EVALUATION OF RESISTANCE TO BIRD CHERRY-OAT APHID (*Rhopalosiphum padi* L.) IN WHEAT GERMPLASM

By

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Abstract: Wheat, *Triticum aestivum*, has been a valuable crop grown around the world and has been cultivated since as early as 7,000 BC (Smith 2005). Because of its worldwide distribution, it is vulnerable to a wide variety of arthropod pests. Notably, bird cherry-oat aphid or BCOA, *Rhopalosiphum padi* (Hemiptera: Aphididae), is an important pest of wheat in many production areas (Dunn et al. 2011). This aphid is considered to be particularly damaging to wheat crops due to its ability to transmit barley yellow dwarf virus (Jiménez-Martínez et al.2004). An important integrated pest management (IPM) tactic against arthropod pests is host plant resistance, which can be identified in experimental wheat germplasm through proper screening techniques. The purposes of this study were to evaluate the contributing factors in a previously developed screening method and to identify and characterize any existing types of host plant resistance in a set of experimental wheat germplasm entries. The factors contributing most to the overall health score of a plant following intense aphid feeding pressure were determined to be percent chlorosis, the height of the tallest leaf, and the height of the newest leaf. The most valuable contributing factors were used to form a generalized equation for score that may be used to screen for host plant resistance or tolerance against BCOA regardless of the wheat germplasm used. A set of 140 experimental germplasm were also screened to identify to any potential resistant characteristics. Six germplasm entries were chosen for further evaluation to determine the type of host plant resistance present. The 6 germplasm entries showed strong tolerance characteristics with average scores of between 1.8 to 3.1, based on a scale of 1-5 where 1 is the highest score. After antixenosis and antibiosis experiments, none of the 6 entries showed significant antixenotic or antibiotic characteristics.

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CHAPTER I

INTRODUCTION

Wheat, *Triticum aestivum*, is one of the most widely grown crops around the world. Wheat began being cultivated in the Middle East around the year 7,000 BC (Smith 2005), but in North America, wheat was first planted around 1529 with the arrival of Spanish settlers in Mexico (Shewry 2009). Since the beginning of its cultivation, wheat has continued to gain importance as a food source for both humans and livestock to many countries and cultures worldwide. In the United States, approximately 1.884 billion bushels of wheat were harvested in the 2018-2019 growing seasons (USDA: ERS 2019). In the United States, wheat has a variety of uses. Most commonly, wheat is grown for grain, but it is also used as forage for cattle and other livestock species. Some producers in the US Southern Plains also utilize a combination of forage and grain harvested for their wheat crop (Epplin *et al.* 2000).

The bird cherry-oat aphid or BCOA, *Rhopalosiphum padi* (Hemiptera: Aphididae), is an important pest of many crops, but is consistently found infesting wheat in most production regions (Dunn *et al.* 2011). The bird cherry-oat aphid is capable of directly damaging wheat plants through their feeding on the phloem of the plants as well as indirectly damaging the plants

through their transmittance of the *Barley yellow dwarf virus*, BYDV. Feeding injury caused by BCOA occurs through probing of the plant, reactions to their saliva, and through the ingestion of the plant's phloem (Goggin *et al.* 2017). Bird cherry-oat aphids are able to cause yield loss to different varieties of wheat (Voss *et al.* 1997) and amount of reduction in yield depends on the growth stage of the wheat at the time of infestation and the density of aphids. Dunn *et al.* (2011) found that bird cherry-oat aphid infestations reduced root and shoot growth in wheat, which can reduce the yield of a wheat crop. The highest reduction in yield, up to 40-60%, occurs when the aphids infest the wheat at the seedling stage, when the plants only have two leaves (Papp and Mesterházy 1993). Feeding injury cause by BCOA can be detected through symptoms it produces in the wheat plant. These symptoms of infestation and injury may include necrosis, chlorosis, leaf rolling, and stunted growth. However, in many cases of bird cherry-oat aphid infestation, there are very few or no visible symptoms of injury (Riedell *et al.* 2003).

While there are several forms of pest control available today in wheat, one effective method is host plant resistance. There are three types of host plant resistance: antixenosis, antibiosis, and tolerance. Antixenosis or non-preference describes when the host plant is undesirable to the pest insect either in the chemical composition of the plant or in the morphology of the plant. Antibiosis occurs when the host plant is detrimental to the biology of the pest insect in some way, whether by shortening the life span or preventing optimum reproduction. Tolerance characterizes when the host plant will continue to grow and produce an acceptable yield despite an infestation by a pest insect (Kogan 1982; Smith 2005).

The first resistant wheat varieties were developed for resistance to Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae) in the United States in the late eighteenth century (Smith 2005). Since these early days of developing plant resistance in wheat to the Hessian fly,

plant breeders have sought to design cultivars that are resistant to other arthropod pests capable of damaging wheat. New varieties of wheat are being developed for resistant characteristics against greenbug (*Schizaphis graminum* Rondani) (Tan *et al.* 2017) as well as resistant against Russian wheat aphid (*Diuraphis noxia* Kurdjumov) (Tolmay *et al.* 2015). The bird cherry-oat aphid is recognized as an important pest of wheat throughout the United States. Thus far, there has not been a wheat variety released that is acceptably resistant to the feeding injury caused by bird cherry-oat aphids.

In order to properly examine potential resistant characteristics in developing wheat germplasm, an appropriate protocol must be followed in order to definitively determine the presence of adequate resistance in a germplasm line. These phenotyping protocols must be accurate, reliable, and repeatable to determine the success of resistance in new varieties. Girvin *et al.* (2017) used a method of preliminary screening of newly developed germplasm involving the infestation of seedlings for 120 days. Those seedlings which were still green following this infestation were selected for further testing of the mode of resistance. Similar phenotyping assays were developed by the Wheat Improvement Team (WIT) at Oklahoma State University. The screening procedure involved the infestation of seedlings of promising germplasm lines. After approximately 21 days, the seedlings were evaluated based on a visual assessment and were classified as having high, moderate, or low resistance. This method requires further investigation in order to confirm the validity and reliability of its evaluation of wheat germplasm.

The objectives of this project were two-fold. The first objective sought to evaluate the contributing factors towards the previously existing method for phenotyping wheat germplasm for BCOA resistance or tolerance by quantifying the following parameters: plant height, number of leaves, percentage of chlorosis, width of leaves, and number of aphids. The second objective

was to identify and characterize any types of host plant resistance in the germplasm entries. The three types of host plant resistance that were tested for were antixenosis, antibiosis, and tolerance.

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CHAPTER II

REVIEW OF LITERATURE

Wheat Production in the United States Southern Plains

The production of cereal crops is an important resource as a food source for both human and livestock consumption across the globe. In the 2018-2019 growing year, over 2,000 million (2 billion) tons of grain seed were produced, with wheat (*Triticum aestivum* L.) contributing 732.2 million tons to that total (IGC 2020). In the United States alone, the 2020-2021 growing year is projected to produce 50.4 million tons of wheat grain (IGC 2020). Wheat is the third most planted crop after corn and soybeans. Approximately 47.8 million acres of land was used in the 2018-2019 growing year to produce wheat in the US. Of the different varieties of wheat grown in the US, winter wheat represents up to 80% of the wheat grown each year (USDA-ERS 2020). The United States is a major exporter of wheat, contributing around 15% of the global wheat exports (USDA-ERS 2020).

A major production area of wheat in the United States is the Southern Great Plains, which

includes Texas, Oklahoma, and Kansas. From 2017-2019, Kansas produced an average of 8.16 metric tons on 2.79 million hectares, yielding 3093 kg/ha. Oklahoma produced 3.02 million metric tons on 1.2 million hectares, yielding 1006 kg/ha and Texas produced 2.11 million metric tons on 951,012 hectares, yielding 898 kg/ha (USDA-NASS 2019). The climate of Oklahoma is optimal for production of hard red winter wheat, which is the most common type of wheat produced. It used for both cattle forage and grain production (USDA-NASS 2018; Luper *et al.* 2005). Between 30 and 50% of winter wheat grown in Oklahoma is used for cattle forage, which helps producers to reduce the cost of grain feed each year (Edwards 2015). Typically, winter wheat is best planted between early September through November and is harvested from May through July (Luper *et al.* 2005). When intended for forage as well as grain production, the wheat is typically planted early in August or September to allow for extra growth before grazing (Luper *et al.* 2005).

Insect Pests and Pathogens of Wheat

There are several significant insect pests of wheat in the Southern Great Plains of the United States. The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), was introduced into the United States in New York in 1779 and has since become a significant pest of wheat across the country (Ratcliffe *et al.* 2000). In the Southern Great Plains, Oklahoma has experienced outbreaks of Hessian fly every year since 2004 with these outbreaks becoming increasingly common and severe (Royer *et al.* 2017). Hessian flies in Oklahoma typically have two generations per year, one in the fall and one in the spring. These insects feed on the stems of

the wheat tiller, causing stunting and discoloration of the leaves and eventually causing death of the tiller when infestations are high (Royer *et al.* 2017). This injury to the wheat plants can lead to damage, yield loss, and decreased grain quality as the plant is weakened and stunted (Buntin 1999).

Several species of aphids are also significant pests of wheat in the Southern Great Plains of the United States, including the greenbug, *Schizaphis graminum* (Rondani), and the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Giles *et al.* 2008). The economic losses caused by these two species of aphids is estimated to be $150 million on average (Giles *et al.* 2008). Aphids cause damage to wheat plants through feeding on the phloem, which can cause necrosis of plant tissue, discoloration, leaf stunting, leaf rolling, and other issues (Cooper *et al.* 2010). These injuries can lead to the death of the plant and a significant reduction in yield. In Oklahoma, the greenbug is considered a key pest for much of the state due to their wide range, ability to severely damage wheat crops, and ability to reproduce quickly (Giles *et al.* 2008; Royer *et al.* 2015). Other aphid species that are notable in the Southern Plains include the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and the English grain aphid, *Sitobion avenae* (F.).

Another way that aphids are able to damage wheat is by transmitting pathogens to the plants during feeding. Wheat is susceptible to barley yellow dwarf (BYD), which is caused by several viruses in the genera *Luteovirus* and *Polerovirus* in the family *Luteoviridae* (Lui *et al.* 2007). Five strains of barley yellow dwarf virus (BYDV) include PAS, MAV, PAV, SGV, GPV, and RMV; two strains of cereal yellow dwarf virus (CYDV) include RPV and RPS (Flanders *et al.* 2006). BYD is an extremely damaging virus infecting grain crops around the world and causing estimated yield losses of 0.19-0.37 bushels per acre (13-25 kg/ha) (Flanders *et al.* 2006;

McKirdy *et al.* 2002). Yield losses can be as high as 80% due to BYDV or CYDV infection (McKirdy *et al.* 2002). There are around 25 species of aphid that transmit BYDV (Halbert and Voegtlin 1995). Of these species, the bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) is considered to cause the most damage due to its transmission of both BYDV and CYDV (Choudhury *et al*. 2017). Other common aphids that are effective in their transmission of BYD include the English grain aphid, corn leaf aphid, and greenbug. Specific aphids are more effective vectors of specific strains of BYDV and CYDV. For instance, BYDV-MAV, -RMV, -SGV, and CYDV-RPV are more efficiently transmitted by *R. padi, R. maidis, S. avenae,* and *S. graminum.* BYDV-PAV is more efficiently transmitted by *S. avenae* and *R. padi* than other species (Choudhury *et al*. 2017).

Key symptoms of BYD in most cereal crops include stunting of the plant, reduction of tillers, reduction of root growth, leaf curling, chlorosis, leaf discoloration, and reduction of both kernel weight and number (Riedell *et al.* 2003). Symptoms can widely vary based on the age and stage of the plant at the time of infection, weather, soil, and strain of the virus (D-Arcy 1995). Each of these symptoms of BYD severely weaken the plant, which can potentially cause the death of the plant as well as drastically reduce the yield (Flanders *et al.* 2006). BYD is transmitted to the phloem of the plant through the saliva of a viruliferous aphid, an aphid carrying the virus, during feeding (Choudhury *et al.* 2017). The aphids become viruliferous when they feed on a plant that was previously infected with the virus (Flanders *et al.* 2006).

Bird Cherry-Oat Aphid Biology and Ecology

The bird cherry-oat aphid, or BCOA, is a well-known and well-studied insect pest of a wide variety of crop plants (Jiménez-Martínez and Bosque-Pérez 2009). This aphid species feeds on the phloem of many different host plants, allowing them to utilize plants in many ecosystems around the globe. Populations of this insect have been found in many areas around the world, but it is particularly common in northern in Europe, in North America, and in New Zealand (Kamran *et al.* 2013; Wiktelius and Pettersson 1985). Bird cherry-oat aphids are one of the most important pests that affect wheat worldwide (Jiménez-Martínez and Bosque-Pérez 2009). BCOA have a nearly global distribution, making it difficult to determine the true geographical origin of this aphid species. One theory is that BCOA has its origin in North America and was spread to Europe and Asia, as it was observed in Europe by Linnaeus during the 18th century (Blackman and Eastop 2017).

The bird cherry-oat aphid is 1.2 to 2.4 mm in length with an oval body shape and body color that varies from a light-yellow green to a dark blue gray. There is typically a red-brown color patch along the posterior of the abdomen, surrounding the bases of the aphids' siphunculi. In addition, their tarsi, leg joints, and antennae are dark brown to black. *R. padi* exhibits a host alternating, holocyclic life cycle. A holocyclic life cycle is one that utilizes both sexual and asexual means of reproduction. A host alternating, holocyclic life cycle indicates that the sexual and the asexual cycles occur on different host plants. The primary host for these aphids is the bird cherry tree, *Prunus padus* (Nielsen and Steenberg 2004). During the winter, BCOA reproduce sexually on the bird cherry tree. The female sexual morph is called an ovipara, while the male sexual morph is simply known as a sexual (Hille Ris Lambers 1966). The sexually

reproducing males and females mate to produce eggs that overwinter. In the spring, the fertilized egg hatches, producing a fundatrix (Hardie 2017). The fundatrix develops and produces alate fundatrigeniae that will leave the primary host in the spring (Hille Ris Lambers 1966). Once the emigrants locate the secondary host, they begin reproducing asexually via parthenogenesis. The secondary hosts for BCOA consist of a wide variety of herbaceous plants, typically grasses (Nielsen and Steenberg 2004; Wiktelius and Pettersson 1985). The aphids inhabit the secondary host throughout the spring and summer. In the midst of autumn, parthenogenic female sexuparae will begin to produce the sexual males and females that return to the primary host for the winter months (Hille Ris Lambers 1966).

The bird cherry-oat aphid does not seem to exhibit a preference between its European and North American primary hosts. In Europe, BCOA overwinters on the bird cherry tree (*Prunus padus*, L.). In North America, they overwinter on the common choke-cherry tree (*Prunus virginiana* L.) (Blackman and Eastop 2017) as well as on bird cherry (Dixon 1971). Further, they have been found to infest the blackthorn, *Prunus spinosa* L., in Asia (Halarewicz and Gabryś 2012). Less commonly, it has been observed to feed on additional hosts such as the dwarf Russian almond, *Prunus tenella* Batsch, the cherry plum, *Prunus cerasifera* Ehrh, and the spring cherry, *Prunus subhirtella* Miq. when their preferred primary host plants are unavailable. Because the aphids seem to survive equally well on these primary hosts, the origins of the aphid have still not been definitively determined.

In the United States, *R. padi* completes a holocyclic life cycle with the common choke-cherry tree or the bird cherry tree serving as the primary host and many species of grasses serving as the secondary host. However, BCOA are also capable of utilizing an anholocyclic life cycle when primary hosts are unavailable. This has been observed in the United Kingdom where

bird cherry trees are not as common as in previous years (Hardie 2017). Likewise, in the Southern Great Plains of the United States, the climate is sufficiently warm enough to allow *R. padi* to maintain a successful anholocyclic life cycle on graminaceous plants alone (Michaud 2008). The mild winters of the Southern Great Plains often allows BCOA to colonize fields of winter wheat throughout the entirety of the growing season. To avoid freezing, the aphids will move to the base of the wheat plant or even below the soil level when temperatures begin to drop in early fall (Elliot and Kieckhefer 1989). The warmer temperatures of Oklahoma along with the behavior of seeking shelter near or in the soil allows bird cherry-oat aphids to survive in the Southern Great Plains without the need for a primary host.

Bird cherry-oat aphids utilize various behaviors for host location. Firstly, aphids will incorporate a range of both visual and olfactory cues in order to locate a host plant (Nam and Hardie 2012). Once the aphid lands on a potential host plant, it must determine if the plant is acceptable to feed upon by examining the chemical composition of volatiles of the plant as well as the structure of the epicuticular wax on the surface of the plant (Nam and Hardie 2012). If the plant is acceptable, the aphid will insert its stylet into the plant in what is known as probing behavior and ingest a small amount of the mesophyll cell contents and the phloem sap to assess the suitability of the host plant for feeding (Halarewicz and Gabryś 2012). Almost all plant species are equipped with defensive compounds selected to deter herbivores from feeding. Aphids must possess the necessary compounds to overcome these. Bird cherry-oat aphids utilize polyphenol oxidase and peroxidase to neutralize the defensive phenolic compounds of the plant (Halarewicz and Gabryś 2012) and beta-cyanoalanine synthetase and rhodanese to combat cyanide compounds (Urbańska *et al.* 2002).

Host Plant Resistance and Bird Cherry-Oat Aphids

One method of controlling pest organisms in a crop is through integrated pest management or IPM. This mode of pest management uses various strategies in order to prevent a population of pests from rising above the economic injury level, which is the lowest pest population that will cause economic damage in a crop (Luckmann and Metcalf 1982). By definition, IPM uses a combination of these control tactics to manage a single pest, thus maximizing the benefits of each method of pest prevention. The first strategy, biological control, seeks to control the pest through natural enemies, predators, and parasitoids of the pest organism. For example, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) is a parasitoid wasp of cereal aphids (Giles *et al.* 2003), which attacks their target hosts by ovipositing their eggs within the host organism. This hymenopteran has been found to be effective at controlling populations of aphids (Giles *et al.* 2003). The second strategy, cultural control, involves using the knowledge of the biology of the target pest insect in order to avoid the heaviest infestations. This can include altering the timing of planting to be earlier or later in the season based on the arrival of immigrant aphids to avoid aphid infestations at the seedling stage of the plants. Other aspects of cultural control can include intercropping (Xu *et. al* 2010), providing a more hospitable environment for the target pest's predators and natural enemies (Chang *et al.* 2017), adding ground cover (natural or artificial) (Chang *et al.* 2017), crop rotation, and soil cultivation. The third strategy, chemical control, uses insecticides to remove pest insects from crop plants. Some examples of insecticide types include pyrethroids, pyrethrins, organophosphates, neonicotinoids, carbamates, and pyridines (Dewer and Denholm 2017).

The fourth strategy is host plant resistance, an important IPM tool that works alone or in combination with other tactics in a more comprehensive IPM programs (Smith 2005). There are three mechanisms of host plant resistance. The first category of plant resistance is known as antixenosis, which occurs when the host plant adversely affects an arthropod's behavior (Smith 2005). Antixenosis was originally known as "nonpreference", defined by Painter (1951) as plant characteristics that cause a response in arthropods that yield less damage in plants with these characteristics as compared to plants without those characteristics.

Antixenotic characteristics could include either morphological or chemical characteristics in the host plant (Smith 2005). Changing the color of a plant could alter the visual perception by the pest and deter the pest from accepting that plant as a host. Changes in the plant's "taste" to the pest is also a possible mechanism to deter a pest from feeding on that plant (Smith 2005). Altering the color and smell can also disrupt the host plant recognition abilities of an insect (Kogan 1982). Other mechanisms of antixenosis in host plants are mechanical, meaning that the surface of the host plant is altered in such a way that makes it more difficult for the arthropod pest to feed on the plant. Methods of mechanical antixenosis can involve an increase in hardness of the surface of a plant. When the surface of a plant is more difficult to pierce, aphids will not be able to access the phloem of the plant easily (van Emden 2017). While arthropods are not likely to develop a resistance to a physical characteristic of a plant, it should be noted that a characteristic that deters one pest may not deter another in plants with multiple pest species. Physical antixenotic characteristics may need to be paired with other chemical deterrents in order to repel multiple pests (Smith 2005).

Antixenosis is a method of resistance that has been used to manage various species of aphid. Kishaba and Manglitz (1965) reported a study in which they compared resistant and

susceptible varieties of alfalfa and sweet clover for control of spotted alfalfa aphid (*Therioaphis maculata*) and sweet clover aphid (*Therioaphis riehmi*). The study found that there was a higher degree of aggregation of these aphid species on the susceptible varieties of alfalfa and sweet clover when compared to the resistant plants. More recently, antixenosis has also been developed for pests of soybean. Diaz-Montano *et al*. (2006) found that of the 240 soybean genotypes evaluated in their study, only 2 entries showed potential antixenotic characteristics against soybean aphid (*Aphis glycines*).

Antixenosis has also been developed as a method to control the many pests of wheat. The cereal leaf beetle (*Oulema melanopus* L.) (Coleoptera: Chrysomelidae) can experience decreased oviposition and decreased larval survival on wheat with an increased trichome density as well as on wheat with long trichomes (Hoxie *et al.* 1975). Likewise, leaf pubescence in wheat has been used to combat Hessian fly. Hessian flies had decreased oviposition and smaller larvae on wheat variety 'Vel', a pubescent variety (Roberts *et al.* 1979). These antixenotic characteristics in wheat have also been developed to combat bird cherry-oat aphids. A study performed in 2015 by Wojcicka found that an increase in wax substances on the surface of a wheat plant successfully prevented BCOA from feeding on that plant (van Emden 2017). Additionally, trichomes on the surface of wheat plants have been found to significantly discourage bird cherry-oat aphids from feeding (Roberts and Foster 1983).

The second category of host plant resistance is known as antibiosis and occurs when a host plant negatively impacts the pest arthropod's biology (Smith 2005). There are several mechanisms through which a host plant can be antibiotic towards a pest. The host plant could contain toxins or inhibitors, harming the pest arthropod directly or inhibiting its ability to successfully mature and reproduce (Smith 2005). Another type of antibiotic resistance occurs

when the host plant contains a reduced amount of nutrients. Without the proper nutrients for growth and reproduction, the arthropod pests will experience a shortened life span or a reduced fecundity. The availability and quality of nutrients in a host plant can both directly affect individual herbivorous insects as well as affect the population density of that insect (Awmack and Leather 2002). Other methods of antibiosis occur when the host plant contains disproportionate amounts of nutrients or when the host plant contains antimetabolites or enzymes that inhibit the insect's ability to properly obtain or digest the nutrients within the plant (Kogan 1982). Much like the development of resistance to pesticides, it is possible for insects to develop a resistance to antibiotic characteristics in plants. An example of this occurring is the development of insect resistance to *Bt* crops such as corn and cotton (Tabashnik *et al.* 2013).

Antibiosis characteristics have been used to combat a variety of aphid species in several different crop plants. For instance, gramine (N, N – dimethyldimethyl indole), an indole alkaloid in barley, has been correlated with resistance to both *R. padi* and *S. graminum* (Smith 2005; Zuniga *et al.* 1985; Zuniga and Corcuera 1986; Kanehisa *et al.* 1990). It is thought that gramine may be a toxin for these aphids. However, other similar studies have yielded differing results, especially with *R. padi* (Smith 2005). Similarly, Lattanzio *et al.* (2000) found that there was a higher flavonoid content in resistant lines of cowpea (*Vigna unguiculata* L. Walp.) than in susceptible lines. The flavonoid compounds found in these resistant lines produce a significant reduction in reproduction rate in black bean aphids (*Aphis fabae*) (Lattanzio *et al.* 2000). Other compounds in resistant lines of cowpea, including ethyl acetate, have been shown to inhibit the growth of the cowpea aphid, *Aphis craccivora* (Walp.) (Annan *et al.* 1996).

Various antibiotic lines of wheat have been developed to provide protection against the many pests of this crop. As described previously, a significant pest of wheat is the cereal leaf

beetle, *O. melanopus*. Simple trichomes on the leaf surface of the wheat have an antibiotic effect on *O. melanopus* by causing mortality in both the egg and the larval stage. The egg stage can become desiccated or become punctured by the trichomes. The larval stage can experience puncture wounds to their alimentary canal after feeding on wheat trichomes (Smith 2005; Wellso 1979; Wellso 1973). Antibiotic wheat has also been developed for resistance to aphids. The resistant genes *Dnx* and *Dn7* in wheat have been shown to significantly minimize the intrinsic rate of increase of *D. noxia* as well as significantly reduce the number of aphids per plant (Lazzari *et al.* 2009). Similarly, wheat varieties with antibiotic characteristics are desirable to defend against BCOA, a common wheat pest. Kazemi and van Emden (1992) correlated resistant cultivars of wheat against BCOA to considerably lowered amounts of alanine, histadine, and theronine. More recently, a study from 2012 found that certain varieties of wheat were able to reduce the life span and the fecundity of bird cherry-oat aphids infesting the plant (Mohamadi *et al.* 2012). According to Mohamadi et al. (2012), the level of antibiotic resistance in a host plant to BCOA has been linked to the amounts of hydroxamic acid produced by the plant.

The third category of host plant resistance is known as tolerance, which refers to the capability of host plants to withstand or recover from high levels of damage incurred by infestations of an arthropod pest (Smith 2005; Hesler 2005). In some forms of tolerance, plants are able to fully recover from any damages or symptoms due to infestations of an arthropod pest following the removal of that pest. In other forms of tolerance, host plants lack the visible symptoms that would normally be present during an infestation of the pest (Smith 2005). In plants with high levels of tolerance, there is a heightened growth rate, photosynthetic rate, level of carbon conserved in the roots, ability to transport carbon from storage to the shoots, and an increase in branching or tillering (Strauss and Agrawal 1999; Smith 2005). There are several

benefits to tolerant varieties of crop plants. With tolerance, arthropod pests are less likely to develop a genetic resistance to any negative effects from the plant host, as is possible with antibiotic crops. This is due to the fact that tolerant plants do not generate any selection pressure in arthropod pest populations (Pierson *et al.* 2010). Additionally, tolerant varieties are less likely to negatively impact the beneficial insects that may be present in a crop system and they raise the economic injury level, which can minimize the risk of premature pest management strategies (Smith 2005; Gutsche *et al.* 2009).

Tolerance characteristics have been identified in many types of crop plants in order to defend against aphid pests. Recently, two lines of sorghum, 'Tx3408' and 'Tx3409', have been registered for tolerance against sugarcane aphids (*Melanaphis sacchari* Zehntner), as they have shown adequately high levels of tolerance both in the greenhouse and in the field. These two lines do not experience the normal symptoms associated with *M. sacchari* infestation and do not experience high populations of aphids as compared to other lines (Mbulwe *et al.* 2016). Another crop in which tolerance to aphid feeding has been developed is barley. A genetic analysis was performed on susceptible cultivar 'Otis' and tolerant cultivar 'Sidney'. Results showed that there was an upregulation of HvPRXAI and HvPRXA2, two peroxidase genes, in the tolerant cultivars, which could potentially be a part of the tolerance characteristics of the cultivar (Gutsche *et al.* 2009). Other crop plants that have cultivars tolerant to aphid feeding pressure include wheat, maize, alfalfa, rice, and rye (Smith and Chuang 2014).

In wheat, tolerance is an important resistance characteristic to protect against the many pests of this crop plant. One of the key features of wheat plants tolerant to aphid feeding is that the genes controlling photosystem and chlorophyll production, which concern photosynthesis, are typically highly expressed (Smith and Chuang 2014). This is supported by a study from Haile

et al. (1999) found that tolerant entry ‘PI262660’ had a greater leaf area, a greater dry weight, and was able to recover full photosynthetic capacity following aphid infestation by *D. noxia* when compared to antibiotic entry ‘PI137739’ and susceptible variety ‘Arapahoe’. Another key element contributing to the tolerant characteristics of wheat is the ability to bear injury from aphid feeding without sustaining a great amount of damage. Tolerant wheat cultivars had fewer cell structural changes and had fewer damages to mesophyll cells caused by feeding of *S. graminum* than compared to susceptible cultivars (Morgham *et al.* 1994). Tolerance in wheat is also used as a management strategy against BCOA. In their experimentation, Papp and Mesterházy (1993) identified several varieties of wheat exhibiting tolerance to bird cherry-oat aphids. The tolerant lines included in their field study experienced a grain yield loss of 26 to 33% compared to their susceptible lines, which experienced a grain yield loss of 58 to 63% (Papp and Mesterházy 1993). This study demonstrates that tolerant characteristics in a crop variety does not guarantee zero yield losses from pest pressures. Tolerant varieties simply experience less yield loss than susceptible varieties. Likewise, growth chamber experiments have found several tolerant wheat varieties in which the shoot lengths of the infested plants did not differ from the un-infested plants after a 300 aphid-day treatment on the infested plants (Hesler 2005).

Host plant resistance can be measured through quantitative means using a plant resistance index (PRI). This method of measuring host plant resistance involves examining characteristics of the three types of resistance present in a variety or germplasm being tested. Once the three host plant resistance assays have been completed, the results can be synthesized into a resistance factor value for each variety being tested. The PRI measures each of the three resistance categories an allows the researcher to explain the overall resistance effect that a plant will have when there are multiple types of resistance present (Razmjou *et al.* 2012; Paudyal *et al.* 2019).

The PRI uses values of tolerance, antibiosis, and antixenosis as values of X, Y, and Z to calculate the overall resistance factor of a plant. Factors used to determine these values could include a damage rating or a percent reduction in root length to represent tolerance, the number of nymphs to represent antixenosis, and the number of nymphs produced or the number of nymphs per female to represent antibiosis (Razmjou *et al.* 2012; Paudyal *et al.* 2019). The values created by a plant resistance index can be normalized in order to classify plants into categories of relative resistance (highly resistant, resistant, moderately resistant, etc.).

Phenotyping Protocols

In order to test the success and efficiency of resistant and tolerant wheat plants, reliable and repeatable phenotyping protocols are needed. One of the earliest records of screening for resistance in wheat comes from an unknown author in the late 1700s who was seeking resistance to Hessian fly (Painter 1951). Later on in the 19th century, the search for resistance to Hessian fly continued in California with the screening of other cereal crops, including wheat, oats, barley, and rye (Wickson 1881; Woodsworth 1891; Kellner 1892). The screening for resistance in cereal crops to *R. padi* began with studies performed by Hsu and Robinson (1962,1963). The initial greenhouse and field studies yielded promising results with many of the tested varieties of barley showing resistance. However, subsequent repetition of these screenings in an environmental chamber yielded contradictory results with few small differences in the varieties (Papp and Mesterházy 1993; Robinson 1964). Since these preliminary forays into screening for resistance to bird cherry-oat aphid, the classification of resistance to this pest insect has produced a wide

range of contradictory results (Hsu and Robinson 1962,1963; Kieckhefer *et al*. 1980; Wiktelius and Pettersson 1985). The persistence of contradictory results in the screening experiments is due largely to the complex plant-insect interactions that occurs between the wheat and the aphid, along with the complicated biology and behavior of the aphid. Several factors that affect the outcomes of screening attempts can include the growth stage of the host plant, the behavior of the pest, the number of pests, and environmental factors (Painter 1951). Additionally, the relationship and interactions between aphid virulence and host plant resistance genes are exceedingly difficult to interpret (Smith and Chuang 2014).

Since the origins of phenotyping resistance to bird cherry-oat aphid in wheat, there have been advances in new phenotyping protocols, seeking to standardize the process. As described previously, the study performed by Papp and Mesterházy (1993) found a significant difference in grain yield between the tolerant and susceptible wheat varieties. This study was a field experiment using a randomized complete block design with infested and uninfested cages. The grain yield was measured with 20 randomly selected heads from each cage; grain yield was compared between the infested and uninfested cages (Papp and Mesterházy 1993). This study was originally intended to study the effects of cereal leaf beetle and was unintentionally infested with *R. padi*. Thus, the infestation levels between cages were not standardized. In 2005, a screening protocol was used that was based on nymphiposition and population growth on wheat accessions. Seven day old plants were randomized and infested with 3 alate *R. padi.* After 24 hours, the adults were removed, and 5 nymphs were allowed to remain. After 14 days, the number of aphids per plant were counted in order to identify potential resistance in the tested wheat accessions (Hesler 2005; Hesler and Tharp 2005).

A similar method was used by Mohamadi *et al.* (2012) in Iran. This study sought to screen 40 lines of wheat in a growth chamber experiment. Wheat seedlings at the 2-3 leaf growth stage were infested with 3 apterous *R. padi* adults. The adults were removed after 24 hours and 3 nymphs were allowed to remain on each plant. After 14 days, the number of aphids per plant were counted and compared between lines. Lines with the highest and lowest numbers of aphids per plant were chosen for further analysis of the mechanism of potential resistance (Mohamadi *et al.* 2012). As with the previously described protocol, this screening technique focuses on the number of aphids on each plant, which would highlight antibiosis and antixenosis characteristics, rather than tolerance characteristics. In another type of screening protocol, 67 lines of wheat were examined for potential resistance to *R. padi*. Two to three plants of each tested line were grown within a growth chamber colony of BCOA, where they were subjected to heavy levels of infestation and feeding pressure from apterous aphids. The plants that were still green at the end of a 120-day infestation period were chosen as having promising resistant characteristics (Girvin *et al.* 2017). With this screening method, antibiosis, antixenosis, and tolerance characteristics could potentially be present in the selected lines still living following heavy aphid feeding pressure.

More recently, a study performed in Brazil sought to evaluate the resistance present in 15 wheat cultivars to *R. padi* by conducting an antixenosis assay, an antibiosis assay, a trichome analysis, a histological analysis, and a gene expression analysis (Jesus Correa *et al.* 2020). The antixenosis assay consisted of a two-choice test without contact, in which 10 aphids were given the choice between a susceptible and a resistant cultivar within a Petri dish without touching the leaves. In the antibiosis assay, each cultivar was infested with two apterous adult aphids. The number of aphids on each plant was examined after 12 days. For the trichome analysis, 4 areas of

1 mm^2 on each side of the leaf of each cultivar was examined for trichome density and length, as trichomes have been found to influence plant resistance to aphids (Roberts and Foster 1983). The histological analysis included visual assessment of leaf epicuticular waxes, mesophyll cell characteristics and vascular bundle sheath characteristics. Lastly, the gene expression analysis examined total RNA extractions from cultivars to compare gene expression levels of selected genes on plants with and without aphid feeding pressure (Jesus Correa 2020). This series of tests gives a relatively complete analysis of resistant characteristics of these cultivars, encompassing both physical and molecular characteristics.

At Oklahoma State University, efforts have been made to develop a screening protocol for resistance in wheat germplasm to bird cherry-oat aphids. A bioassay was developed using seed germination pouches. Five seeds were germinated and grown in each pouch. When seedlings were 7 days old, a leaf of susceptible 'Jagger' wheat with 40 to 60 aphids was placed on the pouch to infest each seedling with 10 to 15 aphids. A 14-day infestation period was shown to be sufficient after 10, 12, 14, 16, and 18-day infestation periods were tested. The root and shoot dry weights after aphid removal were compared between wheat lines (Dunn *et al.* 2007). This protocol was used to screen for resistance in 55 wheat entries from the USDA-ARS National Small Grains Collection, Aberdeen, Idaho which had been reported to be resistant to BYDV (Dunn *et al.* 2011). The seedlings were grown and infested as previously described. After the root and shoot dry weights were collected after 14 days of infestation, each entry was categorized as better than, equal to, or worse than the uninfested control plants (Dunn *et al.* 2011). This screening procedure is effective for identifying tolerance in wheat plants but does not take into account the populations of aphids on each seedling, which is important for identifying potential antibiosis or antixenosis.

Since 2015, experimental lines of wheat from the Wheat Improvement Team (WIT) have been screened and evaluated for resistance and tolerance to bird cherry-oat aphid (BCOA) at Oklahoma State University using a standardized evaluation method (PIP Report 2019). The initial screening process involved replicated evaluation of experimental lines and infesting them with approximately 22 BCOA per seedling from a laboratory colony of aviruliferous aphids maintained in a growth chamber. After 5 to 6 days, the development of a second leaf was an indication of partial resistance in those entries. Approximately 17 to 18 days after infestation, each seedling was visually assessed and visually classified into scores 1-5 based on overall plant health and presence of aphids; criteria included number of leaves, leaf width, and amount of chlorosis (Table 1).

Table 1. Evaluation scale (1-5) and criteria for each score level used to classify wheat cultivars for their resistance to bird cherry-oat aphids.

Chlorosis scale (1-5)	Criteria (Status and Condition of Plant)
1: Highly Resistant	Alive, green, no sign of chlorosis
2: Resistant	Alive, green, chlorosis on the tip of the blade
3: Moderately Resistant	Alive with some green, chlorosis on 80% of leaf area
4: Slightly Resistant	Alive, extensive chlorosis, little green and approaching necrosis
5: Highly Susceptible	Dead

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CHAPTER III

MATERIALS AND METHODS

Modified Phenotyping Protocol

The previous phenotyping protocol was modified to improve efficiency and ensure replication of heavily infested seedlings that would screen out the most tolerant lines. The initial screening process was completed for the 140 germplasm lines received from the WIT. They were evaluated to select the best entries for further assessment of resistance to BCOA. To accomplish this modified evaluation, two seeds of each germplasm entry were planted in conetainers measuring 4 cm in diameter and 20.5 cm in length (Figure 6) and were randomized within a 98 cell black plastic tray measuring 60 cm long by 30 cm wide by 17 cm tall (Figure 7). Entry numbers were denoted using small white tags. Seven replicates of each germplasm entry were planted as well as seven replicates of a susceptible check (cv. 'Jagger') and a resistant check (cv. 'S2'). In order to accommodate all 140 germplasm entries with 7 replicates each, this trial was

done in 4 groups with each group including 12 germplasm entries, the susceptible check, and the resistant check.

When approximately 75% of seedlings had emerged, the trays were infested with approximately 35 aphids per seedling by shaking potted plants that were heavily infested with aphids over the trays. After 24 hours, the level of infestation was estimated to ensure that each seedling had ~35 of aphids. After 16 days, the seedlings were evaluated based on five factors: amount of chlorosis, number of leaves, width of the leaves, height of the plant, and number of aphids. These factors were considered to give each seedling a score from 1 to 5 (Table 1; Figures 1-5). Through a statistical analysis using SAS, the 6 best germplasm entries were chosen for detailed tolerance, antibiosis, antixenosis, and field evaluations.

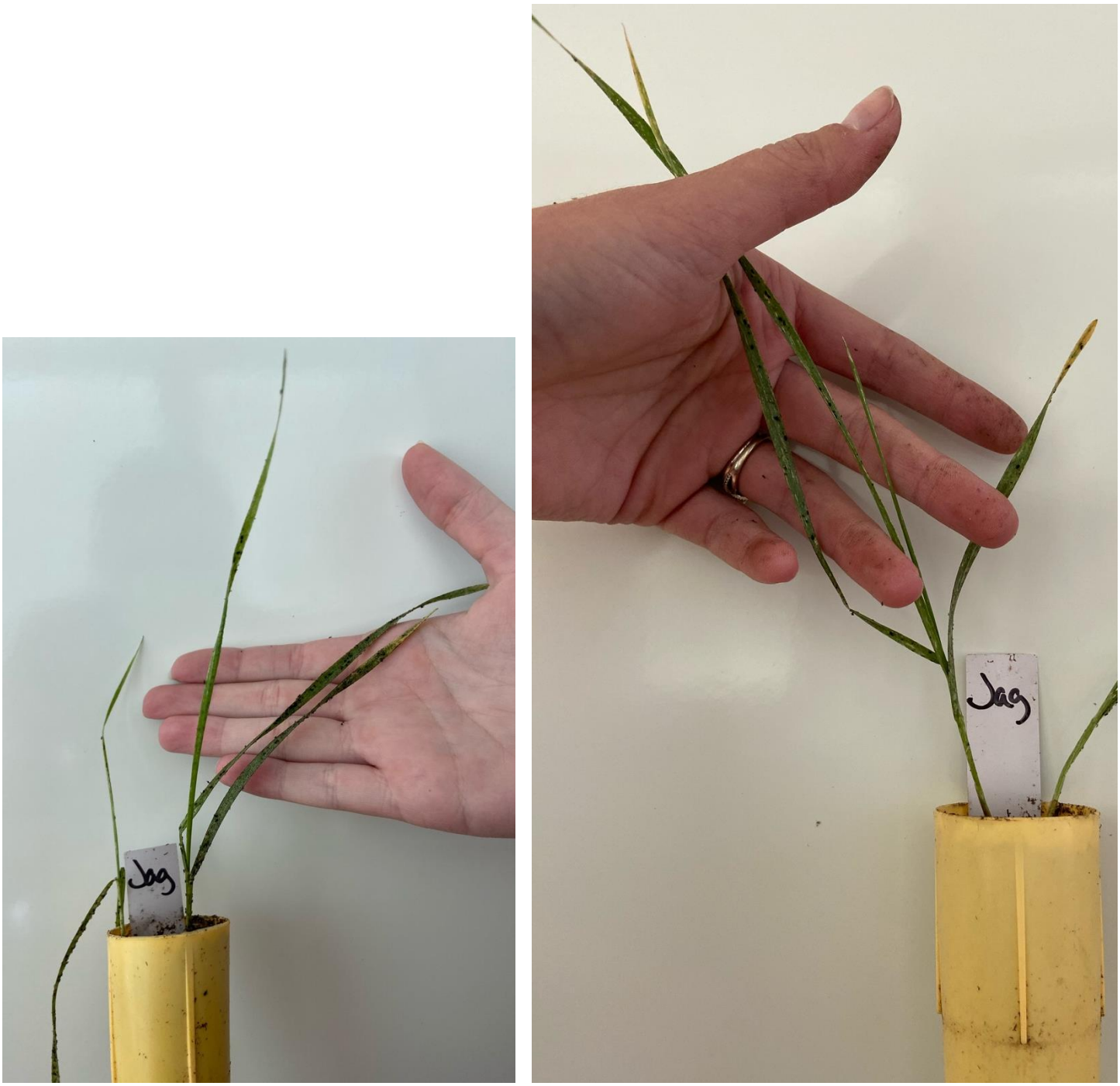


Figure 1a (left) and 1b (right). Visual examples of wheat plants that were assigned a score of 1 (the highest score) for their resistance or tolerance to bird cherry-oat aphid feeding.

Figure 2a (left) and 2b (right). Visual examples of wheat plants that were assigned a score of 2 for their resistance or tolerance to bird cherry-oat aphid feeding.

Figure 3a (left) and 3b (right). Visual examples of wheat plants that were assigned a score of 3 for their resistance or tolerance to bird cherry-oat aphid feeding.

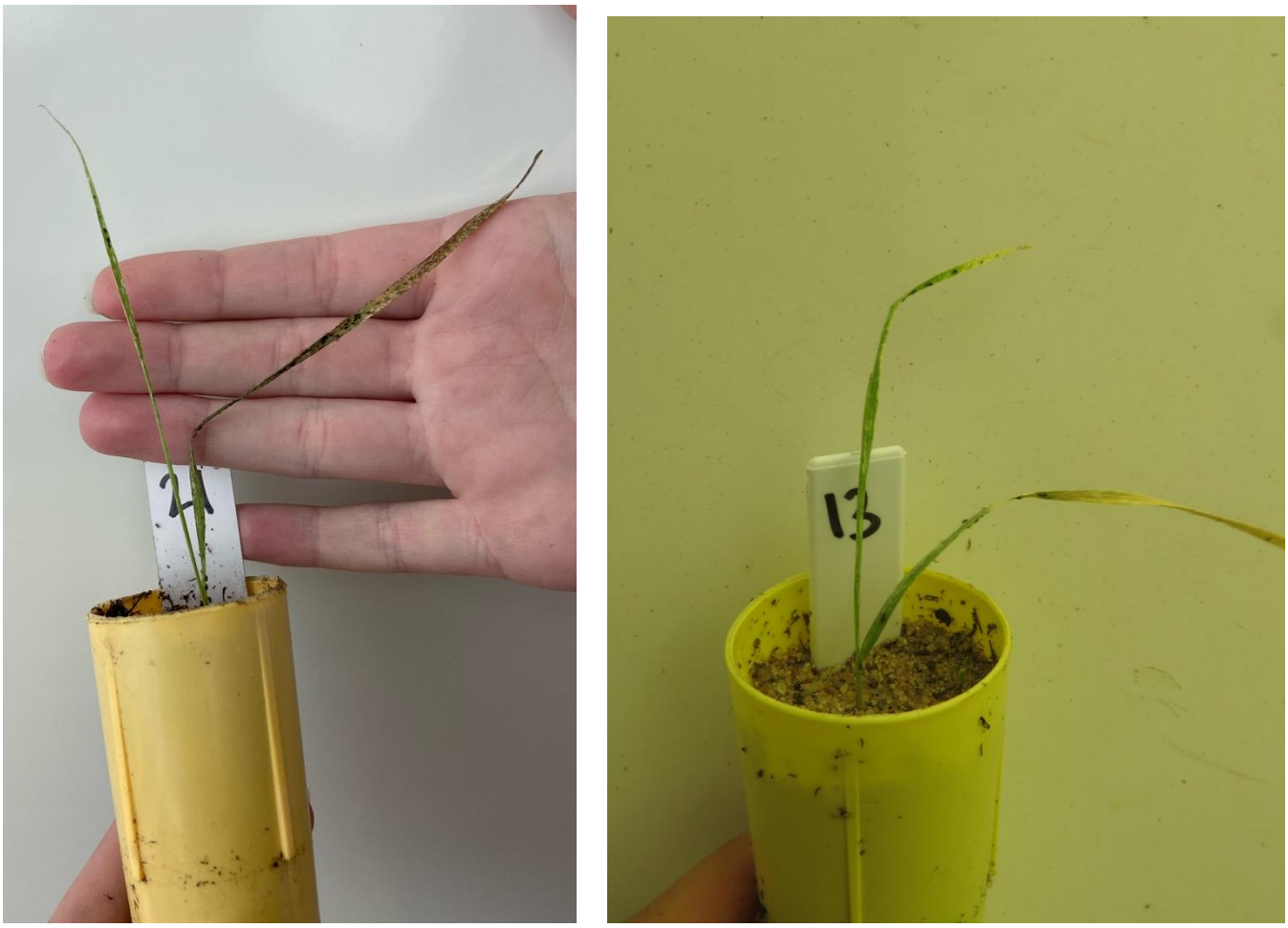
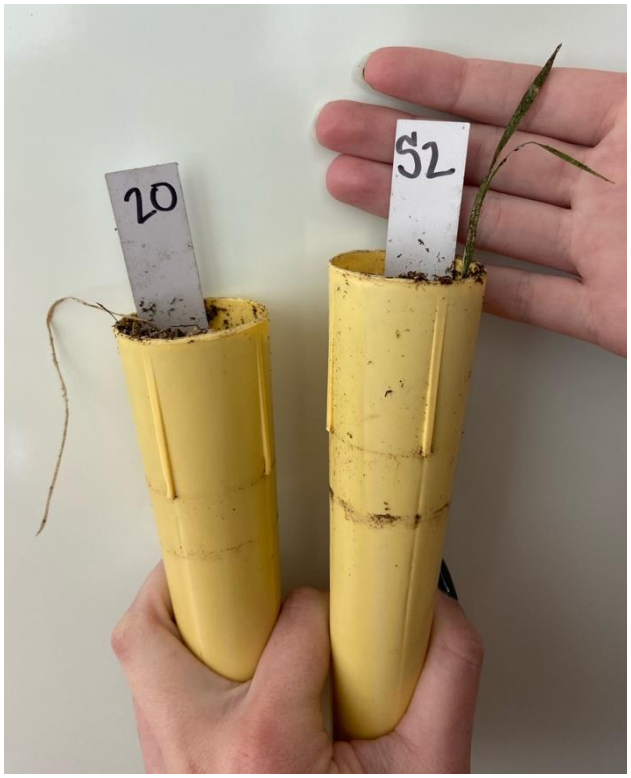


Figure 4a (left) and 4b (right). Visual examples of wheat plants that were assigned a score of 4 for their resistance or tolerance to bird cherry-oat aphid feeding.



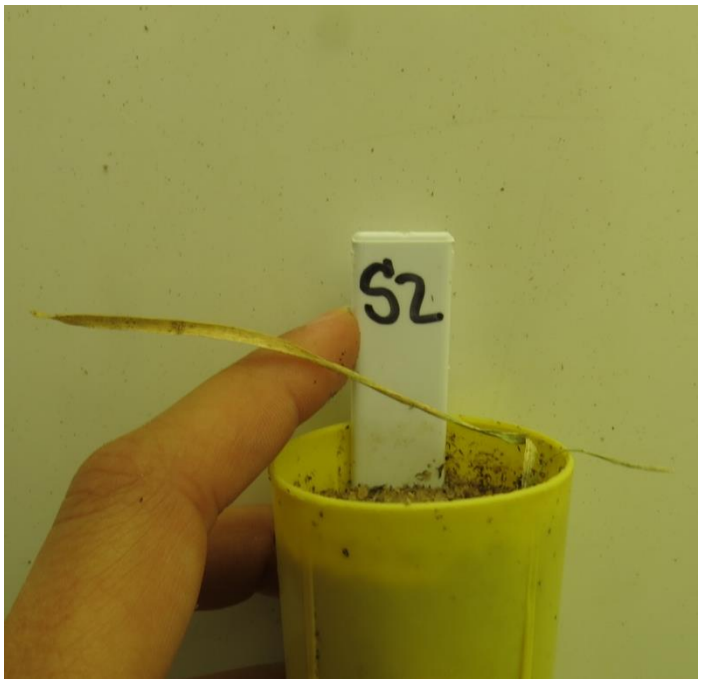


Figure 5a (left) and 5b (right). Visual examples of wheat plants that were assigned a score of 5 for their resistance or tolerance to bird cherry-oat aphid feeding.

Figure 6. Yellow conetainer used for the growth of seedlings in the initial evaluation, tolerance trial, and antibiosis trial.

Figure 7. Conetainers within the 98-cell tray used for randomization.

Quantitative Assessment of Phenotyping Protocol

Preliminary Evaluation: This test was performed during September 2019 using a method similar to the evaluation process. Yellow conetainers were placed in a 98-cell black plastic tray (Figures 6 and 7). Each conetainer was lined with a small piece of paper to cover the hole at the bottom of the cone. The conetainer was then filled with Miracle-Gro® soil mix. The soil was thoroughly watered, and two holes were created in the damp soil. On 23 September, two seeds of each germplasm evaluated were placed in the prepared conetainers and were covered with a thin layer of sand. The trays were then placed in growth chambers (24±2°C, 16:8 LD). The seedlings were allowed to emerge from the soil. Once approximately 75% of the seeds planted had emerged, the tray was infested with aphids. On 28 September, the trays were infested by shaking a pot of heavily infested wheat over the tray of cones.

After 24 hours, the level of infestation was assessed to ensure that each seedling had approximately 35 aphids. This level of infestation was used in order to simulate a heavy level of infestation as could be possible in a field setting. The seedlings were watered every other day for the duration of the test. On 14 October 2019, the trial was terminated, and the data were collected. The data collected consisted of a score from 1 to 5 (1 being the best, 5 being the worst) for each seedling based on a visual assessment of factors, including amount of chlorosis, height of the tallest leaf, height of the newest leaf, number of leaves, width of the leaves, and number of aphids. Once the visual assessment was concluded, quantitative measurements were taken for the following factors: estimated percent chlorosis, height of the tallest leaf (cm), height of the newest growing leaf (cm), number of leaves, width of the widest leaf blade (mm), and the number of

aphids. Analyses were completed using generalized linear models to compare factors among entries. Counted data such as aphid number and leaf number were modeled using a Poisson distribution and continuous responses such as height and blade width were analyzed using a normal distribution. When significant differences were found, Tukey multiple comparisons procedures were used. All tests of significance were done at the nominal $p \leq 0.05$ level. The GLIMMIX Procedure of SAS version 9.4 (SAS Institute, 2014) was used in this analysis.

After this analysis was completed, the data set was used to form an equation that is designed to produce a score after the input of each measured variable. A multiple regression model for predicting score based on chlorosis, height, leaf number and blade width was obtained using stepwise regression methods to obtain the final model equation that predicts resistance scores from quantitative measurements (Montgomery *et al.* 2012).

Expanded Evaluation: To strengthen the reliability of this method of evaluation and scoring, this data set was combined with a second data set. This second data set came from another larger set of germplasm entries from the Wheat Improvement Team at Oklahoma State University. This second set of germplasm entries contained 36 entries, some of which were included in the first data set.

Antibiosis and Antixenosis Trials

For the antibiosis experiment, a group of same age aphids was produced. In order to accomplish this, 30 seedlings of ‘Jagger’ wheat were planted. Each seedling was infested with a single adult aphid. In order to keep the aphids on the seedlings, the cones were covered with

cylindrical plastic tubes. To allow for air flow, these plastic tubes have several mesh-covered holes along their length. After 24 hours, the adult aphids were removed from the seedlings and nymphs born on the same day were allowed to grow to the adult stage.

Nine seeds of each germplasm line were planted in the yellow conetainers used previously and using the same planting method. The germplasm seedlings, including the 6 experimental lines, 'Jagger' and 'Lone Rider', were infested with the same age adult aphids that were raised on the 'Jagger' seedlings. Each cone was covered with the same type of cylindrical plastic tubes as described previously. After 48 hours, adults were removed from the plant along with all nymphs except for one on each seedling. The date of birth of each nymph on each seedling was recorded. Every aphid was examined every 24 hours. The nymphs were allowed to grow to the adult stage. The date of the first nymph born to each adult was recorded. Every 24 hours, the number of nymphs born to each adult was recorded and all nymphs were removed from the plant. The date of death of each adult aphid was recorded. The data were analyzed using the GLIMMIX procedure and a generalized linear model in SAS version 9.4 (SAS Institute, 2014).

In the antixenosis experiment, 10 plastic pots measuring 15.25 cm in diameter were lined with a small piece of copy paper to cover the holes at the bottom of the pots. The pots were then filled with Miracle-Gro® soil mix up to 2.5 cm below the top edge of the pot. The soil was then moistened with water until damp all the way through. Eight holes were made in the soil in a circle approximately 2.5 cm from the edge of the pot. White plastic labels were placed randomly at the edge of the pot in front of each hole to indicate the germplasm entry number (Figure 8). Two seeds of the entry corresponding to the label were placed in each hole in the pot. The seeds were then covered with about 1.25 cm of soil. The soil was then covered with a thin layer of

sand. All of the pots were then placed in a growth chamber (24±2°C, 16:8 LD).

The pots were watered every other day until infestation. The seedlings emerged in approximately three days and were allowed to grow for three more days before they were infested with aphids. Six of the ten pots were chosen for infestation based on uniformity of seedling emergence. The experimental pots were infested with bird cherry-oat aphids from the existing lab colony. Several plants from the colony were removed and shaken over a blank sheet of paper until approximately 200 dislodged aphids were collected. The aphids were then gently shaken to the center of the paper. Prior to infestation, a fresh layer of dry sand was added to the surface of the pots. The aphids were then gently shaken or brushed off using a dry paintbrush into the center of the circle of seedlings in the trial pots. A plastic cylindrical cover was placed over all of the seedlings and was pushed into the soil. The outside of each pot was labeled #1 through #6 using a small square of paper (Figure 9).

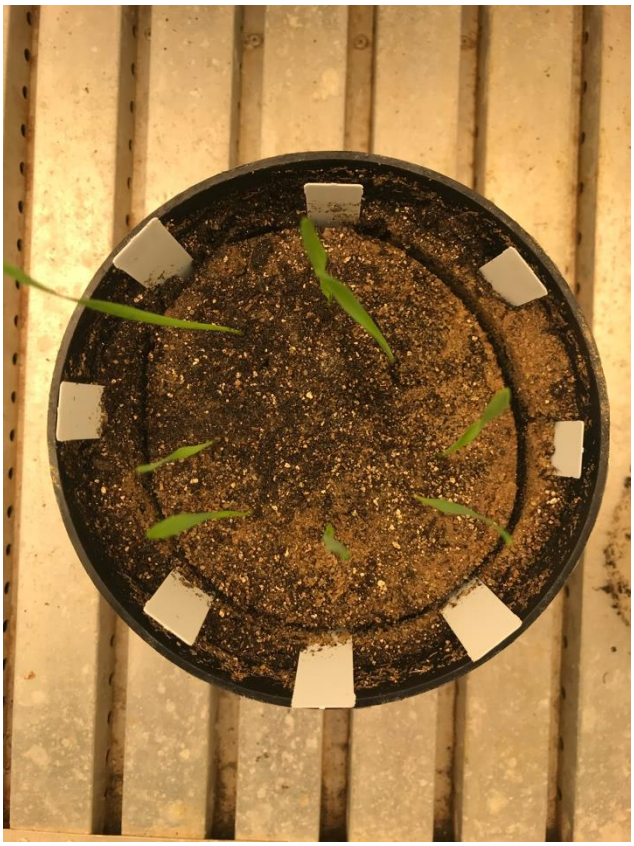


Figure 8. A top view of the antixenosis trial setup demonstrating the planting arrangement of the seedlings in a circle around the perimeter of the pot. For infestation, the aphids were placed in the center of the circle of seedlings.

Figure 9. Antixenosis trial pot setup demonstrating the pot number label, the entry labels, and the seedlings planted in a circle around the perimeter of the pot.

The number of aphids on each seedling was counted after 24, 48, and 72 hours after infestation and was compared among entries using the GLIMMIX procedure of SAS. This trial was replicated a total of three times.

Field Trial to Assess Aphid Populations

A field trial was conducted at the Cimarron Valley Research Station in Perkins, Oklahoma. The trial consisted of 6 rows of wheat germplasm entries with plots measuring 1.52 m by 7.62 m with 31.75 cm between each row. The entries included in this field trial were 15, 110, 120, 122, 130, and Jagger as the susceptible check. The seeds were planted on 25 October 2019. After the seedlings had emerged, four cages constructed from PVC pipe were placed on the two middle rows of each of the six entry plots, held in place with landscaping staples. The cages measured 45 cm wide, 65 cm deep, and 32.5 cm high. The number of seedlings within each cage was counted and recorded. The first three cages in each row were infested on 26 November 2019 with two pots heavily infested with aphids (~2000 aphids) from the existing BCOA colony. The fourth cage in each row was left un-infested. Following infestation, the cages were covered with a sheet of fine mosquito mesh to keep the aphids in and exclude predators. The edges of the mesh were held down with landscaping staples and soil. After 7 days, the initial infestation level was recorded by counting the number of aphids on eight randomly selected plants. The populations of aphids were recorded approximately every 14 days by counting the number of aphids on eight randomly selected plants. The aphid populations were compared among entries using the GLIMMIX procedure of SAS.

In order to determine the approximate feeding injury done to the wheat during this field trial, the aphid days and cumulative aphid days (CAD) were calculated for the period of time that data were collected. Aphid Days were calculated for each experimental case using the following formula:

$$Aphid\ Days = \frac{Aphid\ \#1 + \ Aphid\ \#2}{2} * \ Days\ between\ \#1\ and\ \#2$$

This process was repeated until the CAD were calculated for each of the counting dates (Kieckhefer *et al.* 1995).

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CHAPTER IV

RESULTS

Modified Phenotyping Protocol

To validate the reliability of the evaluation process, 140 experimental germplasm lines previously selected for BCOA resistance, including checks, were examined further for potential tolerant or resistant traits. The entries were selected based on their previous designation of being highly to moderately resistant, having a favorable field performance based on the agronomic characteristics sought by plant breeders, and seed availability. As described previously, each plant in each entry was examined at the termination of the trial and given a score between 1 and 5. The entries selected out of 140 were those with high survivorship of plants and overall scores ranging from 1.8 to 3.1, calculated using the following formula.

$$Average\ score$$

$$= \frac{(\%\ plants\ with\ score\ 1 * 1) + (\%\ plants\ with\ score\ 2 * 2) + (\%\ plants\ with\ score\ 3 * 3) + (\%\ plants\ with\ score\ 4 * 4) + (\%\ plants\ with\ score\ 5 * 5)}{100}$$

The final entries selected from the 140 included 131, 130, 120, 122, 99, and 15 (Table 2).

Table 2. The 6 chosen germplasm entries and their percentage of tested plants that were alive at the termination of the evaluation and the average score those plants received.

Entry Number	% Alive	Average Score
131	100	2.7
130	92.3	3.1
120	92.9	3.0
122	100	2.1
99	100	2.3
15	100	1.8

Quantitative Assessment of Phenotyping Protocol

Preliminary Evaluation: The classification process of the tolerance evaluation was similar to that performed in the initial screening. The purpose of the tolerance trial was to evaluate the factors contributing to the scoring process involved in the initial screening method by collecting and analyzing quantitative measurements. Plant measurements collected included the number of

aphids, an estimation of the percent chlorosis, the number of leaves, the height of the tallest leaf (height 1), the height of the newest leaf (height 2), and the blade width of the widest leaf. The entries were then scored one through five with one being the best score and five being the worst score. The entry that had the greatest number of plants with the best scores was entry 131 due to having the highest percentage of plants with a score of 1 compared to the other entries. There was a great deal of variation in the distribution of scores among the eight entries included in this analysis (Figure 10) and therefore the data set is likely robust enough for evaluation of the factors contributing to the scoring method.

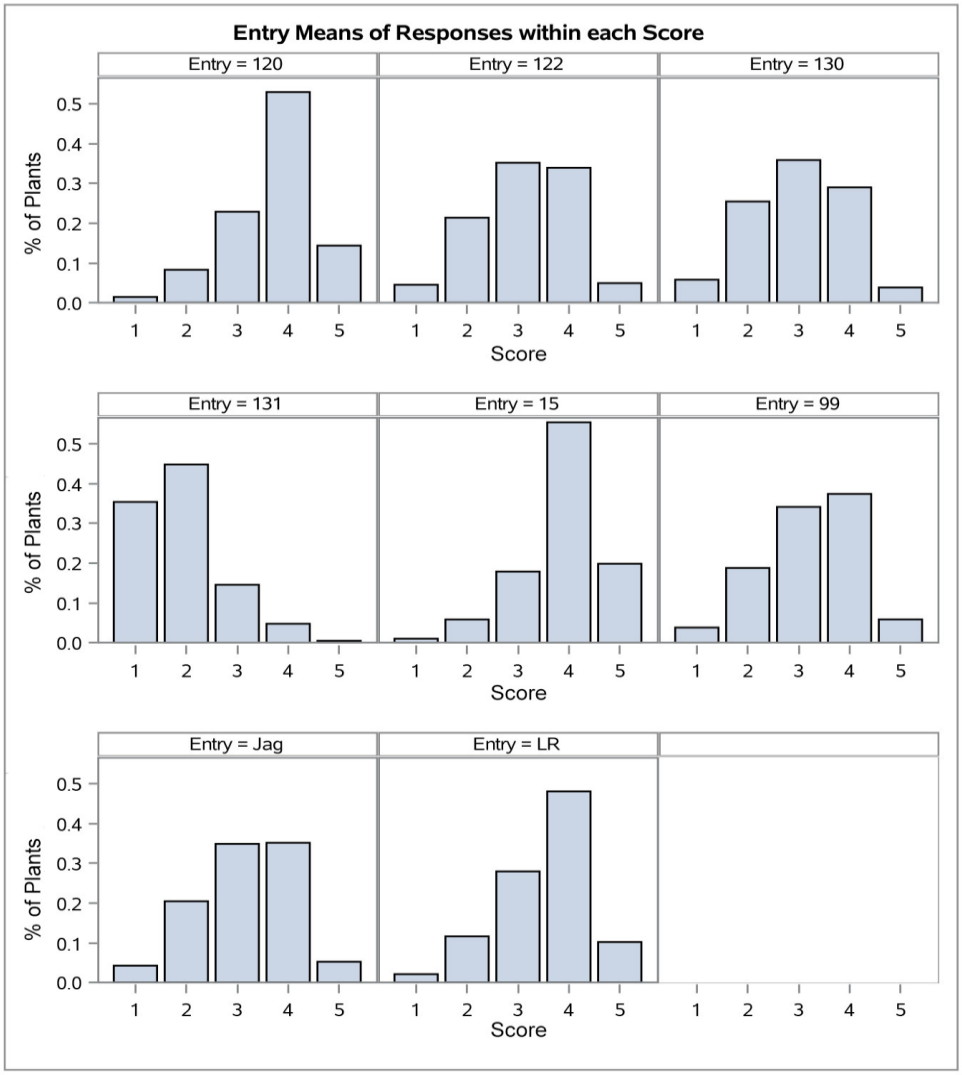

Figure 10. Series of graphs depicting the percent of wheat plants from each entry that were scored in each level (1-5) for their relative level of tolerance to feeding damage by bird cherry-oat aphids.

The ranges of quantitative measurements for each score also varied considerably. By looking at the ranges of each variable for scores 1 through 5, there is a clear separation in some variables, while the separation is not as apparent in other variables. Percent chlorosis has the most variability among the five score levels, while the other variables contain some overlap from one score to the next.

Table 3. The measured variables contributing to the tolerant or resistant score level of wheat plants exposed to feeding pressure by bird cherry-oat aphids presented as average ranges.

	Percent Chlorosis	Height 1 (cm)	Height 2 (cm)	Leaf Number	Blade Width (mm)	Aphid Number
Score 1	20-25	18.6-22.43	7.4-19	3	2-4	173.3-240
Score 2	30-54	17.64-22.34	5.45-10	3	1.95-3	80.95-150
Score 3	36.25-58	17.14-19.12	5.02-8.33	2.67-3	1.8-2.55	67-121.43
Score 4	42.86-62.5	13.43-17.63	1.95-3.13	2.33-2.71	1.56-2.35	47.5-110
Score 5	66.67-100	10.4-15.17	0.5-1.5	2	0.5-1.17	0-65

This data set was used to formulate a general equation to provide a score for any given plant based on a set of measured variables, including percent chlorosis, leaf number, height 1, and height 2. The stepwise regression performed excluded aphid number and blade width as variables due to their lack of significance. The equation for score (p) based on the information in Table 4 is as follows:

$$p = 5.9099 + 0.0154\ (\%\ Chlorosis) - 0.2663\ (Leaf\ \#) - 0.1341\ (Height\ 1) - 0.0828\ (Height\ 2)$$

Table 4. Values of the stepwise regression analysis that was performed with each of the measured variables contributing to score level representing the tolerance or resistance of wheat plants against feeding pressure by bird cherry-oat aphids.

Variable	Parameter Estimate	Standard Error	Pr > F
Intercept	5.9099	0.4269	<.0001
Percent Chlorosis	0.0154	0.0027	<.0001
Leaf Number	-0.2663	0.1309	0.0434
Height 1	-0.1341	0.0158	<.0001
Height 2	-0.0828	0.0182	<.0001

Based on the results of the stepwise regression analysis, five categories of predicted score (*p*) value ranges were calculated that matched the scoring method used in this project. These five ranges of *p* values (Table 5) could be reliably used to assign scores for germplasm evaluated for tolerance to BCOA.

Table 5. Predicted score (*p*) values produced by the score equation as they correlate with the scoring method that is based on a visual assessment of wheat plant characteristics.

Score	*p*
1	$p < 1.5$
2	$1.5 \leq p < 2.5$
3	$2.5 \leq p < 3.5$
4	$3.5 \leq p < 4.5$
5	$4.5 \leq p$

Expanded Evaluation: The averages of each measured variable for each score were combined between the two data sets. Interestingly, all measurements with the exception of leaf numbers, from this expanded group of entries significantly varied among the evaluation scores (Figures 11 and 12). The letters above the bars in these two figures indicate statistical differences according to a Tukey-Kramer separation of means analysis. Bars with different letters indicate a statistical difference.

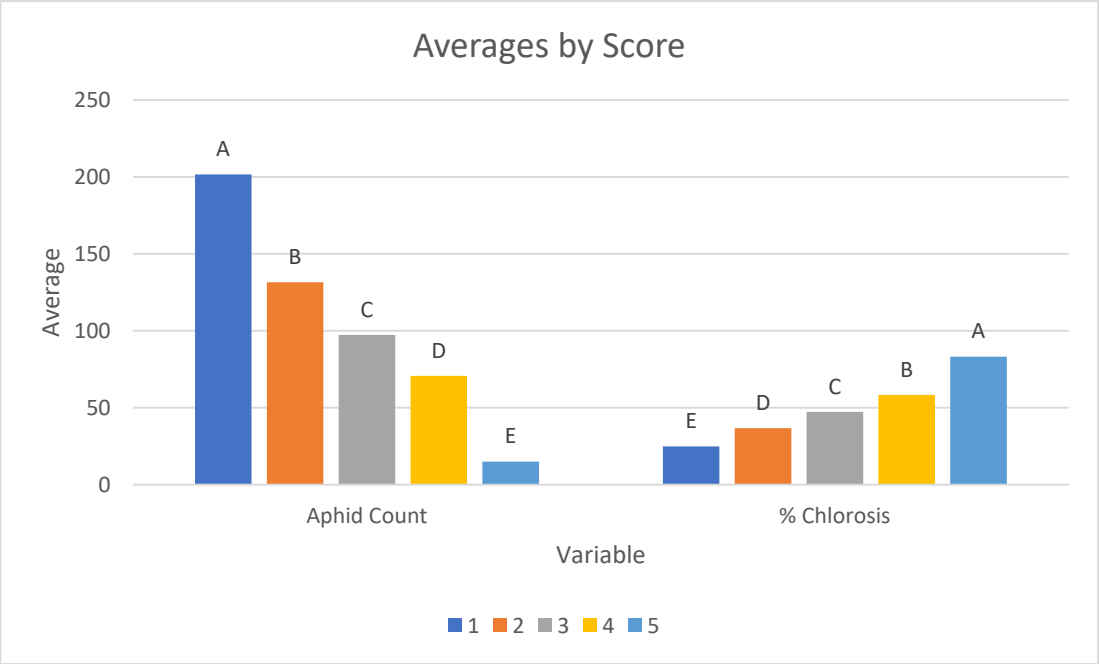

Figure 11. Graph depicting the average values of aphid count and percent chlorosis from wheat plants placed into each score category. The different colors represent each score level (1-5) as denoted by the color legend. Letters at the top of the bars indicate statistical separation of means. Bars with different letters are significantly different.

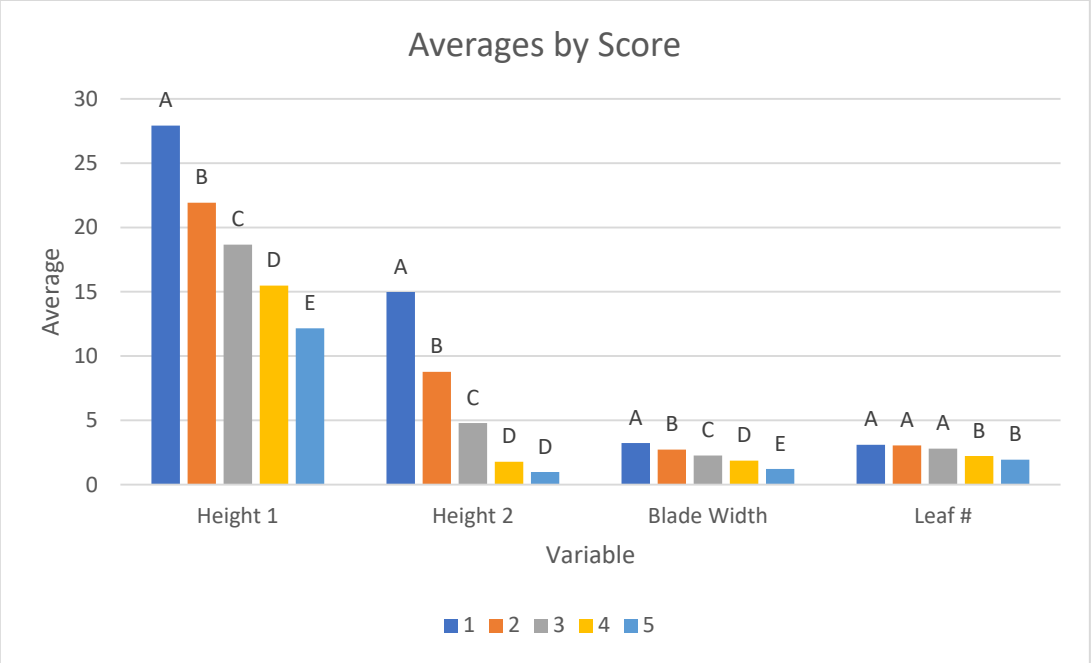

Figure 12. Graph depicting the average values of height 1, height 2, blade width, and leaf number from wheat plants placed into each score category. The different colors represent each score level (1-5) as denoted by the color legend. Letters at the top of the bars indicate statistical separation of means. Bars with different letters are significantly different.

By adding these two data sets together, the methodology used in this project is validated. The combination of the two data sets strengthens the validity of this method by increasing the number of plants evaluated. Unlike the initial analysis of the 8 entries, there are statistical differences between every score for the aphid number, percent chlorosis, height 1, and blade width (Figures 11 and 12). Both analyses indicate that the ranking of the plants on scores of 1 through 5 is a valid method to determine level of resistance or tolerance to aphid pressure. Clearly, with the evaluation of large numbers of entries, aphid number and percent chlorosis have the strongest influence on the final score. The variables that have a strong separation among score values are more important factors contributing to the score of the plant.

Antibiosis and Antixenosis Trials

The purpose of the antibiosis trial was to examine whether or not any of the 6 selected entries have a negative effect on the biological processes of the aphid. If the plant is affecting the biology of the of the aphid, the insect will not be able to grow and reproduce as normal. In this experiment, the days till first nymph birth, the number of nymphs, and the life span of each aphid was recorded and average values of each measurement was calculated (Table 6). Values followed by differing letters are significantly different based on a Tukey-Kramer Grouping analysis.

Table 6. Average values of days until first birth, number of nymphs, and life span of aphids for each germplasm entry in the antibiosis experiment. Letters following values within each column indicate statistical differences. Values with different letters are statistically different.

Germplasm Entry	Days to First Birth	# of Nymphs	Life Span (days)
120	5.71 a	60.57 ab	25.29 ab
122	5.89 a	49.89 bc	25.44 ab
130	5.57 a	47.14 c	18.75 b
131	5.44 a	61.11 a	23.67 ab
15	5.56 a	58.44 abc	26.25 a
99	5.71 a	59.43 abc	20.22 ab
‘Jagger’	5.44 a	54.56 abc	20.89 ab
‘Lone Rider’	5.11 a	58.56 abc	21.00 ab

The results of this experiment showed that there is little, if any, antibiotic effect for any of the experimental lines included in this analysis. The days until the first birth did not statistically differ among any of the germplasm tested. Among the values for the number of nymphs produced, the only significant difference between any germplasm entries occurred between entry 130 and entry 131. Aphids living on entry 131 produced the highest number of nymphs, while those on entry 130 produced the fewest. None of the other aphids living on the other entries were significantly different from each other. For the life span values, the only two entries that statistically differ from one another were entry 15 and entry 130. The aphids feeding on entry 15 experienced the longest life span compared to other aphids living on the other germplasm entries. Aphids living on entry 130 experienced the shortest life spans.

The purpose of the antixenosis trial was to identify any antixenotic effects in any of the selected 6 entries. The number of aphids found on each seedling for each germplasm entry was recorded and the averages of these values was calculated. The separation of these means was calculated using a Tukey-Kramer Grouping analysis in SAS. Figure 13 graphically depicts the number of aphids on each entry at each hour mark for the three repetitions of this experiment. Due to contradictory results compared to the other trials and the low infestation levels of Trial 1, this data was excluded from the remaining analyses.

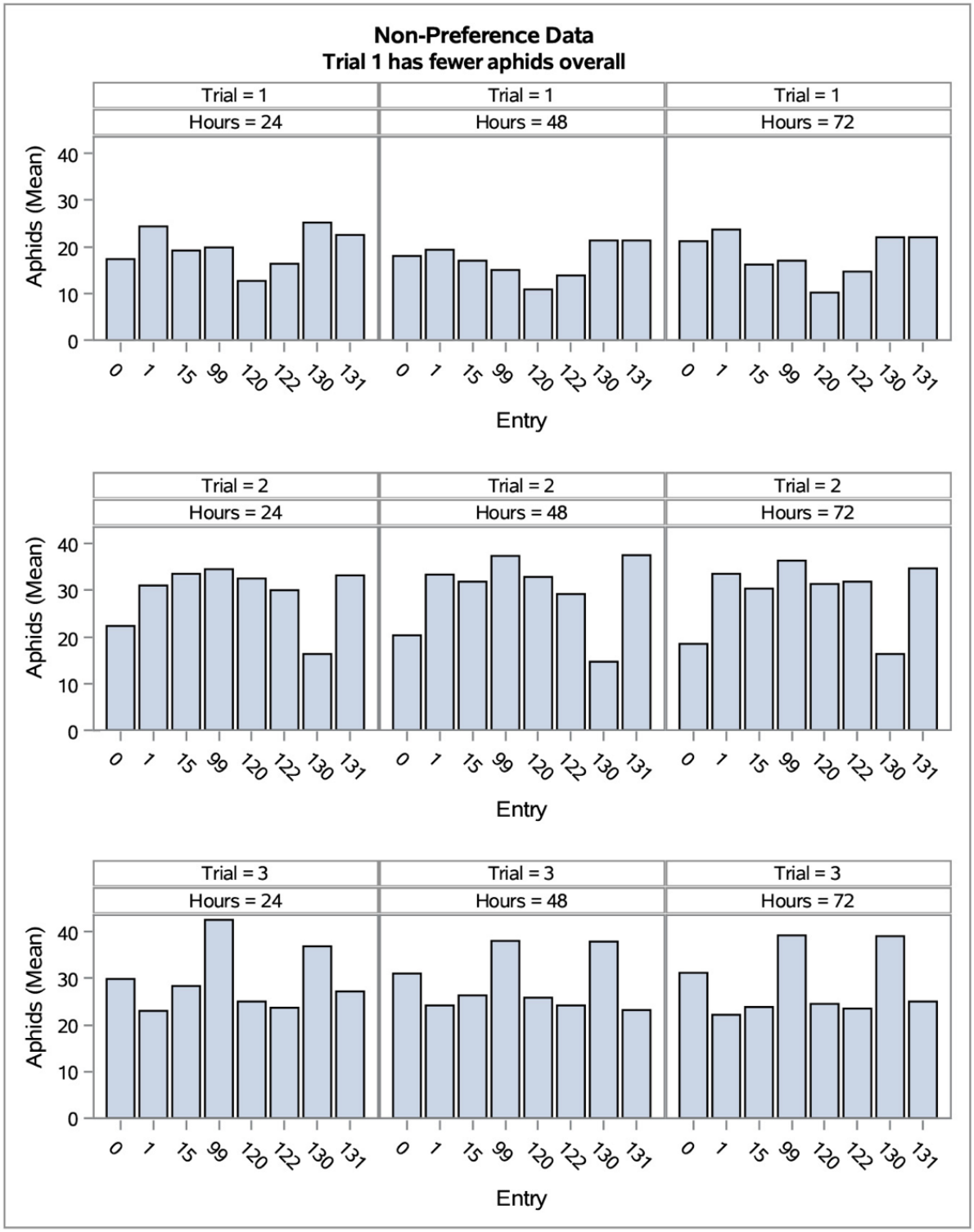

Figure 13. Series of graphs depicting the average number of aphids on each entry at each observation time (24, 48, and 72 hours after infestation) for Trials 1, 2, and 3 in the antixenosis experiment. Entry 0 indicates Jagger and Entry 1 indicates Lone Rider.

Table 7 contains the data for Trials 2 and 3 combined, including the average number of aphids on each entry at 24, 48, and 72 hours after infestation and the standard errors of the means. According to a Tukey-Kramer Grouping separation of means analysis, there was no statistical difference among any entries for the number of aphids per plant at any observation time.

Table 7. Average number of aphids on each entry for Trials 2 and 3 at each observation time after infestation and the standard error of the means.

Entry	Hours after Infestation	Mean Number of Aphids	Standard Error Mean
15	24	30.92	5.45
15	48	29.08	5.45
15	72	27.08	5.45
99	24	38.5	6.52
99	48	37.67	6.52
99	72	37.75	6.52
120	24	28.75	6.08
120	48	29.33	6.08
120	72	27.92	6.08
122	24	26.83	4.83
122	48	26.67	4.83
122	72	27.67	4.83
130	24	26.58	5.26
130	48	26.25	5.26
130	72	27.67	5.26
131	24	30.17	5.98

Entry	Hours after Infestation	Mean Number of Aphids	Standard Error Mean
131	48	30.33	5.98
131	72	29.83	5.98
‘Jagger’	24	26.08	4.49
‘Jagger’	48	25.67	4.49
‘Jagger’	72	24.83	4.49
‘Lone Rider’	24	27.00	4.26
‘Lone Rider’	48	28.75	4.26
‘Lone Rider’	72	27.83	4.26

Field Trial

For the field trial, 5 experimental lines along with Jagger were planted in order to confirm results of growth chamber trials and examine population growth on the various germplasm entries planted. The average number of aphids on each entry and at each sampling date were compared and results were analyzed using SAS. The population of BCOA in the Jagger cages at sampling date 6 (10 February 2020) was greater than all other populations on all other entries (Figure 14). However, because of variation among cages, there was not a statistically significant difference between the Jagger population and the entry 130 at sampling date 6.

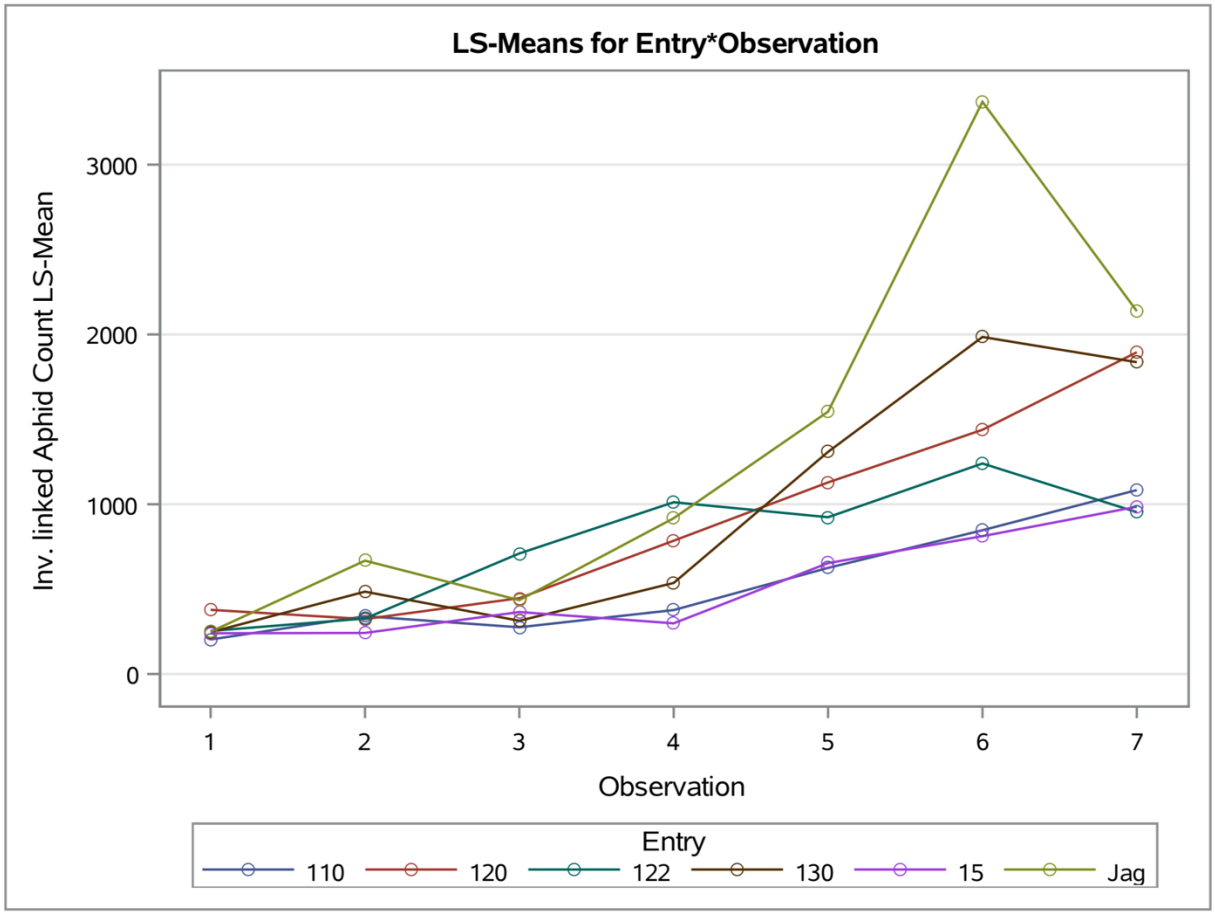

Figure 14. Graph depicting the total number of aphids counted on 8 random plants in each cage of each entry over 7 observation dates.

In order to reflect aphid pressure over time more accurately, the number of aphid days accumulated in each cage and for each entry was calculated. The number of aphid days for each entry was calculated by using the average of the three infested cages. The cumulative aphid days (CAD) was found for each entry by adding the number of aphid days calculated for each observation date (Table 8, Figure 15). The cages of 'Jagger' wheat accumulated the highest CAD. However, none of the entries accumulated significantly different amounts of CAD according to a Tukey-Kramer Grouping separation of means analysis.

Table 8. The CAD for each entry of experimental germplasm, along with 'Jagger', that was planted in the field trial to examine the dynamics of aphid populations on each entry. Values with the same letter following are not significantly different according to a Tukey-Kramer separation of means analysis.

Entry	CAD
15	5687.57 a
110	5943.23 a
120	10095.30 a
122	9006.71 a
130	11023.87 a
Jagger	11717.37 a

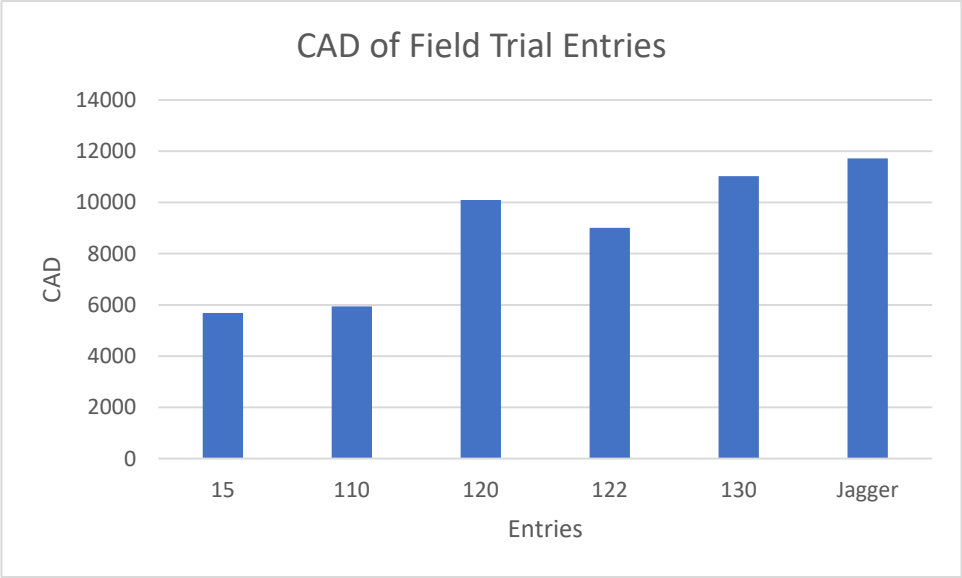

Figure 15. Graph of the cumulative aphid days (CAD) calculated using the average number of aphid days cumulated in the three infested cages for the duration of the field trial for each entry.

CHAPTER V

DISCUSSION

Modified Phenotyping Protocol

The initial screening allowed us to determine the best of 140 experimental germplasm lines provided by the Wheat Improvement Team at Oklahoma State University that had previously shown promising resistant or tolerant characteristics in preliminary field trials. Due to time and resource constraints, not all 140 lines could be thoroughly examined specifically for the type of tolerance or resistance that each entry displayed. Therefore, we selected lines that displayed the most resistant or tolerant characteristics for further evaluation using a set of defined scores based on plant condition and health after 16 to 18 days of infestation by BCOA. Six entries (15, 99, 120, 122, 130, and 131) that displayed the best resistant and tolerant characteristics and had average scores between 1.8 and 3.1 (Table 2) were chosen for further study. This evaluation was successful in confirming the reliability of the methodology. The visual assessment of the plant characteristics was similar to the method used originally by Dr.

Zarrabi and his team in the screening procedure. The characteristics examined in this visual assessment were intended to include a thorough overall assessment of the plant in order to highlight any symptoms of feeding damage on each plant.

Quantitative Assessment of Phenotyping Protocol

The purpose of the tolerance trial was evaluate the contributing factors of the existing method for screening experimental wheat germplasm lines for resistance to BCOA (Objective 1) and to identify existence of inherent tolerance in the germplasm lines (part of Objective 2). To fulfill the first objective, the screening and scoring method previously described (scores 1-5) was evaluated by taking quantitative measurements and analyzing them for their contribution to score value. The measurements taken included number of leaves, width of the widest leaf (mm), height of the tallest leaf (cm), height of the newest leaf (cm), estimated percent of chlorosis, and number of aphids. The addition of these measurements allowed a further understanding of tolerant and resistant characteristics and provided a way to confirm that the method and scoring criteria were appropriate and reliable. If the values of the parameters for the 5 score levels were statistically separated after an examination of the measured variables, then the scoring method would be confirmed as reliable. Under the given experimental conditions, the average of these 6 measured components give a perspective of the characteristics of the most tolerant plants. The ranges of these calculated averages also give a reference for how a tolerant or resistant plant will perform under intense aphid pressure (Table 3). This gives an approximation of how a tolerant or resistant plant of a certain score will appear.

None of the entries showed distinct resistant characteristics. Resistance was determined by having 50 aphids or less at the time of termination. If there were antibiotic or antixenotic characteristics present in the plants, the aphid population would have been lower than those with tolerant characteristics. Plants with more than 50 aphids at the time of termination were determined to be tolerant. The most tolerant plants scored 1, 2, or 3. Of the 6 experimental germplasm entries examined for tolerance traits, entry 131 was determined to be the most tolerant by having the highest percentage of plants given a score of 1 according to the measured characteristics (Figure 10).

Table 4 contains the values that resulted from a stepwise regression analysis performed on the tolerance data, excluding aphid number and blade width based on their lack of significance. The equation resulting from this analysis is as follows:

$$Score = 5.9099 + 0.01154\ (\%\ Chlorosis) - 0.2.663\ (Leaf\ \#) - 0.1341\ (Height\ 1) - 0.0828\ (Height\ 2)$$

This equation is designed to be used for any wheat plant regardless of its germplasm type being evaluated for resistance against BCOA following the procedure outlined in this project. The equation takes each of the measured variables into account in order to produce a score for that plant. The development of this equation will allow the method used in this project to be used by others to evaluate their germplasm lines for resistance and tolerance characteristics.

Alongside this tolerance trial, another set of experimental wheat germplasm was given to Dr. Zarrabi for a screening evaluation for potential resistant characteristics. This second group of experimental germplasm had some overlapping entries with those being examined during this

thesis project. The second group of germplasm entries were evaluated for resistance using the same method as previously described. In order to strengthen the results seen from the 6 chosen germplasm entries of this thesis, the data set from the secondary resistance screening was combined with the data set from this project. The resulting data set was considered to be stronger due to the increased number of data points being analyzed. This was done to validate the scoring method as reliable.

In the graphs depicting the average values for each measured variable according to each score using the combined data set, there are clear statistical differences between the values for each score for aphid number, percent chlorosis, height 1 (height of the tallest leaf), and blade width (Figures 11 and 12). For height 2 (height of the newest leaf) there is a statistical difference between scores 1, 2, 3, and 4. However, there is no difference between scores 4 and 5 for this variable. Likewise, there is not a strong statistical difference in the values for leaf number. Scores 1, 2, and 3 are different from scores 4 and 5. With this information, it is possible to make and inference as to the importance of the correlation that each variable has with the score that a plant is given. Because there is not a strong statistical difference between the variable values of height 2 and leaf number for each score, then these variables likely do not have a strong influence on the overall score of the plant.

In contrast, there is a statistical separation between the values of percent chlorosis and height 1 for each score. Therefore, it is possible that these characteristics strongly influence the overall score of the plant. This is supported by the fact that both percent chlorosis and height 1 were included in the stepwise regression analysis used to produce the score equation. The average values for aphid number were statistically different between scores but did not influence the overall score of the plant. This is evidenced by the stepwise regression analysis that was

performed to produce the score equation. The aphid number variable was left out of the score equation due to its lack of significance in contribution to the score value. Likewise, the average values for blade width were statistically separated between each score. However, this value was also left out of the score equation due to its lack of significance in contribution to the score value.

Antibiosis and Antixenosis Trials

The purpose of the antibiosis trial was to determine if any of the 6 chosen germplasm entries displayed antibiotic characteristics, as dictated by the second objective of this study. This experiment utilized same age aphids and allowed one aphid to feed and reproduce on plants of each entry in order to determine if their biological characteristics differed from those aphids living on the susceptible check, 'Jagger'. The colonies of aphids maintained at Oklahoma State University are regularly maintained on 'Jagger' plants. These plants placed in the colony are eventually killed from the intense aphid feeding pressure, proving that 'Jagger' is a susceptible variety of wheat. The aphid colony is able to feed and reproduce normally on this variety and are not biologically affected by it. Therefore, 'Jagger' serves as a reliable susceptible check.

There was no statistical difference between any of the entries for the length of time needed to become reproductive (Table 6). Aphids living on entry 130 produced the fewest number of nymphs on average. However, this value was only significantly different from entries 131 and 120. Aphids living on entry 131 produced the most nymphs (Table 6). The life span of each aphid was also recorded. Aphids living on entry 130 had the shortest life span. However,

this value was only significantly different from entry 15, which had the longest life span value (Table 6). Aphids living on entry 130 had the shortest life spans and produced the lowest numbers of nymphs on average. This could possibly indicate that entry 130 has a slight antibiotic effect on bird cherry-oat aphids. However, the numerical values for life span and reproduction for entry 130 were not significantly different from the majority of the other entries tested in this experiment. This indicates that the possible antibiotic effect of entry 130 is likely not strong, as the results for the plants in this entry were not extremely different than the other entries. It is likely that none of the entries in this experiment have any significant antibiotic characteristics.

The purpose of the antixenosis trial was to establish the presence of any antixenotic characteristics in the 6 germplasm lines chosen for evaluation, in fulfillment of the second objective. To accomplish this, a choice experiment was performed in which *R. padi* were allowed to choose which of the 8 seedlings to feed on. This type of experiment can be carried out over 24, 48 or 72 hours (Girvin *et al.* 2017; Hesler 2005; Hesler and Tharp 2005; Razmjou *et al.* 2012). This experiment was observed at 24, 48 and 72 hours in order to record any differences in host selection that may occur after the first 24 and 48 hours. Figure 13 shows the results of the average number of aphids on each entry at each of the observation times for all three trials. By comparing Trial 1 with Trials 2 and 3, it is evident that Trial 1 involved a lower total number of aphids than the other two. Trial 1 was infested with approximately 160 aphids (approximately 20 aphids per seedling) while Trials 2 and 3 were infested with approximately 240 aphids (approximately 30 aphids per seedling). Because of this difference, Trial 1 was excluded from all other data analyses.

A comparison between the number of aphids on each entry in trials 2 and 3 shows that the results between these two trials were inconsistent. In Trial 2, entries 99 and 131 had the

highest number of aphids at 48 hours, and entry 99 had the highest number of aphids at 72 hours. Entries 130 and ‘Jagger’ had the lowest numbers of aphids at both 48 and 72 hours. However, in Trial 3, entries 99 and 130 had the highest numbers of aphids at 48 and 72 hours. Entry 131 and ‘Lone Rider’ had the lowest numbers of aphids at 48 and 72 hours. Because these two Trials had produced contradictory results, the two data sets were combined in an effort to determine any commonalities between them. However, even with the two data sets combined, there were no statistically significant differences in the numbers of aphids on any of the entries at any of the observation times (Table 7). Therefore, none of the 6 chosen germplasm entries have displayed any antixenotic characteristics.

Field Trial

The purpose of the field trial was to confirm the results from the lab in a field setting. Though this field trial does not specifically fulfill an objective in this project, it lent valuable information to the results of this project in examining the performance of aphid populations on the entries tested. It should be noted that the entries planted in the field did not exactly match the entries that were tested in the rest of this project. This was due to a shortage of seed. Entry 110 was not tested in the other experiments of this project. Additionally, entry 131, entry 99, and ‘Lone Rider’ were not included in the field trial. It should also be noted that due to the lack of seed, this field trial was not randomized. There was only enough seed to plant two rows of each entry. Ideally, this field trial would have been planted in plots in a Randomized Complete Block Design (RCBD), with the entry plots randomized and replicated. However, due to the

circumstances, the four cages were all placed within the same row for each entry. It should also be noted that parasitized aphids were found within several of the infested cages at multiple sampling dates. The mummies were collected, and the parasitoid wasps were observed to hatch from them, confirming the presence of the wasps. The presence of parasitoids was recorded, but there was not a statistical difference in aphid populations between cages found with wasps and those without wasps.

Figure 14 shows that the peak of the recorded aphid population occurred at sampling date 6 (10 February 2020) in the 'Jagger' cages. However, this value was not significantly different from the next highest population value on entry 130 at the same sampling date. This indicates that there is not a difference in aphid population size between feeding on entry 130 and the susceptible check, 'Jagger'. The population value at sampling date 6 is significantly different from all other entries at that sampling date.

The true evidence of any tolerance traits in these experimental germplasm lines will be evident with the analysis of the yield data. A truly tolerant plant will produce a higher amount of yield at harvest than a susceptible plant despite the pressure of aphid feeding (Papp and Mesterházy 1993). Comparing the yield amounts between the plants in the infested and the uninfested cages will illuminate any tolerant characteristics in these plants. Unfortunately, these plants were not ready for harvest at the conclusion of this project. The yield data will be collected an analyzed by others in the coming months. For the purposes of this project, there are not any notable difference in the aphid population levels between the germplasm entries and the susceptible check. However, visual observation indicates slow kernel forming and filling in plants in the infested cages, regardless of level of tolerance. Plants are still green, and heads are soft compared to plants in the uninfested cages.

Aphid days were calculated to quantify the amount of injury inflicted by the aphids on the wheat plants within each cage. Aphid days were calculated as they accumulated between each observation date. Table 8 contains the values for the cumulative aphid days (CAD) calculated for each entry. The cages containing ‘Jagger’ wheat accumulated the highest number of CAD. The cages of entry 15 wheat accumulated the lowest number of CAD. The cages of ‘Jagger’ experienced more than twice the amount of aphid days experienced by entry 15, but none of the entries experiences statistically different CADs according to a Tukey-Kramer separation of means analysis. This wide variation could have been due to a resistant effect present in entry 15. However, the results of the antibiosis experiment did not reveal any antibiotic characteristics present in entry 15. With this disparity between number of aphid days between ‘Jagger’ and entry 15, if there is a significant difference in yield between these two entries, the difference cannot be attributed to a resistant effect in entry 15.

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CHAPTER VI

CONCLUSION

The first objective in this study was to evaluate the contributing factors existing in the method of screening for host plant resistance in wheat against bird cherry-oat aphids (BCOA) that had been previously developed by Drs. Zarrabi and Giles. To accomplish this, the screening method was performed to quantify the variables that were previously only used in a visual assessment. The parameters included were estimated percent chlorosis, height of the tallest leaf (height 1), height of the newest leaf (height 2), number of leaves, blade width, and estimated number of aphids. These parameters were chosen in order to give a full evaluation of each plant and to measure its overall health after being subjected to intense aphid feeding pressure.

Firstly, the results of this study show that the two variables with the most influence on the overall score of each plant were likely percent chlorosis and height 1. This is due to these factors both having a statistical separation between each score level when the average values were evaluated with a Tukey-Kramer Grouping analysis. This is also evidenced by these variables being included into the score equation when evaluated by the stepwise regression analysis. In a

stepwise regression, the most consistent and significant factor influencing the score is placed first in the equation.

Secondly, this screening method was confirmed to be valid and reliable by the results of this study. When the average values of the measured variables were evaluated with a Tukey-Kramer Grouping analysis, there was a statistical separation between each score level for percent chlorosis, height 1, height 2, and blade width. This indicates that the scoring method and use of 5 score levels is a valid way of determining resistance or tolerance levels against BCOA among wheat germplasm. Aphid number also had a statistical separation between each score level but was left out of the score equation that was created using a stepwise regression analysis. The score equation is as follows for score (p):

$$p = 5.9099 + 0.0154\ (\%\ Chlorosis) - 0.2663\ (Leaf\ \#) - 0.1341\ (Height\ 1) - 0.0828\ (Height\ 2)$$

The score equation generated by the stepwise regression is designed to be used to give a damage rating score for any wheat germplasm being evaluated for resistance against bird cherry-oat aphids using the phenotyping method used in this study. This score equation and a plant resistance index (PRI) are similar in that they give a numerical value as an expression of relative host plant resistance. However, the contributing factors determining the numerical expressions are different. This score equation differs from the use of a PRI in that a PRI is designed to give a rating of overall host plant resistance, which uses antibiosis, antixenosis, and tolerance characteristics as contributing factors (Razmjou *et al.* 2012; Paudyal *et al.* 2019). This type of scale is useful when more than one type of host plant resistance may be present in a variety and gives a comprehensive resistance score to express the combination of each resistance type in one

value. The score equation presented in this study is designed to give a damage rating score that is based on several factors of plant health. It can be inferred that healthier plants with a better score have tolerance or resistance characteristics when compared to susceptible plants.

The phenotyping method used in this study has similarities and differences to methods found in the literature. For example, one phenotyping method examined nymphiposition and aphid population growth on wheat accessions to screen for resistance characteristics. The accessions that had a lower number of aphids were considered to have resistance characteristics (Hesler 2005). This phenotyping method is more suited to screen for plants with antibiosis or antixenosis characteristics rather than tolerance characteristics. A tolerant plant may have a larger number of aphids feeding on it when compared to an antibiotic or antixenotic plant. The screening method used in this study is more suited to screen for both resistance and tolerance characteristics. The screening method used here is based on principles of plant health and will highlight those plants with either resistance or tolerance characteristics due to the idea that both resistant and tolerant plants will appear to be more healthy than susceptible plants following intense aphid feeding pressure.

Likewise, the method used by Mohamadi *et al.* (2012) produced 3 same-age nymphs on each line of wheat and counted the total number of aphids on each plant after 14 days. The lines with the lowest numbers of aphids were selected for further analysis of the mechanism of resistance present in these lines (Mohamadi *et al.* 2012). This phenotyping method is focused more on antibiosis or antixenosis as the mechanism of resistance rather than tolerance. The plants with reductions in aphid population were chosen as having resistance characteristics and were studied further to determine the presence of antibiosis. The phenotyping method used in this study is designed to select for plants with resistance or tolerance characteristics. The

phenotyping method used by Mohamadi *et al.* (2012) would be effective when the lines of wheat being examined are already suspected to be antibiotic or antixenotic. However, in the instance when the mechanism of resistance is unknown, the phenotyping method used in this study would be more effective, as it allows for the selection of both resistance and tolerance characteristics.

The phenotyping method used by Girvin *et al.* (2017) to screen for resistance involved placing plants of various cultivars into a colony of *R. padi*, exposing them to heavy infestation pressure. The plants that were still alive after 120 days of infestation were considered to be resistant or tolerant and were chosen for further study to examine the mechanism of resistance (Girvin *et al.* 2017). This phenotyping method was similar to the method used in this study as it allowed for the selection of plants with either resistance or tolerance and examined plant health as an indicator of resistance or tolerance. However, the infestation levels of the plants in this study was more controlled than in the method used by Girvin *et al.* (2017). Placing the plants within a colony of aphids does not ensure a uniform infestation of each plant. This study infested each plant with approximately 35 aphids to ensure that each plant is receiving approximately equal amounts of aphid feeding pressure.

The second objective in this study was to identify and characterize the type of host plant resistance present in any of the chosen 6 germplasm entries. To accomplish this, a series of three trials were completed to identify antibiosis, antixenosis, or tolerance in any of these 6 entries. The results of the tolerance trial found that most of the chosen germplasm entries displayed tolerant characteristics rather than resistant characteristics. The majority (79.88%) of plants tested in this trial were categorized as tolerant due to having more than 50 aphids at the time of termination. Only 20.12% of plants were categorized as resistant due to having 50 aphids or less at the time of termination. From these results, none of the 6 germplasm entries were expected to

display any antibiotic or antixenotic characteristics. To confirm this, the antixenosis trial was conducted and showed that BCOA did not prefer any of the 6 germplasm entries over the others. The number of aphids on each entry seedling was counted at 24, 48, and 72 hours after infestation. A Tukey-Kramer Grouping analysis showed that there was no statistical difference between the number of aphids on any of the entries at any of the observation times. Therefore, none of the germplasm entries displayed any antixenotic characteristics. Likewise, the antibiosis trial was performed to confirm that none of the entries possessed any antibiotic characteristics. *R. padi* same age nymphs were raised on seedlings of each of the 6 germplasm entries. The variables measured included the number of days until first birth, the number of nymphs produced, and the life span in days. There was a statistical separation between entries 130 and 131, which produced the fewest number of nymphs and the highest number of nymphs, respectively. There was also a statistical separation between entries 130 and 15, which experienced the shortest life span and the longest life span, respectively. There is likely not a strong antibiotic effect in entry 130 due to the fact that these were the only statistical differences among all 6 of the germplasm entries.

The phenotyping method used in this study focused on screening for seedling tolerance to early infestations. The subsequent antibiosis and antixenosis trials were intended to confirm that these mechanisms were not present in these experimental germplasm lines. The infestation of this screening protocol was caused immediately after seedling emergence and was intended to represent a heavy infestation of BCOA, as could happen in a field setting. An early infestation in a field setting is a “worst case scenario”. When seedlings are found to be tolerant to early infestations, this will aid growers in preparing for this possibility. In addition, host plant resistance generally tends to increase with the age of the plant as it becomes stronger (Miller *et*

al. 2003). Tolerance is also able to be used in conjunction with other IPM approaches, which can help to mitigate the risk of damage.

Lastly, a field trial was performed in order to assess the success of the tested germplasm entries in a field setting. Unfortunately, there was not sufficient seed available to test all 6 germplasm entries that were tested in two objectives of this study. The entries included in the field trial included 120, 122, 130, 15, 110, and 'Jagger' as a susceptible check. Of these 5 germplasm entries, there was not enough seed sufficient to plant a field in a Randomized Complete Block Design (RCBD) as there was only about 20 to 25 g of each entry. The variables that were measured in this field study included estimated aphid population in each cage, aphid days, and cumulative aphid days (CAD). Aphid populations were estimated by counting the number of aphids on 8 randomly selected plants in each cage. 'Jagger' had the highest aphid populations at sampling date 6 (10 February 2020) compared to the populations on all other entries. However, this value was not statistically different than the population recorded on entry 130 at sampling date 6. 'Jagger' had the highest number of aphid days at sampling date 7 (27 February 2020), but this value was not statistically different from any of the other entries at this sampling date. 'Jagger' also experienced the highest CAD than any other entry included in this field study. At the conclusion of this thesis project, the wheat in this field trial was not ready for harvest. The yield data will be collected at a future date by members of Dr. Zarrabi's team and will be added to the data from this field trial.

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