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Relationship between Aphid Infestations, Aphid Management Regimes, and the Incidence of Barley Yellow Dwarf Virus in Soft Red Winter Wheat

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I am submitting herewith a thesis written by Clay M. Perkins entitled "Relationship between Aphid Infestations, Aphid Management Regimes, and the Incidence of Barley Yellow Dwarf Virus in Soft Red Winter Wheat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Scott D. Stewart, Major Professor

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(Original signatures are on file with official student records.)

**Relationship between Aphid Infestations, Aphid Management
Regimes, and the Incidence of Barley Yellow Dwarf Virus in Soft Red
Winter Wheat**

**A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

**Clay M. Perkins
December 2018**

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DEDICATION

I would like to dedicate this research to my family. First and foremost, my parents Amy and Paul Perkins. Your love, guidance, and support along the way have meant the world to me. I could not have gotten to where I am today without your help. To my brother Clint, you have always been there for me. Even though we find something to argue about every once in a while, we always bounce back and have each other's back the next minute. I would also like to dedicate this to my grandparents who have always had an interest in my education and what new research I participated in each day. In addition, I would like to dedicate this research to Mr. Eddie Anderson. You took me under your wing before I was in high school and always gave your time when I needed something or had a question. Thank you for instilling a love for both agriculture and the Dyer County Fair in me.

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ABSTRACT

Aphid (Hemiptera: Aphididae) feeding may cause substantial loss of yield and grain quality by transmitting barley yellow dwarf (BYD) in wheat, *Triticum aestivum* L. Neonicotinoid seed treatments and foliar-applied insecticides are the two most common methods to manage aphid infestations and BYD. The overall goal for my research was to refine recommendations on the management of aphids in wheat to provide a consistent return on investment. An analysis was done across 33 insecticide efficacy trials in west Tennessee during the last eleven years to determine how neonicotinoid seed treatments and a late-winter foliar insecticide application affected aphid populations, incidence of BYD, and yield. A significant decrease in aphid populations and incidence of BYD was observed where an insecticide seed treatment, foliar insecticide, or both were used. Average wheat yields were increased by 280 – 381 kg/ha if an insecticide seed treatment or when a foliar insecticide application was made. A factorial experiment was repeated at four locations to examine how variety tolerance to BYD, a neonicotinoid seed treatment, and a foliar insecticide application affected populations of aphids, the occurrence of BYD, and yield. Similarly, experiments with various neonicotinoid seed treatments and foliar insecticide spray regimens were repeated to evaluate treatment effects on aphids, BYD, and yield. Aphid populations and BYD symptomology were consistently reduced by the use of insecticides, particularly foliar insecticides. Quantitative ELISA assays confirmed the presence of BYD in leaf tissue and was also able to detect some treatment effects on the concentration of BYD. Varieties considered tolerant to BYD had reduced symptoms of

BYD, but this difference was not reflected in yield responses, and ELISA assays detected no difference between varieties in the concentration of BYD in leaf tissue. In these tests, foliar applied insecticides provided more benefit than neonicotinoid seed treatments. Collectively, my data show that insecticides should be used to manage aphids and BYD, and foliar-applied insecticides appear to provide more benefit and flexibility than insecticide seed treatments.

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INTRODUCTION

Wheat

Wheat (*Triticum spp.*) is the world's most widely cultivated crop with over 221 million hectares (ha.) produced (USDA). Common bread wheat (*T. aestivum* L.) and durum wheat (*T. durum*) are the most common types of wheat planted, making up nearly 90% of the world's wheat crop. Wheat can be classified as spring or winter; soft or hard; red or white; and also by protein content (Briggle and Curtis 1987). In Tennessee, annual production in soft red winter wheat (*T. aestivum* L.) varies considerably but typically ranges from 100,000 to 200,000 ha. The average yield in Tennessee during 2016 was 4,627 kg/hectare (68 bu/acre) (USDA-NASS).

Internationally, wheat is the most traded cereal crop. Wheat is grown in all continents and is considered to be the most vital cereal crop in the Northern hemisphere along with Australia and New Zealand (Oerke 2006). The majority of the wheat produced is used as human consumption. Bread wheats are used in making breads, cakes, cookies, rolls and pastries while durum wheat is used in making pasta (Wiese 1987). Wheat is the primary food for about 40% of the world's population. Worldwide, it is estimated that two-thirds of the world's population is reliant on wheat as a vital food source (WHO 2012). Wheat can also be used in livestock feed (Briggle and Curtis 1987).

Growers in the southeastern United States typically grow wheat in a double-crop system and follow wheat harvest with the planting of soybeans, *Glycine max* L. Wheat is typically planted in the fall and then harvested in late May or June. Wheat yield is

determined by the number of fertile heads per square meter, the number of kernels per head, and individual kernel weight (Frederick et al. 2001). Wheat planted during the optimum window typically has higher yield potential by increasing tillers, heads, and kernel weight (Darwinkel et al. 1977, Thill et al. 1978). There are multiple insect pests that threaten wheat yield. These include aphids (Homoptera: Aphididae), armyworms (Lepidoptera: Noctuidae), cereal leaf beetle (Coleoptera: Chrysomelidae, *Oulema melanopus* (L.)), and Hessian fly (Diptera; Cecidomyiidae, *Mayetiola destructor* (Say)). Environmental factors and plant diseases may also play a major factor in the yield and quality of wheat.

Barley Yellow Dwarf Virus

Barley yellow dwarf (BYD) is a worldwide economic problem on cereal grains and can cause detrimental effects to yield and grain quality by stunting shoot and root growth (Plumb 1983). As the disease progresses, phloem tissues in the shoot and root are killed in the infected plants (Esau 1957a, b). The causal pathogens of yellow dwarf on species in Poaceae (grasses) are viruses from the family *Luteoviridae* and genus *Luteovirus*. Currently, there are five recognized species of BYD: Barley yellow dwarf virus PAV, Barley yellow dwarf virus MAV, Barley yellow dwarf virus PAS, Barley yellow dwarf kerII, and Barley yellow dwarf kerIII. Barley yellow dwarf PAV is the most prevalent in Tennessee due to the high transmission by the bird cherry-oat aphid, the most commonly found aphid. The BYD virus particles have an icosahedral shape and include a single-stranded, positive RNA genome. The BYD virus is phloem limited within the host plant and cannot be transmitted without the assistance of aphids (Gildow 1987,

Jensen and D'Arcy 1995). Only certain aphids can transmit particular species of BYD. As noted above, the bird cherry-oat aphid and English grain aphid can transmit BYD PAV, whereas the English grain aphid transmits BYD MAV (Gray et. al. 1991). BYD viruses are persistent, but they are also circulative as they do replicate within the aphid.

BYD epidemics in winter wheat in North America have normally been associated with fall transmission by the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Halbert and Pike 1985, Clement et. al. 1986, Araya et. al. 1987, Halbert et. al. 1992). The virus can also be spread in the spring, but this is significantly less important and causes less damage compared with fall transmission (Goulart et al. 1989). However, BYD management requires an understanding of its epidemiology and local vector (aphid) biology which varies geographically (Burnnett 1991). Bird cherry-oat aphid; greenbug, *Schizaphis graminum* (Rondani); English grain aphid, *Sitobion avenae* (F.); and corn leaf aphid, *Rhopalosiphum maidis* (Fitch) have been confirmed as vectors of BYD (McPherson and Brann 1983, Johnson and Hershman 1996). BYD is thought to overwinter in perennial or volunteer grasses, which serve as reservoir hosts of the virus (Jones et al. 1990, McKirdy and Jones 1993). The BYD virus is often spread in the fall when viruliferous aphids fly or get blown into winter wheat out of these host reservoirs (McKirdy and Jones 1996). However, observations in Tennessee suggests that overwintering aphids and their offspring likely transmit BYD during winter months (Stewart, personal communication).

The most typical symptom of BYD infection in wheat is the discoloration of leaves to a yellow color. Certain wheat genotypes can also exhibit orange, red or purple leaf

discoloration, especially at the leaf tips, and leaves may appear water-soaked and develop chlorotic stripes, blotches or mottle, which start at the leaf tip (McKirdy and Jones 1996). These symptoms are very common on the flag leaf. In Tennessee, a pink to reddish leaf discoloration, particularly of leaf tips during the spring, is a consistent indicator of BYD (Stewart, personal communication). Yellowing of leaves may be caused by other diseases or abiotic factors that can confuse the diagnosis of BYD. Patchy stunting of plants is usually associated with early infections of the plant in the fall (Goulart et al. 1989). After heading, tiller stunting and darkening of the wheat heads may be associated with infection of BYD. In addition, the use of enzyme-linked immunosorbent assays (ELISA) can be used to diagnose BYD infection by testing plant tissue. ELISA has become a less costly and time-consuming technique that is now routinely used to detect BYD in aphids (Lister and Rochow 1979). However, virus detection sensitivity corresponds to one million BYD particles per sample (Canning et al. 1996). Virus quantity in aphids depends on aphid species and aphid feeding history (Sadeghi et al. 1997a, Bencharki et al. 2000). Thus, using ELISA techniques on aphids cannot be solely used to confirm BYD infection.

Aphids can acquire a BYD virus within a few seconds to minutes of feeding on an infected plant. However, there is a latent period of a few hours to a few days before the aphid can transmit the virus to a plant (Waterhouse et al. 1988). Fall infestation of aphids in winter wheat may occur at the seedling stage immediately after planting and through the tiller stage before crop dormancy (Kieckhefer and Kantack 1988). Aphid feeding before wheat dormancy is known to cause substantial crop injury and yield loss

(Burton 1986, McPherson et al. 1986). The magnitude of crop damage by aphids depends on the extent of infestation (Kieckhefer et al. 1995), timing of infestation (Kieckhefer and Gellner 1988), growth stage at time of infestation (Pike and Schaffner 1985), and whether or not BYD is transmitted to the crop (McPherson et al. 1986). Stunting and decreased tillering associated with BYD often decreases yield. In addition, a decrease in kernel size and kernel number per head may decrease yield and grain quality (Herbert et al. 1999, Hoffman and Kolb 1998, Weisz et al. 2005). Several scientists have suggested that these environmental stresses, such as severe growing conditions and water stress, interact with aphid or BYD damaged crops to substantially reduce yield and grain quality (Endo and Brown 1962, Fitzgerald and Stoner 1967, Andrews and Paliwal 1983 and 1986, Kieckhefer and Gellner 1992).

Aphids

General Biology

Aphids are small, soft bodied insects and generally somewhat pear shaped. They have sucking mouthparts and feed by sucking sap (phloem) from the plants. Phloem is rich in sugars and poor in proteins which means aphids need to feed on copious amounts of phloem to receive adequate proteins in their diet (Szczepaniec 2013), and thus, excess sugary liquids are excreted as honeydew. Adult aphids may be winged (alates) or wingless. Winged aphids are the likely life stage colonizing wheat. Winged aphids are often produced in response to stressful conditions such as poor weather, overcrowding, and poor food quality (Whitworth and Ahmad 2008). Aphids primarily reproduce asexually through parthenogenesis. Parthenogenesis is the asexual

form of reproduction where females give birth to live young (nymphs) without having to mate. Under optimal environmental conditions, the nymphs of many species in wheat become capable of reproducing within 5 – 10 days (Whitworth and Ahmad 2008). With this quick reproduction cycle, aphid populations can grow rapidly.

There are several aphid species that are found in winter wheat. Some of these species include the bird cherry-oat aphid, English grain aphid, greenbug, corn leaf aphid, and rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki). The first three species above are the most commonly observed in Tennessee (Stewart, personal communication) and have implicated in the transmission of barley yellow dwarf virus (McPherson and Brann 1983, Johnson and Hershman 1996). It is generally believed that direct feeding damage by aphids in wheat only occurs when infestation levels are unusually high. However, infestations of greenbug are potentially more damaging, particularly in seedling wheat (see below). When aphid populations are high, the flag leaf may curl up in a tight corkscrew fashion which might trap the awns and result in a fish hook appearance in the head (Godfrey et al. 2016). Populations of twenty or more per tiller at boot stage to heading stage can drastically decrease yields (Whitworth and Ahmad 2008). In addition, the honey dew that aphids excrete during feeding allows for the opportunity for sooty mold to take place which also can effect wheat and cause problems (Rabbinge et al. 1981).

Bird Cherry-Oat Aphid

Bird cherry-oat aphid is the most predominant species found in winter wheat. Bird cherry-oat aphids are very effective vectors of barley yellow dwarf virus and commonly

invade winter wheat (Yount 1985, Kieckhefer and Kantack 1988). Its color can range from orange green to olive green to dark green and then to greenish black as adults (Godfrey et al. 2016). Adults normally have a dark brown to reddish-orange spot across their abdomen near their cornicles which is a good identifying characteristic. Nymphs are usually a pale green to yellowish-green in color (Whitworth and Ahmad 2008). This species has long antennae and long tube-like cornicles arising from the side of the abdomen protruding toward the rear end. The tips of the antennae, cornicles and legs are black. Bird cherry-oat aphids compare to the greenbug in size at 1.6 mm in length (Whitworth and Ahmad 2008). Their antennae may be more than half the length of their body.

English Grain Aphid

English grain aphid is another common aphid found in wheat. The English grain aphid can be found throughout the United States and southern Canada. This species can feed on a wide host range of wild grasses and may colonize wheat at any stage in development, usually preferring the upper portion of the plant (Michaud 2008). This species is a yellow-green to reddish-brown in color as adults (Godfrey et al 2016). As nymphs, they are similar in shape and color as the adult, however, they are smaller. They have black antennae, cornicles, and leg joints. A wingless adult is about 2.5 mm in length. The majority of the population is anholocyclic and overwinters as nymphs on grasses or winter cereals, but a small proportion is holocyclic and can overwinter as eggs which hatch in March. English grain aphids normally appear later in the season compared to the bird cherry-oat.

Greenbug

Greenbug has been a recognized pest on small grains for over 150 years. These aphids are small (1.6 mm), pale green in color with a dark green stripe running down their abdomen (Szczepaniec 2013). Their cornicles have a black tip along with their legs. Greenbugs form in colonies on the underside of leaves. The greenbug has a host range that includes over seventy graminaceous species (Michels 1986). Although this species is not a common problem in Tennessee, its feeding is more damaging on a per aphid basis because it injects toxins into the host plant. This toxin present in the saliva produces a pathological plant response, resulting in the breakdown of tissues and severe chlorosis as it extracts the nitrogen required for its own growth and reproduction (Michaud 2014).

Management of Aphids and BYD

Aphids are managed most effectively with an integrated pest management (IPM) approach. This means employing a combination of different control tactics to reduce crop loss, rather than depending exclusively on one control tactic. Delaying planting to near or after the fly free date (October 15th in Tennessee) is an important control method that often reduces fall infestations of aphids (Whitworth and Ahmad 2008). Beneficial insects such as parasitic wasps and ladybeetles (Coleoptera: Coccinellidae) can become increasingly effective in controlling aphid populations as temperatures rise in the spring (Whitworth and Ahmad 2008). In addition, varieties with resistance or tolerance to aphids, especially greenbug, and BYD can be used. However, information

is not readily available from most seed companies, and no commercially available wheat varieties are totally resistant to aphids or BYD.

Controlling of weedy hosts such as grasses or volunteer wheat in ditch banks can help minimize the acquisition and transmission of BYD by aphids. However, insecticide use is the primary defense against aphid infestations in small grain crops (Hays et al. 1999). Neonicotinoid seed treatments (ISTs) such as Gaucho® (imidacloprid, Bayer CropScience, Raleigh, NC), Cruiser® (thiamethoxam, Syngenta Crop Protection, Greensboro, NC), and NipsIt Inside® (clothianidin, Valent, Walnut Creek, CA) are recommended control methods for the management of aphids and BYD (Stewart and McClure 2017). Mullins (1993) found that the use of imidacloprid, which exhibits both systemic and contact insecticidal functions, is an excellent aphid management strategy, and foliar insecticide applications may be advisable if populations are above the threshold before the beneficial insects become active. If an IST is not used, a foliar chemical application thirty days after planting and/or late winter may reduce aphid populations and the incidence of BYD (Stewart and McClure 2017, Buntin 2007).

Economic thresholds for aphids in wheat are not well established and vary considerably among states. Suggested treatment thresholds for Tennessee are below (Stewart and McClure 2017).

- *Bird cherry-oat aphid and English grain aphid*: Insecticide seed treatments or a foliar insecticide application for aphid control during the fall (e.g., approximately 30 days after planting) and/or late winter (prior to March) may reduce BYD.

Insecticide applications should be made before aphid populations reach 6 – 8 per linear foot (0.3 m) of row. However, infection with BYD becomes less damaging as plants grow, and applications after jointing are less likely to increase yield.

- *Greenbug*: This aphid species injects a toxin while feeding. Treatment should be made when aphid populations are killing three or more leaves per plant. In addition, if wheat is less than 6 inches (15 cm) tall then treatment should be considered if aphid populations reach 50 or more per linear foot (0.3 m) of row. In wheat 6 – 10 inches (15 – 25 cm) tall, treatment should be made if 200 or more aphids are present per linear foot of row.

Objectives

Recent data collected in Tennessee suggests that the management of aphids and BYD in wheat consistently increases yields, but additional data is needed to better define best management practices. The objectives of my research are to:

- 1) Perform a meta-analysis of previous data from aphid insecticide efficacy trials in Tennessee.
- 2) Evaluate how variety susceptibility, insecticide seed treatments and/or foliar insecticide applications affects aphids, barley yellow dwarf, and wheat yield.
- 3) Test the relative efficacy of existing and new insecticide seed treatments as compared with foliar insecticide applications.

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CHAPTER I
IMPACT OF INSECTICIDE SEED TREATMENTS AND FOLIAR
INSECTICIDES ON APHID INFESTATIONS IN WHEAT, INCIDENCE OF
BARLEY YELLOW DWARF, AND YIELD IN WEST TENNESSEE

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Abstract

Several species of aphids (Hemiptera: Aphididae) infesting wheat may reduce yield by the transmission of barley yellow dwarf (BYD). Neonicotinoid seed treatments and foliar application of insecticides are two common methods to control aphid infestations and reduce BYD. An analysis was done across 33 insecticide efficacy tests performed in west Tennessee during the last 11 years to determine how insecticide seed treatments and/or a late-winter foliar insecticide application affected aphid populations, incidence of BYD, and yield. A significant decrease in springtime aphid populations and incidence of BYD was observed when using a seed treatment, a foliar insecticide application, or both. Average wheat yields were increased by 280 - 381 kg/ha (5.3 - 7.2%) if an insecticide seed treatment was used or when a foliar insecticide application was made. Compared to insecticide seed treatments, average springtime aphid populations and the incidence of BYD were lower where a foliar insecticide was applied. A foliar insecticide application made in addition to insecticide seed treatments increased yield by an average of 196 kg/ha (3.4%). The yield increases over the non-treated control suggest that wheat growers in west Tennessee can use insecticides to manage aphids and prevent transmission of BYD. Consideration of environmental

conditions, whether or not insecticide seed treatments were used, and scouting can be used to help make decisions on when or if to apply foliar insecticides.

Introduction

World wheat (*Triticum* spp.) production is estimated at over 221 million hectares which, makes it the most widely cultivated crop (USDA 2016). In Tennessee, wheat production varies considerably, but typically ranges from 100,000 to 200,000 hectares of soft red winter wheat (*T. aestivum* L.) which is typically planted in the fall (September to November) and harvested in June. The majority of Tennessee's wheat is grown in west and middle Tennessee. Producers in the midsouthern and southeastern United States typically grow wheat in a double-crop system where wheat harvest is followed with planting of soybeans (*Glycine max* L.) or another summer crop. In 2017, the average wheat yield in Tennessee was 4,764 kg/ha (70 bu/acre) (USDA-NASS), and over the last ten years, average yield has ranged from 4,967 kg/ha to 3,403 kg/ha (50 – 73 bu/acre).

Aphids (Hemiptera: Aphididae) are a threat to wheat production not only by feeding on the plant and causing yield loss, but also by transmitting barley yellow dwarf virus (BYD, Family Luteoviridae). BYD virus within the host plant is phloem limited and cannot be transmitted without the assistance of aphids (Gildow 1987, Jensen and D'Arcy 1995). Several aphid species may infest wheat and have been shown to transmit BYD, including the bird cherry-oat aphid, *Rhopalosiphum padi* (L.); greenbug, *Schizaphis graminum* (Rondani); English grain aphid, *Sitobion avenae* (F.); and corn

leaf aphid, *Rhopalosiphum maidis* (Fitch) (McPherson and Brann 1983, Johnson and Hershman 1996).

BYD is the most common and widespread disease of wheat and cereal crops worldwide (Edwards et al. 2000). BYD is a worldwide economic problem not only for wheat but also for other cereal grains, causing loss in yield and grain quality by stunting shoot and root growth (Plumb 1983). The magnitude of crop damage by aphids depends on the extent of infestation (Kieckhefer et al. 1995), timing of infestation (Kieckhefer and Gellner 1988), plant growth stage at time of infestation (Pike and Schaffner 1985), and whether or not BYD is successfully transmitted to the host (McPherson et al. 1986). In North America, the most serious BYD outbreaks have primarily been associated with fall transmission by the bird cherry-oat aphid (Halbert and Pike 1985, Clement et. al. 1986, Araya et. al. 1987, Halbert et. al. 1992). Observations in Tennessee suggest that overwintering aphids and their offspring also transmit BYD during the late winter months which, also results in yield loss (Stewart, pers. comm.).

Symptomology of BYD infection include orange, red or purple leaf discoloration, especially at the leaf tips (McKirdy and Jones 1996). A consistent indicator of BYD in Tennessee is an observable pink to reddish discoloration of leaf tips, especially during the spring. Reported yield loss associated with BYD in wheat has been variable. Kieckhefer and Kantack (1988) reported that high aphid populations can directly reduce yield up to 50%. Patterson et al. (1990) reported typical yield losses from BYD range from 2 - 10% in the United States. However, another study demonstrated that BYD

could reduce wheat yield by 46%, and yield loss was 58% when infested with bird cherry-oat aphids (Riedell et al. 1961). A different study demonstrated that wheat yield could be decreased by 34% when infected with BYD virus (Herbert et al. 1999).

Aphids and BYD are managed most effectively by implementing an integrated pest management (IPM) approach including region appropriate planting dates and use of tolerant varieties to BYD. Insecticides are commonly used to control aphid populations in wheat (Hays et al. 1999). Neonicotinoid seed treatments such as Gaucho® (imidacloprid, Bayer CropScience, Raleigh, NC), Cruiser® (thiamethoxam, Syngenta Crop Protection, Greensboro, NC), Poncho (clothianidin, Bayer CropScience), and NipsIt Inside® (clothianidin, Valent, Walnut Creek, CA) are a recommended control method for the management of aphids and BYD (Stewart and McClure 2017). If insecticide seed treatments are not used, a foliar chemical application thirty days after planting and/or in late winter may reduce aphid populations and the incidence of BYD (Buntin 2007).

Materials and Methods

Design

An analysis was performed across 33 experiments in west Tennessee from 2006 – 2017 to evaluate the impact of insecticide seed treatments and foliar insecticide applications on aphid infestations, incidence of BYD, and yield (Table 1). Not all experiments contained both treatment factors, and neither did they all have complete, balanced data of treatment effects on aphid populations, incidence of BYD, or yield. All experiments except one were performed at the West Tennessee Research and

Education Center in Jackson. Tests were both small-plot and large-plot and all were arranged in a randomized complete block design. Individual plots in small-plot tests were 1.5 m wide by 9 m long. Plots in large-plot tests were 7.7 m wide and ranged between 30.5 and 300 m long. Planting dates ranged from late September until late November, with the median planting date of approximately 17 October. All tests were planted at a target seeding rate of 2.5 - 3.0 million seeds per hectare and a row spacing of 19 cm. Average planting depth was 2.5 - 3.8 cm. Although several varieties were used during the 11-year period, P26R10 or P26R22 (Pioneer Hi-Bred International, Inc., Johnston, IA) were used in the majority of these experiments. These varieties are commonly planted in the region and are mid-range in their sensitivity to BYD based on evaluations made in Tennessee (Allen et al. 2012, Allen et al. 2013). Wheat was fertilized and managed for weeds based on standard recommendations made by the University of Tennessee (Main et al. 2008).

Treatment Factors

Among these experiments, treatments included 1) plots not treated with insecticide, 2) one to four neonicotinoid seed treatment entries, 3) a foliar-applied insecticide treatment, and 4) a foliar insecticide application made on wheat having a base insecticide seed treatment. In each test, a single variety was planted (Table 1). Only seed treatments tested at rates labeled for aphid control were included in the analyses. If an experiment contained a foliar insecticide application, a single treatment was applied between 31 January and 25 February. Karate Z at a rate of 27.3 g ai/ha

(lambda-cyhalothrin, Syngenta Crop Protection) was normally used, but Baythroid XL at a rate of 13.2 g ai/ha (beta-cyfluthrin, Bayer CropScience) was used in some tests.

Data Collection

Aphid counts were taken as numbers per 0.91 - 1.52 m of row (3 - 5 row ft). Counts were often taken at various times throughout the fall and spring, but assessments were consistently made in March approximately thirty days after any springtime foliar insecticide application and these data were used in these analyses. Ratings on the incidence of BYD were subsequently made between Feekes stage 8 (just before boot) to 10.5 (flowering) by counting the number of flag leaves showing distinct symptoms of BYD, primarily characterized by reddish or pink coloration on the leaf tips, in the entire plot (small plot) or in an area ranging from 9.3 - 13.9 m² (large plots) (Wise et. al. 2011). Timing of this rating was based upon the optimum occurrence of symptomology as judged by the researcher. For small plot experiments, whole-plot yield data was collected using a research-grade plot combine. A yield monitor was used in large plot tests, but only the center 4.6 m of each plot was harvested.

Data Analysis

Matching aphid counts, BYD ratings, and yield were not collected on all tests (Table 1). Thus, we only made comparisons among tests that were balanced in respect to data on aphid numbers, incidence of BYD, or yield when evaluating treatment effects. Response variables reported include spring aphid densities (numbers/row m), frequency of leaves showing BYD symptomology (numbers/10 m²), and yield (kg/ha). We did not attempt to compare the efficacy of different insecticide seed treatments, in part because

rates varied both within and between tests. Data were analyzed using Proc GLIMMIX (SAS Institute, Cary, NC) using a protected LSMEANS for detection of main effects ($\alpha = 0.05$). Similar to the approach of North et al. (2016), treatment factors were fixed effects within the model with test, replicate, and replicates nested with test considered random effects, thus helping to make inferences across a wide range of environments (Carmer et al. 1989, Blouin et al. 2011). Log transformations were performed on aphid and BYD numbers prior to analyses to satisfy the assumptions for analysis of variance. We also present data on the frequency that significant ($\alpha = 0.05$) treatment responses were observed in individual tests based on standard analysis of variance methods (Proc GLIMMIX, LSMEANS). The cost of neonicotinoid seed treatments varies between \$24.70 and \$37.10 per hectare assuming a seedling rate of 134 kg/ha (2 bu/acre); whereas, the costs of a foliar insecticide application varies from \$11.10 to \$18.60 per hectare with the range depending mostly on application costs (Smith et al. 2017 and pers. comm. with local retailers). These values were used in calculating the approximate economic benefits of treatment.

Results

Effects of insecticides on number of aphids

Aphid densities during the fall were not routinely collected and not consistently collected at a specific time point after planting. However, when analyzed across eight trials, fall aphid density was 78% lower when an insecticide seed treatment was used ($F = 37.21$; $df = 1, 75$; $P < 0.001$). Spring aphid densities were significantly reduced by the

use of insecticide seed treatments, a foliar insecticide application, or a combination of both. Although we did not consistently distinguish between the type of aphids present when making ratings, bird cherry-oat aphids and English grain aphids were the predominate aphids observed, with bird-cherry oat aphid being most common in the fall and a comparable mix of these species in the spring. Compared with plots not treated with insecticide, insecticide seed treatments reduced aphid populations by an average of 86% (Table 2). A late-winter foliar insecticide application caused a similar reduction (90%) in aphid populations. For springtime counts, insecticide seed treatments significantly reduced aphid numbers in 10 of 12 individual tests (Table 2). A foliar insecticide application significantly reduced aphid numbers in 15 of 22 tests. Finally, a foliar insecticide application significantly reduced aphid numbers in 4 of 6 tests where a base insecticide seed treatment was also used.

Effects of insecticides on the incidence of barley yellow dwarf virus

Similar to aphid populations, symptomology of BYD was reduced by the use of insecticides. When used alone, insecticide seed treatments reduced the visual incidence of BYD by an average of 60% (Table 2) compared with seed not treated with insecticide; whereas, a foliar insecticide reduced the incidence of BYD by over 84%. Very low incidence of BYD was observed when a foliar insecticide application was made in addition to an insecticide seed treatment. The incidence of BYD was significantly reduced in individual tests by insecticide seed treatments (6 of 10) or a foliar insecticide application (13 of 17). In 4 of 7 tests, applying a foliar insecticide on top of a base insecticide seed treatments also significantly reduced BYD.

Yield protection provided by insecticides

Relative to a non-treated wheat, insecticide seed treatments alone resulted in a yield increase of 280 kg/ha (5.3%, 4.2 bu/acre), but a statistically significant yield increase was not observed in any of 11 individual tests (Table 2, Figure 1). Similarly, yield was increased by an average of 381 kg/ha (7.2%, 5.7 bu/acre) when a foliar insecticide application was used instead of insecticide seed treatments, with a significant increase observed in only 2 of 18 individual tests. A negative yield response (not significant) of a foliar insecticide application was only observed in one test (Fig. 1), and that was when an insecticide seed treatment was also used. When a foliar insecticide application was made to wheat that had a base insecticide seed treatment, yield was increased by an average of 196 kg/ha (3.4%, 2.9 bu/acre). This was significant across seven tests ($P = 0.015$, Table 2), and a significant increase was observed in three individual tests (Figure 1).

Comparison of insecticide seed treatments versus foliar insecticide usage

For tests where there was a direct comparison of insecticide seed treatments versus a late-winter foliar insecticide application, average springtime aphid numbers were 75% lower where a foliar insecticide was applied (Table 3). Similarly, visual symptomology of BYD was 39% lower when a foliar insecticide was applied compared with an insecticide seed treatment. This difference was not significant in any individual tests ($n=17$). Wheat that was treated with a foliar insecticide application yielded 170 kg/ha (2.5 bu/acre) more than wheat treated with insecticide seed treatments, as this difference was significant ($P = 0.030$) when analyzed across tests, however significant differences were not observed in any individual test.

Discussion

Collectively, these data indicated that insecticide seed treatments or a late-winter foliar insecticide application (or both) increased yield and reduced aphid density and the transmission of BYD virus relative to non-treated wheat. Because aphid counts were taken in the spring, relatively soon after the foliar insecticide application, it is not surprising that aphid counts were relatively low compared with insecticide seed treatments. However, there was opportunity for aphid transmission of BYD before the foliar insecticide application. Thus, aphid densities may not fully reflect the benefits of insecticide seed treatments in protecting wheat yields. Counts of aphids were only occasionally made during the fall because infestations are generally low, averaging about 4.2 aphids/m in plots not treated with insecticides across tests, thus making it difficult to separate treatment differences. However, the use of an insecticide seed treatment reduced aphid densities in the spring by an average of 86%, similar to the 78% reduction of aphid numbers observed in the more limited number of fall samples. This suggests that colonization of aphids during the fall and/or early winter months is parental source of springtime aphid infestations in Tennessee, as it is unlikely that insecticide seed treatments provide residual control past the winter months.

This summary of experiments in Tennessee demonstrates that the management of aphid infestations in wheat increased yields by about 5 – 10% depending upon the insecticide treatment regime. On average, where direct comparisons were made, a late-winter foliar application reduced aphid numbers and the incidence of BYD more than insecticide seed treatments alone. The average yields of wheat treated with a foliar

insecticide were 170 kg/ha higher than where only a seed treatment was used. Applying a late-winter foliar insecticide application in addition to insecticide seed treatments provided a similar yield increase (averaging 196 kg/ha) compared with only using a foliar application alone, and there were several individual tests where this increase was statistically significant.

Presumably yields were protected because insecticides reduced the incidence of BYD rather than the direct benefits of aphid control or other pests that may have been present. Aphid populations generally did not reach economically damaging levels apart from BYD infection in these tests. It is possible that seed treatments and foliar insecticides were controlling other pests and therefore impacting yield results. Neonicotinoid seed treatments are labeled for control of Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), although at rates higher than those typically used in our tests, and foliar pyrethroid insecticide may also impact this pest (Flanders et al. 2013). The most common variety used in our tests, P26R10, has shown good resistance to Hessian fly. However, another variety we commonly used, P26R22, has low resistance to Hessian flies (Noland et al. 2017). Application of insecticides could also potentially affect other pests such as cereal leaf beetles, *Oulema melanopus* (Coleoptera: Chrysomelidae), or armyworm, *Mythimna unipuncta* (Lepidoptera: Noctuidae). Cereal leaf beetles and armyworms are rarely observed in Tennessee wheat fields until March or later, but the impact of our treatments on these pests was not assessed. However, economically damaging infestations of these other pests were not apparent.

Climate ultimately influences the occurrence of aphids, and thus BYD, in wheat. Perry et al. (2000) reported that environmental factors and the strain of BYD influence yield reductions of small grains. Bockus et al. (2015) found the greatest economic losses occurred when plants are infested with viruliferous cereal aphids at early leaf development stages. Our data indicates that some aphids survive the winter in Tennessee, serving as the parents of springtime aphid populations. Unlike more northern geographies, aphid survival during the winter likely increases the risk of BYD transmission during warm winter days when wheat is essentially dormant. Thus, results in Tennessee are almost certainly not applicable to all geographies and are only directly relevant to those growing winter wheat

The range of planting dates in our experiments reflect local production practices. If growers plant before the recommended planting window (October 15, Stewart and McClure 2017), they would likely increase the risk of infection with BYD, and early planted wheat may benefit more from the use of an insecticide seed treatment. The University of Tennessee's current recommendations for the management of aphids and BYD state that if a seed treatment is not used, a foliar insecticide application during the fall (approximately 30 days after planting) and/or late winter (prior to March) may also reduce BYD (Stewart and McClure 2017).. These recommendation also suggest that insecticide applications should be made before aphid populations exceed 6-8 per row foot; otherwise, any BYD transmission may have already occurred.

Our data would generally support these recommendations. In practice, pest managers are coached to scout for aphid infestations in the fall and treat if aphids are

found in sufficient numbers (> 3 per row ft., Stewart, personal comm.). If aphid infestation remains low, as often occurs during years with cool falls or when wheat is late planted, growers are encouraged to delay any applications until late winter, typically January or February. Other areas in the South also promote fall and/or late-winter applications of insecticide to control aphids and BYD. For example, The University of Kentucky recommends a foliar insecticide application 30 days post-emergence when numbers exceed 9.8 aphids per row meter (3/row foot) and from 30 to 60 days post-emergence when counts exceed 19.7 aphid per row meter (6/row foot) (Johnson and Townsend 1999). Similarly, the University of Georgia's recommendations discuss the potential benefits of both seed treatments and foliar insecticide application to prevent BYD, and they suggest different timings of a foliar application based on geographic location within the state (Noland et al. 2017). Further, these recommendations suggest managing BYD may be more valuable in the northern parts of Georgia. Implicit in these recommendations is that managing aphid infestations and BYD may be less beneficial in warmer climates where aphid colonization may continue over a longer period of time.

Assuming the most expensive treatment option of insecticide seed treatments (\$37/ha) and an approximate yield response to this treatment of 300 kg/ha, expected economic returns would be \$6.50/ha at a relatively modest commodity price of \$147 per metric ton (\$4/bu). The economic returns for a foliar application would be nearly three times higher considering insecticide and application costs (\$11.10 - 18.60/ha) are approximately one-half that of insecticide seed treatments. Based on our average results, a positive return of investment would be made by making a foliar insecticide

application even when insecticide seed treatments were used because yields were increased by an average of 196 kg/ha when this application was made. It should be considered that a foliar application in the fall, or a fall and late-winter application, may offer similar or better economic returns than insecticide seed treatments. This is particularly true if these foliar applications can be co-applied with herbicides or foliar fertilizer applications, thus reducing application costs. Resistance or tolerance to aphids and BYD varies among wheat varieties (Irwin and Thresh 1990, De Wolf et al. 2017), and the economic benefits of managing aphids with insecticides may vary depending on the variety grown. The two varieties most commonly used in our tests were moderately susceptible to BYD based on ratings of symptomology in variety trials performed in Tennessee (Allen et al., 2012 and Allen et al., 2013). Thus, our results are probably representative of a 'typical' variety, but more research is needed to fully define how variety selection affects the value of aphid and BYD management in wheat.

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Appendix

Table 1. Summary of 33 tests analyzed over an eleven year period in west Tennessee. Treatment factors included insecticide seed treatments (IST), foliar insecticide applications, insecticide seed treatment plus a foliar insecticide application. Plot size (small or large), data collected, planting date, and wheat variety are also shown.

Test ID	Treatment Factors			Data Collected ^d	Plot Size	Planting Date	Variety
	IST ^a	Foliar ^b	IST + Foliar ^c				
1	I, T	LC	T + LC	A	S	Sep. 28, 2005	Coker 9663
2	I, T	LC		A	S	Oct. 2, 2006	Pioneer
3	T	LC		A, B, Y	S	Oct. 15, 2008	Pioneer
4	T	LC		A, B, Y	L	Oct. 22, 2008	Pioneer
5	T	LC		A, B, Y	S	Oct. 15, 2008	Pioneer
6	T	LC		Y	S	Oct. 15, 2008	Pioneer
7	T	LC		Y	S	Oct. 15, 2008	Pioneer
8	I, I+C			A, Y	S	Nov. 6, 2009	Pioneer
9		LC		A, Y	L	Oct. 2007	Unknown
10	I, T	LC		B, Y	L	Oct. 2007	Unknown
11		LC		A, Y	L	Oct. 22, 2010	Progeny
12		LC		A, Y	L	Oct. 15, 2010	Pioneer
13	I	LC	I + LC	A, Y	L	Oct. 22, 2010	Progeny
14	I, C,			A, Y	S	Oct. 28, 2010	Dixie
15	I, T			A, B, Y	S	Oct. 10, 2010	Oaks
16		LC		A, B	L	Oct. 17, 2011	Pioneer
17		LC		A, B, Y	L	Oct. 24, 2012	Unknown
18		LC		A, B, Y	L	Oct. 24, 2012	Pioneer
19	I	BC	I + BC	A, B, Y	S	Oct. 21, 2013	Unknown
20	C	LC	C + LC	A, B, Y	L	Oct. 21, 2013	Pioneer
21		LC		A, B, Y	L	Oct. 21, 2013	Pioneer
22		LC		A	S	Oct. 21, 2014	Pioneer
23		LC		A, B, Y	L	Oct. 21, 2014	Pioneer
24	I			A, B, Y	S	Oct. 22, 2014	Pioneer
25	T	LC	T + LC	A, B, Y	L	Oct. 22, 2014	Pioneer
26		LC		A, Y	L	Oct. 15, 2010	Pioneer
27	I	LC	I + LC	A	S	Oct. 16, 2015	Pioneer
28	I	LC	I + LC	A, B, Y	L	Oct. 16, 2015	Pioneer
29		LC		A, B, Y	L	Oct. 16, 2015	Pioneer
30	I, T	BC	I + BC	A, B, Y	S	Oct. 16, 2015	Pioneer
31	T	BC	T + BC	B, Y	L	Oct. 12, 2011	USG 3251
32	I	LC	I + LC	A, B, Y	L	Oct. 18, 2016	Pioneer
33		LC		A, B, Y	L	Oct. 18, 2016	Pioneer

^aInsecticide seed treatments included in tests with I = imidacloprid, T = thiamethoxam, and C = clothianidin.

^bFoliar insecticide applications where LC = lambda-cyhalothrin and BC = beta-cyfluthrin.

^cIndicates base insecticide seed treatment (I, T, or C) used for test of insecticide seed treatments in combination of foliar insecticide applications (LC or BC).

^dA = aphid density (spring rating), B = barley yellow dwarf (spring rating), and Y = yield.

Table 2. Average number of aphids during the spring, incidence of barley yellow dwarf, and yield for wheat treated or not treated with insecticide seed treatments (IST), treated or not treated with a foliar insecticide application, or treated with insecticide seed treatments but with or without a foliar insecticide application. Data were collected across 33 tests over an eleven year period in west Tennessee.

Aphids (no./meter row)						
Insecticide	Treated	Not Treated	F-value	df	P-value	Frequency of Response ^a
IST	10.1	74.6	214.1	1, 121	< 0.0001	10 / 12
Foliar	7.5	71.7	175.9	1, 77	< 0.0001	15 / 22
IST ± Foliar	4.0	20.0	48.69	1, 23	< 0.0001	4 / 6
Barley Yellow Dwarf (no. flag leaves /10 m ²) ^b						
IST	14.2	35.3	76.89	1, 107	< 0.0001	6 / 10
Foliar	6.1	39.0	63.30	1, 60	< 0.0001	13 / 17
IST ± Foliar	1.6	8.4	29.02	1, 60	< 0.0001	4 / 7
Yield (kg/ha)						
IST	5262	4982	21.40	1, 164	< 0.0001	0 / 11
Foliar	5262	4881	83.38	1, 83	< 0.0001	2 / 18
IST ± Foliar	5807	5611	6.20	1, 62	0.0154	3 / 7

^aNumber of individual tests with a significant insecticide response where aphid numbers or BYD was reduced by treatment or yield was increased (P < 0.05).

^bSymptomatic flag leaves.

Table 3. Direct comparison between wheat treated with an insecticide seed treatments (IST) and a late-winter applied foliar insecticide application on numbers of aphids, incidence of barley yellow dwarf, and yield Data were collected across 33 tests over an eleven year period in west Tennessee.

	IST	Foliar	F-value	df	P-value
Aphids (no./meter row)	25.1	6.3	35.59	1, 92	< 0.0001
Barley Yellow Dwarf ^a	8.9	5.4	4.88	1, 95	0.0296
Yield (kg/ha)	5217	5387	4.83	1, 133	0.0297

^aSymptomatic flag leaves per 10 m².

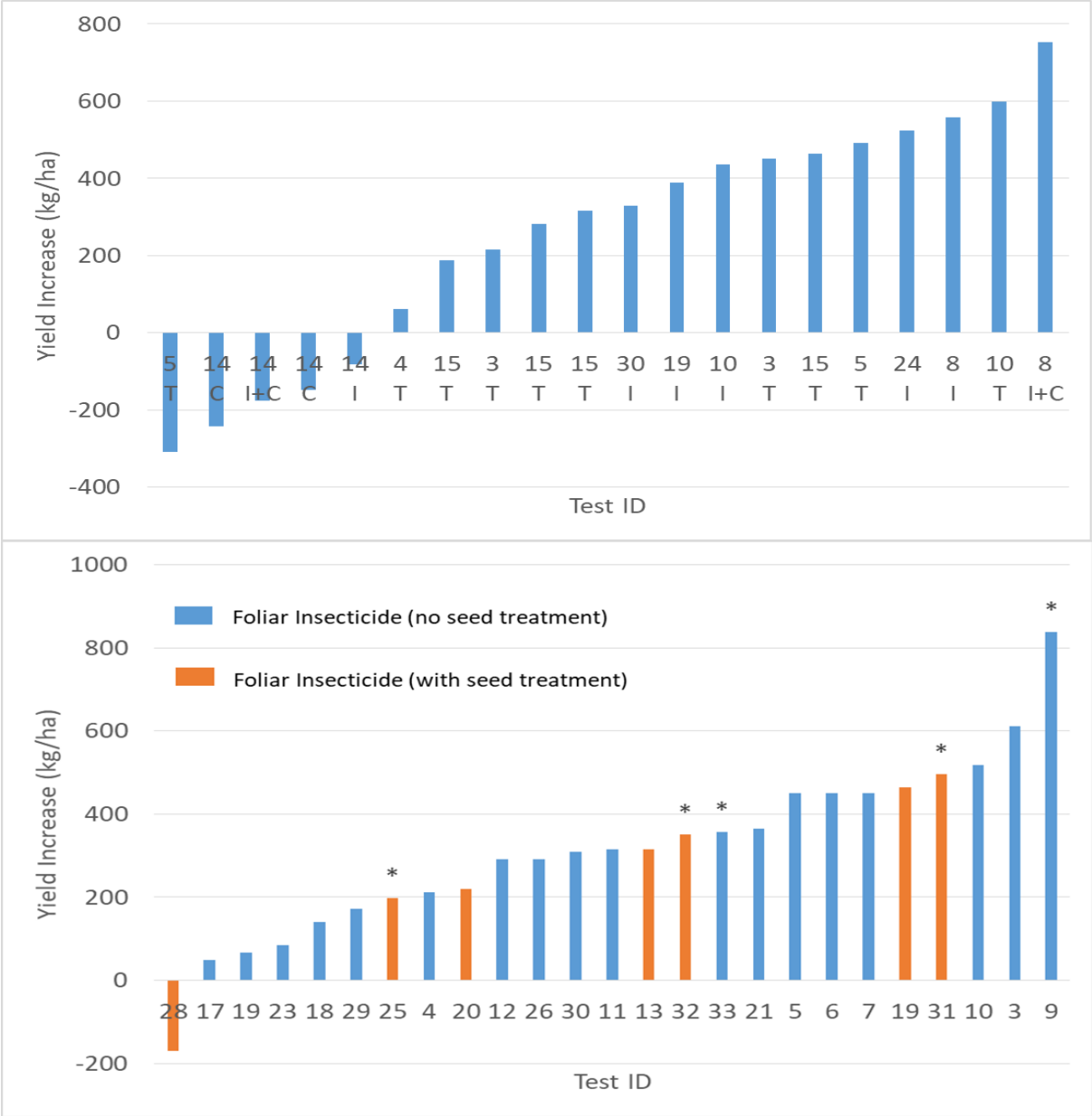


Figure 1. Mean yield response (kg/ha), by test ID as shown in Table 1.1, for wheat treated with an insecticide seed treatment (top) or when a foliar insecticide application was made during late winter (bottom). C, I and T on the x-axis indicates clothianidin, imidacloprid, and thiamethoxam seed treatment. Asterisks indicate significant increases within individual tests ($P < 0.05$).

CHAPTER II
MANAGEMENT OF APHIDS (HEMIPTERA: APHIDIDAE) AND THE
INCIDENCE OF BARLEY YELLOW DWARF IN SOFT RED WINTER
WHEAT (*TRITICUM AESTIVUM* L.)

Abstract

Aphids (Hemiptera: Aphididae) transmit barley yellow dwarf (BYD) in wheat, *Triticum aestivum* L., and thus may cause a substantial loss of yield and grain quality. Foliar applied insecticides and neonicotinoid seed treatments are the two most common forms of control of aphid infestations and BYD incidence. A factorial experiment was repeated at four locations to examine how variety tolerance to BYD, a neonicotinoid seed treatment, and a foliar insecticide application affected populations of aphids, the occurrence of BYD, and yield. Similarly, experiments with various neonicotinoid seed treatments and foliar insecticide spray regimens to evaluate treatment effects on aphids, BYD, and yield. Treatment responses varied considerably across locations. However, aphid populations and symptoms of BYD were consistently reduced by the use of insecticides, particularly a foliar-applied application. Quantitative ELISA assays confirmed the presence of BYD in leaf tissue and was also able to detect some treatment effects on the concentration of BYD in leaf tissue. The use of resistant varieties that were considered tolerant to BYD reduced the visual symptoms of disease, but this difference was not reflected in yield responses to insecticide treatment or quantitative ELISA assays of BYD concentration in leaf tissue. Yield response to insecticide seed treatment were variable, but generally, a foliar-applied application provided more benefit than an insecticide seed treatment and this would provide flexibility in timing any insecticide applications based on need.

Introduction

Soft red winter wheat, *Triticum aestivum* L., is grown on 100,000 to 200,000 hectares in Tennessee, with approximately 68% of wheat being grown in the western part of the state (USDA, 2017). The previous five year average wheat yield in Tennessee is 4,681 kg/ha (USDA-NASS). Winter wheat in Tennessee is typically planted in the fall (late September to November) and harvested in June. The majority of wheat producers in the midsouthern and southeastern United States grow wheat in a double crop system where soybean, *Glycine max* L., is planted following the harvesting of wheat.

Aphids (Hemiptera: Aphididae) feed on the phloem of wheat, but more significantly, they may also transmit BYD, a common and widespread disease (Edwards et al. 2000). In Tennessee, the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) and English grain aphid, *Sitobion avenae* (F.) are the most common observed infesting wheat (Perkins et al. 2018), but the greenbug, *Schizaphis graminum* (Rondani), and corn leaf aphid, *Rhopalosiphum maidis* (Fitch), may also be found in wheat (McPherson and Brann 1983, Johnson and Hershman 1996). In North America, the most serious outbreaks of BYD have been primarily reported from fall transmission by the bird cherry-oat aphid (Halbert and Pike 1985, Clement et. al. 1986, Araya et. al. 1987, Halbert et. al. 1992). It is generally agreed that the most serious impacts of BYD on yield occur when infection occurs during the seedling stage, prior to jointing (Pike and Schaffner 1985, Kieckhefer and Gellner 1988, Halbert et. al. 1992). In Tennessee, research from Perkins et al. (2018) indicated that overwintering aphids and their offspring may transmit

BYD during the late winter or early spring, also resulting in yield loss (Perkins et al. 2018).

BYD infection in wheat is characterized by stunted root and shoot growth, leading to poor grain quality and yield loss (Plumb 1983). Besides patchy stunting of wheat (Goulart et. al. 1989), orange, red, or purple leaf discoloration are common symptoms of infection (McKirdy and Jones 1996). The use of enzyme-linked immunosorbent assays (ELISA) can be used to confirm BYD infection in plant tissue. These immunoassays are used extensively in plant virus detection (Cooper and Edwards 1986). ELISA tests can also be used as a relatively inexpensive and rapid way to detect the presence of BYD within aphids (Lister and Rochow 1979).

Aphids and BYD are managed most effectively by implementing an integrated pest management (IPM) approach which includes region appropriate planting dates and utilizing tolerant varieties to BYD. Delaying planting to near or after the “fly free” date (October 15th in Tennessee) is an important control method because it can reduce fall aphid infestations (Whitworth and Ahmad 2008). In addition, controlling of weedy hosts such as grasses or volunteer wheat in ditch banks can help minimize the acquisition and transmission of BYD by aphids. However, insecticides are the most common control tactic used for aphid management in wheat (Hays et al. 1999). Neonicotinoid seed treatments such as imidacloprid (Gaucho 600®, Bayer CropScience, Raleigh, NC), thiamethoxam (Cruiser 5F®, Syngenta Crop Protection, Greensboro, NC), clothianidin (Poncho®, Bayer CropScience and NipsIt Inside®, Valent, Walnut Creek, CA) are a recommended control methods for the management of aphids and BYD

(Stewart and McClure 2017). Perkins et al. (2018) reported that aphid populations were reduced by an average of 86% with the use of an insecticide seed treatment and found a corresponding reduction in BYD symptoms. Others have reported that foliar-applied insecticides can reduce populations of aphids and the incidence of BYD (e.g., Buntin 2007), and Perkins et al. (2018) found that aphid populations were reduced by on average of 90% when a foliar insecticide application was made in late winter, and this reduced symptoms of BYD by an average of 84%.

Perkins et al. (2018) reported that BYD symptoms can be reduced by 60% with the use of neonicotinoid seed treatment and up to 84% when a foliar insecticide was applied in late winter. McKirdy and Jones (1996) also reported that the spread of BYD was reduced by 75% by a foliar insecticide application, and when an insecticide seed treatment was used with a follow up foliar application, BYD incidence was reduced by 88%. Typical yield losses caused by BYD range from 2-10% (Patterson et al. 1990), but yield losses over 30% and as high as 50% have been associated with BYD (Riedell et al. 1961, Kieckhefer and Kantack 1988, Herbert et al. 1999). In Tennessee, Perkins et al. (2018) reported that managing aphid infestation with insecticides increased wheat yields by an average of 280 - 577 kg/ha depending on whether an insecticide seed treatment, a late-winter foliar insecticide application, or both were applied. The objective of this research was to further evaluate how varieties, insecticide seed treatments, and foliar insecticide applications might influence infestations of aphids, the incidence and concentration of BYD, and yield.

Materials and Methods

Experiment 1 – Factorial of Varieties, Insecticide Seed Treatment, and Foliar Insecticide

Design. Experiments were performed during 2016 and 2017 growing seasons to evaluate how variety susceptibility, an insecticide seed treatment (IST), and/or foliar insecticide applications affected aphid populations, the incidence and concentration of BYD, and wheat yield. During the 2016-2017 growing season, identical tests were conducted at the East Tennessee Plant Sciences Unit (Knoxville), the Research and Education Center at Milan, TN (Milan), and at the R.R. Foil Plant Science Research Center at Mississippi State University (Starkville). Plots at Knoxville were planted on 17 October, at Milan on 19 October, and at Starkville on 6 November. In the 2017-2018 growing season, these tests were repeated at the West Tennessee Research and Education Center in Jackson, TN (Jackson), Milan, and Starkville with planting dates of 18 October, 14 November, and 26 October respectively. Due to poor and uneven stand establishment, data from the Starkville location planted in 2016 and the Milan location planted in 2017 were eliminated from the data set.

Wheat at all locations was planted at a targeted seeding rate of three million seeds per hectare at a depth of 1.9 cm below the soil surface. Plots were 9.1 m long and 1.5 m wide at the Knoxville, Jackson, and Starkville locations. In Milan, plots were 9.1 m long and 2.4 m wide. The test in Knoxville was irrigated with a water-reel to help with germination. All tests were managed for high yields, with fertility, herbicides and fungicides applied based on recommended production standards (Raper 2014). All seed

were treated with a fungicide treatment of trifloxystrobin and triadimenol (Trilex Advanced, Bayer CropScience) at a rate of 47.32 mL/cwt.

Treatments were arranged in a 2 x 2 x 2 factorial within a randomized complete block with four replications, except at the Jackson location, which had six replications. Wheat variety was considered factor A and consisted of a BYD resistant and BYD susceptible variety. In the first year, Milton (University of Missouri) was designated as the resistant variety, and the susceptible variety was USG3438 (UniSouth Genetics, Dickson, TN). The Milton variety yielded poorly, and USG3438 was not available the next year. Thus, in the second year of testing, Pioneer 26R10 (Pioneer Hi-Bred International, Inc., Johnston, IA) and Mcalister (Dixie Wheat, Cash, AR) were selected as the resistance and susceptible variety, respectively. The classification of 'resistant' or 'susceptible' was based on the relative level of BYD symptoms observed in Tennessee variety performance reports of Allen et al. (2012, 2013). These were visual BYD ratings from a range of 1 – 5 where 1 represents no disease and 5 corresponds to greater than 95% of plants being affected by disease. Ratings from these tests were: Milton = 1.0 (2012), 1.0 (2013); USG3438 = 4.3 (2012), 4.3 (2013); Mcalister = 3.8 (2012), 3.8 (2013); and Pioneer 26R10 = 2.8 (2012), 2.8 (2013).

Factor B was insecticide seed treatment (IST), either not treated or treated with imidacloprid (Gaucho 600, 39.1 g ai/100 kg of seed). Factor C was late-winter foliar insecticide application, either not treated or treated with lambda-cyhalothrin (Karate Z or Warrior II, 33.6 g ai/ha, Syngenta Crop Protection,) applied with a CO₂ backpack sprayer calibrated to deliver a spray volume of 112 l/ha. Application dates were Feb. 3,

2017 (Knoxville), Feb. 9, 2017 (Milan), Feb. 20, 2018 (Jackson), and March 8, 2018 (Starkville).

Experiment 2 – Insecticide Seed Treatments and Foliar Insecticide Applications

Design. As above, tests were done during the 2016-2017 and 2017-2018 growing seasons to determine the relative efficacy of several insecticide seed treatments and foliar insecticide applications for aphid and BYD management. All tests were done at the same locations and planted and managed as described above. The variety used in all tests was Pioneer 26R10. Treatments were arranged as a randomized complete block design with four replications, except for Jackson, which had six replications. During the 2016-2017 growing season, the five treatments included a non-treated control, a foliar application of lambda-cyhalothrin (Karate Z or Warrior II, at a rate of 33.6 g ai per ha) at 30 days after planting and late-winter (3 Feb. – 8 March) or seed treatments consisting of imidacloprid (Gaucho 600), thiamethoxam (Cruiser 5F), and clothianidin (Poncho 600) all applied at a rate of 39.1 g ai per 100 kg of seed (Table 13). In the 2017-2018 growing season, these treatments were repeated but three additional treatments were included. These were foliar applications of lambda-cyhalothrin (33.6 g ai/ha) consisting of a fall-only application made about 30 days after planting, a late-winter application on the same dates as reported in the previous experiment, and a late-winter application made at the same time in combination with the imidacloprid seed treatment (Table 14). As with the other experiment, data from the Starkville and the Milan locations were eliminated from the data set due to poor and uneven stand establishment.

Data Collection

The data collected in both experiments were similar. Stand density counts were taken at about 30 days after planting by counting the number of plants in 0.09 m² (1 square foot) at two locations in each plot. Aphid counts were taken as numbers per 0.91 - 1.52 m of row, depending upon the test. Counts were taken at various times, but the only assessments used in analyses were those made in late February or March, approximately 20-30 days after the late-winter foliar insecticide applications were made. Ratings on the incidence of BYD were made between Feekes stage 8 (just before boot) to 10.5 (flowering) by counting the number of flag leaves in the entire plot showing distinct symptoms of BYD, primarily characterized by reddish or pinkish coloration on the leaf tips. The timing of BYD ratings was based on the optimum occurrence of symptoms as judged by the researcher. Whole-plot yield data were collected using combines designed for small-plot research.

In addition to the ratings of BYD based on visual symptoms, an enzyme-linked immunosorbent assay (ELISA) was used to qualify and quantify the severity of BYD PAV infection in selected treatments of some tests. Leaf samples were collected at approximately Feekes stage 4 (early jointing) and between Feekes stage 9 and 10.5. Ten random flag leaves were collected from each plot of the designated treatments. ELISA was performed on leaf tissue collected from the following test locations: Milan IST (collected 11 April, 2017), Knoxville IST (collected 3 February, 2017), Knoxville Factorial (collected 25 April, 2017), Starkville Factorial (collected 5 April, 2018), and the Jackson IST and Factorial tests (collected 7 May, 2018). Samples were stored in a -80°C freezer until ready for analysis. A homogenized 0.05 g sample of 10 random

collected leaf tips from each plot was placed in a 2 mL tube with a steel bead. Samples was submerged in liquid nitrogen for one minute and then placed in a TissueLyser LT (Qiagen, Hilden, Germany) and ran for 3 minutes at 30 Hz to homogenize the sample. Samples were then placed back in the -80°C freezer until the ELISA was performed. The ELISA methods followed a protocol designed for the detection of BYD PAV (m184.3, Agdia, Inc. 2018). ELISA reflectance readings for each sample, and positive and negative controls, were measured using an accuSkan FC Microplate Photometer (Fisher Scientific, Hampton, NH) at a wavelength of 405 nm.

Data Analysis

Response variables included spring aphid densities (numbers/row m), frequency of leaves showing BYD symptoms (numbers/10 m²), ELISA (absorbance values for BYD PAV), and yield (kg/ha). For each experiment (Factorial and IST/Foliar), data were analyzed both across and within test locations. For the experiment that included multiple insecticide seed treatment and foliar insecticide applications, three additional treatments were added during the second year. Thus, an across-test analyses was done for treatments common across both years, and a separate across-test analysis was done for all treatments during the second year.

All data was analyzed using a general linear mixed model of analysis of variance PROC GLIMMIX of SAS (Version 9.4, SAS Institute, Cary, NC). Treatment factors, including location, were considered fixed effects in the model and replication was considered random. Replication was considered a random effect. Means were estimated using LSMEANS and separated using Fisher's protected least significant

difference ($\alpha=0.05$). Mean separation of treatment effects on aphid numbers was done after a Log10 transformation to satisfy the assumptions for analysis of variance. Two and three way interactions between main effects and location, unless specifically mentioned, were not statistically significant ($P > 0.05$). For ELISA results, absorbance values are reported after subtracting the average value of two negative controls for each assay.

A substantial level of bird depredation on the grain occurred in the Starkville location in both experiments. Three plots were omitted from the yield data prior to analysis. These plots were 96%, 94% and 46% below the trial average yield, and 32 – 95% less than the yield from the next lowest yielding plot in the test.

Results

Experiment 1 – Factorial of Varieties, Insecticide Seed Treatment, and Foliar Insecticide

Interactions, especially interactions across test locations, were common for aphid counts and incidence of BYD (Table 4). Thus, most data was analyzed and presented by location in tables. Interactions among main effects (seed treatment, foliar insecticide) were not significant unless specifically mentioned ($P > 0.05$). Data are also presented from across-tests analyses when there were no confounding interactions.

Treatment effects on stand density. Stand densities varied among location ($F = 54.2$; $df = 2,113$; $P < 0.001$), with denser stands at the Knoxville location (390.1 plant/m²), similar plant densities at the Milan (331.6 plant/m²) and Jackson (332.1 plant/m²) locations, and less dense stands at the Starkville location (251.0 plant/m²). There was also an interaction of location and seed treatment ($F = 7.31$; $df = 2,113$; P

<0.001). In three of four locations, stand densities were significantly higher if seed was not treated with imidacloprid (Table 5). Not surprisingly, the foliar insecticide application did not affect stand density as it was made after stands were established and after stand count data were collected ($F = 0.26$; $df = 1,113$; $P = 0.612$). A variety effect was observed on stand density where the resistant variety had a denser stand than the susceptible variety ($F = 11.10$; $df = 2,113$; $P < 0.001$).

Treatment effects on aphids. Across all locations, the vast majority of aphids observed in these experiments were bird-cherry oat aphids, particularly for observation made prior to when any foliar insecticides were applied. English grain aphids were also present and composed a higher percentage ($\approx 40\%$ overall) of the population in the later ratings which are presented in this paper. Significant interactions were found between both main effects and location on aphid numbers (Table 4). At Knoxville and Starkville, aphid numbers were lower, but not significantly lower, where a seed treatment was used (Table 6). The insecticide seed treatment (imidacloprid) significantly reduced aphid numbers at the Jackson location, and interestingly, more aphids were found at the Milan location where the seed treatment was used. The foliar application of lambda-cyhalothrin substantially reduced aphid numbers ranging from 81 – 97%, depending upon location (Table 6). There was a significant interaction between seed treatment and foliar insecticide effects on the number of aphids found at the Jackson location ($F = 8.92$; $df = 1,35$; $P = 0.005$), where aphid numbers were more dramatically reduced by the foliar insecticide application in wheat not having an insecticide seed treatment compared with wheat having the seed treatment (Fig. 2). Interestingly, in the first year of

the study, the BYD resistant variety (Milton) tended to have more aphids, and this difference was significant at the Milan location (Table 6). Varietal effects on aphid numbers were not significant the following year.

Treatment effects on barley yellow dwarf virus. Interactions of both main effects and location were evident (Table 4). In Knoxville, the insecticide seed treatment significantly reduced the visual symptoms of BYD by about 45% (Table 7). A previous rating in late February showed an even larger reduction (66%, data not shown). Seed treatment did not affect the symptoms of BYD at the Milan and Jackson locations, and BYD was higher in Starkville where the seed treatment was used. Similar to its effect on aphids, the early February application of lambda-cyhalothrin substantially reduced symptoms of BYD at all locations except Starkville (Table 7). The varieties considered resistant to BYD (Milton and Pioneer) showed significantly less symptoms of BYD at all locations (Table 7).

There was a three-way interaction of main effects on BYD at the Milan location ($F = 8.48$; $df = 1,19$; $P = 0.009$). This interaction was primarily driven by observing more symptoms of BYD on the susceptible variety (USG) when the imidacloprid seed treatment was used, and this was not observed in the resistant variety (Milton) (Fig. 3). At the Jackson location, there was a two-way interaction between seed treatment and foliar insecticide applications on the level of BYD observed ($F = 4.0$; $df = 1,35$; $P = 0.050$). There was a greater reduction in the symptoms of BYD when the foliar insecticide was applied to wheat not having an insecticide seed treatment compared with seed treated with imidacloprid (Fig. 4). An interaction between variety and seed

treatment was found at the Starkville location ($F = 7.11$; $df = 1,21$; $P = 0.014$). The imidacloprid seed treatment had little effect on BYD in the resistant variety (Pioneer), but it significantly increased symptoms of BYD on the susceptible variety (Mcalister) (Fig. 5).

For the factorial experiment, statistical analyses of ELISA results were only ran by location because only selected treatments were sampled and this varied by location. Relative to the negative control, BYD PAV was detected in leaf tissue at all locations. Of 79 leaf samples across three experiments, 69 samples had greater absorbance values than the negative controls. At the Knoxville location, the relative concentration of BYD PAV was 72% lower ($P = 0.004$, Table 4) in plots treated with lambda-cyhalothrin (absorbance = 0.15 ± 0.08) compared with those not sprayed with insecticide (absorbance = 0.53 ± 0.08). The difference between plots treated with the imidacloprid seed treatment versus those without a seed treatment approached significance ($P = 0.089$, Table 4), with a 36% lower concentration of BYD PAV where the seed treatment was used. Variety had no significant effect on the concentration of BYD PAV detected, nor were there interactions among the main effects (Table 4). At the Jackson location, BYD PAV concentrations trended lower in samples from the resistant variety (Pioneer, absorbance = 0.02 ± 0.01) compared with the susceptible variety (Mcalister, absorbance = 0.31 ± 0.14), but this difference was not quite significant ($F = 4.23$, $df = 1,15$, $P = 0.057$). The main effect of seed treatment was not significant ($F = 0.00$, $df = 1,15$; $P = 0.989$), and there was not an interaction between these main effects ($F = 0.00$; $df = 1,15$; $P = 0.947$). There were no treatment effects on BYD PAV concentrations in ELISA

assays of leaf samples collected from Starkville ($P > 0.31$ for all effects, data not shown).

Treatment effects on yield. When data were analyzed across all tests, yield was not significantly affected by insecticide seed treatment or the late-winter applications of lambda-cyhalothrin, but there was an effect of variety and a variety by location interaction (Table 4). The varieties yielded differently at all locations. In the first year, the BYD resistance variety (Milton) yielded poorly compared with the susceptible variety (USG). In the second year of testing, the BYD resistance variety (Pioneer) yielded more than the susceptible variety (Mcalister).

When tests were analyzed separately, insecticide seed treatment again did not affect yield (Table 8). However, yield at the Jackson location was significantly increased by 195 kg/ha in plots treated with a foliar application of lambda-cyhalothrin ($P = 0.002$). A significant yield increase of 246 kg/ha was also observed at the Starkville location ($P = 0.046$), and a similar difference at the Milan location approached significance ($P = 0.052$). At Starkville, there was an interaction of the seed treatment and foliar insecticide factors ($F = 4.42$; $df = 1,18$; $P = 0.050$) because a yield increase from an application of lambda-cyhalothrin was only observed where imidacloprid was applied to the seed (Fig. 6).

A substantial level of lodging occurred shortly before harvest that may have affected yield data at the Knoxville location. Based on visual estimates, about 11% more lodging was observed where an insecticide seed treatment was used compared with plots not having a seed treatments (Table 9). Similarly, lodging was 19% higher in plots

treated with a foliar insecticide application. There was no interaction between the seed treatment and foliar insecticide factors ($F = 0.27$; $df = 1,21$; $P = 0.611$), and the amount of lodging was similar in both varieties (Table 9). As previously mentioned, bird depredation occurred at the Starkville location, adding considerable variability to yield data that may have masked some treatment effects.

Experiment 2 – Insecticide seed treatments and foliar insecticide applications

There was a strong interaction between treatment and test location on estimates of BYD symptoms (Tables 10 and 11). Thus, for consistency with the previously experiment, most data are presented by location in tables. Data are also presented from across-tests analyses if there were no confounding interactions.

Treatment effects on stand density. Unlike the previous experiment, none of the insecticide seed treatments had a significant effect on stand density compared with plots not having a seed treatment ($F = 0.23$; $df = 4,65$; $P = 0.918$). There was not a treatment by location interaction ($F = 1.10$; $df = 12,65$; $P = 0.377$). Like to the previous experiment, there were similar difference in stand density among locations ($F = 59.0$; $df = 3,65$; $P < 0.001$). The Milan and Jackson locations had statistically similar stand density, averaging 346.6 and 345.5 plants/m² respectively. Stand density was significantly higher at the Knoxville location (380.1 plants/m²), and the Starkville location had substantially lower stand density (233.3 plants/m²).

Effects of insecticides on aphids. Treatments and location had very significant effects on aphid populations when analyzed across trials. There was not an interaction between treatment and location when the across-test analysis was done for treatments

that were common to both years of the test (Table 10). However, a significant treatment by location interaction on aphid numbers was found for the across-test analysis of the two locations in 2017, where additional treatments were added (Table 11).

At each location individually, any treatments receiving a late-winter application of lambda-cyhalothrin had significantly fewer aphids compared to plots not treated with any insecticide (Tables 12, 13 and 14). Generally, insecticide seed treatments had a minimal effect on aphid infestation in February or March. However, aphid numbers in plot where an imidacloprid seed treatment used were significantly lower than plots with a clothianidin seed treatment (Table 12).

Within location, treatment responses varied. None of the insecticide seed treatments significantly affected aphid numbers at the Knoxville and Milan locations (Table 13). At the Jackson location, imidacloprid was the only treatment that significantly reduced aphid numbers (Table 14). Interestingly, insecticide seed treatments tended to increase springtime aphid numbers compared with plots not treated with insecticide at the Starkville location, and this difference was significant for thiamethoxam (Table 14). The fall only application of lambda-cyhalothrin also did not significantly reduce springtime aphid populations, and in Starkville, aphid populations were actually higher where the fall application was made.

Effects of insecticides on barley yellow dwarf virus. On average across all locations, symptoms of BYD were greatly reduced if a foliar application of lambda-cyhalothrin was made in late winter, and generally, insecticide seed treatments also reduced the symptoms of BYD compared with plots not treated with insecticide (Tables

12, 13, and 14). However, as previously mentioned, there were a highly significant treatment by location interactions (Tables 10 and 11). In Knoxville, the only seed treatment that significantly reduced BYD was imidacloprid (Table 13). Seed treatments did not reduce symptoms of BYD at the Milan or the Jackson locations (Table 13 and 14). In contrast, both clothianidin and thiamethoxam seed treatments reduced BYD compared with plots not treated with insecticide at the Starkville location (Table 14). Treatments that included a fall application of lambda-cyhalothrin, which were added the second year of the study, substantially reduced symptoms of BYD at the Starkville location (Table 14). Of particular interest at this location, the late winter application of lambda-cyhalothrin did not have a significant effect on BYD.

For the ELISA assays, there was an effect of location ($F = 17.52$; $df = 2,28$; $P < 0.001$) on the relative concentrations of BYD detected in leaf tissue, but because assays were run separately for each location, this may have resulted from differences in sample preparation. When analyzed by location, there were no effects of treatment on the concentration of BYD detected in leaf samples ($P > 0.08$ for all). However, treatment effects were apparent when data were analyzed across locations ($F = 3.55$; $df = 2,28$; $P = 0.042$), and there was not a location by treatment interaction ($F = 1.78$; $df = 4,28$; $P = 0.161$). The relative concentration of BYD in plots that were not treated with an insecticide was 2.9-fold higher (absorbance = 0.48 ± 0.17) than in plots where an imidacloprid seed treatment was used (absorbance = 0.16 ± 0.07). The concentration of BYD in plots not treated with insecticide was also numerically higher (1.9-fold) compared with those where an application of lambda-cyhalothrin was made

(absorbance = 0.24 ± 0.14). This difference was not significant, and also, there was not a significant difference in the concentration of BYD between the seed-applied and foliar-applied insecticide treatments.

Treatment effects on yield. No significant differences in yield or location by yield interactions were found when data were analyzed across locations (Table 10, 11 and 12). However, when tests were analyzed separately, a significant response was observed at the Jackson location. At this location, plots not treated with insecticide had less yield (5973 kg/ha) than all other treatments (Table 14). There was no significant difference between the yield of plots treated with only a seed treatment (6026 – 6174 kg/ha), and collectively, plots treated with lambda-cyhalothrin tended to yield more (6121 – 6356 kg/ha) than other treatments.

As in the other experiment, there was substantial lodging observed at the Knoxville location. Wheat that was not treated with insecticide had the least amount of lodging, but this difference was not significant compared with the imidacloprid or clothianidin seed treatments. However, compared with other treatments, significantly more lodging was observed where foliar insecticide was applied and where the thiamethoxam seed treatment was used (Table 13). Also as observed in the other experiment, a substantial level of bird depredation occurred at the Starkville location. Yield ranged from 1559 – 3972 kg/ha among individual plots, and this added considerable variability to the yield data.

Discussion

Perkins et al. (2018) previously reported a consistent reduction in springtime aphid infestations, observed across multiple tests in the same geography, when a neonicotinoid seed treatment was used (see also Chapter I). However, the impact of insecticide seed treatments on springtime aphid infestations was mixed in this study. In the factorial experiment (Table 6), aphid populations were reduced by the imidacloprid seed treatment only at the Jackson location. In contrast, the numbers of aphids found at the Milan and Starkville location was higher where a seed treatment was used. The results in the other experiment, evaluating different insecticide seed treatments and foliar spray regimens was similarly inconsistent (Table 13 and 14), again with seed treatments increasing aphid populations at the Starkville location. It is possible that the use of the insecticide seed treatment reduced aphid colonization in the fall, and consequently reduced the establishment of beneficial arthropod populations. Thus, aphid populations may have rebounded to relatively higher levels during late winter and spring where a seed treatment was used. Fall counts of aphids were not done because previous experience suggested populations would be too low to assess treatment effects. However, Perkins et al. (2018) reported that neonicotinoid seed treatments had negative effects on populations of aphids in the fall, and other data also shows these seed treatments are efficacious on aphids found in wheat (Elbert et al. 1991, Altmann and Elbert 1992, Hernandez 1999, Joshi and Sharma 2009).

Insecticide seed treatments may have reduced the viability of seed based on stand density estimates at 3 of 4 locations in the factorial experiment (Table 5). This

may be phytotoxicity or physical damage to the seed during the treating process, but this same effect was not observed in the other experiment. Also, stand density was higher where an insecticide seed treatment was used in the factorial test at Milan. It is unknown if this was related to pest injury, which would have likely been root feeding insects as signs of above ground insect injury was minimal.

The late-winter application of lambda-cyhalothrin consistently reduced aphid populations, with statistically significant reductions observed in both experiments and at all locations (Tables 6, 13 and 14). It is not surprising that, relative to insecticide seed treatments, fewer aphids were present where this application was made as these ratings were made in the early spring, relatively soon after lambda-cyhalothrin was applied. In the experiment where lambda-cyhalothrin was applied only in the fall (Table 14), aphid populations during the spring were either not significantly affected (Jackson) or were higher (Starkville) compared with plots not treated with insecticide (Table 14). In this way, the fall application was similar to the effects observed from an insecticide seed treatment.

Treatment effects on barley yellow dwarf (BYD) were less variable but somewhat mirrored aphid control. Seed treatments did not affect BYD ratings at Jackson or Milan, generally decreased the symptoms of BYD in Knoxville, and increased symptoms of BYD at Starkville in the factorial test where imidacloprid was used (whereas clothianidin and thiamethoxam seed treatments significantly reduced symptoms of BYD at Starkville, Tables 7, 13 and 14). ELISA confirmed the presence of BYD PAV at all locations. In the experiment evaluating different insecticide seed treatments, the

concentration of BYD PAV in leaf tissue was significantly reduced by the use of an insecticide seed treatment when data were analyzed across locations (only imidacloprid tested). BYD PAV concentrations from plots having an insecticide seed treatment (imidacloprid) trended similarly lower in the factorial test at Knoxville ($P = 0.089$).

Similar to aphids, the effect of a foliar applications of lambda-cyhalothrin on symptoms of BYD were more consistent. Treatments with a late-winter application of lambda-cyhalothrin showed less symptomology of BYD, except for both experiments at the Starkville location where seed treatments had no significant effect. Interestingly, in Starkville, it was the fall application of lambda-cyhalothrin that had obvious effects on the symptoms of BYD (Table 14). This suggests, when taken together with the aphid counts, that most of the BYD infection occurred in the fall after any effects of insecticide seed treatments had diminished but before the late-winter application was made. The ELISA showed that the late-winter insecticide application significantly reduced the concentration of BYD PAV in leaf tissue from the Knoxville factorial test. For the other experiment in Knoxville, the concentration of BYD PAV in leaf tissue from plots not treated with insecticides was higher, but not significantly so, compared with those treated with a foliar application. Overall, the ELISA provided confirmation that insecticide treatments could reduce the magnitude of BYD PAV infestation. However, a larger, pooled sample of leaf tissue or increased replication might have better detected treatment effects.

Both the varieties considered susceptible in our tests, USG 3438 and Mcalister, showed substantially more symptoms of BYD (Table 7). Resistance or tolerance to

aphids and BYD varies among wheat varieties (Irwin and Thresh 1990, De Wolf et al. 2017), and thus, the economic benefits of managing aphids with insecticides may vary depending on the variety grown. We chose resistant and susceptible based on previously reported assessments of BYD symptomology. It is unknown whether the tolerance of these varieties to BYD truly differ, or if some varieties simply express symptoms of infection more obviously, as the symptoms assessed in these tests were related to leaf color and not stunting or other effects on plant development. The ELISA did not show significant differences in the concentration of BYD PAV in leaf tissue between varieties, although the difference between the resistant variety (Pioneer 26R10) and the susceptible variety (Mcalister) was nearly significant at the Jackson location ($P = 0.057$). Although there were significant differences in yield among varieties, the yield response to seed treatment or a foliar application of lambda-cyhalothrin was statistically similar across varieties (Table 4). This suggests the varieties used in this study may not be truly resistant or susceptible to BYD, but that some may visually express BYD symptomology more than others; although further research would be needed to verify this conclusion. In addition, we only screened for the PAV species. If other BYD species were present, this would confound our conclusions. However, the bird cherry-oat aphid is the most prevalent aphid in our area which is found in transmitting the PAV species (Perkins et. al. 2018).

Positive effects of insecticide treatment on yield were detected at Jackson and Starkville (experiment 1) and Jackson (experiment 2), and the yield response at Milan (experiment 1) was nearly significant ($P = 0.052$). In these tests, either the foliar

application of lambda-cyhalothrin was the only factor that increased yield, or the yield increase associated with the foliar application was generally higher than that observed with seed treatments (Table 14). Bird depredation had serious impact on yield data collected at the Starkville location, potentially masking or compromising yield responses in both experiments. It was unfortunately that a significant level of lodging occurred at the Knoxville location where aphid populations and symptoms of BYD were most pronounced. Lodging resulted from higher stand densities and what appeared to be an over-application of nitrogen fertilizer. Lodging was generally worse where insecticides were used, and we believe this had a more negative effect on yield where insecticides were used. This does suggest that lodging could be a potential negative effect of aggressively managing aphids to prevent infection with BYD, making varietal selection a potentially important component of an IPM program.

Presumably, the yield responses observed in some tests were the result of insecticides reducing aphid populations and a corresponding reduction of BYD. It is possible that insecticides were controlling other pests and therefore impacting yield. For example, neonicotinoid seed treatments are labeled for the control of Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), and foliar insecticide applications can also impact this pest (Flanders et al. 2013). However, meaningful infestations of Hessian fly or other pests were not observed. A positive, statistically significant yield response to insecticide treatment was only observed in 3 of 8 test tests in these experiments. However, taken collectively with Perkins et al. (2018), these data suggest there is significant value to managing aphids and limiting infection of BYD in

Tennessee. Planting date and weather conditions will obviously influence the colonization patterns and density of aphid populations in wheat, and thus the incidence of BYD. Perry et al. (2000) reported that environmental factors and the strain of BYD will influence yield reduction in wheat and other small grains. Therefore, results from these are almost certainly not applicable to all geographies and only relevant for winter wheat in the Midsouth region.

Our data and those reported in Perkins et al. (2018) suggest that foliar insecticide applications generally provided better plant protection than an insecticide seed treatment. This may be true even for fall infestations of aphids, as evidenced by the benefits of a fall application of lambda-cyhalothrin on BYD at the Starkville location. A foliar-based management approach provides flexibility in deciding if and when an application is needed, and it would be less costly than an insecticide seed treatment (Perkins et al. 2018). Scouting for the presence of aphids in wheat during the fall would support decisions on making a fall-applied application, and it is expected that scouting would be particularly important in warm falls or where wheat was planted early. Studies have shown that the greatest economic losses caused by aphid infestations and associated infection with BYD occur when plants are infested with viruliferous aphids at early leaf development stages (Bockus et al. 2015). Data collected in Tennessee, including Perkins et al. (2018), show that a late-winter insecticide application consistently increase yield, indicating that transmission of BYD during warm winter days or early spring, while wheat is still in winter dormancy, is also important.

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Appendix

Table 4. Type III fixed effects for numbers of aphids, symptomology of barely yellow dwarf (BYD), yield, and an ELISA quantification of BYD in a factorial experiment of insecticide seed treatment (IST), a late-winter foliar insecticide application, and wheat variety.

Effect	Aphids			BYD			Yield			ELISA BYD (Knoxville)		
	F-Value	df	P-Value	F-Value	df	P-Value	F-Value	df	P-Value	F-Value	df	P-Value
Local	2.62	2, 113	0.077	226.8	2, 113	< 0.001	268.55	2, 109	< 0.001	.	.	.
Variety	8.46	2, 113	< 0.001	69.95	2, 113	< 0.001	57.12	2, 109	< 0.001	2.38	1, 21	0.138
Variety*Local	0.34	2, 113	0.716	17.97	2, 113	< 0.001	4.33	2, 109	0.002	.	.	.
IST	3.17	1, 113	0.078	2.41	1, 113	0.123	0.19	1, 109	0.667	3.17	1, 21	0.089
Local*IST	4.51	2, 113	0.013	15.63	2, 113	< 0.001	0.76	2, 109	0.469	.	.	.
Foliar	199.4	1, 113	< 0.001	115.2	1, 113	< 0.001	1.54	1, 109	0.217	10.79	1, 21	0.004
Local*Foliar	11.65	2, 113	< 0.001	47.65	2, 113	< 0.001	0.57	2, 109	0.565	.	.	.
Variety*IST	1.70	2, 113	0.188	2.33	2, 113	0.102	0.15	2, 109	0.857	1.64	1, 21	0.214
Variety*Foliar	1.08	2, 113	0.344	23.64	2, 113	< 0.001	0.82	2, 109	0.445	1.37	1, 21	0.255
IST*Foliar	0.25	1, 113	0.617	3.94	1, 113	0.050	0.15	1, 109	0.698	1.75	1, 21	0.200
Var*IST*Fol	1.72	3, 113	0.167	1.79	3, 113	0.153	0.82	3, 109	0.485	0.33	1, 21	0.573

Table 5. Main effects of an insecticide seed treatment (imidacloprid), a late-winter foliar insecticide application (lambda-cyhalothrin), and variety on stand density of wheat.

Plants per Square Meter						
Treatment Factors	Location	Treated	Not Treated	F-value	df	P-value
Seed Treatment	Knoxville	372.5	407.5	9.07	1, 21	0.007
	Milan	345.6	317.5	4.39	1, 19	0.049
	Jackson	321.8	342.4	4.67	1, 35	0.038
	Starkville	228.5	273.4	9.03	1, 21	0.007
Foliar Insecticide	Knoxville	391.9	388.1	0.10	1, 21	0.756
	Milan	325.9	336.9	0.75	1, 19	0.396
	Jackson	33.7	330.5	0.11	1, 35	0.744
	Starkville	247.7	254.3	0.20	1, 21	0.658
Variety	Knoxville	Milton	USG	8.39	1, 21	0.009
	Milan			6.91	1, 19	0.017
	Jackson	Pioneer	Mcalister	4.27	1, 35	0.046
Starkville			20.53	1, 21	< 0.001	

Table 6. Main effects of an insecticide seed treatment (imidacloprid), a late-winter foliar insecticide application (lambda-cyhalothrin), and variety on the numbers of aphids found in wheat.

Aphids per Meter Row							
Treatment Factors	Location	Treated	Not Treated	F-value	df	P-value	
Seed Treatment	Knoxville	6.32	13.89	2.16	1, 21	0.156	
	Milan	4.37	2.15	4.82	1, 19	0.041	
	Jackson	1.06	3.59	10.13	1, 35	0.003	
	Starkville	2.08	2.74	1.30	1, 21	0.267	
Foliar Insecticide	Knoxville	0.63	19.58	37.75	1, 21	< 0.001	
	Milan	0.51	6.01	56.67	1, 19	< 0.001	
	Jackson	0.62	4.03	32.49	1, 35	< 0.001	
	Starkville	0.82	9.60	105.20	1, 21	< 0.001	
Variety	Knoxville	Milton	11.67	8.54	1.82	1, 21	0.192
		USG	4.61	1.91	14.50	1, 19	0.001
	Jackson	Pioneer	3.21	1.44	3.28	1, 35	0.079
		Mcalister	5.50	4.92	1.17	1, 21	0.291
	Starkville	Pioneer					
		Mcalister					

Table 7. Main effects of an insecticide seed treatment (imidacloprid), a late-winter foliar insecticide application (lambda-cyhalothrin), and variety on the numbers of symptomatic BYD leaves observed in wheat.

BYD Leaves per 10 m²						
Treatment Factors	Location	Treated	Not Treated	F-value	df	P-value
Seed Treatment	Knoxville	39.38	71.23	28.09	1, 21	< 0.001
	Milan	12.41	12.84	0.37	1, 19	0.550
	Jackson	8.28	10.73	1.33	1, 35	0.257
	Starkville	73.98	56.72	8.27	1, 21	0.009
Foliar Insecticide	Knoxville	13.06	97.63	198.18	1, 21	< 0.001
	Milan	4.3	20.95	50.65	1, 19	< 0.001
	Jackson	6.61	12.40	7.42	1, 35	0.010
	Starkville	65.82	64.88	0.02	1, 21	0.877
		Milton	USG			
Variety	Knoxville	31.13	79.48	64.75	1, 21	< 0.001
	Milan	3.95	21.31	57.53	1, 19	< 0.001
	Jackson	3.32	15.69	33.80	1, 35	< 0.001
	Starkville	46.36	84.33	40.04	1, 21	< 0.001
			Pioneer	Mcalister		

Table 8. Main effects of an insecticide seed treatment (imidacloprid), a late-winter foliar insecticide application (lambda-cyhalothrin), and variety on yield of wheat.

Yield (kg/ha)						
Treatment Factors	Location	Treated	Not Treated	F-value	df	P-value
Seed Treatment	Knoxville	4204	4419	0.51	1, 20	0.483
	Milan	5125	5011	2.08	1, 19	0.166
	Jackson	5784	5690	2.43	1, 35	0.128
	Starkville	3089	2902	2.76	1, 18	0.114
Foliar Insecticide	Knoxville	4244	4372	0.18	1, 20	0.673
	Milan	5152	4984	4.31	1, 19	0.052
	Jackson	5838	5643	10.89	1, 35	0.002
	Starkville	3119	2873	4.74	1, 18	0.043
		Milton	USG			
Variety	Knoxville	3578	5038	22.34	1, 20	< 0.001
	Milan	4668	5468	115.19	1, 19	< 0.001
		Pioneer	Mcalister			
	Jackson	6208	5273	241.97	1, 35	< 0.001
	Starkville	3280	2711	25.47	1, 18	< 0.001

Table 9. Main effect on percent lodging observed at the Knoxville factorial test for wheat treated or not treated with an insecticide seed treatment (imidacloprid), a foliar insecticide application (lambda-cyhalothrin), or when using a resistant (Milton) or susceptible (USG) variety.

		Lodging (%)				
Treatment Factors	Location	Treated	Not Treated	F-value	df	P-value
Seed Treatment	Knoxville	41.4	30.1	2.46	1, 21	0.132
Foliar Insecticide	Knoxville	45.3	26.2	6.95	1, 21	0.015
		Milton	USG			
Variety	Knoxville	37.7	33.8	0.29	1, 21	0.599

Table 10. Type III fixed effects across all four test locations for numbers of aphids, symptomology of barely yellow dwarf (BYD), yield, and an ELISA quantification of BYD. Treatments included imidacloprid, clothianidin, and thiamethoxam seed treatment, and a foliar application of lambda-cyhalothrin made in the fall and late winter.

<u>Effect</u>	<u>Aphids</u>			<u>BYD</u>			<u>Yield</u>			<u>ELISA BYD</u>		
	<u>F- Value</u>	<u>df</u>	<u>P- Value</u>	<u>F- Value</u>	<u>df</u>	<u>P- Value</u>	<u>F- Value</u>	<u>df</u>	<u>P- Value</u>	<u>F- Value</u>	<u>df</u>	<u>P- Value</u>
Treatment	25.07	4, 65	< 0.001	13.44	4, 65	< 0.001	0.62	4, 65	0.651	3.55	2, 28	0.042
Local	10.97	3, 65	< 0.001	48.98	3, 65	< 0.001	324.1 3	3, 65	< 0.001	17.52	2, 28	< 0.001
Trt*Local	1.58	12, 65	0.121	4.40	12, 65	< 0.001	0.35	12, 65	0.975	1.78	4, 28	0.161

Table 11. Type III fixed effects across two test locations in 2017 for numbers of aphids, symptomology of barely yellow dwarf (BYD), yield, and an ELISA quantification of BYD. Treatments included imidacloprid, clothianidin, and thiamethoxam seed treatments, an imidacloprid seed treatment plus a foliar application of lambda-cyhalothrin (late winter), and lambda-cyhalothrin applied in the fall, late winter, or fall and late winter.

Effect	Aphids			BYD			Yield			ELISA		
	<u>F-</u> Value	<u>df</u>	<u>P-</u> Value	<u>F-</u> Value	<u>df</u>	<u>P-</u> Value	<u>F-</u> Value	<u>df</u>	<u>P-</u> Value	<u>F-</u> Value	<u>df</u>	<u>P-</u> Value
Treatment	9.81	7, 59	< 0.001	8.78	7, 59	< 0.001	0.81	7, 59	0.584	0.93	2, 10	0.426
Local	26.57	1, 59	< 0.001	93.97	1, 59	< 0.001	1601	1, 59	< 0.001	.	.	.
Trt*Local	2.23	7, 59	0.045	4.83	7, 59	< 0.001	0.45	7, 59	0.864	.	.	.

Table 12. Analysis across four locations of insecticide seed treatments and foliar-applied applications of lambda-cyhalothrin effects on aphids, symptoms of barley yellow dwarf virus, and yield.

<u>Treatment</u>	<u>Aphids</u>^a	<u>BYD</u>^b	<u>Yield</u>^c
Untreated	10.20 ab	34.38 a	4415 a
Imidacloprid	6.31 b	19.49 b	4444 a
Clothianidin	11.42 a	20.86 b	4457 a
Thiamethoxam	8.97 ab	28.10 ab	4354 a
Foliar (Fall+Spring)	0.63 c	2.53 c	4570 a

Means not followed by a common letter are significantly different ($P < 0.05$).

^a Number of aphids per meter row

^b Number of symptomatic flag leaves per 10 m²

^c Yield, kg/ha

Table 13. Numbers of aphids, incidence of barley yellow dwarf (BYD), and yield in soft red winter wheat as affected by seed treatments or a foliar insecticide application of lambda-cyhalothrin.

	Knoxville 2016 - 2017				Milan 2016 - 2017		
<u>Treatment</u>	<u>Aphids^a</u>	<u>BYD^b</u>	<u>Yield^c</u>	<u>Lodging</u>	<u>Aphids</u>	<u>BYD</u>	<u>Yield</u>
Not Treated	23.50 a	82.85 a	4036	26.50 b	23.50 a	9.35 a	5179
Imidacloprid	7.11 a	37.84 bc	4015	32.50 b	7.11 a	6.03 ab	5172
Clothianidin	20.82 a	72.99 ab	4096	33.75 b	20.82 a	10.21 a	5078
Thiamethoxam	8.87 a	51.83 ab	3854	52.50 a	8.87 a	3.94 ab	5401
Foliar (Winter)	0.45 b	4.12 c	4056	47.50 a	0.45 b	1.85 b	5313
F-Value	8.44	7.16	0.19	9.82	18.90	2.20	0.96
df	4, 12	4, 12	4, 12	4, 12	4, 12	4, 12	4, 12
P-Value	0.002	0.004	0.940	< 0.001	< 0.001	0.130	0.46 2

Means not followed by a common letter are significantly different (P < 0.05).

^a Number of aphids per meter row

^b Number of symptomatic flag leaves per 10 m²

^c Yield, kg/ha

Table 14. Number of aphids, incidence of barley yellow dwarf (BYD), and yield in soft red winter wheat as affected by seed treatments and foliar insecticide applications of lambda-cyhalothrin.

<u>Treatment</u>	Jackson 2017 - 2018			Starkville 2017 - 2018		
	<u>Aphids^a</u>	<u>BYD^b</u>	<u>Yield^c</u>	<u>Aphids</u>	<u>BYD</u>	<u>Yield</u>
Not Treated	5.27 a	6.93 ab	5973 d	5.34 bc	38.56 a	2462
Imidacloprid	1.83 bcd	8.61 a	6026 c	9.28 ab	25.65 ab	2556
Clothianidin	5.51 a	8.37 a	6033 c	7.53 ab	20.98 b	2199
Thiamethoxam	2.57 a	6.34 abc	6174 abc	17.08 a	21.52 b	2388
Imid.+Foliar (Winter)	0.44 cd	1.55 cd	6121 bc	1.41 cd	26.36 ab	2509
Foliar (Fall)	2.01 abc	0.96 d	6329 ab	16.92 a	3.41 c	2415
Foliar (Winter)	0.36 d	2.99 bcd	6356 a	1.89 cd	29.41 ab	2542
Foliar (Fall+Winter)	0.38 cd	0.72 d	6221 abc	0.84 d	3.59 c	2690
F-Value	5.43	3.75	3.05	6.83	5.20	0.33
df	7, 35	7, 35	7, 35	7, 21	7, 21	7, 21
P-Value	< 0.001	0.004	0.013	< 0.001	0.002	0.929

Means not followed by a common letter are significantly different ($P < 0.05$).

^a Number of aphids per meter row

^b Number of symptomatic flag leaves per 10 m²

^c Yield, kg/ha

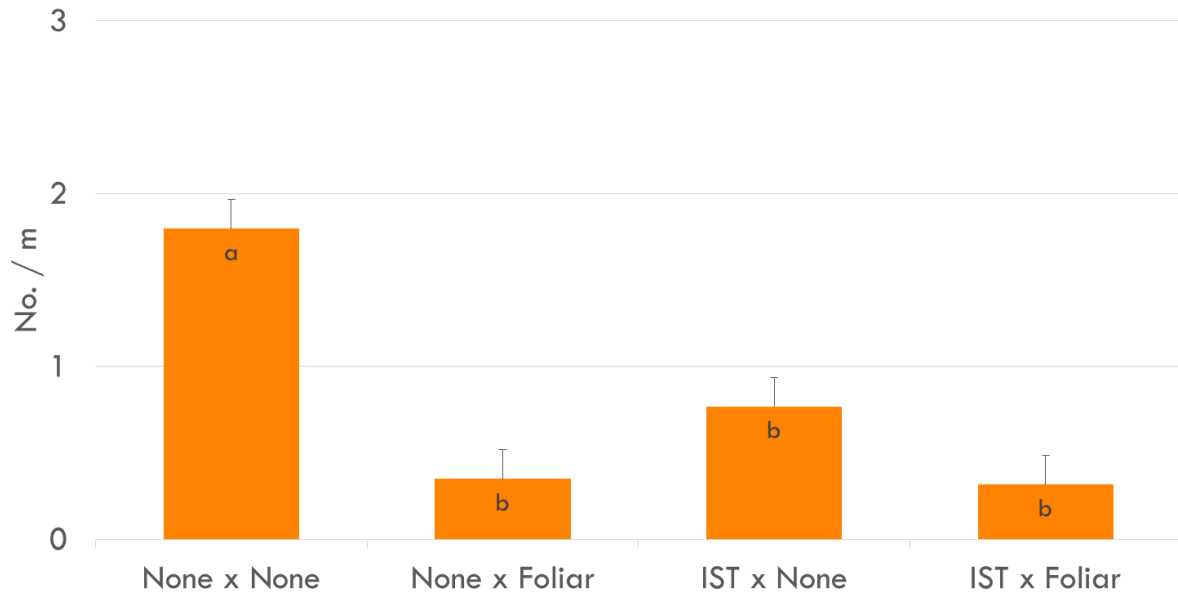


Figure 2. Effect of insecticide seed treatment (IST, imidacloprid) and a foliar insecticide application (lambda-cyhalothrin) on mean number of aphids present at the Jackson location. Means not having a common letter are statistically different ($P < 0.005$).

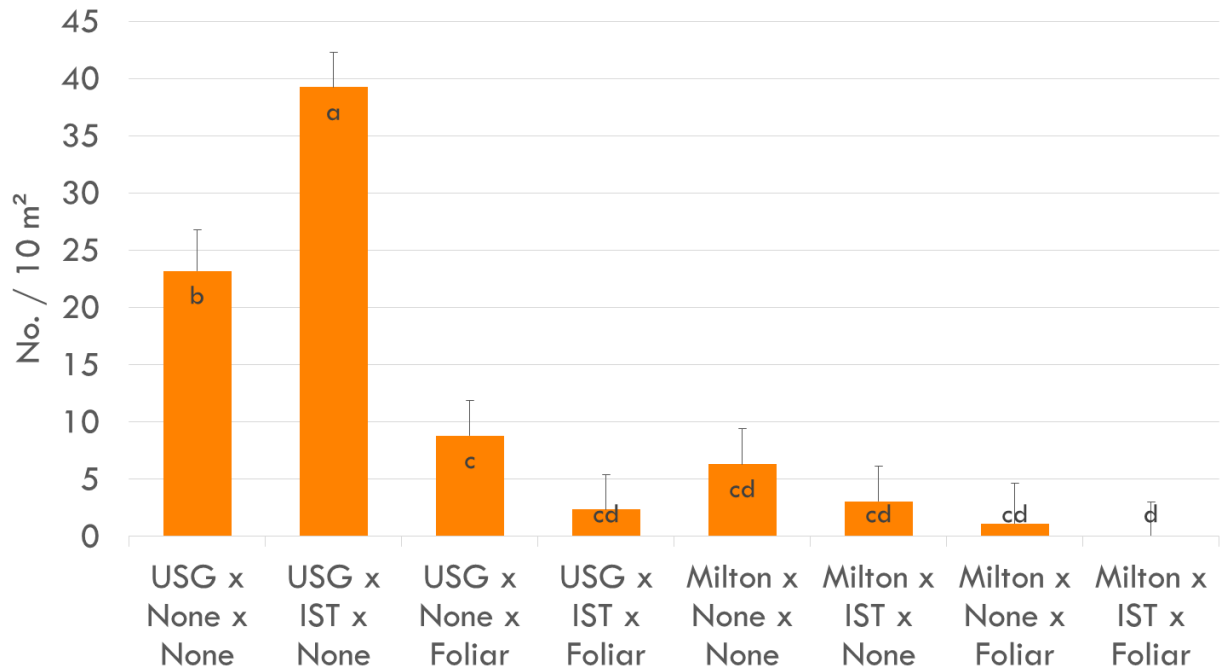


Figure 3. Effect of variety (USG and Milton), insecticide seed treatment (IST, imidacloprid) and a foliar insecticide application (lambda-cyhalothrin) on mean number of flag leaves showing symptomology of barely yellow dwarf virus at the Milan location. Means not having a common letter are statistically different ($P < 0.009$)

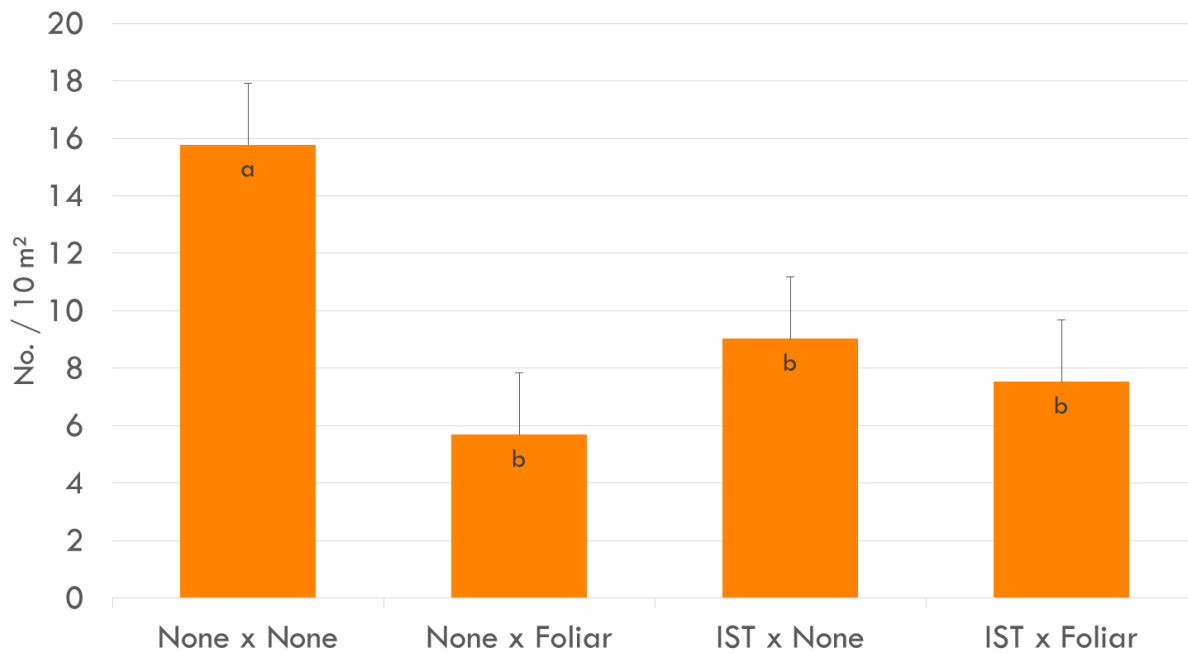


Figure 4. Effect of insecticide seed treatment (IST, imidacloprid) and a foliar insecticide application (lambda-cyhalothrin) on mean number of leaves showing symptomology of barley yellow dwarf virus at the Jackson location. Means not having a common letter are statistically different ($P < 0.05$).

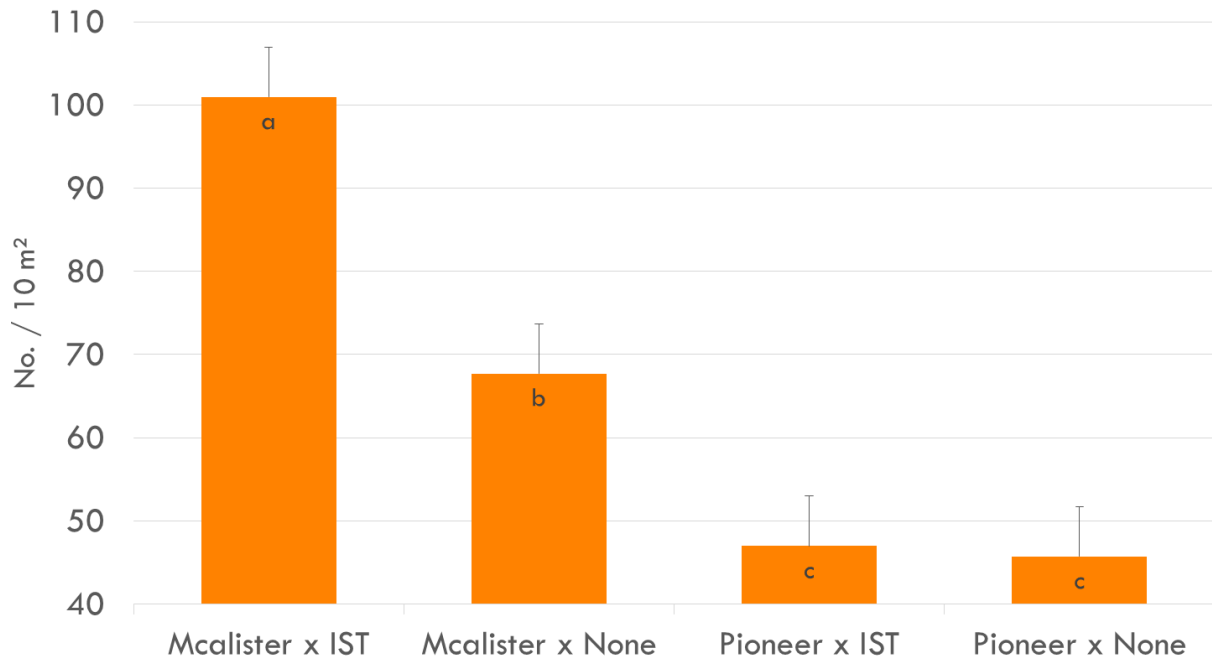


Figure 5. Effect of variety and an insecticide seed treatment (IST, imidacloprid) on the mean number of leaves showing symptomology of barley yellow dwarf virus at the Starkville location. Means not having a common letter are statistically different ($P < 0.014$).

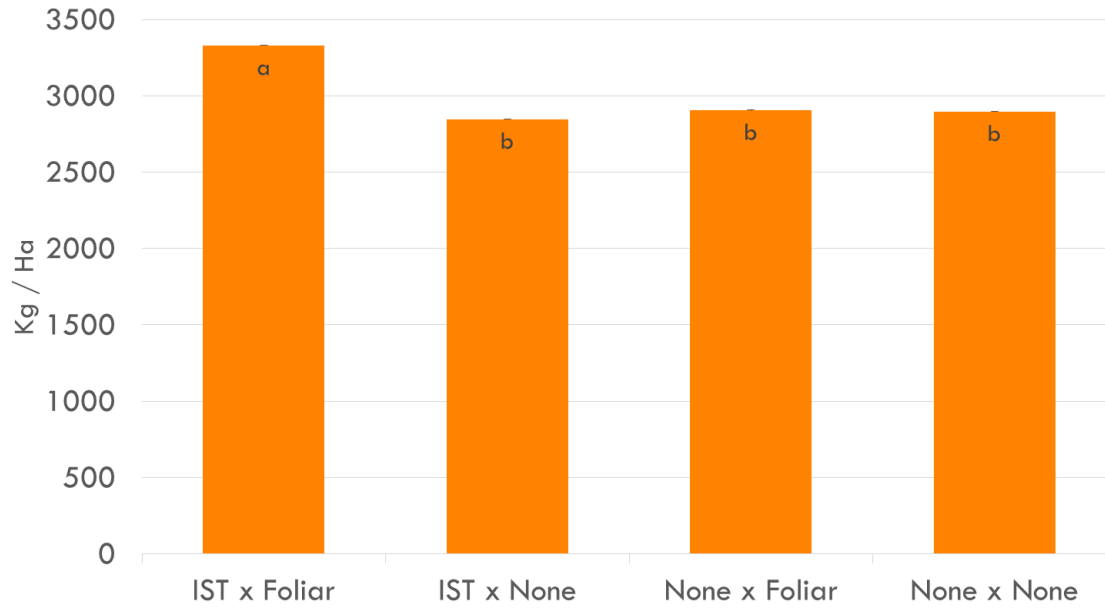


Figure 6. Effect of insecticide seed treatment (IST, imidacloprid) and a foliar insecticide application (lambda-cyhalothrin) on yield at the Starkville location. Means not having a common letter are statistically different ($P < 0.05$).

CONCLUSION

The overall goal for my research was to refine recommendations on the management of aphids in wheat to provide a consistent return on investment. My first objective was to analyze data across 33 experiments performed in west Tennessee from 2006-2017 to evaluate the impact of insecticide seed treatments and/or foliar insecticide applications on aphid infestations, the incidence of BYD, and yield. Insecticide seed treatments reduced aphid densities in the spring by an average of 86%, similar to the 78% reduction of aphid numbers observed in a more limited number of fall samples. This suggests that colonization of aphids during the fall and/or early winter months is parental source of springtime aphid infestations in Tennessee, as it is unlikely that insecticide seed treatments provide residual control past the winter months. A late-winter foliar application resulted in a 90% reduction in aphid populations. On average, where direct comparisons were made, a late-winter foliar application reduced aphid numbers and the incidence of BYD more than a neonicotinoid seed treatment alone. Symptoms of BYD were reduced by an average of 60% where neonicotinoid seed treatment were used; whereas, a foliar insecticide application reduced BYD by over 84%. Across all tests, the use of insecticides increased yield by 5 – 10% depending upon the insecticide treatment regime. The average yields of wheat treated with a foliar insecticide were 170 kg/ha higher than where only a seed treatment was used. An insecticide seed treatment alone increased yield by 280 kg/ha (5.3%, 4.2 bu/acre) compared to non-treated wheat. Similarly, yield was increased by an average of 381 kg/ha (7.2%, 5.7 bu/acre) when a foliar insecticide application was used

compared to non-treated wheat. The combination of using an insecticide seed treatment and a late-winter foliar insecticide application increased yield by about 10%.

My second objective was to evaluate how a combination of treatment factors including variety susceptibility, an insecticide seed treatment (IST), and/or foliar insecticide applications affected aphid populations, the incidence of BYD, and wheat yield. Identical experiments were repeated at multiple locations. Springtime aphid populations were only significantly reduced at one location when a neonicotinoid seed treatment was used. However, a late-winter foliar insecticide application reduced aphid populations by 81 – 97%, depending upon location. A seed treatment significantly reduced symptoms of BYD at only one location. Similar to its effect on aphids, a late-winter application of lambda-cyhalothrin substantially reduced symptoms of BYD at all locations. In addition, the varieties considered resistant to BYD (Milton and Pioneer 26R10) showed significantly less BYD symptomology compared to the susceptible varieties (USG 3438 and Mcalister). However, quantitative ELISA assays did not detect differences in BYD concentrations between varieties. ELISA assays did consistently showed the presence of BYD in leaf tissue, and insecticide treatments reduced the concentration of BYD in leaf tissue in some tests. In this experiment, insecticide seed treatments had no significant effect on wheat yields. However, yield at the Jackson location was significantly increased by 195 kg/ha in plots treated with a foliar application of lambda-cyhalothrin. A significant yield increase of 246 kg/ha was also observed at the Starkville location, and a similar difference at the Milan location approached significance. A substantial level of lodging occurred before harvest at the Knoxville

location, with more lodging where insecticides were applied. Lodging appeared to more negatively affect yield where insecticides were applied.

My last objective was to determine the relative efficacy of several neonicotinoid seed treatments and foliar insecticide applications for aphid and BYD management. Tests were done at multiple locations. Generally, neonicotinoid seed treatments had minimal effect on aphid populations in late February or March and modest effects on symptoms of BYD. In contrast, a late-winter application of lambda-cyhalothrin substantially reduced aphid populations and the symptoms of BYD, in general, with the exception of the Starkville location where a fall application more greatly reduced symptoms of BYD. Insecticides reduced concentrations of BYD detected with EILSA assays of leaf tissue. There was no statistical difference in yield when analyzed across locations. However, at the Jackson location, plots treated with lambda-cyhalothrin generally yielded more (6121 – 6356 kg/ha) than other treatments, and plots not treated with any insecticides had a significantly lower yield (5973 kg/ha) than all other treatment. As mentioned above, lodging at the Knoxville appeared to affect yield results. Bird depredation at the Starkville location may have also masked yield responses to treatment.

Collectively, these data suggest that foliar insecticide applications generally provided better plant protection than an insecticide seed treatment. This may be true even for fall infestations of aphids, as evidenced by the benefits of a fall application of lambda-cyhalothrin on BYD at the Starkville location. A foliar-based management approach provides flexibility in deciding if and when an application is needed, and it

would be less costly than an insecticide seed treatment. Scouting for the presence of aphids in wheat during the fall would support decisions on making a fall-applied application, and it is expected that scouting would be particularly important in warm falls or where wheat is planted early. Conventional wisdom is that the greatest economic losses caused by aphid and associated infection with BYD occur when plants are infested with viruliferous aphids during the fall. My data show that a late-winter insecticide application consistently increased yield, indicating that transmission of BYD during warm winter days or early spring, while wheat is still in winter dormancy, can also be important.

VITA

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