the stylet food and salivary canals are ionically charged, allowing the electricity to pass from the plant through the insect (Pettersson et al. 2007, Backus et al. 2016). Electrical currents follow the path of least resistance. In this system, electricity flows through both the food and salivary canals within the stylets, continuing through the insect tissues, across the cuticle, passing into the conductive glue or paint, then into the gold wire (Figure 2.7) (Walker

2000). The constant electrical signal from the plant becomes variable (i.e. fluctuation in voltage) due to insect feeding (Backus et al. 2016), producing the visible waveform on the computer monitor that is recorded.

The design of first generation of EPG monitors were influenced by radio technology and utilized AC (alternating current) electrical signals. This system employed low amplifier sensitivity, or input impedance of 106 Ohms (McLean and Weigt 1968; Backus et al. 2016). The low Ri output signals produced by this system were caused primarily by the production of electrical resistance, or variable resistance, (Ra) of ionic charges in the saliva or sap fluids as they passed through the insect stylets (Walker 2000). Or more simply, the electrical current cannot pass as easily through the insect as it does through copper wire, causing the insect-plant interface to act as an electrical resistor; making this interface the biological component of the system.

Early recorders were able to detect information pertaining to the beginning and end of stylet probing, stylet pathway mechanisms (secretion of saliva and salivary sheath formation), stylet movement, including: extension, retraction, partial withdrawal, and contact with plant fluids (phloem and xylem) (Backus et al. 2005). Few AC monitors could detect biological voltage in the circuit. This occurred for several reasons: 1) the use of low Ri levels resulted in practically no electromotive force, or 'emf' (i.e. streaming potential or biological voltage) output, 2) the use of slow-response strip chart recorders, which were standard at the time, 3) the original use of bandpass filters found in several AC designs were discovered to block the emf signal output (Backus et al. 2000; Tjallingii 2000; Backus and Bennett 2009; Backus et al. 2016).

The second generation of EPG monitors were developed in the late 1960s to early 1970s. Advancements in electronics, such as improved solid-state transistors (called operation amplifiers or more simply "op amps") led to more highly sophisticated amplifiers and recording systems, first introduced by Schaefers (1966). However, with the work of Tjallingii (1978), this generation

of EPG monitors were vastly improved. Tjallingii's improvements included: DC (direct current) applied signal (leading to the second generation moniker of "DC monitor" or "DC system"), utilization of op amps in printed circuits, the implementation of Faraday cages to block environmental electronic "noise," (see Figure 2.6) and FM tape recorders and/or rapid-response strip chart recorders. Additionally, Tjallingii included higher amplifier sensitivity, with Ri of either 109 or 1013 Ohms (Tjallingii 1978, 1985; Backus et al. 2016). The modern understanding of EPG theory is based on analysis and concepts presented by Tjallingii (1978, 1985). Here, Tjallingii introduced the concept of blending resistance (R, previously Ra) and emf components into one output signal (Walker 2000; Backus et al. 2016). These components are considered to be the electrical origins of a waveform and thus, are the foundation upon which modern EPG science is built.

The emf component at the plant-insect interface is comprised of two mechanisms. The first occurs when the stylet tip ruptures the plant cell membrane, interrupting the charge separation between the internal and external cell domains. This disruption in the cell membrane produces two electrical effects that are visible in the waveform output: 1) abrupt voltage drops, occurs when the stylets pierce the cell membrane; and 2) positive or negative voltage levels, indicating stylet tip position (intracellular vs. extracellular). The second emf mechanism is comprised of miniscule voltages that occur in conductive fluids by charge separation as the fluids pass through the stylet canals (Walker 2000; Backus et al. 2016). These streaming potentials produce waveforms of regular pattern, caused primarily by the consistent pumping action of the insect's cibarial pump (Figure 2.8) (Walker 2000). Detecting both the R component and the emf component simultaneously was made possible by this EPG monitor's increased Ri level (Backus et al. 2016).

Understanding the electrical origin of the signal is paramount to discerning its biological meaning (Backus et al. 2016). Indeed, Tjallingii (1978, 1985) developed the theory of the

sigmoidal emf responsiveness curve, or R/emf responsiveness curve (Backus and Bennett 2009). This theory describes and graphically depicts the relationship between the proportion of emf output signal from the insect (0-100%) in relation to the Ri level (106-1013 Ohms) (Figure 2.9) (Backus et al. 2016). Lower Ri levels produce less emf component in the total signal output, while lower emf proportions result in higher R proportions in the output signal, due to the reciprocal nature of the R/emf components within the total output signal (Backus et al. 2000; Walker 2000; Backus and Bennett 2009; Backus et al. 2016). The responsiveness curve shifts right or left depending on the size of the insect. Larger insects, such as leafhoppers, have larger food and salivary canals, and thus greater conductivity. Utilizing lower Ri levels on these insects results in detection of more emf in the total signal. Smaller insects, such as aphids, require higher Ri levels to detect emf in the output signal. The greatest number of waveforms are detected at the Ri level that results in a 50:50 ratio of R and emf components. When the R/emf components are balanced, the greatest representation of probing behaviors of the insect are achieved (Backus et al. 2016).

The third generation of EPG monitors was named the “universal” EPG monitor because of its ability to screen a variety of insects. Designed by Backus and Bennett (2009), this system utilizes an AC or DC applied signal, selectable Ri levels (106-1010 or 1013 Ohms, respectively), and modern technological components. The controls of this system are more sensitive, scalable, and include a voltage offset knob, allowing the user to correct inverted signal outputs and high resolution dials. These improvements make it possible to categorize the output signal as either emf or R derived (Backus et al. 2013, Pearson et al. 2014). All of these components make it possible for users to precisely reproduce and tailor settings to the needs of specific insect species. It is because of this capability that it has been possible to create a catalogue of output waveforms that quantify feeding behaviors, based on the characteristics of output signals produced at

different Ri levels from different insects (Backus et al. 2013; Pearson et al. 2014; Backus et al. 2016).

Distinct waveforms have been identified for aphids and can be correlated to probing behaviors (Figure 2.10) (Backus et al. 2016). In terms of biological meaning of EPG output, the waveforms are correlated with specific stylet locations and behaviors within the plant tissues. These behaviors include parameters such as stylet tip location within vascular or non-vascular tissues, stylet movement, salivation, and depth of the probe (Walker 2000, Backus et al. 2016). These correlations have been determined through histological studies of stylet sheaths and direct observation of insect feeding behavior on transparent diets (Walker 2000, Backus et al. 2016). Stylet depth within the plant is considered to be a strong R component, as the voltage level increases or decreases depending upon their position (i.e. intercellular vs. extracellular) (Jiang and Walker 2001; Backus et al. 2005; Backus et al. 2016). Another key characteristic is called an X wave (Backus et al. 2009), which is an easily recognizable output signal that is characterized by a repeating pattern (McLean and Kinsey 1967). These waves are considered as landmark waveforms, indicating contact and penetration of the insect stylet into a preferred feeding site within the plant; typically this signals the beginning of long-term ingestion (Backus et al. 2016). Currently, only insects that produced salivary sheaths produce an X wave (McLean and Kinsey 1967; Backus et al. 2009; Backus et al. 2016).

Aphids have served as a model study species for EPG and nearly all aphid species studied display similar waveforms with distinct feeding behaviors (Backus et al. 2016). As a result, aphid EPG waveforms have been characterized and divided into three discernable stages: 1) pathway phase, 2) phloem phase, and 3) xylem phase, with an addition of a transition phase (i.e. X wave) that switches between pathway and phloem. These three stages are then further broken down into their own categories with distinct waveforms (Backus et al. 2016). Identified by Tjallingii (1978), the pathway phase is comprised of waveforms A, B, C, F and potential drop (pd) (Figure 2.10 A,

B). The phloem phase is characterized by the E1 (phloem salivation) and E2 (phloem ingestion) waveforms (Figure 2.10 G, H), while the xylem phase is solely characterized by the G waveform (Tjallingii 1987) (Figure 2.10 E).

Waveforms A, B, and C are observed after the aphid's initial stylet penetration into the plant. This penetration causes an immediate, positive spike in voltage (Tjallingii 1978; Backus et al. 2016). Because these waveforms occur in a consecutive sequence, they are termed "pathway activities" (Tjallingii and Hogen Esch 1993; Backus et al. 2016). During this pathway phase, several intracellular probes occur, and these probes are observed as sudden voltage drops called potential drops (pd), with a typical potential drop lasting approximately 5-15 seconds. It is now understood by EPG scientists that potential drops indicate when the stylets have punctured epidermal cell membranes (Backus et al. 2016) and distinguish between intercellular and intracellular stylet location (Tjallingii 1985). Potential drops are divided into three sub-types: I, II, and III. Sub-type I is characterized by a sudden drop of the waveform; sub-type II is characterized by a brief period of a stable waveform at a lower voltage level; sub-type III is characterized by an abrupt rise of the waveform back to the original level (Figure 2.10 D). The final waveform of the pathway phase, waveform F, is believed to be the result of the stylets trying to withdraw from tight cellular spaces and typically does not result in phloem feeding (Backus et al. 2016)

Studies evaluating aphid feeding behavior and/or virus transmission (McLean and Kinsey 1964; Scheller and Shukle 1986; Sylvester 1980) identified the X wave as a landmark waveform that indicating the onset of phloem feeding by aphids and is considered to be phloem conditioning behavior. These waveforms always precede phloem phase waveforms E1 (phloem salivation) and E2 (phloem ingestion) (Backus et al. 2016). Waveform E1 (phloem salivation) typically begins immediately after the last X wave and is an emf-dominated waveform. The E2 (phloem ingestion) waveform is R-dominated, thus it is more easily affected by the substrate voltage (Tjallingii 1990;

Backus et al. 2016). Both E1 and E2 waveforms are recognized by their regular, repetitive peaks, with E2 always following E1, never the other way around (Backus et al. 2016).

Xylem ingestion is represented by the G waveform. This waveform is seen occasionally in apterous (wingless) aphids, and typically if they have been starved for a period of time (Spiller et al. 1990). The G waveform is similar to the E2 waveform, but can be distinguished by a higher amplitude, a more sinuous shape, and a more irregular pattern and is considered to be extracellular feeding. Powell and Hardie (2002) did note that alate (winged) aphids tended to display more G waveforms during feeding and concluded this may indicate an evolutionarily derived benefit of dispersal morphs in some species of aphids.

The importance of EPG in discerning the feeding behaviors of insects can be applied across many disciplines. Electropenetrography can be used as a major tool in host plant resistance evaluations. The aphid feeding behaviors evaluated with EPG on resistant or susceptible plant varieties has been demonstrated to serve as an initial means of discerning the level and type of resistance of plants to aphid infestation or for locating the site of resistance (Nielson and Don 1974; Lohar and Kawada 1987; Peters et al. 1988; Montllor and Tjallingii 1989; van Helden and Tjallingii 1993; Caillaud et al. 1995; Annan et al. 2000; Kaloshian et al. 2000; Zehnder 2001; Sandanayaka et al. 2003; Wang et al. 2004; Le Roux et al. 2008; Lightle et al. 2012; Koch et al. 2015, Backus et al. 2016).

Miao et al. (2011) used EPG to determine the impact of transgenic wheat on different aphid species. Their results indicated that wheat expressing snowdrop lectin (Galanthus nivalis agglutinin (GNA)) affected the feeding behavior of *Sitobion avenae* and *Schizaphid graminum*, but not *Rhopalosiphum padi* and suggested that transgenic wheat expressing GNA provids some protection against aphid feeding. Nam and Hardie (2012) used EPG to determine probing and larviposition behavior of *R. padi* on both primary and secondary hosts. Additionally, a study by

Prado and Tjallingii (2001) involving *R. padi* correlated stylet probing events with transmission of BYDV and concluded that phloem salivation (E1 waveform) indicated when BYDV virions are inoculated into the plant. It is because of this variety of applications that EPG has become a valuable asset to host plant resistance studies.

Plant Resistance and Rhopalosiphum padi Management

Plant resistance to insects has been documented since the late 1700s for various crop-pest interactions. The earliest of these accounts was documented by an unknown author in a farm article evaluating Hessian fly resistance in wheat (Painter 1951) and the first studies on Hessian fly resistance in several crop species (ie: wheat, barley, rye, oats) were performed in California in the late 1800s (Wickson 1881; Woodworth 1891; Kellner 1892). Any research related to insect resistance to plants requires an understanding of the possible biological plasticity of the insect being studied (Painter 1951). The earliest resistance screening attempts regarding BCOA in cereals were performed by Hsu and Robinson (1962, 1963). These screenings were performed in greenhouse and field experiments on several barley varieties and resulted in 43 varieties demonstrating varying levels of resistance (Papp and Mesterhazy 1993). However, when the varieties were rescreened in an environmental chamber, differences could not be found among the varieties (Robinson 1964; Papp and Mesterhazy 1993). Assays performed by Markkula and Roukka (1972) comparing wheat, barley and oat did not yield any differences in measurable resistance. Yet other studies have demonstrated significant differences in BCOA injury/damage among various wheat varieties (Leszczynski et al. 1985; Niraz et al. 1985; Havlícková 1988; Heyer and Wetzel 1988; Radchenko 1989; Krivchenko and Radchenko 1990; Papp and Mesterházy 1993). The screening processes implemented in these studies involved several biological measures to determine plant resistance, including: developmental time, number of progeny, and aphid weight. However, using these measures to classify resistance to BCOA frequently resulted in contradictory results (Hsu and Robinson 1962, 1963; Rautapää 1970;

Markkula and Roukka 1972; Kieckhefer et al. 1980; Lowe 1980; Wiktelius and Pettersson 1985). The high occurrence of contradictory outcomes between replications and among studies in regard to a plant's ability to resist BCOA's feeding damage implies this insect is highly plastic in its behavior and biology, with dynamic plant relationships (Taheri et al. 2010).

It is understood that various environmental factors and plant growth stages influence insect-plant interactions and can alter pest numbers, behavior, and dynamics; often resulting in damage and/or injury to the plant. It is largely known in entomological studies that resistance varies by plant growth stage; with differences in plant age lending to inconsistent resistance results (Painter 1951). The cause and effect relationship between aphid virulence and the effect of resistance genes within the plant is consistently difficult to interpret (Smith and Chuang 2014). Wheat or barley seedlings have traditionally been used to evaluate resistance to bird cherry-oat aphid, as this insect tends to naturally infest young plants. However, Painter (1951) noted that if resistance measures are recorded before or after the seedlings have completely absorbed the contents of the endosperm, the ensuing results may be contradictory. Indeed, plant maturity by resistance interactions such as these have been observed in chinch bugs on sorghum (Dahms 1948). A definitive designation of resistance is problematic for several reasons, including lacking a basic understanding of the biology of the insect of interest and proper plant breeding and selection techniques of host plants.

Our current understanding of the complexity of plant resistance to aphids is a work in progress, providing only basic knowledge of the concept. Resistance measurements are typically determined by comparing insect populations and/or plant damage across different varieties of a crop (ie: wheat) to a known susceptible variety (Painter 1951). To qualify as resistant, a plant variety endures more or less feeding/injury, but always more than a susceptible variety (Painter 1951). There are three types of plant resistance to insect damage: antixenosis, antibiosis, and tolerance (Painter 1951; Kogan and Ortman 1978; Panda and Khush 1995; Razmjou et al. 2012).

Antixenosis can be categorized as non-preference, which disrupts colonization of the plant by a pest organism. Antibiosis negatively affects the biology of the pest organism (Smith 2005; Razmjou et al. 2012). Tolerance can be defined as supporting the pest population without suffering economic injury (Kogan and Ortman 1978; Smith 1989; Razmjou et al. 2012).

Modern agricultural systems with expansive momocultures are speculated to be at a higher risk of aphid attack (Wittstock and Gershenzon 2002; Smith and Chuang 2014). Years of advantageous plant breeding have resulted in the development of aphid resistant plant cultivars across many crop species, and these aphid resistant crops offer extensive benefits, both economically and environmentally, to producers. However, aphids are continually overcoming resistance (Smith and Chuang 2014). The same biological (genetic) plasticity that allows aphids to be resistant to insecticides also allows them to express virulence to plant genes that are designed for aphid resistance (Devonshire and Field 1991). Prior plant breeding techniques focused on high levels of antibiosis for aphid resistance, which encouraged resistance and increased aphid virulence (Dogimont, Bendahmane, Chovelon and Boissot 2010), whereas cultivars expressing tolerance as a means of resistance tend to be more stable (Marimuthu and Smith 2012). Based on these observations, several researchers have expressed that the discovery and implementation of new aphid resistance genes that harbor a moderate amount of antibiosis resistance, or that have the ability to control tolerance resistance, would be of the most benefit (Dogimont et al. 2010).

In order to establish suitable integrated aphid management programs, the implementation of aphid resistant cultivars paired with other management techniques is paramount (Smith and Chuang 2014). Enhancing the biodiversity in cropping systems would aid in integrated pest management (IPM), by preventing pest outbreaks; outbreaks of pest populations are less likely to occur in diverse natural systems (Benyus 1997; Altieri 1999; Shoffner and Tooker 2013). It has been suggested that polyculture diminishes the frequency of pest infestations and outbreaks by

way of associated resistance, altered pest dispersal, and facilitating the top-down effects of natural enemies (Tahvanainen and Root 1972; Andow 1991; Siemann 1998; Landis et al. 2000; Shoffner and Tooker 2013). In regard to the bird cherry-oat aphid, the effect of additional primary host plants in agroecosystems is largely unexplored in the literature.

The closest comparisons can be gleaned from studies involving the soybean aphid. Similarly to BCOA, the soybean aphid (*Aphis glycines* Matsumura) is a holocyclic and heteroecious aphid species (Blackman and Eastop 2000). This aphid utilizes *Rhamnus cathartica* or buckthorn, as its primary host in North America (Voegtlin et al. 2005; Crossley and Hogg 2015) and utilizes soybean, *Glycine max* L. Merr., as its secondary host. However, according to the literature it is widely unknown whether this aphid species is able to overwinter on additional plant species, with few overwintering locations actually identified (Crossley and Hogg 2015). Voegtlin et al. (2005) found that *R. cathartica* is the primary, critical host to the soybean aphid. Without the presence of this host species, the soybean aphid is unable to complete its life cycle. Based on this example, it can be inferred that the alternative host plant species of the bird cherry-oat aphid have the potential to be just as critical to its life cycle, biology, and ultimately fitness in winter wheat agroecosystems.

BCOA that colonize emerging winter wheat seedlings each fall in the Southern Great Plains can cause significant damage and damage potential may be related to their heteroecious biology within individual fields or within an agricultural landscape: i.e. host-plant alternating results in variable behavior and fitness of BCOA populations. It has been suggested that understanding the relationship between landscape composition (from which migrant pest species are originating) and their influence on crop colonization is integral to the improvement of pest management approaches (Wissinger 1997; Bianchi et al. 2006; Carrière et al 2014; Gilabert et al. 2017). BCOA has been known to utilize pasture and grassland as a refuge in Australia (Barrow and Wallwork 1992; Gilabert et al. 2017). While in France, Gilabert et al. (2017) argues that

BCOA migrants collected in wheat originate primarily from maize, with little evidence supporting BCOA migrants originating from surrounding grasslands. However in the U.S., with particular reference to Kansas and Oklahoma, BCOA was believed to be unable to utilize maize or sorghum as food source (Michaud 2008), and had not been documented in sorghum until 2015, when a colony was found co-infesting sorghum with the sugarcane aphid, (*Melanaphis sacchari*) in Kansas (personal observation, Brian McCornack, Kansas State University, Manhattan, KS). Subsequent studies by Michaud et al. (2017) revealed that BCOA can not only develop on sorghum that has been previously infested with sugarcane aphid, but that the presence of sugarcane aphid appears to facilitate this ability. This report suggests feeding by sugarcane aphids increases the nutritional quality of sorghum, and thus makes it a more suitable host for other aphid species.

Conversely, recent surveys of Oklahoma sorghum fields found no evidence of BCOA infestation either with or without the presence of sugarcane aphid (personal communication with Casi Jessie, Oklahoma State University, Stillwater, OK). Additionally, aphid population surveys conducted by Anstead (2000) found scant evidence of BCOA in native grasses of the Southern Great Plains as well, discovering only one colony on johnson grass (*Sorghum halepense*) in Kansas. This narrative ultimately concludes that there is no consensus as to the origination of BCOA migrants in the Southern Great Plains, with the primary refuge landscape of this species remaining largely unknown. Thus, it is plausible that producers who harbor other plant hosts in their landscape may therefore influence BCOA ecology.

Landscape origination may also influence not only the genetics of migratory aphids, but also their virulence potential (Vialatte et al. 2007). Indeed, because of the high biological plasticity demonstrated by this organism, bioassay results regarding resistant cultivars have historically been highly variable (personal communication with Dolores Mornhinwig, USDA-ARS, Stillwater, OK). A standardized rearing procedure mimicking typical heteroecious aphid

behaviors has the potential to result in healthier, more morphologically consistent BCOA that are more representative of wild populations and can be utilized in bioassays on wheat and barley germplasm. Maintaining aphid body size and fecundity in an artificial environment (i.e. lab colonies) is difficult but may be integral for producing reliable, trustworthy bioassay results. Factors facilitating reduced fitness in BCOA laboratory colonies and inconsistencies in bioassay results are largely unknown; standardizing laboratory rearing protocols for bird cherry-oat aphid may establish greater consistency in regard to both issues.

The encompassing hypothesis of this dissertation is to evaluate the importance of host-plant composition on bird cherry-oat aphid biology, fitness, and behavior. Studies examining the impact of host-plant composition on bird cherry-oat aphid biology and feeding behavior are presented here. Conclusions reached within this dissertation will be discussed, as well as implications for bird cherry-oat aphid utilization in future wheat and barley germplasm resistance screenings.

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Figure 2.1 Circulative pathway of virions of persistently transmitted the Barley Yellow Dwarf Virus through an aphid vector (Modified from Power and Gray 1995; D'Arcy and Domier 2000).

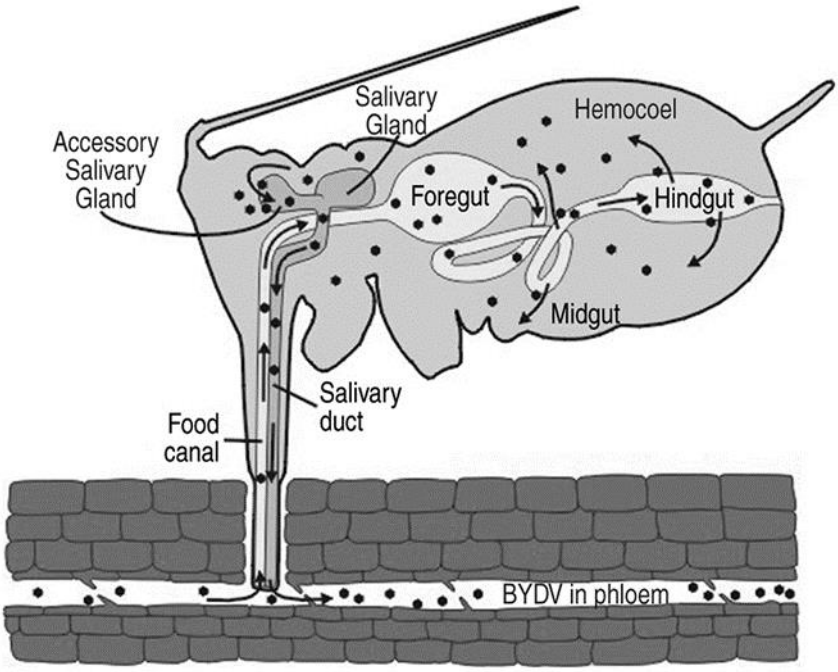


Figure 2.2 General aphid morphology. A) Typical aphid body with rostrum (Ro) visible, flagellum (Fl) of antenna, and coxa (Cx). B) Sagittal view of an generalized aphid head showing how the labial sheath (Sh) protracts to allow the stylet bundle (Sb) access to the plant. C) Close up of internal morphology of the rostrum with outer labial sheath (Lb), modified mandibles (Mnd) and maxilla (Mx) that form the stylet bundle, with internal food (Fc) and salivary (Sc) canals. D) Cross section of the stylets showing how the madibular (Md) and maxillary (Mx) stylets fit together to form the food (Fc) and salivary (Sc) canals.

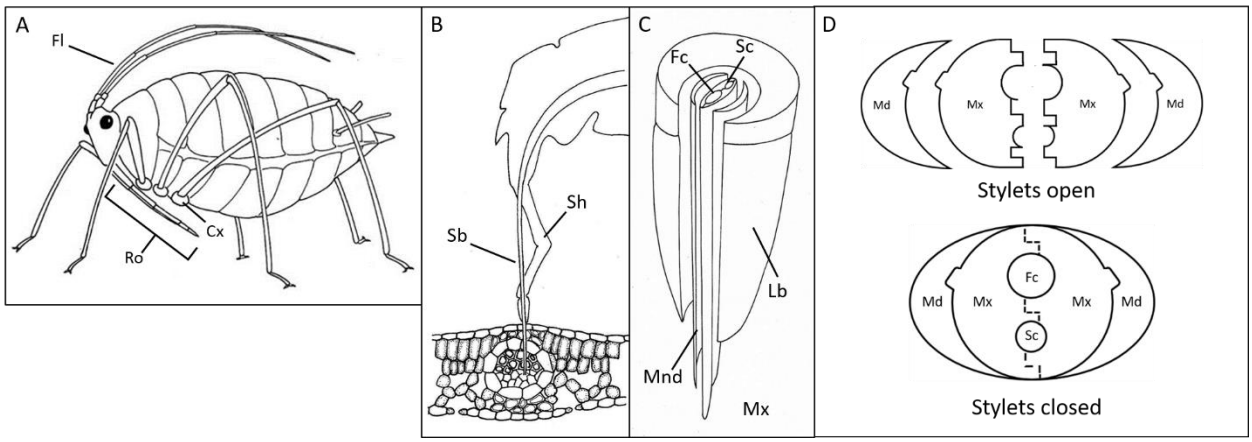


Figure 2.3 Heteroecious aphid lifecycle (modified from Williams and Dixon 2007).

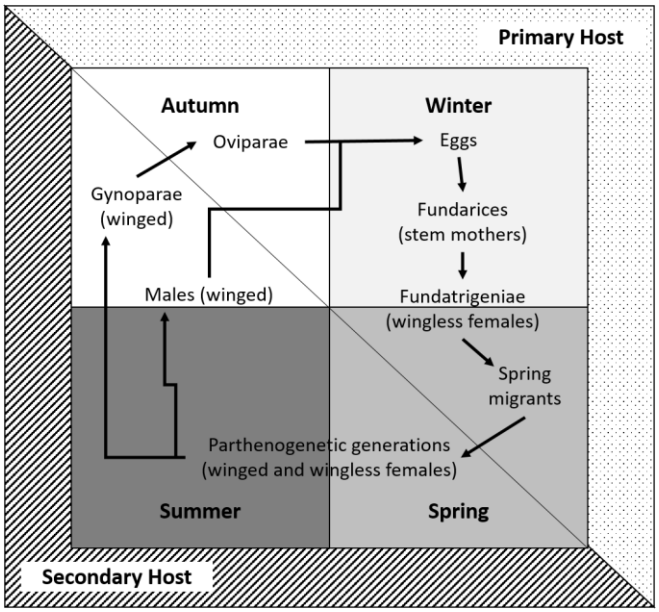


Figure 2.4 Monoecious aphid lifecycle (modified from Williams and Dixon 2007).

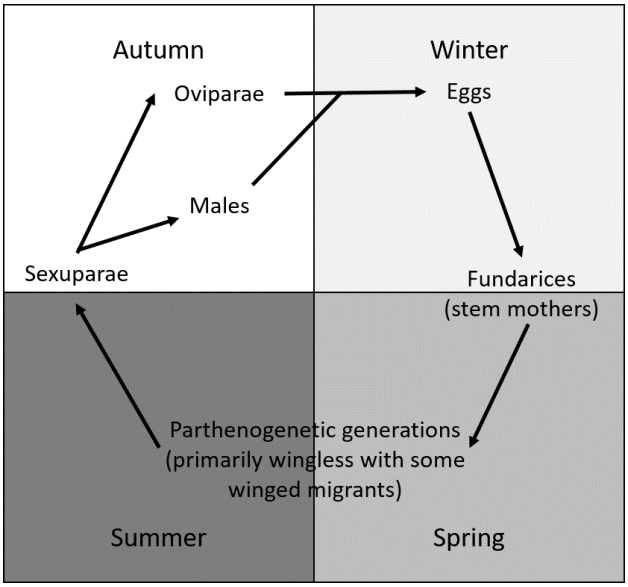


Figure 2.5 General life cycle of the bird-cherry oat aphid in Europe (From Resh and Card 2009)

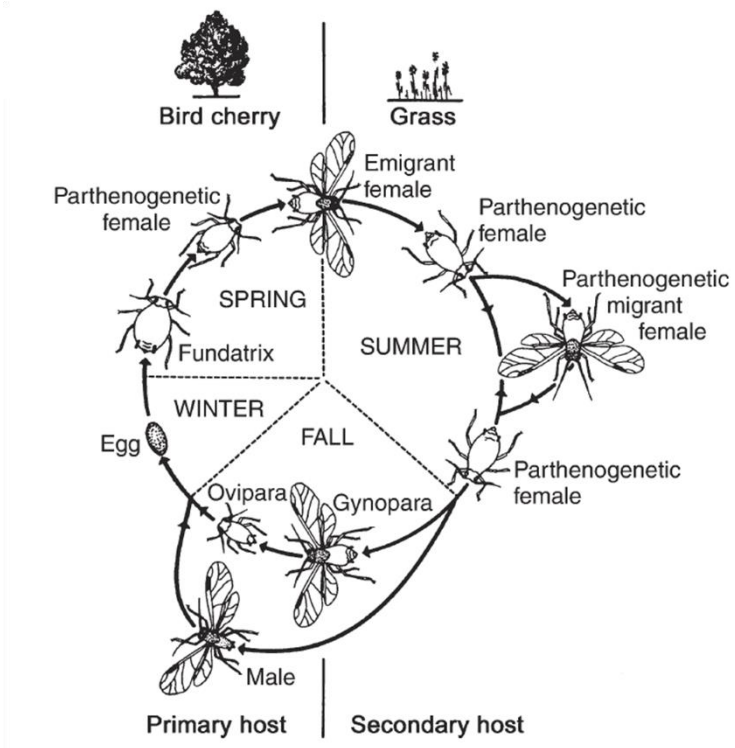


Figure 2.6 The basic components of an EPG system (modified from Walker 2000).

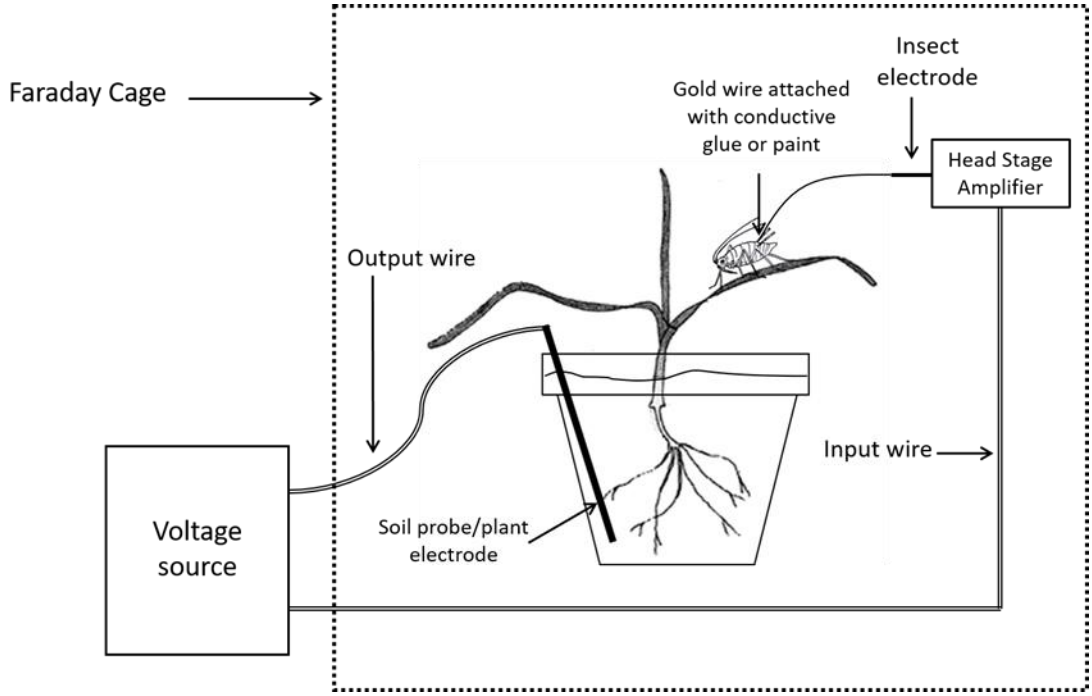


Figure 2.7 Diagram from Walker (2000) depicting the pathway of the electrical current (black arrows) through the plant and insect. a) Overview of the system. b) close up of the electrical current pathway. The plant probe would be in the soil a considerable distance away from the insect (as not to cause injury to the feeding insect) and is only depicted this way for clarity of the pathway.

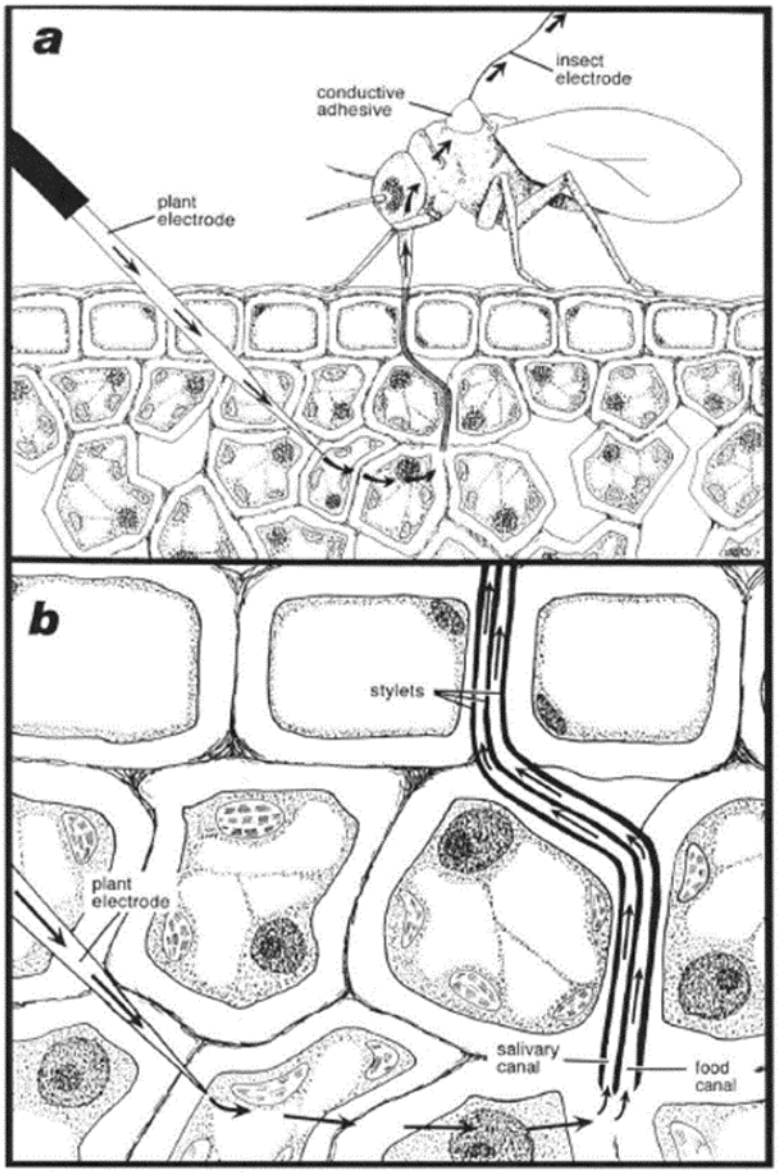


Figure 2.8 Sagittal view of a generalized aphid head showing the internal morphological structures (modified from Dixon 1973).

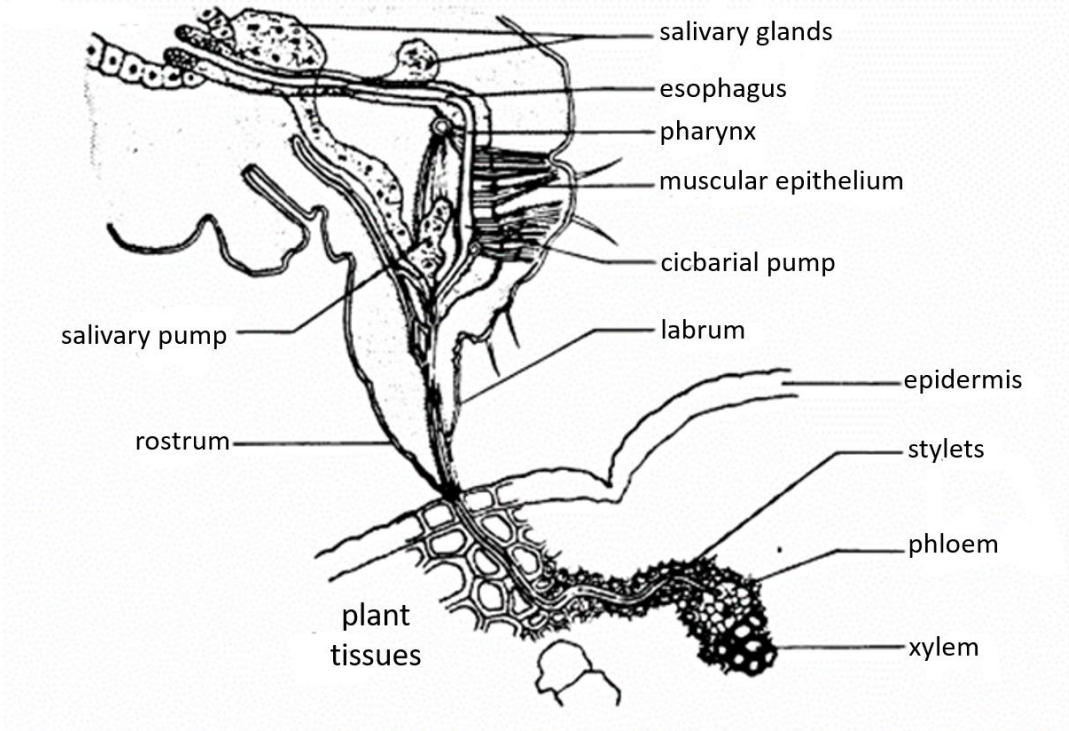


Figure 2.9 R/emf responsiveness curve theory (Tjallingii 1978, 1985a; Backus and Bennett 2009; Backus et al 2016). As input resistance (Ri) increases, the percentage of signal from emf increases and vice versa.

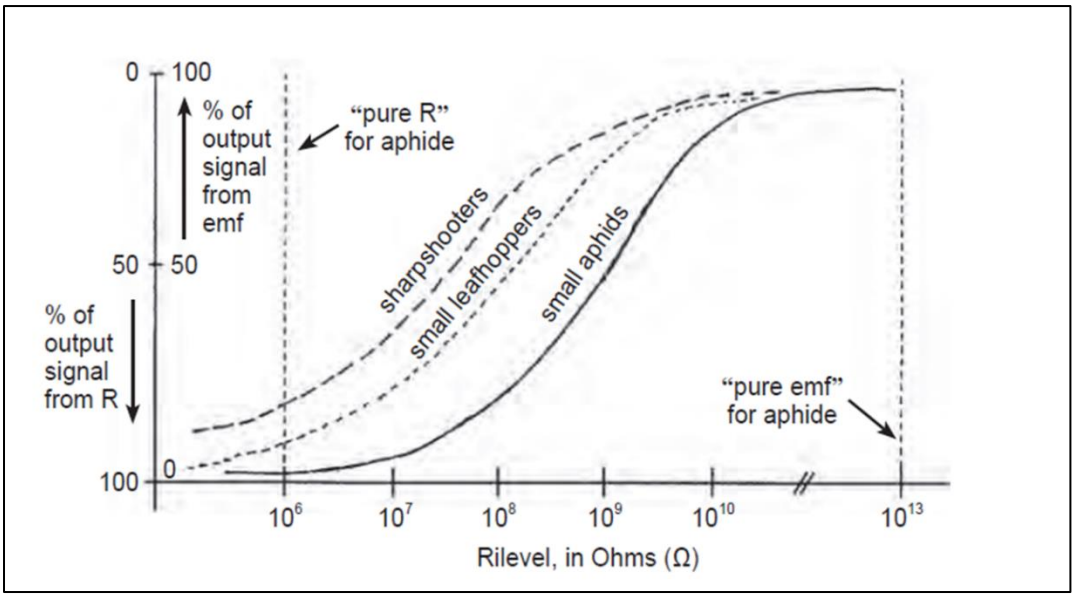
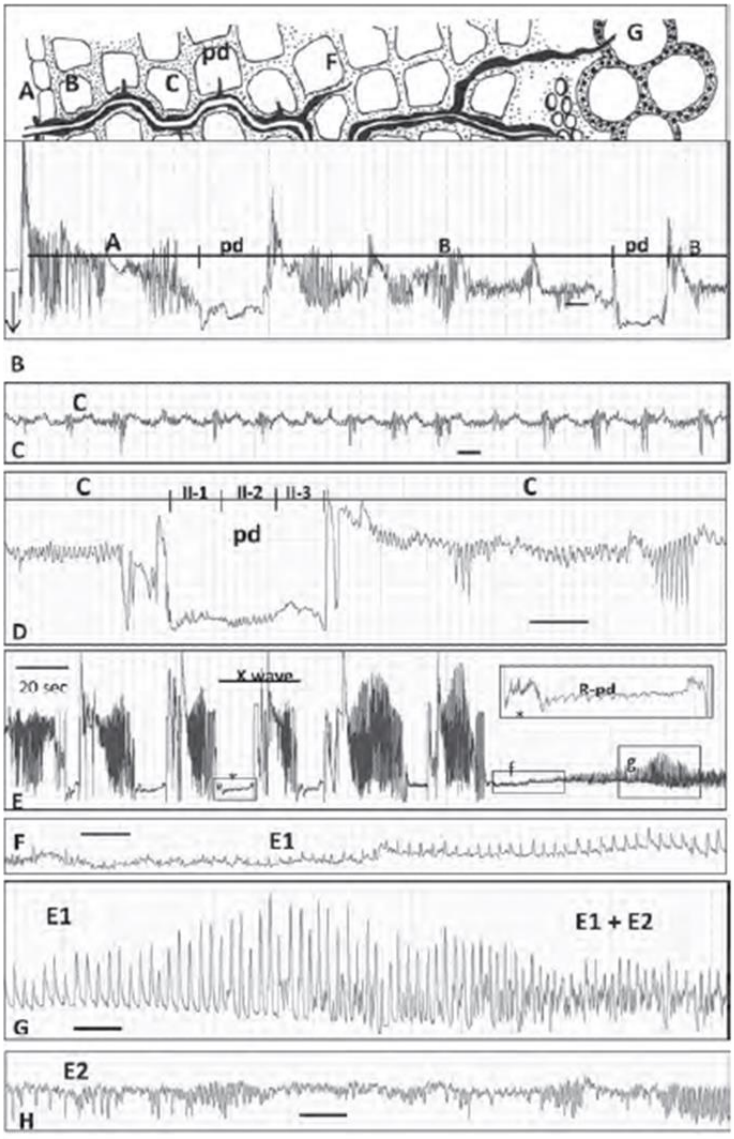


Figure 2.10 General aphid EPG waveforms discerned from *A. pisum*, pea aphid, stylet probing and ingestion using an AC-DC EPG monitor. A) Entire pathway overview from stylet insertion to phloem ingestion with locations of each waveform origin labeled. B) Start of probing with a noticeable voltage spike, arrow indicates baseline voltage which correlates to non-feeding and/or no stylet insertion. C) Pathway with undefined waveforms. D) Section of pathway with pd, includes pd subphases I, II, and III. E) Compressed X waves. F) Beginning of E1 waveform, showing progession of the wave shape over time. G) E1 transitioning into E2 waveforms. H) E2 waveforms, typically appearing much later in the probe (Backus et al. 2016).



CHAPTER III

HOST PLANT COMPOSITION EFFECTS ON BIRD CHERRY-OAT APHID (*RHOPALOSIPHUM PADI* L.) FITNESS

Introduction

The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is a highly polyphagous, heteroecious aphid species that is capable of utilizing a wide variety of plants as a host (Blackman and Eastop 2007). This species seasonally alternates between its primary host (typically a tree species) and a secondary host (typically a grass species) (Dixon 1971), but is able to use a variety of broadleaf and grass species as well (Blackman and Eastop 2007). Bird cherry-oat aphid readily feeds on several cereal crop plant species, making it one of the most economically important insect pests in the world. This status is based on its propensity for transmitting viral pathogens and the ability to cause direct feeding damage to plants (Vickerman and Wratten 1979; Kieckhefer et al. 1980; Elliott, Kieckhefer, and Walgenbach 1990; Halarewicz and Gabryś 2012). Indeed, *R. padi* is a known vector of viral infections which include several strains of the barley yellow dwarf virus (BYDV), a prevalent and potentially severe viral infection of wheat and other cereals (Choudhury et al. 2017), cereal yellow dwarf virus (CYDV), and maize dwarf mosaic virus (MDMV), a viral disease of perennial grasses and small grains within the Gramineae family (Thongmeearkom et al. 1976).

The United States is the third largest producer of wheat in the world, with production estimates for the 2017-18 growing season upwards of 47 million tonnes (IGC 2017).

Rhopalosiphum padi is one of the most commonly found aphids on wheat in the U.S., and current anagement efforts are primarily focused on preventative and curative insecticide use (Royer 2016; Royer and Giles 2017). Development of R. padi resistant wheat cultivars, however, would protect yield and allow for reduced management inputs and there have been considerable efforts aimed at identifying resistant germplasm sources and developing regionally adapted cultivars (Aradottir et al. 2017; Khan et al. 2017; Girvin et al. 2017). In laboratory evaluations conducted in Pakistan, Khan et al. (2017) identified *R. padi* antibiosis resistance to a locally adapted wheat variety. However, recent laboratory and field phenotypic studies on locally adapted and historical wheat cultivars in Europe did not reveal any resistance to *R. padi* (Aradottir et al. 2017). Most recently in the U.S., Girvin et al. (2017) evaluated hard red and soft white wheat cultivars from Kansas, and identified resistance that either suppressed *R. padi* populations or tolerated the effects of its feeding.

Only recently have the interactions between aphids and plants been explored on a molecular basis. Both plant resistance genes and defense response genes have been identified in plants, with results indicating that aphids induce these defense-signaling pathways (Smith and Boyko 2007). Plant defensive-signaling pathways are dependent upon salicylate and jasmonate signaling molecules (Kaloshian 2004). There is evidence that these molecules are either directly synthesized by aphids or are byproducts of endosymbiont bacteria within the aphids themselves, however the origins of these signaling molecules is not well understood (Urbanska et al. 1998; Miles 1999; Forslund et al. 2000; Smith and Boyko 2007). The variety of resistance sequences currently identified between different aphid-plant interactions suggests that several mechanisms may be involved in order for resistant plants to recognize aphid feeding. These differences in mechanisms may be plant specific, resulting in differences in both defense signaling and response gene pathways among plants (Smith and Boyko 2007). For example, in aphid resistant wheat, β-glucosidase sequences are highly up-regulated, but are down-regulated in aphid resistant sorghum

(Park et al. 2005; Boyko et al. 2006), and an ADP-ribosylation factor is up-regulated in apple plants resistant to aphids, but is down-regulated in resistant sorghum (Park et al. 2005). Interactions such as these demonstrate the need to study specific aphid-plant systems, as using a single generalized model may not be representative of all aphid-plant interactions (Smith and Boyko 2007).

Germplasm screening assays for resistance are typically artificially infested with laboratory-reared aphids and there is little consistency in the literature regarding *R. padi* rearing protocols, with this aphid being raised in a variety of growing conditions and on a variety of cereal hosts. Hesler and Tharp (2005) raised their *R. padi* colony on barley at 20±1°C, with a photoperiod of 13: 11 (L:D), while Aradottir et al. (2017) also raised their colony on barley, but at 22±1°C with a photoperiod of 16:8 (L:D). Razmjou et al. (2012) raised their *R. padi* on wheat at 25±1°C, with a photoperiod of 14:10 (L:D), while Gray et al. (1998) preferred the rearing methods outlined by Rochow (1969); aphids were reared on barley at approximately 21°C, but without a defined photoperiod. As a potential result of rearing condition variability, *R. padi* used in germplasm resistance evaluations may not be reliable among phenotyping trials and may not be representative of the effects of wild populations (i.e. behavior and fitness).

Because of the heteroecious nature of *R. padi*, multiple host plants within a rearing environment may be essential for this species to maximize fitness. By mimicking a more naturally diverse environment, appropriate host plant complexity may allow for the production of aphids that are more representative of aphids that regularly colonize wheat fields. Heterogeneous plant compositions can also increase the possibility of associational resistance. Associational resistance occurs when non-crop plant species are in close proximity to crop plant species and cause a decrease in the amount of pest damage the crop sustains by either influencing the crop quality or by altering herbivore host plant selection and/or feeding behavior (Tahvanainen and Root 1972; Dahlin and Ninkovic 2013). Interactions among plants and insects can be dynamic

and changes in host plant quality can greatly affect herbivorous insect fitness (Pettersson et al. 2007; Dhalin and Ninkovic 2013).

Highly reduced body size of aphids in colonies of *R. padi* were observed in 2013 during wheat germplasm resistance evaluations (personal observation). As discussed previously, *R. padi* colonies used for germplasm resistance evaluations are produced on single cultivar grass species that represent only a portion of this aphid's life cycle (Dixon 1971). Indeed, *R. padi* colonies currently used for Oklahoma State University wheat germplasm screenings are produced on a single susceptible cultivar ('Jagger') and these aphids are significantly smaller than naturally occurring counterparts that invade winter wheat fields in the fall (personal observation). Smaller aphids have reduced capacity to feed, reproduce, and cause plant injury (Michaud 2012), and perhaps, host plant complexity within heteroecious habitats has the potential to alter feeding behavior and reproduction. The objective of this study was to determine if increasingly complex host plant habitats increase the fitness of *R. padi*. We hypothesized that the addition of suitable host plant species to *R. padi* rearing cages, in an effort to mimic heterogeneous complexity of agroecosystems, would increase aphid fitness. We evaluated aphid size, weight, and fecundity as measures of fitness.

Methods for Evaluating Effects of Habitat Complexity on Rhopalosiphum padi Fitness

Aphid source colony. *Rhopalosiphum padi* were collected from a wheat field in Hennessey, Oklahoma in 2013 and were raised on continuous wheat (cultivar 'Jagger') grown in 15cm plastic pots. The colony was caged and held at 24.4±0.9°C (ambient room temperature of the laboratory) under 40W full spectrum florescent lights (F40DSGN50, LEDVANCE, LLC, Ontario, Canada) with a photoperiod of 16:8 (L:D) and 43±1.2% RH. Plants were watered every other day and fertilized bi-weekly. Fresh, uninfested wheat plants (approx. 50 plants per pot,

grown to the two leaf stage) were added weekly. This colony will be referred to as the "source colony" throughout the remainder of this chapter and subsequent chapters.

Host Plant Composition Evaluation

Greenhouse trial. All plant species used were grown in a greenhouse with a photoperiod of 14:10 (L:D) at 18°C. Planting flats (2" x 2" x 2") were cut into 2x2 cell sections and filled with a 1:1 mixture of potting soil (Professional growing mix, Sungro® Horticulture, Agawam, MA) and absorbent clay (Absorb-N-Dry, Balcones Minerals Co., Flatonia, TX). In Europe, *R. padi* has been found colonizing *Capsella bursa-pastoris*, commonly called Shepherd's purse (Blackman and Eastop 2006). This weed species is found throughout the United States and Oklahoma and is considered a winter annual, but can survive all year in milder climates (Azulai et al. 2014). Shepherd's purse (Horizon Herbs, LLC, Williams, OR) seeds were sewn approximately two weeks before wheat and barley due to a longer germination time. All plant species were over planted and thinned upon emergence to one plant per cell prior to aphid infestation. Treatments consisted of different combinations of host plants for a total of 7 different treatments: wheat alone, barley alone, shepherd's purse alone, wheat with barley, wheat with shepherd's purse, barley with shepherd's purse, and a combination of wheat, barley, and shepherd's purse. Flats were transferred to a second greenhouse at the USDA-ARS facility (Stillwater, OK) once the wheat and barley plants reached the two leaf growth stage and shepherd's purse reached the seedling rosette growth stage (Figure 3.1). The temperature and RH of the second greenhouse could not be manually regulated, thus trials were performed in February 2016 in order to take advantage of cooler weather. The green house maintained an average temperature of 24.1±9°C but with highly variable RH (49.3±40%).

Experimental flats were infested with five healthy alate (winged) aphids from the source colony and arranged on a greenhouse bench in a randomized complete block design. Alates are

the aphid life stage responsible for migrating and establishing new colonies (Dixon 1971), and were selected for this evaluation as representative colonizing aphids. Aphids were collected via aspiration into a micro centrifuge tube from the source aphid colony. The micro centrifuge tube was then placed in the center of the flat and opened to allow aphids to roam freely in an effort avoid host-plant selection bias. Each flat was then covered with a small plastic cage with a fine mesh top to allow airflow (Figure 3.2), placed on a plastic tray, with the edges of the cages covered with sand to prevent aphid escape. Plants were watered and fertilized as needed throughout the duration of the study to ensure vigorous plant growth.

Aphids were allowed to reproduce uninterrupted for a period of five days. On day 5 alates were removed from the cages. Aphids remaining in the cages were allowed to continue reproducing for another five days, and on day 10, individual plants were cut at the base, or just below the soil surface, and placed separately in a labeled Ziploc bag. Care was taken to not dislodge aphids from plants. Any aphids that did fall off, were recovered with a fine paintbrush and placed in bags. Bags were placed in a cooler on ice where they remained until they were processed. Aphid counts and weights were recorded for each plant within each treatment. Aphids were chilled in a refrigerator in order to reduce mobility before live weights were measured for each aphid. This experiment was replicated eight times.

Environmental growth chamber trial. The host plant composition evaluation was repeated in an environmental chamber to provide greater control over temperature and humidity. All plants were grown and treated in the same manner as described for the greenhouse evaluation. At the seedling stage, all flats were moved to an environmental growth chamber that was maintained at 18±2°C, 65±10% RH and a photoperiod of 14:10 (L:D) at the environmental growth chamber facility at Oklahoma State University (Figure 3.3). Flats were again infested with 5 alate aphids obtained from the source colony. Aphids were allowed to reproduce undisturbed for a period of 10 days. Unlike the greenhouse evaluation, alates were not removed from the

cages in order to prevent disturbance that could cause aphids to drop off the plants. At the end of the 10 days, plants were removed as described previously and processed. The number of aphids from each treatment and total weight of aphids per treatment were recorded.

For both the greenhouse evaluation and the environmental growth chamber evaluation, aphid counts and weights were square root transformed prior to comparing differences using three factor ANOVA (SAS 9.4, SAS Institute Inc., Cary, NC). In addition, for the greenhouse study, the weight per aphid per treatment was compared between aphids collected from single host plant species within treatments. For example, aphid weight of aphids originating from the shepherd's purse only treatment were compared to the weight of aphids originating from shepherd's purse plants within host plant combination treatments. During the 10d evaluation, *R. padi* were observed moving within experimental cages and feeding on all plants. Within plant species comparisons of aphid weights among treatments allowed for a more direct evaluation of how habitat complexity and utilization influenced potential fitness. Results were compared using three factor ANOVA (SAS 9.4, SAS Institute Inc., Cary, NC). The purpose of this analysis was to further illustrate the impact of multiple host plants on aphid fitness.

Results

Host Plant Composition Treatment Effects

Environmental Chamber Evaluation. In the highly controlled environmental chamber experiment, when comparing total aphid weight per treatment, the shepherd's purse only treatment produced significantly lighter aphids than the wheat only treatment (t = -2.38, df = 30.1, P = 0.0238), the barley only treatment (t = -2.15, df = 30, P = 0.0396), and the wheat-barley treatment (t = -2.69, df = 30.1, P = 0.0116). All other host plant combinations resulted in similar weights among aphids (Figure 3.4). For total aphid production, the shepherd's purse only treatment produced significantly fewer aphids than the wheat only treatment (t = -2.98, df = 30, P

= 0.0056), the wheat-barley treatment (t = -2.39, df = 30, P = 0.0233), the barley-shepherd's purse treatment (t = -2.54, df = 30, P = 0.0164) and the wheat-shepherd's purse treatment (t = -2.23, df = 30, P = 0.0331). The difference between the number of aphids produced from the shepherd's purse only treatment and the barley only treatment was nearly significant (t = -2.03, df = 30, P = 0.0517). There were no significant differences in total aphid production per treatment among any of the other treatments (Figure 3.5).

Greenhouse Evaluation. In the greenhouse study, relative humidity varied greatly and daylight varied based on time of year which may have enhanced the negative effect of shepherd's purse (and perhaps barley) on aphid fitness (Figures 3.6, 3.7). When comparing total aphid weight per treatment, the shepherd's purse only treatment again produced significantly lighter aphids than the wheat only treatment (t = -3.57, df = 46, P = 0.0008) and the wheat-barley treatment (t = -2.52, df = 46, P= 0.0154) (Figure 3.6). Results for the total number of aphids produced per treatment indicated there was a significant difference between shepherd's purse only and wheat only (t = -2.04, df = 46.1, P = 0.0472) but not barley only (t = 0.74, df = 46.1, P = 0.4660). However, there was a significant difference in treatments involving barley. The total number of aphids per treatment produced from the barley only treatment was significantly lower than the wheat only treatment (t = -2.70, df = 46, P = 0.0097). The barley only treatment also produced significantly fewer aphids than the wheat-barley treatment (t = -2.59, df = 46, P = 0.0129) (Figure 3.7). In such variable conditions, the addition of shepherd's purse and/or barley to host plant combinations clearly results in negative consequences for *R. padi*.

Individual Effect of Host Plants on Aphid Weight

Greenhouse Evaluation. When comparing the weight per aphid per treatment of the wheat only treatment to the weight per aphid of aphids originating from wheat plants within each host plant composition treatment, there is a highly significant effect of the presence of other host

plants on aphid weight. Aphids originating from wheat plants within mixed host plant treatments weighed significantly less than those that originated from the wheat only treatment ($P < 0.0001$) (Table 3.1). A similar effect occurred on barley. Aphids originating from barley plants within mixed host plant treatments weighed significantly less than those that originated from barley only ($P < 0.0001$) (Table 3.2). While the shepherd's purse comparisons were not significant ($P = 0.0762$), the negative trend of the effect of host plant combinations can be seen for each comparison involving shepherd's purse (Table 3.3). These results indicate a negative effect of additional host plant availability and utilization on aphid weight.

Discussion

Rhopalosiphum padi is a highly polyphagous aphid species that is able to utilize a wide variety of plant species as host plants (Blackman and Eastop 2007). However, despite being able to utilize alternate plant species, simultaneous availability and utilization may not be optimal for this aphid species. The results of this study resulted in a rejection of the hypothesis that the inclusion of additional suitable host plants within *R. padi* rearing cages would increase their fitness. In particular, despite observations of this aphid species colonizing weed species such as *Capsella bursa-pastoris* (Blackman and Eastop 2006), results indicated that inclusion of *C. bursa-pastoris* as an additional and optional food source was in fact detrimental to aphid fitness.

Results indicated what appears to be an antagonistic relationship when multiple host plant types are available for aphids to feed on. Results from both the greenhouse and environmental growth chamber evaluations indicated an overall detrimental effect of shepherd's purse on aphid weight and reproduction. These results mirror findings by Dhalin and Ninkovic (2013) who demonstrated complex host plant environments had a negative impact on *R. padi* performance. They concluded that additional host plants cause changes within primary host plants that result in a significant reduction in aphid reproduction. This effect can be seen in my evaluation when

examining within treatment combinations. *Rhopalosiphum padi* produced fewer offspring on average when feeding on shepherd's purse when compared to wheat or barley. This effect was not only seen in the solitary plant treatments (i.e. wheat only, barley only, shepherd's purse only) but were also seen in all host plant combinations that included shepherd's purse. This observation indicates a possible interaction between the plants that resulted in decreased suitability of wheat and barley within mixed host plant treatments.

One explanation for the lower weights of aphids collected from shepherd's purse may involve its nutritional suitability as a host. While this evaluation does indicate that *R. padi* can survive on shepherd's purse and readily reproduce, it may not be nutritionally sufficient to support optimal aphid growth. The polyphagous nature of this aphid species has been well documented (see Chapter 2) and it has been documented to alternate hosts when the nutrient quality of the host either declines or is suboptimal to begin with, triggering a migration either away from the primary host to a secondary host, or vice versa (Dixon 1971). Bergman et al. (1991) demonstrated that spotted alfalfa aphids, *Therioaphis maculata*, feeding on alfalfa that had not been infested prior to their introduction to the plant, preserved their energy stores instead of using it for reproduction. Indeed, the authors concluded that *T. maculata* required sustained feeding and plant host conditioning for optimal nutrient utilization, growth, and reproduction to occur. Similarly, populations of *R. padi* in Kansas require prior infestation by *Melanaphis sacchari* (sugar cane aphid) in order to successfully utilize sorghum as a food source (Michaud et al. 2017). This observation may imply that host plant conditioning is a necessity for successful colonization of *R. padi* on other plant species as well. Perhaps similar host plant conditioning activities were required for optimal *R. padi* growth and reproduction to occur in this study; in mixed plant conditions, aphids moved among plants and potentially did not feed long enough to condition individual plants for optimal growth and reproduction.

Another plausible explanation for observations of reduced fitness in more complex host plant compositions involves the relationship between the natal experience effect on the aphids and their ability to disperse. The alate aphids utilized in this evaluation were raised on wheat only and were used to infest the experimental host-plant composition treatments to represent aphids that were leaving wheat vegetation. However, Stamps and Davis (2006) explored the adaptive effects of natal experiences and explained that an organism may be influenced later in life by experiences gained in the natal habitat, in this case the wheat only environment. These experiences may influence the organism's habitat preference (i.e. host-plant selection) and their ability to recognize cues from their preferred host in a heterogeneous plant population. These affects may make the individual more or less capable of detecting these cues, and thus affecting their ability to locate the host and potentially their subsequent performance on a new host (Dahlin and Ninkovic 2013; Gilabert et al. 2017). This idea may lend some insight into why *R. padi* utilized in this evaluation did not perform well when transferred into a new habitat containing shepherd's purse or barley. These aphids may have lacked the necessary preconditioning required for adapting to these alternate host plants. Successful colonization of a new plant, or lack thereof, might be dependent upon which plant the migrant aphid originated from (Klingauf 1987).

Gilabert et al. (2017) concluded that variables at both the local and landscape level had a significant impact on the rate at which *R. padi* colonized wheat crops. Diverse agroecosystems have been demonstrated to reduce populations in crops of herbivorous insects by interfering with their ability to detect integral volatiles (i.e. olfactory cues) from host plants or masking them entirely and due to the increased incidence of natural enemies within these landscapes (Dahlin and Ninkovic 2013). The impact of host plant composition on *R. padi* in Oklahoma is largely unknown, as are the host plant(s) used as a refuge. Some assumptions have been made that *R. padi* can overwinter at the base of wheat plants, or even just below the soil surface (Elliott and Kieckhefer 1989). Another possibility is *R. padi* have assumed a largely monoecous, anholocyclic

life cycle and no longer require an alternate host in this region of the U.S. (Michaud 2008). More research is required on the basic biology and ecology of *R. padi* in the Southern Great Plains in order to accurately discern the impact of host plant complexity on this organism.

Conclusion

For this study, the impact of host plant complexity on *R. padi* fitness was evaluated. I compared seven different host plant compositions in green house and environmental chamber trials. By comparing the weight and number of *R. padi* produced within these treatments, I was able to reject the primary hypothesis that the availability and utilization additional suitable host plants to aphid rearing cages would increase fitness. Overall, aphids produced in treatments including shepherd's purse as a host were smaller and weighed less than those produced without the presence of this host. The negative effect of shepherd's purse was apparent when weight of aphids from individual host plant compositions were compared to their same counterparts in the mixed host compositions. The data revealed what may be an antagonistic relationship between wheat, barley, and/or shepherd's purse when grown in close proximity and the resulting negative effect on aphid fitness. Factors including natal experience effects, plant-plant interactions, lack of host plant conditioning, and/or host plant composition effects may have impacted *R. padi* fitness. The lack of information regarding basic *R. padi* biology and ecology in the Southern Great Plains limits our biological understanding of this aphid species, and thus how we optimize rearing procedures that produce laboratory colonies of *R. padi* that are representative of wild populations.

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Table 3.1 Greenhouse study of individual host plant effect on *R. padi* weight. Three factor ANOVA comparisons of the mean weight per aphid per host plant within treatments. Aphids originating from the wheat only treatment were compared to aphids originating from wheat plants within each host plant composition treatment. Host plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

Host Plant Composition	Weight (mg)	SE		*P* -value
W	0.570	± 0.022	a	<.0001
W/B	0.273	± 0.010	b	
W/SP	0.228	± 0.036	b	
W/B/SP	0.140	± 0.018	c	

Table 3.2 Greenhouse study of individual host plant effect on *R. padi* weight. Three factor ANOVA comparisons of the mean weight per aphid per host plant within treatments. Aphids originating from the barley only treatment were compared to aphids originating from barley plants within each host plant composition treatment. Host plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

Host Plant Composition	Weight (mg)	SE		*P* -value
B	0.457	± 0.078	a	<.0001
W/B	0.204	± 0.034	b	
B/SP	0.182	± 0.031	bc	
W/B/SP	0.060	± 0.028	c	

Table 3.3 Greenhouse study of individual host plant effect on *R. padi* weight. Three factor ANOVA comparisons of the mean weight per aphid per host plant within treatments. Aphids originating from the shepherd's purse only treatment were compared to aphids originating from shepherd's purse plants within each host plant composition treatment. Host plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

Host Plant Composition	Weight (mg)		SE	*P* -value
SP	0.454	±	0.037	0.0762
W/SP	0.403	±	0.158	
B/SP	0.262	±	0.034	
W/B/SP	0.157	±	0.039	

Figure 3.1 Planting flat configuration for the environmental chamber and greenhouse studies. Seedling stage of wheat, barley and shepherd's purse. The vial in the center was the point of origin for *R. padi* alates.

Figure 3.2 Individual cage set up within the greenhouse.

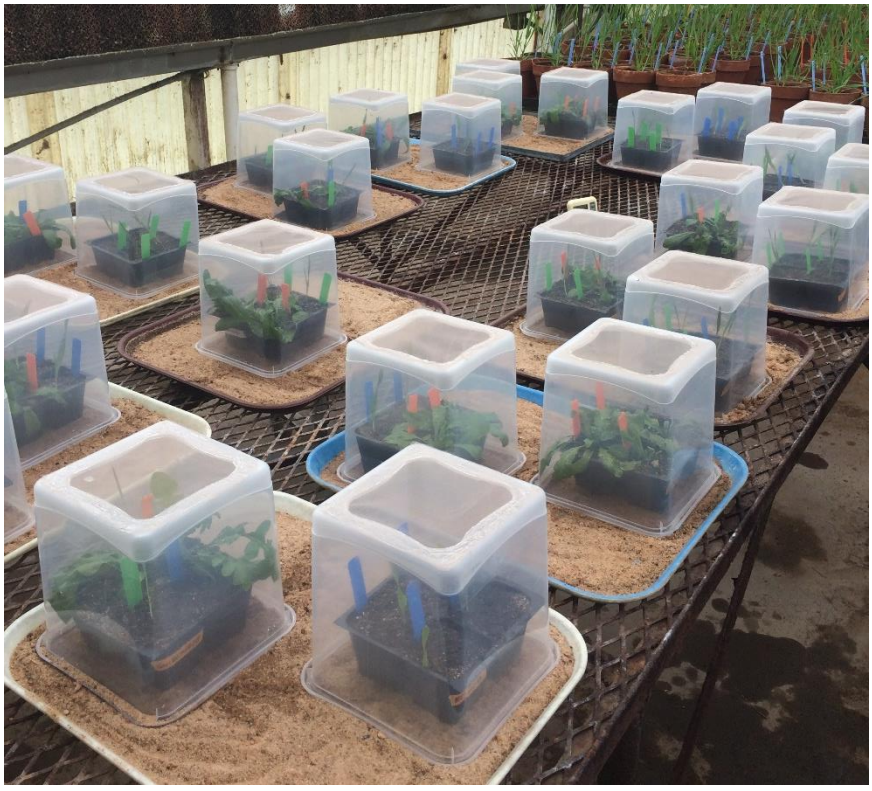


Figure 3.3 Individual cage set up within the environmental chamber.

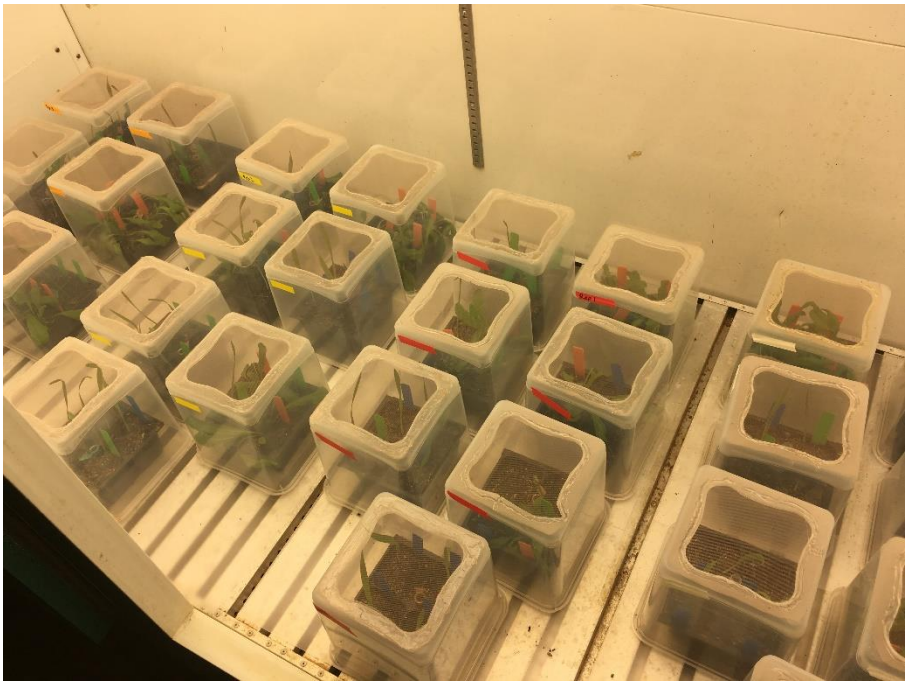


Figure 3.4 Environmental chamber evaluation of host plant complexity effects on *R. padi* weight. Three factor ANOVA comparisons of the mean aphid weight per treatment. Plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

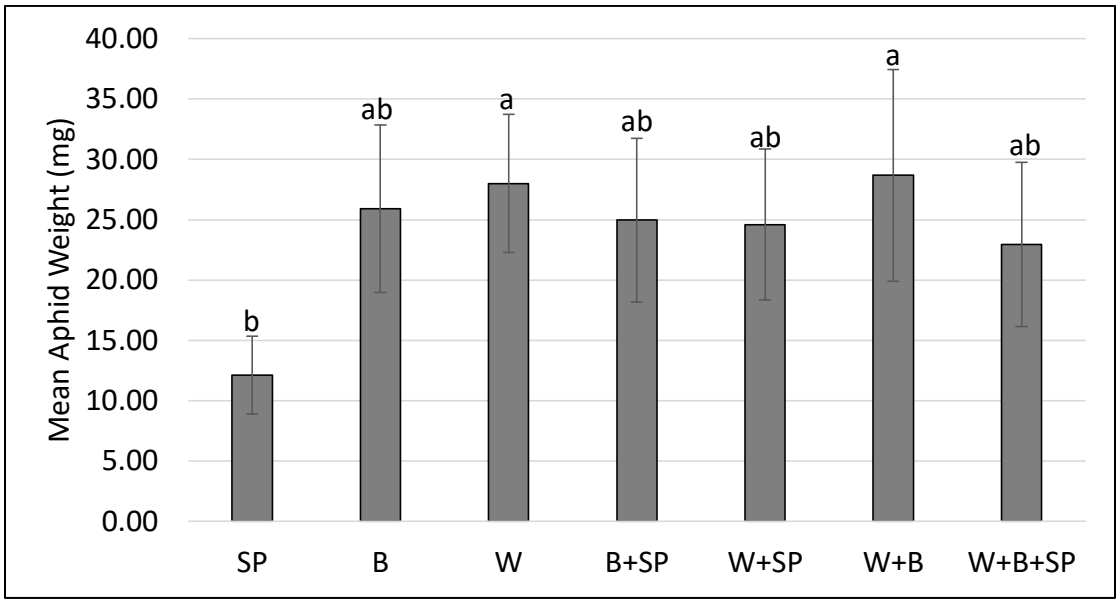


Figure 3.5 Environmental chamber evaluation of host plant complexity effects on total number of *R. padi* produced per treatment. Three factor ANOVA comparisons of the mean number of aphids produced per treatment. Plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

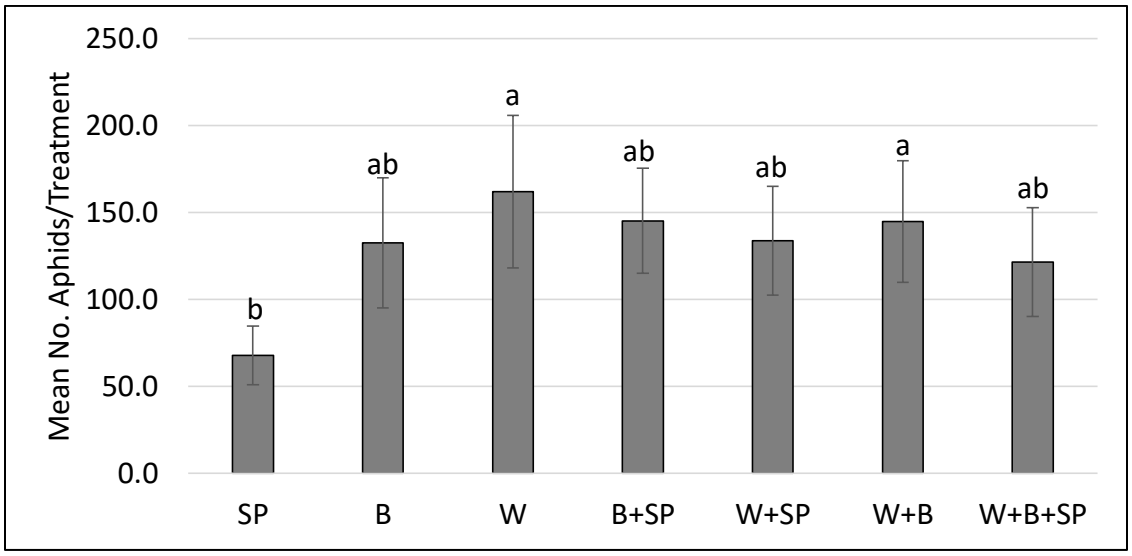


Figure 3.6 Greenhouse evaluation of host plant complexity effects on *R. padi* weight. Three factor ANOVA comparisons of the mean aphid weight per treatment. Plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.

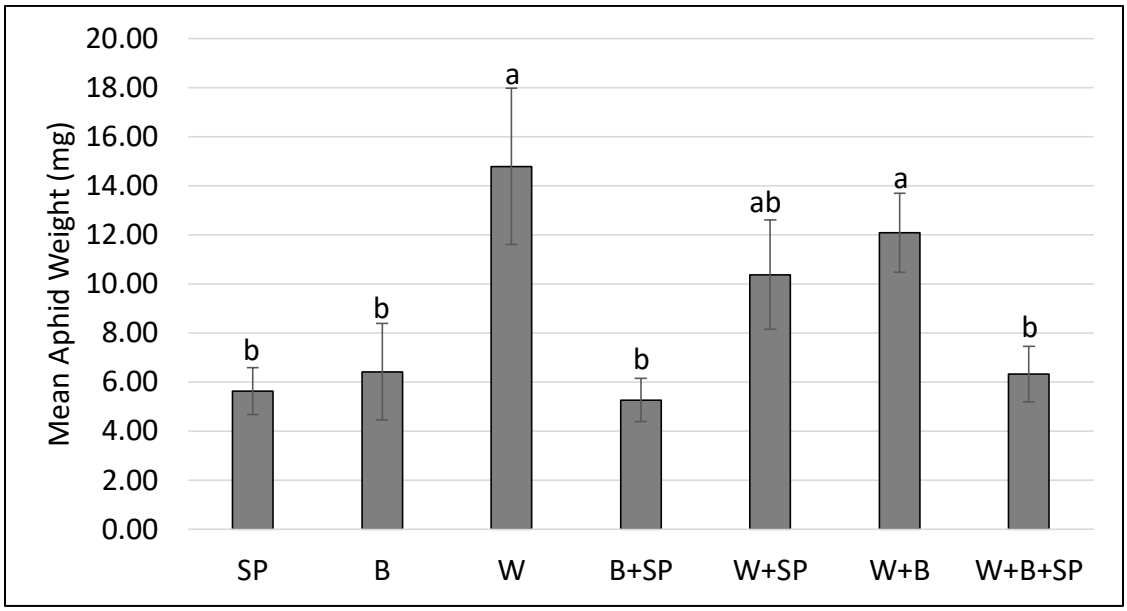
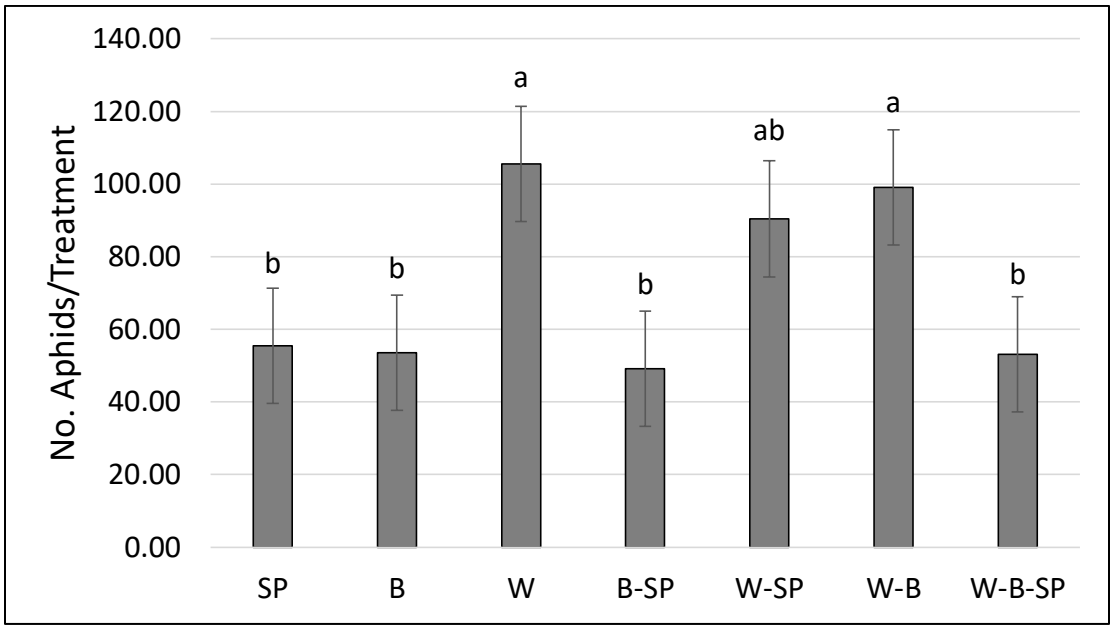


Figure 3.7 Greenhouse evaluation of host plant complexity effects on total number of *R. padi* produced per treatment. Three factor ANOVA comparisons of the mean number of aphids produced per treatment. Plant designations: wheat (W), barley (B), shepherd's purse (SP). Means with the same letter designation are not significantly different at $\alpha = 0.05$.



CHAPTER IV

ASSESSSMENT OF SALIVARY SHEATH COUNTS AND ELECTROPENETROGRAPHY TO EVALUATE BIRD CHERRY-OAT APHID (*RHOPALOSIPHUM PADI* L.) PROBING ACTIVITY ON WHEAT

Introduction

Wheat is an important commodity for the U.S., with exports of 26.5 million tonnes projected for the 2017-18 production season (IGC 2017). The Southern Great Plains of the U.S., specifically the states of Oklahoma and Texas, are significant producers of wheat. Combined, these two states harvested over 226 million bushels of wheat in 2016 (USDA-NASS 2017). Winter wheat, *Triticum aestivum* L., is the most commonly grown crop in Oklahoma and its uses range from human food products to livestock feed and forage, with upwards of 6 million acres of Oklahoma land sown to hard red winter wheat annually (Luper et al. 2005).

Of the many pathogens that infect wheat, barley yellow dwarf (BYD) is one of the most detrimental viral diseases of small grains worldwide (Flanders et al. 2006). Comprised of a suite of luteoviruses, these pathogens are persistently transmitted in a non-propagative manner by several aphid vectors (Waterhouse et al. 1987; Gray et al. 1991). In particular, as one of the most commonly found aphids in wheat in Oklahoma, *Rhopalosiphum padi* L., the bird cherry-oat aphid (Royer 2016), is of economic importance due to its propensity for transmitting several strains of BYDV and causing direct feeding damage (Stern 1967; Jiménez-Martínez et al. 2004; Royer 2016). Current management efforts are primarily focused on preventative and curative insecticide

use (Royer 2016; Royer and Giles 2017), but the development of *R. padi* and BYDV resistant wheat cultivars that would protect yield and allow for reduced management inputs remains as an important worldwide goal (Aradottir et al. 2017; Khan et al. 2017; Girvin et al. 2017).

Quantifying the feeding behavior of aphids among plant germplasm sources is a fundamental component of describing plant resistance to the insects themselves but also to the pathogens they transmit (Purcell and Almeida 2005). Aphids inflict physical injury to plants by puncturing plant cells with their stylets and by disrupting apoplasmic transport of fluids throughout the plant by deposition of saliva (Miles 1999). Aphid feeding behavior (i.e. stylet activity, salivation, and ingestion) cannot be directly observed because the feeding sites lie within the host plant. However, methods have been developed to quantify homopteran insect feeding behavior by examining salivary sheaths left behind in leaf tissue. Previous efforts to understand homopteran insect feeding behavior involved staining salivary sheath flanges, the portion of the sheath visible on the leaf surface (Bowling 1979, Viator et al. 1983; Marion-Poll et al. 1987). Whole tissue sectioning of salivary sheaths formed the basis of early descriptions of homopteran feeding behavior. These methods were modified by Backus et al. (1988) for use with leafhopper salivary sheaths, resulting in the development of a method that provided researchers the ability to observe the entire salivary sheath within leaf tissues, instead of just the outer flange. The advantage of staining is that sheaths can be visualized without paraffin or resin sectioning of the plant (Backus et al. 1988). While this method allows researchers to use sheath counts as a quantitative measure of homopteran insect injury, it does not determine where the insect feeds within the plant or what feeding behaviors were occurring at the time of feeding.

The use of electropenetrography (EPG) allows researchers to monitor the feeding behavior of homopteran insects within plants during active feeding. EPG is a technique that has been evolving since the late 1950s when McLean and Kinsey (1965) first developed an electronic monitoring system for studying aphid feeding behavior, which allowed for major breakthroughs

describing homopteran insect behavior. Modern EPG techniques incorporate an insect with piercing-sucking mouthparts (such as an aphid) and a plant into an electrical circuit. This is achieved by inserting an electrical probe (attached to a voltage source) into the soil of a potted plant and fixing a fine wire (connected to an electrical resistor) to the insect. Upon inserting its stylets, the insect completes the circuit, resulting in fluctuations of the electrical signal, known as the EPG signal, which result in waveforms that can then be recorded (Tjallingii 1978, 1985; Pettersson et al. 2007). Distinct waveform patterns have been recorded for aphids and correlate to probing, as well as stylet position within the plant (Tjallingii et al. 1985Pettersson et al. 2007; Backus et al. 2016). Several EPG parameters can be used to describe aphid feeding behavior including: number of probes and probe duration, duration of phloem salivation, duration of phloem ingestion, total feeding duration, as well as other stylet pathway parameters (Halarewicz and Gabryś 2012; Backus et al. 2016).

The origins of this study stem from observations of laboratory reared *R. padi.* Utilized as the source colony in evaluations conducted in Chapter 3, these aphids had significantly smaller body sizes than aphids reared on wheat in less crowded conditions under optimal temperatures (i.e. experimental colony from Chapter 3). Dixon (1985) stressed the importance of rearing aphids under standardized conditions to avoid undermining aphid fitness and subsequent interpretation of aphid-plant bioassays. This recommendation has been largely ignored in the literature regarding *R. padi*, with colonies being reared in a variety of conditions (See Chapter 3). The results from Chapter 3 indicate that *R. padi* raised uncrowded on wheat, under optimal environmental conditions, are larger and presumably more fit than the source colony aphids reared under conditions consistent with methods used by Oklahoma State University for rearing *R. padi* for use in germplasm resistance evaluations. I speculate that these differences may influence *R. padi* feeding behavior. The objective of this study is to quantify host-plant feeding

behaviors for *R. padi* reared under different conditions. It is hypothesized that differences in *R. padi* rearing conditions will result in differences in feeding behaviors.

Methodology

Aphid Colonies. The source *R. padi* colony was reared at 24.4±0.9°C (ambient room temperature of the laboratory) under 40W full spectrum florescent lights (F40DSGN50, LEDVANCE, LLC, Ontario, Canada) with a photoperiod of 16:8 (L:D) and 43±1.2% RH. The experimental *R. padi* colony was reared in an environmental growth chamber that was maintained at 18±2°C, 65±10% RH with a photoperiod of 14:10 (L:D). Both colonies were raised on a susceptible wheat cultivar ('Jagger'). Plants were watered and fertilized as necessary to facilitate optimal plant growth. To quantify apparent differences between *R. padi* colonies, antennal length and hind leg lengths were measured and recorded for 10 apterous adult aphids selected at random from each colony. Antennal length was measured from the base of the flagellum to the tip. Hind legs were measured from the coxa to the end of the tarsal claw (Figure 2.2). Means for each measure were compared using GLIMMIX (SAS 9.4, SAS Institute Inc., Cary, NC). Experimental aphids reared on uncrowded wheat in highly controlled environmental chamber conditions were significantly larger than aphids from the source colony in based on antennal length ($P = 0.0057$) and hind leg length ($P < 0.0001$) (Table 4.1). These morphological measurements have been correlated with aphid size and fitness in previous studies (Dixon et al. 1982; Dixon 1985).

***R. padi* Salivary Sheath Evaluation.** Wheat plants were grown in 2"x2"x2" planting flats. Two wheat seeds were planted per cell, thinned to one plant after emergence, and grown to the two leaf stage. Aphid exclusion cages were constructed by cutting the end off of a clear 2.0mL micro tube which was then taped to a large twist tie (Figure 4.1). Cages were slipped over the first leaf of a wheat plant, with the twist tie acting as a support. The bottom hole was then stuffed with a small ball of cotton. Each tube was infested with 5 apterous (wingless) adult aphids from either the source colony or the experimental colony, or no aphids (control). The top hole

was then stuffed with another small ball of cotton to prevent aphids from escaping. The cotton allowed the cages to be closed without damaging the wheat leaves or impeding plant growth. One assay consisted of 25 plants: 10 plants infested with *R. padi* adults from the source colony, 10 plants infested with *R. padi* adults from the experimental colony, and 5 untreated controls. Plants were randomly assigned to treatments and a total of three replications were performed. Flats were placed in an environmental chamber at 18±1° C, 65±10% RH, and a photoperiod of 14:10 (L:D) for 24hrs. Individual wheat plants were then cut just below and above the exclusion cage and aphids were gently removed with a fine-haired paintbrush from each leaf section before being transferred into its correspondingly labeled 2.0mL micro tube. Tubes were filled with 2.0mL of McBride's stain as prepared by Backus et al. (1988) and capped. Leaves were allowed to soak in the stain at room temperature for 24hrs. After 24hrs, leaves were removed and placed into individual glass petri dishes with corresponding labels. Petri dishes were then filled with clearing agent (Hooper 1986; Backus et al. 1988) to clear the leaves of stain, while leaving any salivary sheaths located within them stained a vivid pink color. In order to speed up the clearing process, Petri dishes were immediately autoclaved for 10 minutes at 120°C (Backus et al. 1988).

After cooling, leaf sections were gently removed from the clearing agent with soft forceps and placed onto a glass microscope slide. A second slide was placed on top in order to flatten and stabilize the leaf section for microscope viewing. Great care was taken during this process, as leaves became very soft and fragile during the clearing process and damaged easily. Salivary sheaths are clearly distinguished from the surrounding leaf tissue due to their pink color, deep purple-colored flange, and their orientation within the leaf, as they tended to run antiparallel to leaf venation. These characteristics made it possible to differentiate them from the vascular structures of the plant (Figure 4.2). All salivary sheaths present were counted and recorded for each labeled leaf. Sheath count means between colonies were square root transformed and compared PROC MIXED (SAS 9.4, SAS Institute Inc., Cary, NC).

Electropenetrography Evaluation. When an aphid begins feeding, a distinct voltage spike can be observed. This spike is characterized as a probe (Figure 2.10 B). Stylet pathway activities include potential drops, which are characterized by a sudden drop in voltage. Potential drops represent intracellular cell membrane punctures by the stylets as they move through the plant (Figure 2.10 D). These cells are often not feeding sites, causing the aphid to withdraw the stylets and continue pathway activities. When the stylets reach the xylem phloem, an X-wave is produced when the aphid begins ingesting the xylem sap (Figure 4.8 A, 4.9 A). When the stylets reach the phloem sap and the aphid begins salivating into the phloem sieve element, an E1 waveform is produced (Figure 2.10 F, G). The phloem salivation waveform (E1) transitions into the E2 waveform, indicating active phloem ingestion (Figure 2.10 G, H) (Tjallingii 1978, 1985; Backus et al. 2016). The number and/or duration of each of these parameters were recorded. Time was recorded and reported in seconds.

Certain parameters required distinct characteristics in order to be counted. For example, potential drops have three distinct subphases, subphases I, II, III, each with their own waveform. Potential drops were only counted if all three subphases were present. Capacitance tails often seen on a potential drop can indicate a suboptimal tether or electrical connection (personal communication with Astri Wayadande, Oklahoma State University, Stillwater, OK). However, these tails were consistently observed in all potential drops recorded for both aphid colonies. Additionally, if aphids remained in E2 for ≥10 minutes, it was considered to be sustained ingestion. This duration was selected based on studies by Prado and Tjalligii (1994) that demonstrated when *R. padi* fed for 10 minutes or more, it resulted in more efficient BYDV acquisition.

When evaluating the EPG recordings for this study, only aphids that fed for a period of 8 hours were used. Aphids that fed for shorter durations (due to falling off the plant, breaking free of the tether, or death) were not evaluated. As a result, out of the 57 apterous adult aphids

recorded (28 from the source colony and 29 from the experimental colony) only 36 of the recordings were usable. A total of 18 recordings for each aphid colony were analyzed. Each recording took 2-4 hours to analyze and was evaluated for specific aphid feeding behavior parameters including: time to first probe, duration of probe, number of probes performed per recording, number of potential drops, time to first potential drop, xylem feeding duration, time to first phloem contact, phloem salivation duration, phloem ingestion duration, and time to sustained ingestion.

In order for a recording to be successful, *R. padi* from both colonies had to be precisely positioned on the plant. This aphid species prefers to feed at the base of wheat plants (Whitworth and Ahmad 2008) and did not perform well if positioned too high on the leaf. Aphids also preferred to be oriented parallel to vascular plant structures, and would often spend an extended period of time trying to readjust themselves if not started in this position at the onset of the recording.

Electropenetrography (EPG) recordings were started between 8-10am, with each recording lasting a duration of 8hrs. As with the salivary sheath evaluation, feeding behavior of aphids from the standard colony and from the experimental colony were compared. EPG electrode tethering was modified for *R. padi* after procedures described by Carpane et al. (2011) for corn leaf hoppers. A manual aspirator was used to hold aphids in place while the tether was attached to the dorsal surface of the abdomen with silver print paint (Ladd Research Industries, Williston, VT). A small loop was bent into the end of the gold wire in order to provide greater surface area to ensure a more secure attachment and greater electrical connectivity. Once tethered to the electrode, the aphid was allowed to dangle freely for approximately 20 mins; this dangling period served as the starvation period. An individual aphid could perform feeding behaviors multiple times throughout the duration of the recording or not at all, which explains the high degree of variation among EPG parameter data (Table 4.6).

All recordings were performed on a four-channel Universal AC-DC EPG monitor. Over the duration of the study, standard EPG recording settings consisted of 200-mV direct current (DC) substrate voltage, input impedance of $10^9\,\Omega$, and an amplification (gain) of 500x90x1 or 500x140x1. The aphid and plant were placed inside a Faraday cage (2'x2'x4') in order to reduce background electrical interference and connected as described in Carpane et al. (2011). An analog-to-digital board (model DI-710 UHD, Dataq Instruments, Akron, OH) was used to convert output waveforms to a digital format. An acquisition rate of 100 samples per second per channel was recorded with Windaq software (Dataq Instruments, Akron, OH) and stored on a computer (Backus et al. 2016). Time to first probe, duration of probe, number of probes performed per recording, number of potential drops, time to first potential drop, xylem sap feeding duration, time to first phloem sap contact, phloem sap salivation duration, phloem sap ingestion duration, and time to sustained ingestion were compared between colonies using independent t-tests (SAS 9.4, SAS Institute Inc., Cary, NC).

Results

***R. padi* salivary sheath evaluation**. Salivary sheaths from both colonies were easily discernable under a microscope (80x magnification) from the surrounding leaf tissue as expected by their pink color, distinct flange, and orientation (Figure 4.2). Salivary sheaths identified during this evaluation had several variations. Sheaths were singular (Figure 4.3), branched (Figure 4.4), or had multiple sheaths originating from one flange (Figure 4.5). Sheaths often terminated in vascular tissue. Structures on the leaf surface (what appeared to be small hairs or trichomes) were found in all treatments and were easily differentiated from salivary sheaths or flanges by their orange color and lack of an extension into the leaf tissues.

Salivary sheath count analysis revealed a highly significant difference ($P = 0.0003$) between the number of stylet sheaths produced by the *R. padi* source colony and the experimental

R. padi colony, with the experimental colony producing significantly more salivary sheaths than the source colony (Figure 4.6). Sheath counts have been used as a quantitative measure of feeding by homopoteran insects (Bowling 1979; Viataor et al. 1983; Marion-Poll et al. 1987; Backus et al. 1988), thus it can be inferred by these results that aphids from the experimental colony feed (or attempt to feed) more frequently than aphids from the source colony.

***R. padi* electropenetrography evaluation**. References for EPG waveform designations and their biological behavior correlation can be found in Table 4.2. EPG waveforms recorded from both the source colony (Figure 4.8) and the experimental colony (Figure 4.9) were consistent with waveforms previously recorded for *R. padi* (Golawska et al. 2014) and were consistent with previously established general EPG waveform patterns for aphid feeding behaviors (Tjallingii 2000; Backus et al. 2016) (Figure 4.7). However, not all waveforms recorded could be correlated to specific aphid activities (Figures 4.10 and 4.11). Tjallingii (1978) classifies such waveforms as "unknown feeding behaviors," as they follow no known pattern.

Aphids from both colonies were observed spending time settling at the beginning of each recording, often performing labial dabbing (testing the leaf surface) or a series of test probes before the initial probe. Stylet dabbing and test probes were not recorded as their typical duration was ≤1 second, and did not meet the predetermined ≥5 seconds duration criteria for a probe. Analysis revealed a significant difference between the time it took aphids to begin probing (P = 0.0224) (Table 4.3), with the source colony aphids taking longer to begin feeding. Aphids from the experimental colony took significantly longer to reach sustained ingestion (P = 0.0216), taking nearly twice as long as the source colony to begin prolonged feeding.

Aphids from the experimental colony performed significantly more probes than the source colony (P = 0.0044) (Table 4.3), and the data obtained here is consistent with the results obtained from the salivary sheath staining evaluation (Figure 4.6). Mean probe duration per aphid

was nearly significantly different between the two colonies ($P = 0.0581$) (Table 4.5) with the source colony spending more time per probe. While total probing duration per treatment was higher for experimental aphids, but this difference was not significant (P = 0.0772) (Table 4.5).

There was no significant difference in the pathway activities (i.e. behaviors correlated to stylet movement within the plant to reach vascular tissue) including potential drops (pds). EPG parameters including time to first pd (Table 4.3), number of pds per probe (Table 4.4), and total pathway duration (Table 4.5) were evaluated. Pds recorded from both colonies were visually similar, with no recognizable difference in pd waveform characteristics (Figure 4.10 A, B).

In EPG monitoring, two different waveforms are associated with the phloem: E1 and E2. The E1 waveform is correlated with phloem salivation behavior in aphids. During this time, aphids are salivating into the phloem sieve tube, preparing for ingestion, but are not ingesting phloem sap (Morris and Foster 2008). Analysis revealed that there was no significant difference between the colonies for the amount of time it took for aphids to first contact the phloem ($P = 0.8628$), with one aphid from the source colony never reaching the phloem throughout the duration of the recording (Table 4.3). However, the total number of E1 events performed by the experimental colony was significantly higher than the source colony ($P = 0.0021$) (Table 4.4). However, the duration of E1 events were not significantly different between the two colonies ($P = 0.5487$) (Table 4.5).

The E2 waveform is correlated with phloem ingestion (Kimmins and Tjallingii 1985; Prado and Tjallingii 1994; Reese et al. 2000; Morris and Foster 2008). Aphids from the source colony spent significantly more time ingesting phloem than aphids from the experimental colony ($P = 0.0464$) (Table 4.5). Source colony aphids also spent significantly more time in combined contact with phloem elements (E1 and E2) (Table 4.5) than the experimental colony ($P = 0.0477$)

and took significantly less time to reach sustained ingestion than aphids from the experimental colony ($P = 0.0216$) (Table 4.3).

Discussion

EPG and *Rhopalosiphum padi.* This study is the first time aphids have been evaluated using a Universal AC-DC electropenetrography (EPG) monitor. The resulting *R. padi* waveforms were consistent with those previously recorded for this species (Golawska et al. 2014) and for aphids in general using older techniques (Tjallingii 2000; Backus et al. 2016). Electropenetrography is a well-established technique for studying the feeding behaviors of piercing-sucking insects, providing objective visualizations of feeding activities within the plant (Tjallingii and Hogen Esch 1993). By incorporating the insect and plant into an electrical circuit (completed by insertion of the stylets into the plant), changes in voltage amplitudes produced during insect feeding can be converted into digital waveforms and can be correlated to aphid feeding behaviors (Walker 2000). This technique can be utilized as a major tool in host plant resistance evaluations. Indeed, aphid feeding behaviors evaluated with EPG on resistant or susceptible plant varieties have been demonstrated to serve as an initial means of discerning the level and type of resistance of plants to aphid infestation or for locating the site of resistance (Nielson and Don 1974; Lohar and Kawada 1987; Peters et al. 1988; Montllor and Tjallingii 1989; van Helden and Tjallingii 1993; Caillaud et al. 1995; Annan et al. 2000; Kaloshian et al. 2000; Zehnder 2001; Sandanayaka et al. 2003; Wang et al. 2004; Le Roux et al. 2008; Lightle et al. 2012; Koch et al. 2015, Backus et al. 2016).

Despite its advantages, EPG is not a technique without challenges. EPG requires substantial practice by the operator in both insect preparation and set up, and to properly evaluate EPG output waveforms. Of the aphids successfully recorded in this study, there were several dozen more that did not make it past the tethering process. Being a small insect, *R. padi* are

difficult to tether. Looping the end of the wire before attaching it to an aphid was much more successful than using a minute ball of paint on the end of the wire, as it provided more surface area for attachment (personal communication with Astri Wayadande, Oklahoma State University, Stillwater, OK). Because *R. padi* seem to have more waxy secretions on their exoskeletons, silver glue (1:1:1 of water, silver flake, and Elmer's glue) typically used for attaching EPG tethers, would not stick. Although less desirable due to its potentially chronic toxic effects on insects (Hagler and Jackson 2001), silver print paint was used to tether aphids in this study. However, before the advent of silver glue, silver print paint was the standard used in EPG studies. All aphids in this study were tethered in this manner, thus any potential toxic effects were consistent for all aphids. Additionally, although aphids attached to EPG tethers have been demonstrated to exhibit reduced longevity and fecundity (Tjallingii 1986), this has not been shown to affect probing or phloem penetration behaviors or the waveform patterns produced (Tjallingii 1986; Annan et al. 1997; Morris and Foster 2008). The most obvious effect of the EPG tether on aphids is a reduction in mobility, making the position of the aphid on the plant integral to success of an EPG recording, as was discovered early on in this project.

***Rhopalosiphum padi* feeding behavior.** The findings of these studies support the hypothesis that differences in rearing conditions for *R. padi* can influence their feeding behavior. Differences in environmental rearing conditions have been cited as one of the potential factors responsible for inconsistent results in previous barley yellow dwarf transmission studies involving *R. padi* (Gray et al. 1991). The impact of environment on aphid biology and feeding behavior is an important factor when considering evaluation development of aphid resistant sources. Aberrations in aphid feeding behavior may influence the ability of cereal cultivars to withstand feeding injury and/or transmission of pathogens by these insects. These variations in aphid feeding behavior have the potential to skew the outcomes of such studies by potentially increasing the likelihood of concluding that resistance is occurring. Indeed, inconsistencies

regarding the identification of *R. padi* resistant cultivars has been a reoccurring and problematic issue (Hsu and Robinson 1962, 1963; Rautapää 1970; Markkula and Roukka 1972; Kieckhefer et al. 1980; Lowe 1980; Wiktelius and Pettersson 1985). Comparisons of aphid feeding behaviors, specifically related to the number of probes, the time spent salivating into phloem, and the time spent ingesting phloem or xylem sap, are all facets of aphid feeding behavior that have the potential to influence the outcomes of *R. padi* host plant resistance bioassays.

Potential for plant injury and BYDV transmission. *Rhopalosiphum padi* reared continuously uncrowded on wheat at ideal environmental conditions (i.e. experimental colony) were larger with high fitness (based on results of Chapter 3, Table 4.1). In comparison, source colony aphids were substantially smaller and would be predicted to be less fit, thus causing less plant injury (Michaud 2012). Fitness of source colony aphids was not measured. However, indicators of feeding injury and potential for BYDV transmission were quantified for this study.

Differences in probing behaviors between these two aphid colonies obtained through the salivary sheath staining evaluation as well as the EPG evaluation, suggests that the potential for plant injury and BYDV transmission are different between aphid colonies. Aphids from the experimental colony probed more frequently than the standard colony, supporting the data obtained from the salivary sheath staining evaluation. Additionally, aphids from the experimental colony initiated probing more quickly than source colony aphids, indicating that the source colony aphids may be more reluctant to begin feeding. However, once source colony aphids began feeding, they reached sustained ingestion more quickly than the experimental colony.

Aphids from the experimental colony spent more time salivating into the phloem than ingesting phloem, while the source colony spent more time ingesting phloem than salivating into the phloem. Considering that *R. padi* is an efficient vector of barley yellow dwarf virus (BYDV) strains BYDV-RPV and BYDV-PAV (Gourmet et al. 1994), this difference in phloem feeding

activity may be important for researchers studying BYDV resistance in cereals. This virus is a persistently transmitted luteovirus that must be acquired through the phloem and cycle through an aphid vector (Figure 2.1) in order to become infective (Katis et al. 2007). Gray et al. (1991) noted that although the acquisition of persistently transmitted viruses by an aphid can happen in less than 30 minutes (Watson and Mulligan 1960; Tamada 1970; Leonard and Holbrook 1978), the ability of an aphid to efficiently transmit the virus to healthy plants requires extended access to the phloem.

The source colony spent more overall time in phloem elements (i.e. phloem salivation and phloem ingestion combined), but had significantly fewer E1 events. This is an important observation and may indicate that the experimental colony would be better suited for use in BYDV transmission and resistance studies, as increased access to the phloem provides greater opportunity for transmission of BYDV. Indeed, the longer an aphid has access to the phloem, the greater the efficiency of BYDV acquisition (Tjallingii 1994) and the greater the concentration of virus is within that aphid (Paliwal and Sinha 1970; Tamada and Harrison 1981). Because aphids from the source colony spent significantly more time ingesting phloem sap, I infer that they may be more likely to acquire BYDV, but may not perform this behavior frequently enough for effective BYDV transmission into healthy plants. Overall, EPG results indicate that differences in phloem feeding activities may be important during phenotypic studies evaluating sources of resistance. Results indicate that the most "fit" experimental colony aphids (i.e. larger and higher reproduction) may not be best for plant injury evaluations, but "stressed" source colony aphids appear more likely to feed over extended periods of time and induce plant injury.

Additionally, the total duration of xylem bouts was not significantly different between the two colonies. However, these observations are still worth noting in relation to predictions for phenotype assays of plant resistance. The experimental colony accessed the xylem more times over the course of 8hrs than the source colony (Table 4.6). Unlike the phloem, xylem sap is

mostly composed of water (Tyree and Sperry 1989) and in drought conditions, this difference in feeding behavior could largely impact the ability of the host plant to defend itself against insect attack. Even if the host plant were able to withstand the mechanical injury inflicted by aphid feeding, the removal of xylem sap may result in plant stress and apparent injury due to lack of water. Water stress in wheat causes the stomata to close in an effort to conserve water. With the stomata closed, photosynthesis ceases, as the exchange of carbon dioxide required to make essential sugars is no longer occurring. The resulting depletion of sugar resources would eventually impede plant growth and ultimately, reduced plant growth leads to yield reduction (Macpherson 2017). Indeed, one of the few noticeable impacts of *R. padi* feeding on wheat often mimics the effect of drought (Bruehl 1961; Rochow 1961; Burnett 1984; D'Arcy 1995). This effect has also been observed in potted wheat plants in laboratory-reared colonies (personal observation).

Conclusion

Rhopalosiphum padi, the bird cherry-oat aphid, is an economically important aphid due to its propensity for transmitting several strains of BYDV and causing direct feeding damage (Stern 1967; Jiménez-Martínez et al. 2004) to wheat (Royer 2016). Understanding how the feeding behaviors of this aphid might be influenced by colony rearing conditions is integral for investigations examining resistance in cereals. Of the numerous insect feeding behaviors that can be evaluated with electropenetrography, fifteen of these parameters were selected for this study and several important feeding behavior parameters indicated that colony conditions may influence plant injury and BYDV transmission. Differences between the number of probes, the time spent salivating into phloem, and the time spent ingesting phloem or xylem sap, are important components of aphid feeding behavior that have the potential to influence the outcomes of *R. padi* host plant resistance bioassays. It has been demonstrated here that variation in environmental rearing conditions between colonies of *R. padi* can lead to altered feeding behavior

and these differences should be considered when evaluating the impact of aphid feeding on host plants during screenings of resistant plant sources.

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Table 4.1 Morphological measurements of *R. padi* from each colony evaluated in the salivary sheath study and the EPG study. Means with the same letter designation are not significantly different at $\alpha = 0.05$. Lengths were compared with GLIMMIX.

Colony	Antenna Length(µm)	Hind Leg Length(µm)
Experimental	1054.8±31.2[a]	1320.2±30.0[a]
Source	804.5±30.1[b]	950.7±25.2[b]

Table 4.2 EPG waveforms and their feeding behavior correlations (modified from Tjallingi 2000; Golawska et al. 2014; Backus et al. 2016).

Waveform Designation	Behavior Correlation
Baseline	No stylet insertion and/or non-probing
Probe (≥2V above baseline)	Stylets inserted, probe initiated
Pathway	Stylets moving intercellularly
Potential Drop (pd)	Intracellular puncture during stylet pathway
X-wave	Transition phase alternating between pathway and phloem phases
E1	Phloem salivation
E2	Phloem ingestion
E1+E2	Transition from phloem salivation to ingestion
G	Xylem ingestion

Table 4.3 Mean±SE comparisons of *R. padi* from two different colonies for EPG parameters correlated to the onset of probing, intracellular stylet pathway activities (potential drops), phloem contact, and phloem ingestion. $\alpha = 0.05$. All time durations are reported in seconds. Means were compared with t-tests.

EPG Parameter	*N*	Aphid Colony		*P* -value
		Source	Experimental	
Time to 1st Probe	18	685.30±124.8	326.30±81.26	0.0224
Time to 1st Potential drop	18	98.27±21.59	91.32±12.03	0.7803
Time to 1st Phloem Contact	17	281.90±1153.00	5032.10±871.80	0.8628
Time to Sustained Ingestion	17	925.50±1140.20	11017.30±1760.30	0.0216

Table 4.4 Mean±SE comparisons of *R. padi* from two different colonies for major numerical EPG parameters: total number of probes, total number of potential drops, and total number of E1 events. $\alpha = 0.05$. Means were compared with t-tests.

EPG Parameter	Aphid Colony				*P* -value
	N	Source	*N*	Experimental	
Total Probes	109	6.06±0.85	230	12.78±1.95	0.0044
Total Potential drops per Probe	1035	9.58±1.27	1749	9.27±1.47	0.8711
Total E1 Events	46	2.56±0.54	107	5.94±0.85	0.0021

Table 4.5 Mean±SE comparisons of *R. padi* from two different colonies for additional EPG parameters. $\alpha = 0.05$. All time durations are reported in seconds. Means were compared with t-tests.

EPG Parameter	Aphid Colony		*P* -value
	Source	Experimental	
Mean Probe Duration	6880.81±1671.98	3294.66±740.15	0.0581
Total Probing Duration/Treatment	23167.85±1379.28	26904.27±1419.98	0.0772
Total Pathway Duration	827.47±147.58	960.34±270.94	0.6694
Total Xylem Duration	173.31±58.22	355.01±116.38	0.1717
Xylem Duration/Treatment of Aphids that fed in Xylem	283.62±79.38	456.45±138.61	0.3253
E1 Duration/Treatment	187.28±54.90	237.60±62.33	0.5487
E2 Duration/Treatment	5357.48±1659.45	1624.60±711.18	0.0464
Total Phloem Contact Duration (E1 + E2)	5544.75±1650.58	1859.13±703.55	0.0477

Table 4.6 Raw data of *R. padi* individuals measured for all EPG parameters. All time durations are reported in seconds.

Colony	Insect	Total Probes	Mean Probe Duration	Σ Total Probe Duration	Mean Potential Drops/Probe	Mean Total Pathway Duration	Mean Xylem Duration	Mean E1 Duration	Mean E2 Duration	Mean Total Phloem Contact (E1+E2)
Source	1	9	892.92	8036.3	0.8889	344.87	309.28	0	0	0
Source	2	8	1788.71	14309.71	5.625	569.46	189.09	217.67	806.91	1024.57
Source	3	8	2626.79	21014.34	9	732.33	250	140.92	1503.55	1644.47
Source	4	7	3061.72	21432.06	5.5714	410.26	0	133.49	2512.68	2646.17
Source	5	7	3694.32	25860.25	5.4286	606.55	0	386.08	2698.59	3084.67
Source	6	6	4350.77	26104.6	8	527.86	158.8	41.02	3505.76	3546.78
Source	7	4	6984.21	27936.85	9.75	686.95	962.53	4.7	5314.03	5318.73
Source	8	10	2204.62	22046.21	18.1	1210.86	146.65	338.92	423.42	762.34
Source	9	2	14048.04	28096.07	14	3048.09	569.8	11.77	10988.18	10999.95
Source	10	7	3446.71	24126.99	6.7143	639	0	182.3	2609.37	2791.66
Source	11	2	14163.18	28326.35	7	464.5	0	15.04	13506.04	13521.08
Source	12	6	1678.37	10070.2	7.6667	712.88	201.87	633.21	119.97	753.18
Source	13	2	14141.44	28282.87	17	1209.38	37.95	829.71	10804.37	11634.08
Source	14	3	8253.01	24759.04	20.6667	1109.35	167.43	189.69	1937.94	2127.63
Source	15	3	9409.29	28227.88	6.3333	497.93	0	12.36	8814.98	8827.34
Source	16	1	28656.85	28656.85	8	569.85	0	22.15	28064.85	28087
Source	17	9	2844.8	25603.24	5.7778	353.17	0	3.85	2482.82	2486.67
Source	18	15	1608.76	24131.42	17	1201.21	126.25	208.11	341.13	549.24
Experimental	1	12	2205.54	26466.44	9.4167	541.86	136.69	430.25	1056.82	1487.07
Experimental	2	8	3455.42	27643.36	20.875	1857.6	1142.65	327.71	127.46	455.17
Experimental	3	9	2847.94	25631.44	13.7778	744.48	759.5	88.59	1255.36	1343.95
Experimental	4	8	3337.07	26696.55	7.5	1287.16	167.75	547.12	441.07	933.06
Experimental	5	26	891.56	23180.5	6.2692	587.9	166.42	30.36	81.7	112.07
Experimental	6	11	2504.54	27549.98	2.8182	295.79	0	320.09	1726.85	2046.94
Experimental	7	12	2134.5	25614.04	7.3333	547.52	21.08	14.47	1550.06	1564.53
Experimental	8	11	2470.02	27170.18	3.6364	682.97	0	386.08	1377.92	1764
Experimental	9	5	5631.85	28159.23	15.8	1519.11	1839.16	1039.59	1210.45	2250.05
Experimental	10	9	2097.68	18879.08	9.1111	779.76	647.81	214.79	453.44	668.22
Experimental	11	7	6964.14	48748.96	26.1429	5231.92	421.31	232.99	357.97	590.97
Experimental	12	34	590.55	20078.82	5.9412	390.78	76.61	25.73	29.37	55.1
Experimental	13	15	1658.51	24877.59	6.3333	377.83	0	19.68	1243.88	1263.56
Experimental	14	2	14218.84	28437.67	10	715.78	0	26.68	13476.39	13503.06
Experimental	15	28	917.3	25684.34	3.6429	265.91	30	32.29	561.56	593.85
Experimental	16	13	2041.53	26539.89	6.7692	596.61	486.67	157.24	787.87	945.11
Experimental	17	11	2443.42	26877.6	4.5455	349.89	482.15	3.64	1601.55	1605.19
Experimental	18	9	2893.47	26041.25	6.8889	513.27	12.43	379.47	1903	2282.46

Figure 4.1 Exclusion cages designed to isolate aphids on single wheat leaves.

Figure 4.2 Two single branched *R. padi* salivary sheaths within wheat leaf tissue. Sheaths often ran antiparallel to vascular structures, making them clearly identifiable. Arrows indicate the dark purple-colored flanges. The flange is located on the leaf surface, with the salivary sheath extending into the plant below it.

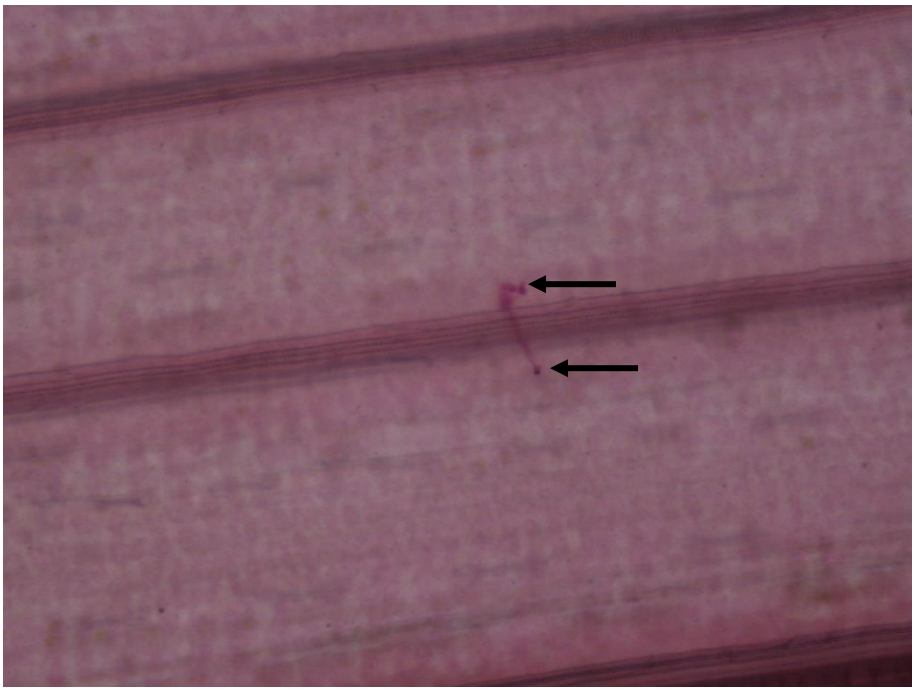


Figure 4.3 Single *R. padi* salivary sheaths within wheat leaf tissue.

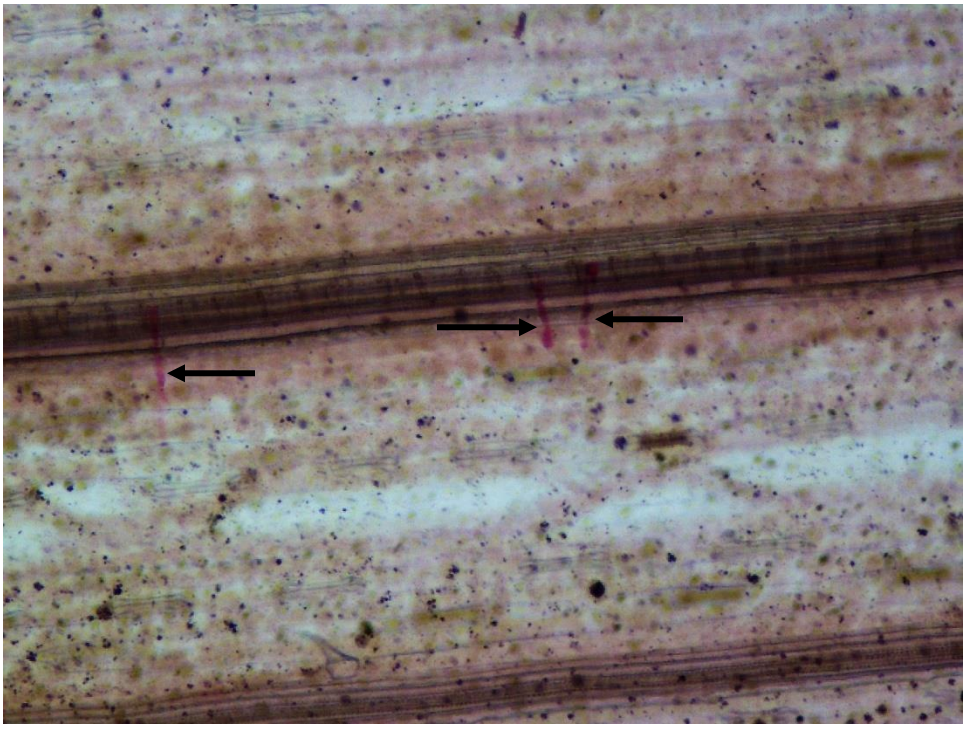


Figure 4.4 Branched *R. padi* salivary sheath within leaf tissue.

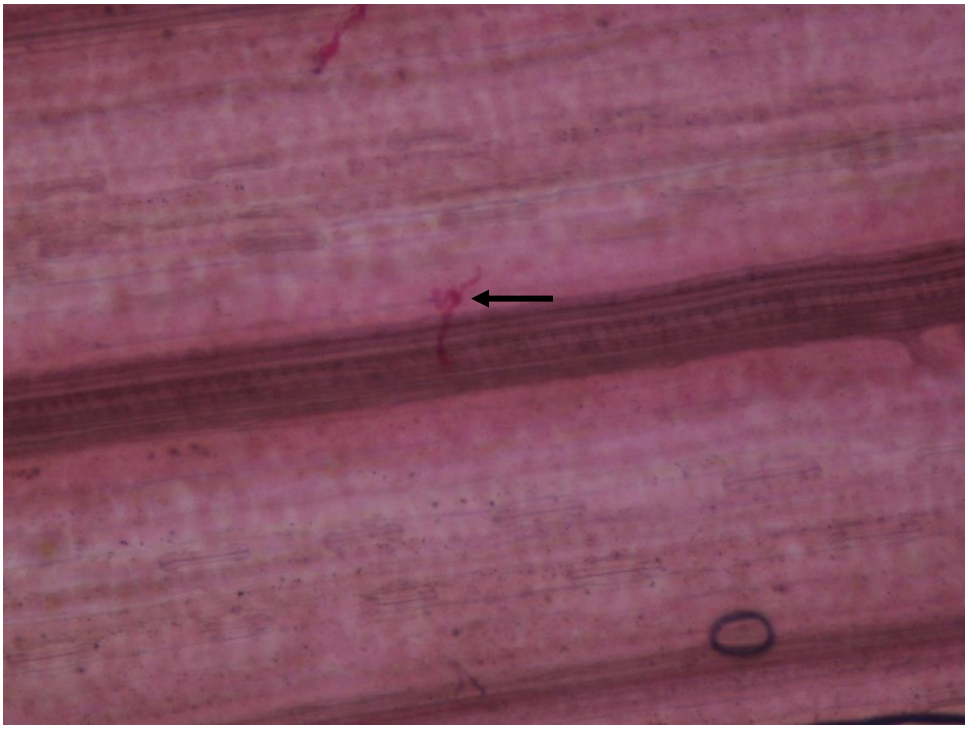


Figure 4.5 Multiple *R. padi* salivary sheaths originating from one flange.

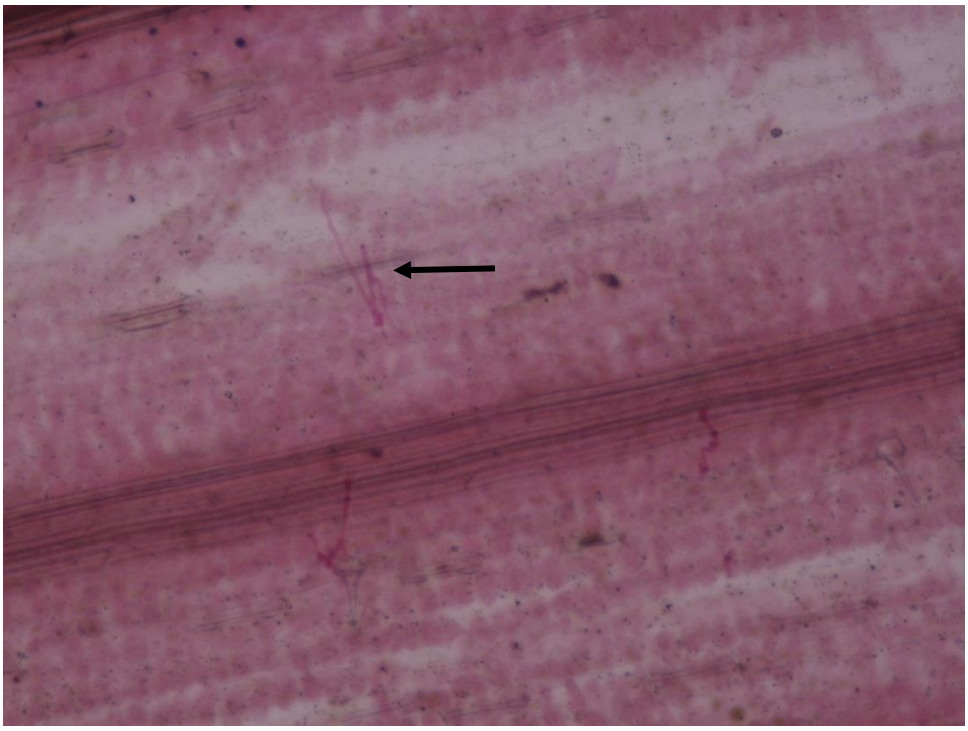


Figure 4.6 *R. padi* salivary sheath staining study results. Mean number of salivary sheaths per treatment. Means with the same letter designation are not significantly different at $\alpha = 0.05$.

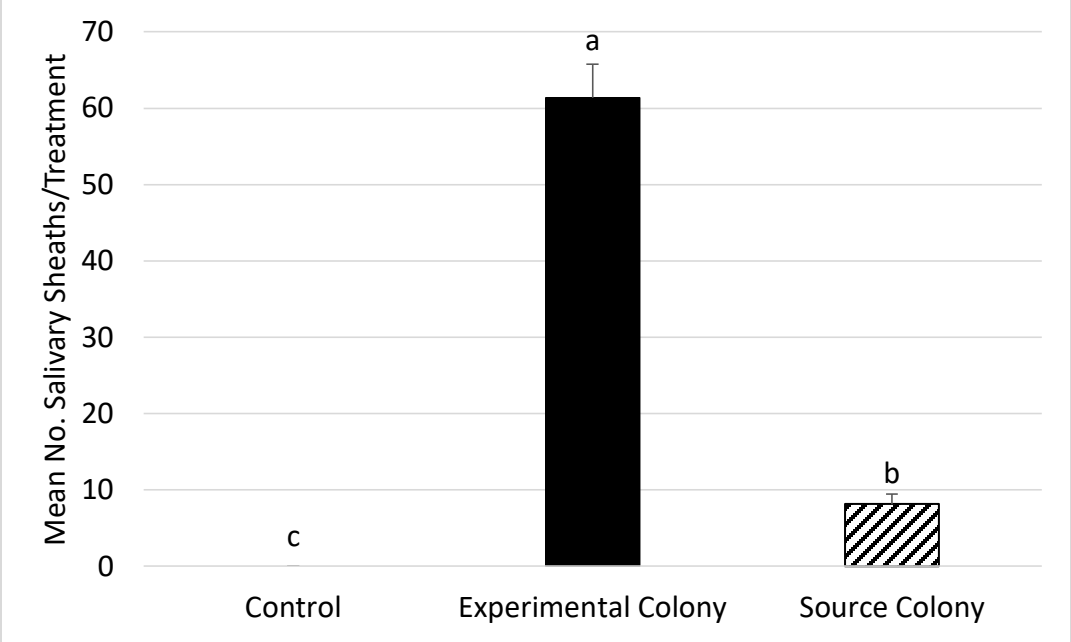
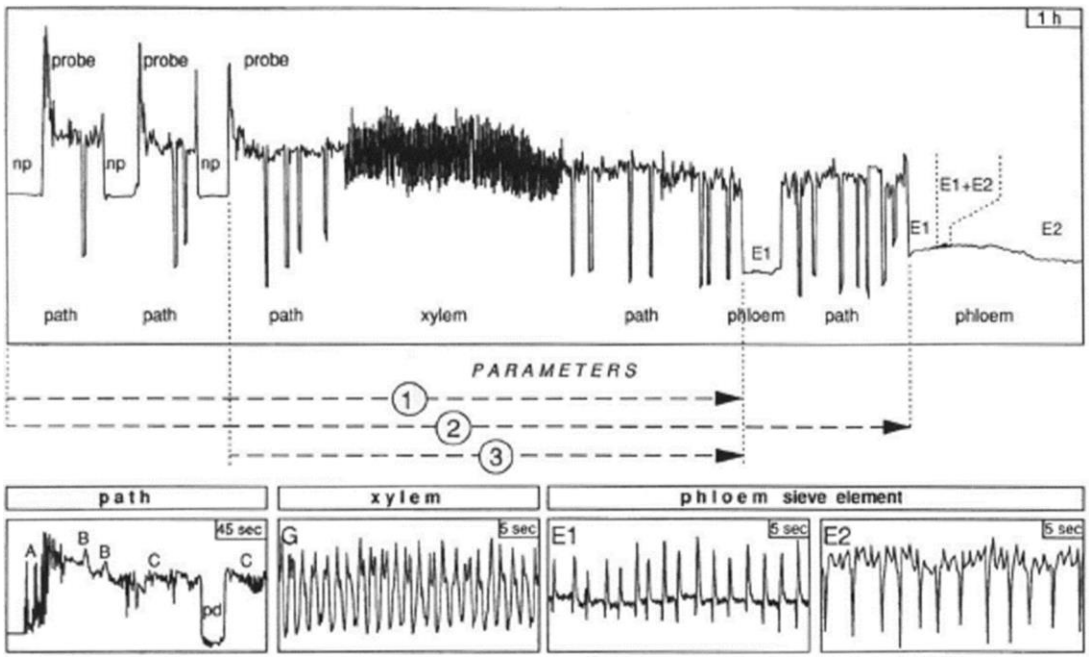


Figure 4.7 Generalized EPG waveforms established for aphids. Top: main features of aphid feeding from initial plant contact to phloem ingestion with Non-probing (np), stylet insertion (probe), stylet pathway, xylem ingestion (G), phloem salivation (E1) transitioning (E1+E2) to phloem ingestion (E2). Lower left: Pathway with stylet contact (A), stylet sheath salivary material secretion (B), and (C) with undefined pathway activities and a potential drop (pd) indicating an intercellular puncture. Lower center: Decompressed xylem waveform (G). Lower right: Decompressed phloem salivation waveform (E1) and phloem ingestion waveform (E2). Parameters: 1) Time to first phloem contact from beginning of recording, 2) Time to sustained ingestion from beginning of probe, and 3) Time to first phloem contact from the beginning of the probe (from Tjallingii 2000).



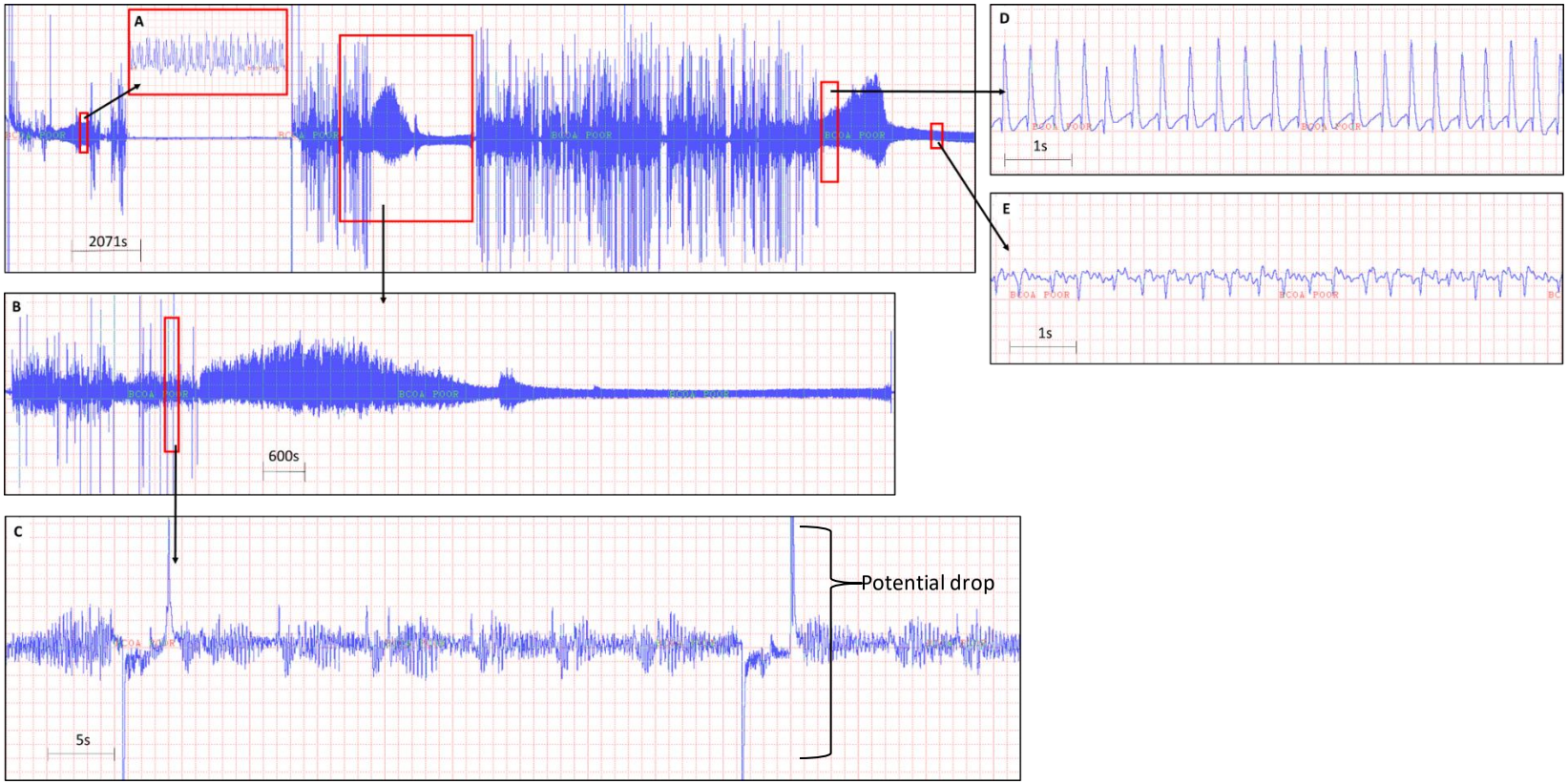


Figure 4.8 A complete overview of an 8hr recording of one apterous *R. padi* adult from the source colony with samples of major feeding waveforms. Box A: Expanded xylem ingestion waveforms. Box B: Expansion of one probe. Box C: Expansion of pathway within a probe, with two potential drops (pds), indicating intracellular puncturing, and X-waves. X-waves are a precursor to phloem contact. Box D: Expansion of E1 waveforms, indicating phloem salivation. Box E: Expansion of E2 waveforms, indicating phloem ingestion.

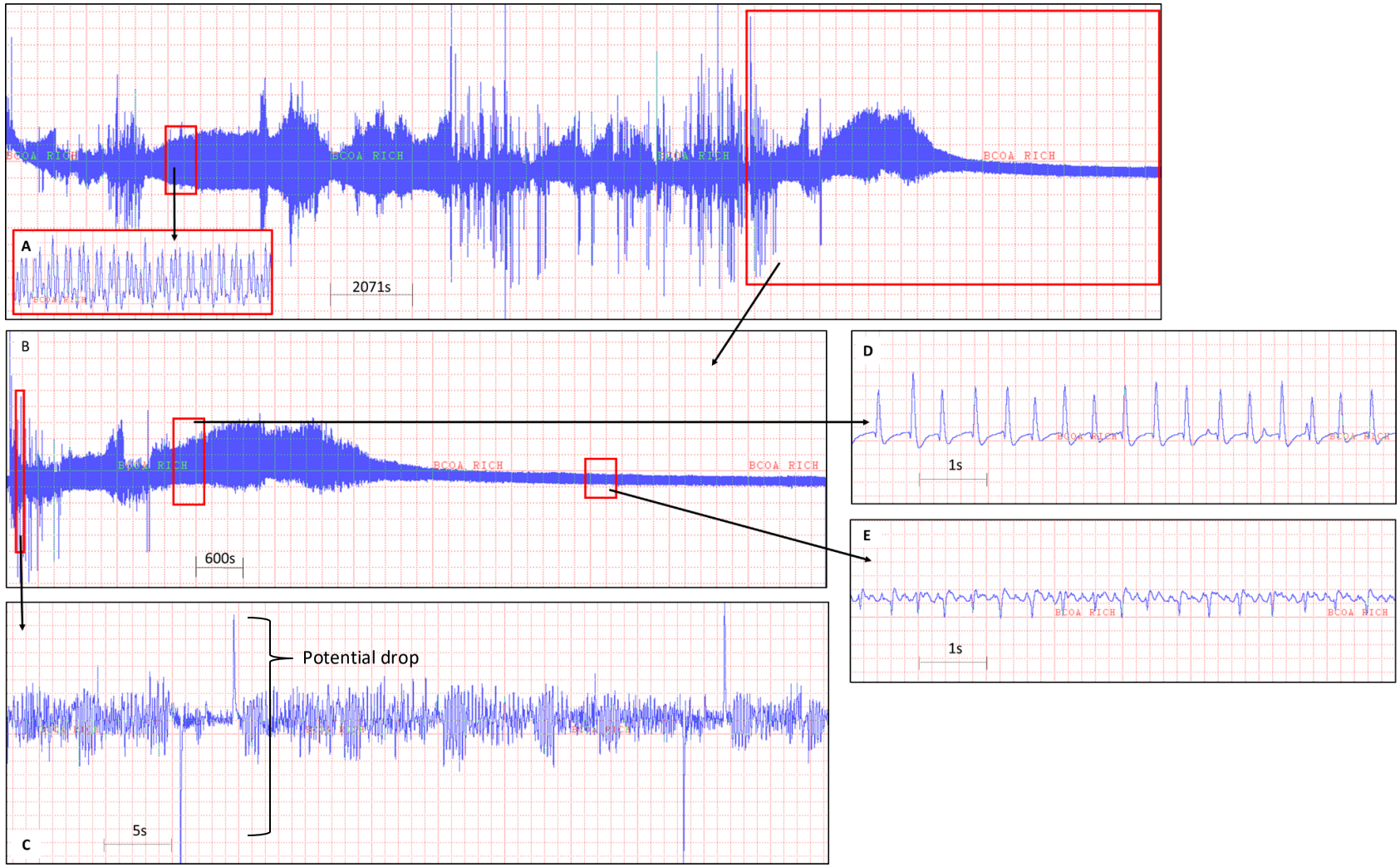


Figure 4.9 A complete overview of an 8hr recording of one apterous *R. padi* adult from the experimental colony with samples of major feeding waveforms. Box A: Expanded xylem ingestion waveforms. Box B: Expansion of one probe. Box C: Expansion of pathway within a probe, with two potential drops (pds), indicating intracellular puncturing, and X-waves. X-waves are a precursor to phloem contact. Box D: Expansion of E1 waveforms, indicating phloem salivation. Box E: Expansion of E2 waveforms, indicating phloem ingestion.

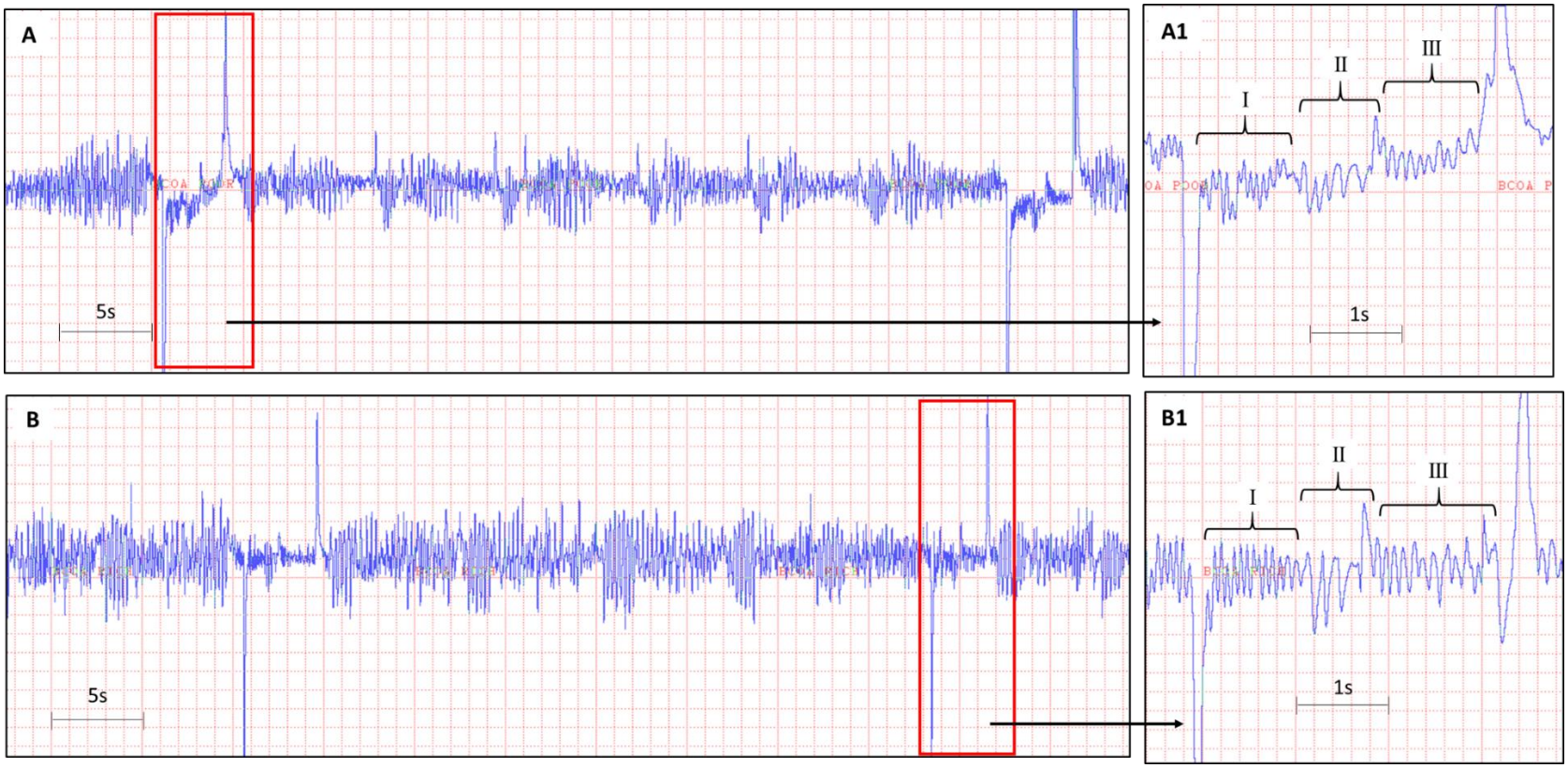


Figure 4.10 Comparison of potential drops (pds) recorded from both the source colony (A) and the experimental colony (B). Although pds had large capacitance tails, the downward spike at the beginning and upward spike at the end, this was a consistent feature of all pds recorded for all aphids. Capacitance tails can indicate a suboptimal tether or connection. Fully expanded pds from the source colony (A1) and the experimental colony (B1) have clear sub phases I, II, and III.

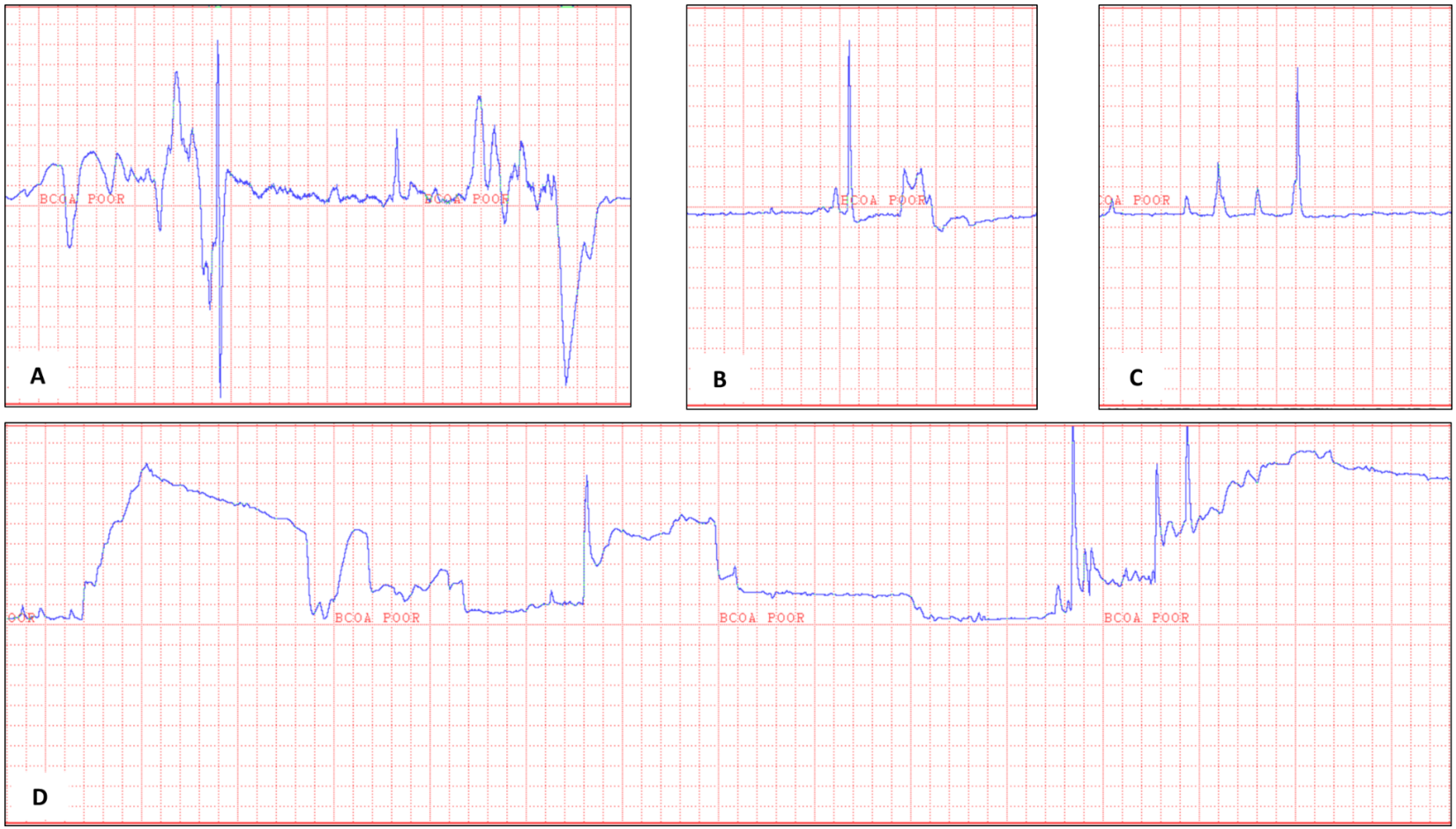
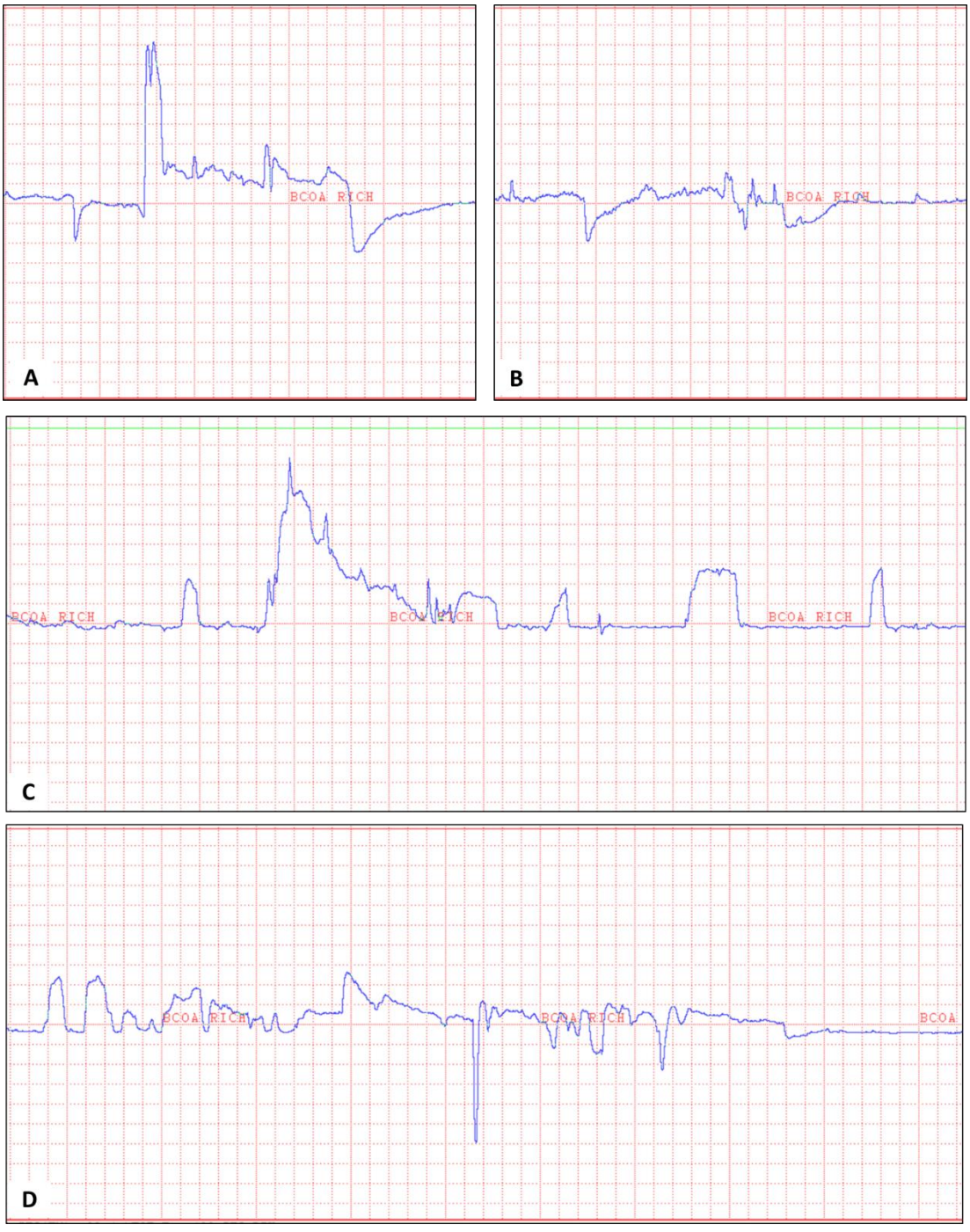


Figure 4.11 Examples of indiscernible waveforms recorded from the *R. padi* source colony. These waveforms followed no know pattern established for *R. padi* or for aphids in general. They could not be correlated to any known aphid feeding behaviors. Each division equals 0.400 seconds.

Figure 4.12 Three examples of indiscernible waveforms recorded from the *R. padi* experimental colony. These waveforms followed no know pattern established for *R. padi* or for aphids in general. They could not be correlated to any known aphid feeding behaviors. Each division equals 0.400 seconds.



CHAPTER V

CONCLUSION

The first objective of this study was to determine if increasingly complex host plant habitats resulted in the most fit *Rhopalosiphum padi*. I hypothesized that the addition of suitable host plant species to *R. padi* rearing cages, in an effort to mimic the heterogeneous complexity of agroecosystems, would increase aphid fitness. I compared seven different host plant compositions in greenhouse and environmental chamber trials. By comparing the weight and number of *R. padi* produced within these treatments, I was able to reject my primary hypothesis that the availability and utilization of additional suitable host plants to aphid rearing cages would increase fitness. Conversely, this study revealed that the addition of shepherd's purse (*Capsella-bursa pastoris*) to *R. padi* rearing habitats resulted in significantly reduced aphid fitness. Aphids produced in treatments including shepherd's purse as a host plant were not only smaller, but also weighed less than those produced without the presence of this host plant. Indeed, the data revealed a potentially antagonistic relationship between wheat, barley, and/or shepherd's purse when grown in close proximity and resulted in a negative effect on aphid fitness. Factors including natal experience effects, plant-plant interactions, lack of host plant conditioning, and/or host plant composition effects may have impacted *R. padi* fitness in this study.

The second objective of this study was to quantify host-plant feeding behaviors for *R. padi* reared under different conditions. The results from the first objective indicated that experimental *R. padi* reared uncrowded on wheat, under ideal environmental conditions, were larger and had high fitness. In comparison, *R. padi* from a source colony were substantially smaller and would be predicted to be less fit, and thus cause less plant injury. I hypothesized that differences in rearing conditions had the potential to influence *R. padi* feeding behavior and these behaviors were quantified using salivary sheath staining and electropenetrography (EPG) techniques. The results of this study supported the hypothesis that differences in rearing conditions of *R. padi* may impact feeding behaviors, as significant differences were revealed for typical feeding behaviors between aphid colonies. Of the fifteen EPG parameters evaluated, significant differences were observed between colonies for the number of probes, the time spent salivating into phloem, and the time spent ingesting phloem or xylem sap. Each of these measures are facets of aphid feeding behavior that have the potential to influence the outcomes of *R. padi* host plant resistance bioassays.

Results indicated that the most "fit" experimental colony aphids (i.e. larger and with higher fitness) may not be the best for plant injury evaluations, but because of their propensity to initiate more feeding attempts, may be highly beneficial in barley yellow dwarf virus (BYDV) transmission study evaluations. Alternatively, the "stressed" source colony, reared under crowded and less optimal environmental conditions appear more likely to feed for extended periods of time and induce plant injury. These differences should be considered when evaluating the impact of aphid feeding on host plants during screening of resistant plant sources.

APPENDICES

Appendix A: Statistical analysis outputs for Chapter 3 evaluations

Environmental chamber: total aphids per host plant treatment comparisons

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	SRAPHIDS
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	6	1 2 3 4 5 6
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	26
Columns in Z	6
Subjects	1
Max Obs Per Subject	42

Number of Observations

Number of Observations Read	42
Number of Observations Used	42
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	201.23021015	
1	1	187.01505382	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	6.5096
Residual	6.3386

Fit Statistics

-2 Res Log Likelihood	187.0
AIC (smaller is better)	191.0
AICC (smaller is better)	191.4
BIC (smaller is better)	190.6

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W	1	30	2.21	0.1472
B	1	30	1.34	0.2555
W*B	1	30	4.40	0.0445
SP	1	30	0.12	0.7288
W*SP	1	30	0.60	0.4438
B*SP	1	30	0.01	0.9070
W*B*SP	0	.	.	.

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	7.9275	1.4633	13.8	5.42	<.0001
W*B*SP	0	1	0	10.8734	1.4633	13.8	7.43	<.0001
W*B*SP	0	1	1	11.6223	1.4633	13.8	7.94	<.0001
W*B*SP	1	0	0	12.2628	1.4633	13.8	8.38	<.0001
W*B*SP	1	0	1	11.1741	1.4633	13.8	7.64	<.0001
W*B*SP	1	1	0	11.4036	1.4633	13.8	7.79	<.0001
W*B*SP	1	1	1	10.5572	1.4633	13.8	7.21	<.0001

Tests of Effect Slices

Effect	W	B	SP	Num DF	Den DF	F Value	Pr > F
W*B*SP	0	0		0	.	.	.
W*B*SP	0	1		1	30	0.27	0.6102
W*B*SP	1	0		1	30	0.56	0.4597
W*B*SP	1	1		1	30	0.34	0.5647
W*B*SP	0		0	0	.	.	.
W*B*SP	0		1	1	30	6.46	0.0164
W*B*SP	1		0	1	30	0.35	0.5589
W*B*SP	1		1	1	30	0.18	0.6743
W*B*SP		0	0	0	.	.	.
W*B*SP		0	1	1	30	4.99	0.0331
W*B*SP		1	0	1	30	0.13	0.7178
W*B*SP		1	1	1	30	0.54	0.4694

W	B	SP	MNAphids	SEAphids
0	0	1	67.833	16.8016
0	1	0	132.667	37.4706
0	1	1	145.167	30.2130
1	0	0	162.000	43.9060
1	0	1	133.833	31.2628
1	1	0	144.833	34.9136
1	1	1	121.500	31.4120

W	SP	B	MNAphids	SEAphids
0	0	1	132.667	37.4706
0	1	0	67.833	16.8016
0	1	1	145.167	30.2130
1	0	0	162.000	43.9060
1	0	1	144.833	34.9136
1	1	0	133.833	31.2628
1	1	1	121.500	31.4120

B	SP	W	MNAphids	SEAphids
0	0	1	162.000	43.9060
0	1	0	67.833	16.8016
0	1	1	133.833	31.2628
1	0	0	132.667	37.4706
1	0	1	144.833	34.9136
1	1	0	145.167	30.2130
1	1	1	121.500	31.4120

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	SRAPHIDS
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	6	1 2 3 4 5 6
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	8
Columns in Z	6
Subjects	1
Max Obs Per Subject	42

Number of Observations

Number of Observations Read	42
Number of Observations Used	42
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	201.23021015	
1	1	187.01505382	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	6.5096
Residual	6.3386

Fit Statistics

-2 Res Log Likelihood	187.0
AIC (smaller is better)	191.0
AICC (smaller is better)	191.4
BIC (smaller is better)	190.6

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W*B*SP	6	30	1.83	0.1258

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	7.9275	1.4633	13	5.42	<.0001
W*B*SP	0	1	0	10.8734	1.4633	13.8	7.43	<.0001
W*B*SP	0	1	1	11.6223	1.4633	13.8	7.94	<.0001
W*B*SP	1	0	0	12.2628	1.4633	13.8	8.38	<.0001
W*B*SP	1	0	1	11.1741	1.4633	13.8	7.64	<.0001
W*B*SP	1	1	0	11.4036	1.4633	13.8	7.79	<.0001
W*B*SP	1	1	1	10.5572	1.4633	13.8	7.21	<.0001

Differences of Least Squares Means

Effect	W	B	SP	_W	_B	_SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	0	1	0	-2.9459	1.4536	30	-2.03	0.0517
W*B*SP	0	0	1	0	1	1	-3.6949	1.4536	30	-2.54	0.0164
W*B*SP	0	0	1	1	0	0	-4.3353	1.4536	30	-2.98	0.0056
W*B*SP	0	0	1	1	0	1	-3.2466	1.4536	30	-2.23	0.0331
W*B*SP	0	0	1	1	1	0	-3.4762	1.4536	30	-2.39	0.0233
W*B*SP	0	0	1	1	1	1	-2.6297	1.4536	30	-1.81	0.0805
W*B*SP	0	1	0	0	1	1	-0.7489	1.4536	30	-0.52	0.6102
W*B*SP	0	1	0	1	0	0	-1.3894	1.4536	30	-0.96	0.3468
W*B*SP	0	1	0	1	0	1	-0.3007	1.4536	30	-0.21	0.8375
W*B*SP	0	1	0	1	1	0	-0.5303	1.4536	30	-0.36	0.7178
W*B*SP	0	1	0	1	1	1	0.3162	1.4536	30	0.22	0.8293
W*B*SP	0	1	1	1	0	0	-0.6404	1.4536	30	-0.44	0.6627
W*B*SP	0	1	1	1	0	1	0.4483	1.4536	30	0.31	0.7599
W*B*SP	0	1	1	1	1	0	0.2187	1.4536	30	0.15	0.8814
W*B*SP	0	1	1	1	1	1	1.0651	1.4536	30	0.73	0.4694
W*B*SP	1	0	0	1	0	1	1.0887	1.4536	30	0.75	0.4597
W*B*SP	1	0	0	1	1	0	0.8591	1.4536	30	0.59	0.5589
W*B*SP	1	0	0	1	1	1	1.7056	1.4536	30	1.17	0.2499
W*B*SP	1	0	1	1	1	0	-0.2296	1.4536	30	-0.16	0.8756
W*B*SP	1	0	1	1	1	1	0.6169	1.4536	30	0.42	0.6743
W*B*SP	1	1	0	1	1	1	0.8465	1.4536	30	0.58	0.5647

Environmental chamber: total aphids weight per host plant treatment comparisons

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	Weight
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	6	1 2 3 4 5 6
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	26
Columns in Z	6
Subjects	1
Max Obs Per Subject	42

Number of Observations

Number of Observations Read	42
Number of Observations Used	42
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	304.55041665	
1	2	290.67893647	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	123.83
Residual	123.09

Fit Statistics

-2 Res Log Likelihood	290.7
AIC (smaller is better)	294.7
AICC (smaller is better)	295.1
BIC (smaller is better)	294.3

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W	1	30.1	2.59	0.1178
B	1	30.1	2.50	0.1240
W*B	1	30	2.56	0.1198
SP	1	30	0.52	0.4752
W*SP	1	30.1	0.47	0.4966
B*SP	1	30.2	0.30	0.5850
W*B*SP	0	.	.	.

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	12.1213	6.4150	13.9	1.89	0.0798
W*B*SP	0	1	0	25.9002	6.4150	13.9	4.04	0.0012
W*B*SP	0	1	1	24.9608	6.4150	13.9	3.89	0.0016
W*B*SP	1	0	0	26.8651	6.2043	12.5	4.33	0.0009
W*B*SP	1	0	1	24.6017	6.4150	13.9	3.84	0.0018
W*B*SP	1	1	0	30.2851	6.7667	16.3	4.48	0.0004
W*B*SP	1	1	1	22.9378	6.4150	13.9	3.58	0.0031

Tests of Effect Slices

Effect	W	B	SP	Num DF	Den DF	F Value	Pr > F
W*B*SP	0	0		0	.	.	.
W*B*SP	0	1		1	30	0.02	0.8844
W*B*SP	1	0		1	30.1	0.13	0.7174
W*B*SP	1	1		1	30.1	1.18	0.2856
W*B*SP	0		0	0	.	.	.
W*B*SP	0		1	1	30	4.02	0.0541
W*B*SP	1		0	1	30.3	0.27	0.6089
W*B*SP	1		1	1	30	0.07	0.7968
W*B*SP		0	0	0	.	.	.
W*B*SP		0	1	1	30	3.80	0.0608
W*B*SP		1	0	1	30.1	0.42	0.5213
W*B*SP		1	1	1	30	0.10	0.7543

W	B	SP	MNWeight	SEWeight
0	0	1	12.1213	3.22190
0	1	0	25.9002	6.93811
0	1	1	24.9608	6.78011

1	0	0	28.0080	5.71709
1	0	1	24.6017	6.25004
1	1	0	28.6850	8.78562
1	1	1	22.9378	6.81383

W	SP	B	MNWeight	SEWeight
0	0	1	25.9002	6.93811
0	1	0	12.1213	3.22190
0	1	1	24.9608	6.78011
1	0	0	28.0080	5.71709
1	0	1	28.6850	8.78562
1	1	0	24.6017	6.25004
1	1	1	22.9378	6.81383

B	SP	W	MNWeight	SEWeight
0	0	1	28.0080	5.71709
0	1	0	12.1213	3.22190
0	1	1	24.6017	6.25004
1	0	0	25.9002	6.93811
1	0	1	28.6850	8.78562
1	1	0	24.9608	6.78011
1	1	1	22.9378	6.81383

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	Weight
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	6	1 2 3 4 5 6
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	8
Columns in Z	6
Subjects	1
Max Obs Per Subject	42

Number of Observations

Number of Observations Read	42

Number of Observations Used	42
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	304.55041665	
1	2	290.67893647	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	123.83
Residual	123.09

Fit Statistics

-2 Res Log Likelihood	290.7
AIC (smaller is better)	294.7
AICC (smaller is better)	295.1
BIC (smaller is better)	294.3

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W*B*SP	6	30.1	1.54	0.1999

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	12.1213	6.4150	13.9	1.89	0.0798
W*B*SP	0	1	0	25.9002	6.4150	13.9	4.04	0.0012
W*B*SP	0	1	1	24.9608	6.4150	13.9	3.89	0.0016
W*B*SP	1	0	0	26.8651	6.2043	12.5	4.33	0.0009
W*B*SP	1	0	1	24.6017	6.4150	13.9	3.84	0.0018
W*B*SP	1	1	0	30.2851	6.7667	16.3	4.48	0.0004
W*B*SP	1	1	1	22.9378	6.4150	13.9	3.58	0.0031

Differences of Least Squares Means

Effect	W	B	SP	_W	_B	_SP	Estimate	Standard Error	DF	t Value	Pr > |t|

W*B*SP	0	0	1	0	1	0	-13.7788	6.4055	30	-2.15	0.0396
W*B*SP	0	0	1	0	1	1	-12.8395	6.4055	30	-2.00	0.0541
W*B*SP	0	0	1	1	0	0	-14.7438	6.1944	30.1	-2.38	0.0238
W*B*SP	0	0	1	1	0	1	-12.4803	6.4055	30	-1.95	0.0608
W*B*SP	0	0	1	1	1	0	-18.1637	6.7576	30.1	-2.69	0.0116
W*B*SP	0	0	1	1	1	1	-10.8165	6.4055	30	-1.69	0.1017
W*B*SP	0	1	0	0	1	1	0.9393	6.4055	30	0.15	0.8844
W*B*SP	0	1	0	1	0	0	-0.9649	6.1944	30.1	-0.16	0.8773
W*B*SP	0	1	0	1	0	1	1.2985	6.4055	30	0.20	0.8407
W*B*SP	0	1	0	1	1	0	-4.3849	6.7576	30.1	-0.65	0.5213
W*B*SP	0	1	0	1	1	1	2.9623	6.4055	30	0.46	0.6471
W*B*SP	0	1	1	1	0	0	-1.9043	6.1944	30.1	-0.31	0.7606
W*B*SP	0	1	1	1	0	1	0.3592	6.4055	30	0.06	0.9557
W*B*SP	0	1	1	1	1	0	-5.3242	6.7576	30.1	-0.79	0.4369
W*B*SP	0	1	1	1	1	1	2.0230	6.4055	30	0.32	0.7543
W*B*SP	1	0	0	1	0	1	2.2634	6.1944	30.1	0.37	0.7174
W*B*SP	1	0	0	1	1	0	-3.4200	6.6156	30.3	-0.52	0.6089
W*B*SP	1	0	0	1	1	1	3.9273	6.1944	30.1	0.63	0.5309
W*B*SP	1	0	1	1	1	0	-5.6834	6.7576	30.1	-0.84	0.4070
W*B*SP	1	0	1	1	1	1	1.6638	6.4055	30	0.26	0.7968
W*B*SP	1	1	0	1	1	1	7.3472	6.7576	30.1	1.09	0.285

Greenhouse: total aphids per host plant treatment comparisons

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	SRTotalAphids
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	4	1 2 3 4
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	8
Columns in Z	4
Subjects	1
Max Obs Per Subject	56

Number of Observations

Number of Observations Read	56
Number of Observations Used	56
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	251.76766668	
1	2	251.03656374	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0.4635
Residual	7.0190

Fit Statistics

-2 Res Log Likelihood	251.0
AIC (smaller is better)	255.0
AICC (smaller is better)	255.3
BIC (smaller is better)	253.8

---------- Day=10 ----------

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W*B*SP	6	46.1	2.56	0.0317

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	7.2062	0.9476	27.7	7.60	<.0001
W*B*SP	0	1	0	6.2593	0.9966	31.1	6.28	<.0001
W*B*SP	0	1	1	6.6015	1.0594	34.2	6.23	<.0001
W*B*SP	1	0	0	9.8327	0.9966	31.1	9.87	<.0001
W*B*SP	1	0	1	8.8192	0.9966	31.1	8.85	<.0001
W*B*SP	1	1	0	9.6858	0.9966	31.1	9.72	<.0001
W*B*SP	1	1	1	6.8941	0.9966	31.1	6.92	<.0001

Differences of Least Squares Means

Effect	W	B	SP	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	0	1	0	0.9469	1.2882	46.1	0.74	0.4660
W*B*SP	0	0	1	0	1	1	0.6046	1.3395	46.6	0.45	0.6538
W*B*SP	0	0	1	1	0	0	-2.6265	1.2882	46.1	-2.04	0.0472
W*B*SP	0	0	1	1	0	1	-1.6130	1.2882	46.1	-1.25	0.2168
W*B*SP	0	0	1	1	1	0	-2.4797	1.2882	46.1	-1.92	0.0604
W*B*SP	0	0	1	1	1	1	0.3121	1.2882	46.1	0.24	0.8096
W*B*SP	0	1	0	0	1	1	-0.3423	1.3725	46.2	-0.25	0.8042
W*B*SP	0	1	0	1	0	0	-3.5734	1.3247	46	-2.70	0.0097
W*B*SP	0	1	0	1	0	1	-2.5599	1.3247	46	-1.93	0.0595
W*B*SP	0	1	0	1	1	0	-3.4266	1.3247	46	-2.59	0.0129
W*B*SP	0	1	0	1	1	1	-0.6348	1.3247	46	-0.48	0.6341
W*B*SP	0	1	1	1	0	0	-3.2312	1.3725	46.2	-2.35	0.0229
W*B*SP	0	1	1	1	0	1	-2.2176	1.3725	46.2	-1.62	0.1130
W*B*SP	0	1	1	1	1	0	-3.0843	1.3725	46.2	-2.25	0.0294
W*B*SP	0	1	1	1	1	1	-0.2925	1.3725	46.2	-0.21	0.8322
W*B*SP	1	0	0	1	0	1	1.0135	1.3247	46	0.77	0.4481
W*B*SP	1	0	0	1	1	0	0.1469	1.3247	46	0.11	0.9122
W*B*SP	1	0	0	1	1	1	2.9386	1.3247	46	2.22	0.0315
W*B*SP	1	0	1	1	1	0	-0.8666	1.3247	46	-0.65	0.5162
W*B*SP	1	0	1	1	1	1	1.9251	1.3247	46	1.45	0.1529
W*B*SP	1	1	0	1	1	1	2.7917	1.3247	46	2.11	0.0406

Day	W	B	SP	MNTotal Aphids	SETotal Aphids
5	0	0	1	8.714 a	2.9818
5	0	1	0	9.500 a	3.3004
5	0	1	1	11.667 a	3.1091
5	1	0	0	16.625 a	2.1039
5	1	0	1	14.375 a	2.3975
5	1	1	0	21.750 a	5.1261
5	1	1	1	11.875 a	3.7580
10	0	0	1	55.889 b	9.5531
10	0	1	0	51.500 b	14.7938
10	0	1	1	49.143 b	12.7847
10	1	0	0	103.500 a	20.8198
10	1	0	1	88.125 ab	20.5187
10	1	1	0	97.000 ab	13.7866
10	1	1	1	51.000 b	11.0032

Total weight per host plant treatment comparisons

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	WEIGHT
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Rep	4	1 2 3 4
Batch	2	1 2
W	2	0 1
B	2	0 1
SP	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	26
Columns in Z	8
Subjects	1
Max Obs Per Subject	56

Number of Observations

Number of Observations Read	56
Number of Observations Used	56
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	316.98692872	
1	1	312.74272947	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep*Batch	5.9332
Residual	22.1222

Fit Statistics

-2 Res Log Likelihood	312.7
AIC (smaller is better)	316.7
AICC (smaller is better)	317.0
BIC (smaller is better)	316.9

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
W	1	42	9.63	0.0034
B	1	42	2.09	0.1554
W*B	1	42	1.23	0.2730
SP	1	42	5.74	0.0212
W*SP	1	42	1.94	0.1715
B*SP	1	42	0.17	0.6854
W*B*SP	0	.	.	.

Least Squares Means

Effect	W	B	SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	5.6374	1.8727	42	3.01	0.0044
W*B*SP	0	1	0	6.4155	1.8727	42	3.43	0.0014
W*B*SP	0	1	1	5.2696	1.8727	42	2.81	0.0074
W*B*SP	1	0	0	14.7974	1.8727	42	7.90	<.0001
W*B*SP	1	0	1	10.3813	1.8727	42	5.54	<.0001
W*B*SP	1	1	0	12.0928	1.8727	42	6.46	<.0001
W*B*SP	1	1	1	6.3198	1.8727	42	3.37	0.0016

Differences of Least Squares Means

Effect	W	B	SP	_W	_B	_SP	Estimate	Standard Error	DF	t Value	Pr > |t|
W*B*SP	0	0	1	0	1	0	-0.7781	2.3517	42	-0.33	0.7424
W*B*SP	0	0	1	0	1	1	0.3678	2.3517	42	0.16	0.8765
W*B*SP	0	0	1	1	0	0	-9.1600	2.3517	42	-3.90	0.0003
W*B*SP	0	0	1	1	0	1	-4.7439	2.3517	42	-2.02	0.0501
W*B*SP	0	0	1	1	1	0	-6.4554	2.3517	42	-2.74	0.0089
W*B*SP	0	0	1	1	1	1	-0.6824	2.3517	42	-0.29	0.7731
W*B*SP	0	1	0	0	1	1	1.1459	2.3517	42	0.49	0.6286
W*B*SP	0	1	0	1	0	0	-8.3819	2.3517	42	-3.56	0.0009
W*B*SP	0	1	0	1	0	1	-3.9657	2.3517	42	-1.69	0.0991
W*B*SP	0	1	0	1	1	0	-5.6773	2.3517	42	-2.41	0.0202
W*B*SP	0	1	0	1	1	1	0.09575	2.3517	42	0.04	0.9677
W*B*SP	0	1	1	1	0	0	-9.5278	2.3517	42	-4.05	0.0002
W*B*SP	0	1	1	1	0	1	-5.1116	2.3517	42	-2.17	0.0354
W*B*SP	0	1	1	1	1	0	-6.8231	2.3517	42	-2.90	0.0059
W*B*SP	0	1	1	1	1	1	-1.0501	2.3517	42	-0.45	0.6575
W*B*SP	1	0	0	1	0	1	4.4161	2.3517	42	1.88	0.0674
W*B*SP	1	0	0	1	1	0	2.7046	2.3517	42	1.15	0.2566
W*B*SP	1	0	0	1	1	1	8.4776	2.3517	42	3.60	0.0008

W*B*SP	1	0	1	1	1	0	-1.7115	2.3517	42	-0.73	0.4708
W*B*SP	1	0	1	1	1	1	4.0615	2.3517	42	1.73	0.0915
W*B*SP	1	1	0	1	1	1	5.7730	2.3517	42	2.45	0.0183

Tests of Effect Slices

Effect	W	B	SP	Num DF	Den DF	F Value	Pr > F
W*B*SP	0	0		0	.	.	.
W*B*SP	0	1		1	42	0.24	0.6286
W*B*SP	1	0		1	42	3.53	0.0674
W*B*SP	1	1		1	42	6.03	0.0183
W*B*SP	0		0	0	.	.	.
W*B*SP	0		1	1	42	0.02	0.8765
W*B*SP	1		0	1	42	1.32	0.2566
W*B*SP	1		1	1	42	2.98	0.0915
W*B*SP		0	0	0	.	.	.
W*B*SP		0	1	1	42	4.07	0.0501

The Mixed Procedure

Tests of Effect Slices

Effect	W	B	SP	Num DF	Den DF	F Value	Pr > F
W*B*SP		1	0	1	42	5.83	0.0202
W*B*SP		1	1	1	42	0.20	0.6575

W	B	SP	MNWeight	SEWeight
0	0	1	5.6374	0.95526
0	1	0	6.4155	1.96863
0	1	1	5.2696	0.88175
1	0	0	14.7974	3.18093
1	0	1	10.3813	2.23728
1	1	0	12.0928	1.60771
1	1	1	6.3198	1.12891

W	SP	B	MNWeight	SEWeight
0	0	1	6.4155	1.96863
0	1	0	5.6374	0.95526
0	1	1	5.2696	0.88175
1	0	0	14.7974	3.18093
1	0	1	12.0928	1.60771
1	1	0	10.3813	2.23728
1	1	1	6.3198	1.12891

B	SP	W	MNWeight	SEWeight
0	0	1	14.7974	3.18093
0	1	0	5.6374	0.95526
0	1	1	10.3813	2.23728
1	0	0	6.4155	1.96863
1	0	1	12.0928	1.60771
1	1	0	5.2696	0.88175

1	1	1	6.3198	1.12891

Greenhouse: Weight per aphid per host plant comparisons

---------- Plant=B ----------

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	WtPerAphid
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	8	1 2 3 4 5 6 7 8
Trt	4	B B/SP W/B W/B/SP

Dimensions

Covariance Parameters	2
Columns in X	5
Columns in Z	8
Subjects	1
Max Obs Per Subject	32

Number of Observations

Number of Observations Read	32
Number of Observations Used	32
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-24.67543797	
1	1	-24.67543797	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0
Residual	0.01802

Fit Statistics

-2 Res Log Likelihood	-24.7
AIC (smaller is better)	-22.7
AICC (smaller is better)	-22.5
BIC (smaller is better)	-22.6

---------- Plant=B ----------

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	28	12.32	<.0001

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B	0.4572	0.04746	28	9.63	<.0001
Trt	B/SP	0.1818	0.04746	28	3.83	0.0007
Trt	W/B	0.2041	0.04746	28	4.30	0.0002
Trt	W/B/SP	0.06049	0.04746	28	1.27	0.2130

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B	B/SP	0.2754	0.06712	28	4.10	0.0003
Trt	B	W/B	0.2531	0.06712	28	3.77	0.0008
Trt	B	W/B/SP	0.3967	0.06712	28	5.91	<.0001
Trt	B/SP	W/B	-0.02231	0.06712	28	-0.33	0.7421
Trt	B/SP	W/B/SP	0.1214	0.06712	28	1.81	0.0814
Trt	W/B	W/B/SP	0.1437	0.06712	28	2.14	0.0412

---------- Plant=SP ----------

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	WtPerAphid
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	8	1 2 3 4 5 6 7 8
Trt	4	B/SP SP W/B/SP W/SP

Dimensions

Covariance Parameters	2
Columns in X	5
Columns in Z	8
Subjects	1
Max Obs Per Subject	32

Number of Observations

Number of Observations Read	32
Number of Observations Used	32
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	8.01861412	
1	1	7.93992026	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0.002577
Residual	0.05535

Fit Statistics

-2 Res Log Likelihood	7.9
AIC (smaller is better)	11.9
AICC (smaller is better)	12.4
BIC (smaller is better)	12.1

---------- Plant=SP ----------

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

Trt	3	21	2.64	0.0762

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B/SP	0.2618	0.08509	27.8	3.08	0.0047
Trt	SP	0.4536	0.08509	27.8	5.33	<.0001
Trt	W/B/SP	0.1569	0.08509	27.8	1.84	0.0758
Trt	W/SP	0.4033	0.08509	27.8	4.74	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B/SP	SP	-0.1918	0.1176	21	-1.63	0.1180
Trt	B/SP	W/B/SP	0.1049	0.1176	21	0.89	0.3828
Trt	B/SP	W/SP	-0.1415	0.1176	21	-1.20	0.2423
Trt	SP	W/B/SP	0.2966	0.1176	21	2.52	0.0198
Trt	SP	W/SP	0.05023	0.1176	21	0.43	0.6737
Trt	W/B/SP	W/SP	-0.2464	0.1176	21	-2.09	0.0485

---------- Plant=W ----------

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	WtPerAphid
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	8	1 2 3 4 5 6 7 8
Trt	4	W W/B W/B/SP W/SP

Dimensions

Covariance Parameters	2
Columns in X	5
Columns in Z	8
Subjects	1
Max Obs Per Subject	32

Number of Observations

Number of Observations Read	32

Number of Observations Used	32
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-64.47417531	
1	1	-67.30235820	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0.001266
Residual	0.003084

Fit Statistics

-2 Res Log Likelihood	-67.3
AIC (smaller is better)	-63.3
AICC (smaller is better)	-62.8
BIC (smaller is better)	-63.1

---------- Plant=W ----------

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	21	90.31	<.0001

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	W	0.5702	0.02332	22.3	24.45	<.0001
Trt	W/B	0.2726	0.02332	22.3	11.69	<.0001
Trt	W/B/SP	0.1402	0.02332	22.3	6.01	<.0001
Trt	W/SP	0.2280	0.02332	22.3	9.78	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|

Trt	W	W/B	0.2976	0.02777	21	10.72	<.0001
Trt	W	W/B/S	0.4301	0.02777	21	15.49	<.0001
Trt	W	W/SP	0.3422	0.02777	21	12.32	<.0001
Trt	W/B	W/B/SP	0.1325	0.02777	21	4.77	0.0001
Trt	W/B	W/SP	0.04461	0.02777	21	1.61	0.1231
Trt	W/B/SP	W/SP	-0.08788	0.02777	21	-3.16	0.0047

Plant	Trt	MnWt Per Aphid	SEWt Per Aphid
B	B	0.45722	0.07808
B	B/SP	0.18184	0.03126
B	W/B	0.20415	0.03391
B	W/B/SP	0.06049	0.02806
SP	B/SP	0.26181	0.03430
SP	SP	0.45356	0.03707
SP	W/B/SP	0.15694	0.03944
SP	W/SP	0.40333	0.15766
W	W	0.57022	0.02211
W	W/B	0.27265	0.01000
W	W/B/SP	0.14017	0.01801
W	W/SP	0.22804	0.03552

The Mixed Procedure

Model Information

Data Set	WORK.FOUR
Dependent Variable	WtPerAphid
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
Rep	8	1 2 3 4 5 6 7 8
Trt	7	B B/SP SP W W/B W/B/SP W/SP

Dimensions

Covariance Parameters	2
Columns in X	8
Columns in Z	8
Subjects	1
Max Obs Per Subject	56

Number of Observations

Number of Observations Read	56
Number of Observations Used	55
Number of Observations Not Used	1

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-95.64227525	
1	2	-100.56318259	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0.001379
Residual	0.004527

Fit Statistics

-2 Res Log Likelihood	-100.6
AIC (smaller is better)	-96.6

AICC (smaller is better)	-96.3
BIC (smaller is better)	-96.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	6	41.1	48.35	<.0001

The Mixed Procedure

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B	0.5192	0.02877	39.2	18.05	<.0001
Trt	B/SP	0.2420	0.02717	36.4	8.91	<.0001
Trt	SP	0.4536	0.02717	36.4	16.69	<.0001
Trt	W	0.5702	0.02717	36.4	20.99	<.0001
Trt	W/B	0.2506	0.02717	36.4	9.22	<.0001
Trt	W/B/SP	0.1347	0.02717	36.4	4.96	<.0001
Trt	W/SP	0.2362	0.02717	36.4	8.69	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	B	B/SP	0.2772	0.03495	41.3	7.93	<.0001
Trt	B	SP	0.06566	0.03495	41.3	1.88	0.0673
Trt	B	W	-0.05099	0.03495	41.3	-1.46	0.1521
Trt	B	W/B	0.2686	0.03495	41.3	7.69	<.0001
Trt	B	W/B/SP	0.3845	0.03495	41.3	11.00	<.0001
Trt	B	W/SP	0.2830	0.03495	41.3	8.10	<.0001
Trt	B/SP	SP	-0.2116	0.03364	41	-6.29	<.0001
Trt	B/SP	W	-0.3282	0.03364	41	-9.76	<.0001
Trt	B/SP	W/B	-0.00859	0.03364	41	-0.26	0.7996
Trt	B/SP	W/B/SP	0.1073	0.03364	41	3.19	0.0027
Trt	B/SP	W/SP	0.005821	0.03364	41	0.17	0.8635
Trt	SP	W	-0.1167	0.03364	41	-3.47	0.0012
Trt	SP	W/B	0.2030	0.03364	41	6.03	<.0001
Trt	SP	W/B/SP	0.3188	0.03364	41	9.48	<.0001
Trt	SP	W/SP	0.2174	0.03364	41	6.46	<.0001
Trt	W	W/B	0.3196	0.03364	41	9.50	<.0001
Trt	W	W/B/SP	0.4355	0.03364	41	12.95	<.0001
Trt	W	W/SP	0.3340	0.03364	41	9.93	<.0001
Trt	W/B	W/B/SP	0.1159	0.03364	41	3.44	0.0013
Trt	W/B	W/SP	0.01442	0.03364	41	0.43	0.6705
Trt	W/B/SP	W/SP	-0.1015	0.03364	41	-3.02	0.0044

Trt	MnWt Per Aphid	SEWtPer Aphid
B	0.52253	0.049392
B/SP	0.24200	0.029171
SP	0.45356	0.037068

W	0.57022	0.022114
W/B	0.25060	0.009398
W/B/SP	0.13472	0.015497
W/SP	0.23618	0.013954

Appendix B: Statistical analysis outputs for Chapter 4 evaluations

Salivary Sheath Count Comparisons

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	SRSHEATH
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Treatment	3	C P R

Dimensions

Covariance Parameters	3
Columns in X	4
Columns in Z	12
Subjects	1
Max Obs Per Subject	75

Number of Observations

Number of Observations Read	75
Number of Observations Used	75
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	248.92373863	
1	3	245.60515339	0.00002262
2	1	245.60390969	0.00000004
3	1	245.60390740	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep	0
Rep*Treatment	0.2364
Residual	1.4490

Fit Statistics

-2 Res Log Likelihood	245.6
AIC (smaller is better)	249.6
AICC (smaller is better)	249.8
BIC (smaller is better)	247.8

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	4	106.11	0.0003

Least Squares Means

Effect	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	C	8.88E-16	0.4188	4	0.00	1.0000
Treatment	P	2.5845	0.3565	4	7.25	0.0019
Treatment	R	7.6777	0.3565	4	21.54	<.0001

Differences of Least Squares Means

Effect	Treatment	_Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	C	P	-2.5845	0.5500	4	-4.70	0.0093
Treatment	C	R	-7.6777	0.5500	4	-13.96	0.0002
Treatment	P	R	-5.0931	0.5042	4	-10.10	0.0005

Treatment	MNNum Sheaths	SENum Sheaths
C	0.0000	0.00000
P	8.1667	1.30875
R	61.3667	4.41588

EPG parameter comparisons

Variable: TotProbes

Trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
Poor	18	6.0556	3.6051	0.8497	1.0000	15.0000
Rich	18	12.7778	8.2999	1.9563	2.0000	34.0000

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	34	-3.15	0.0034
Satterthwaite	Unequal	23.194	-3.15	0.0044

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	17	17	5.30	0.0013

Variable: Time2FirstPr

Trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
Poor	18	685.3	529.3	124.8	108.7	1950.6
Rich	18	326.3	344.8	81.2585	35.6500	1115.6

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	34	2.41	0.0215
Satterthwaite	Unequal	29.223	2.41	0.0224

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	17	17	2.36	0.0860

Variable: Time2SusIng

Trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
Poor	18	5925.5	4837.4	1140.2	0	18517.9
Rich	18	11017.3	7468.5	1760.3	472.0	27195.9

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	34	-2.43	0.0206
Satterthwaite	Unequal	29.129	-2.43	0.0216

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F

Folded F	17	17	2.38	0.0821

Variable: Time2FirstE1

Trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
Poor	17	5281.9	4754.0	1153.0	713.0	15040.9
Rich	18	5032.1	3698.9	871.8	438.3	12153.9

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	33	0.17	0.8628
Satterthwaite	Unequal	30.227	0.17	0.8639

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	16	17	1.65	0.3144

Variable: TotE1Events

Trt	N	Mean	Std Dev	Std Err	Minimum	Maximum
Poor	18	2.5556	2.3066	0.5437	0	10.0000
Rich	18	5.9444	3.5887	0.8459	1.0000	14.0000

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	34	-3.37	0.0019
Satterthwaite	Unequal	28.998	-3.37	0.0021

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	17	17	2.42	0.0770

Printout of the per insect means of relevant variables:

Obs	Trt	Insect	MEANProbeDur	SumTotProbeDur	MEANTotPD_Probe	MEANTotPathDur	MEANXylemDur	MEANE1Dur	MEANE2Dur	MEANTotE1E2
1	Poor	1	892.92	8036.30	0.8889	344.87	309.28	0.00	0.00	0.00
2	Poor	2	1788.71	14309.71	5.6250	569.46	189.09	217.67	806.91	1024.57
3	Poor	3	2626.79	21014.34	9.0000	732.33	250.00	140.92	1503.55	1644.47
4	Poor	4	3061.72	21432.06	5.5714	410.26	0.00	133.49	2512.68	2646.17
5	Poor	5	3694.32	25860.25	5.4286	606.55	0.00	386.08	2698.59	3084.67
6	Poor	6	4350.77	26104.60	8.0000	527.86	158.80	41.02	3505.76	3546.78
7	Poor	7	6984.21	27936.85	9.7500	686.95	962.53	4.70	5314.03	5318.73
8	Poor	8	2204.62	22046.21	18.1000	1210.86	146.65	338.92	423.42	762.34
9	Poor	9	14048.04	28096.07	14.0000	3048.09	569.80	11.77	10988.18	10999.95
10	Poor	10	3446.71	24126.99	6.7143	639.00	0.00	182.30	2609.37	2791.66
11	Poor	11	14163.18	28326.35	7.0000	464.50	0.00	15.04	13506.04	13521.08
12	Poor	12	1678.37	10070.20	7.6667	712.88	201.87	633.21	119.97	753.18
13	Poor	13	14141.44	28282.87	17.0000	1209.38	37.95	829.71	10804.37	11634.08
14	Poor	14	8253.01	24759.04	20.6667	1109.35	167.43	189.69	1937.94	2127.63
15	Poor	15	9409.29	28227.88	6.3333	497.93	0.00	12.36	8814.98	8827.34
16	Poor	16	28656.85	28656.85	8.0000	569.85	0.00	22.15	28064.85	28087.00
17	Poor	17	2844.80	25603.24	5.7778	353.17	0.00	3.85	2482.82	2486.67
18	Poor	18	1608.76	24131.42	17.0000	1201.21	126.25	208.11	341.13	549.24
19	Rich	1	2205.54	26466.44	9.4167	541.86	136.69	430.25	1056.82	1487.07
20	Rich	2	3455.42	27643.36	20.8750	1857.60	1142.65	327.71	127.46	455.17
21	Rich	3	2847.94	25631.44	13.7778	744.48	759.50	88.59	1255.36	1343.95
22	Rich	4	3337.07	26696.55	7.5000	1287.16	167.75	547.12	441.07	933.06
23	Rich	5	891.56	23180.50	6.2692	587.90	166.42	30.36	81.70	112.07
24	Rich	6	2504.54	27549.98	2.8182	295.79	0.00	320.09	1726.85	2046.94
25	Rich	7	2134.50	25614.04	7.3333	547.52	21.08	14.47	1550.06	1564.53
26	Rich	8	2470.02	27170.18	3.6364	682.97	0.00	386.08	1377.92	1764.00
27	Rich	9	5631.85	28159.23	15.8000	1519.11	1839.16	1039.59	1210.45	2250.05
28	Rich	10	2097.68	18879.08	9.1111	779.76	647.81	214.79	453.44	668.22
29	Rich	11	6964.14	48748.96	26.1429	5231.92	421.31	232.99	357.97	590.97
30	Rich	12	590.55	20078.82	5.9412	390.78	76.61	25.73	29.37	55.10
31	Rich	13	1658.51	24877.59	6.3333	377.83	0.00	19.68	1243.88	1263.56
32	Rich	14	14218.84	28437.67	10.0000	715.78	0.00	26.68	13476.39	13503.06
33	Rich	15	917.30	25684.34	3.6429	265.91	30.00	32.29	561.56	593.85
34	Rich	16	2041.53	26539.89	6.7692	596.61	486.67	157.24	787.87	945.11
35	Rich	17	2443.42	26877.60	4.5455	349.89	482.15	3.64	1601.55	1605.19
36	Rich	18	2893.47	26041.25	6.8889	513.27	12.43	379.47	1903.00	2282.46

2

Trt	MNProbe Dur	SEProbe Dur	PVALUE
Poor	6880.81	1671.98	0.0581
Rich	3294.66	740.15	

3

Trt	MNTot ProbeDur	SETot ProbeDur	PVALUE
Poor	23167.85	1479.28	0.0772
Rich	26904.27	1419.98	

5

Trt	MNTotPD_ Probe	SETotPD_ Probe	PVALUE
Poor	9.58459	1.27638	0.8711
Rich	9.26675	1.46615	

10

Trt	MNTot PathDur	SETot PathDur	PVALUE
Poor	827.472	147.577	0.6694
Rich	960.341	270.941	

12

Trt	MNXylem Dur	SEXylem Dur	PVALUE
Poor	173.314	58.221	0.1717
Rich	355.013	116.383	

13

Trt	MNE1Dur	SEE1Dur	PVALUE
Poor	187.277	54.9002	0.5487
Rich	237.598	62.3321	

14

Trt	MNE2Dur	SEE2Dur	PVALUE
Poor	5357.48	1659.45	0.0464
Rich	1624.60	711.18	

15

Trt	MNTot E1E2	SETot E1E2	PVALUE
Poor	5544.75	1650.58	0.0477
Rich	1859.13	703.55	

6

Trt	MNTime2First PD	SETime2First PD	PVALUE
Poor	98.2749	21.5923	0.7803
Rich	91.3242	12.0330	

9

Trt	E2Prop	SEE2Prop	PVALUE
Poor	0.8725	0.0786	0.3692
Rich	0.8069	0.0930	

12

Trt	MNXylem Dur	SEXylem Dur	PVALUE
Poor	283.605	79.383	0.3253
Rich	456.445	138.607	

VITA

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Doctor of Philosophy

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