Potential Interaction between Pre-and Post-Emergence Herbicides and the Efficacy of Insecticide and Fungicide Seed Treatments in Cotton

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Cory Joseph Vineyard

December 2015

Acknowledgements

First of all, I would like to thank Jesus Christ, our savior, for allowing me the opportunity to continue my education and giving me the will power and knowledge to achieve this honor. I would also like to thank my wonderful family and friends for always being there for me and always encouraging me to continue on when things are rough. I would also like to thank Dr. Stewart for giving me this opportunity to continue my studies and for his guidance and teaching. I would also like to thank my fellow committee members, Drs. Larry Steckel and Heather Kelly, for their guidance and support as well.

I would also like to thank the individuals of the West Tennessee Research and Education Center for the time they have put into my research. I would like to give a special thanks to Sandy Steckel, and the rest of the "bug crew" for their effort in helping me complete my research and for their fellowship during my time in Jackson. I would also like to thank my professors and fellow classmates in Knoxville for their kind support and friendship as well.

Abstract

An apparent reduction in the performance of neonicotinoid seed treatments in controlling thrips, especially in cotton, has been observed, which has coincided with the increased use of both pre- and post-emergence herbicides to control glyphosate-resistant weeds. The objective of this research was to evaluate the potential interactions of both pre- and post-emergence herbicides on the efficacy of insecticide and fungicide seed treatments in cotton

Aldicarb along with imidacloprid and thiamethoxam seed treatments reduced thrips numbers compared with non-treated seed. However, thiamethoxam did not provide effective control. Some pre-emergence herbicide treatments reduced plant health. While there was evidence that thrips injury and herbicides could both negatively affect plant health, there was little indication of interactions between these factors, indicating that the efficacy of thrips treatments was the primary factor affecting yield. Fungicide seed treatments also generally improved seedling health and improved stand density in all tests. In these tests, negative effects of pre-emergence herbicides on plant health were also observed, but these data indicated no interaction with fungicide seed treatments.

Tests were also conducted to determine the potential interaction of insecticide seed treatments and post-emergence herbicides. In 2013, applications of glufosinate or glufosinate plus s-metolachlor caused substantial plant injury as evidenced by low vigor ratings, decreased plant biomass and height, and yield. While there was clear evidence that thrips and post-emergence herbicides, especially glufosinate, negatively affected plant health, there was little evidence of interactions between these two factors.

The role of microbial decay in the diminishing activity of neonicotinoid insecticides use as seed treatment was also tested. Sterilizing the soil reduced the concentrations of the imidacloprid and thiamethoxam metabolites indicating that soil microbes can degrade both

insecticides. However, the amounts of degradation to secondary metabolites were relatively low, and it is unlikely that microbial metabolism of either insecticide would appreciably impact their performance as seed treatments.

Collectively, these data indicate the negative impacts of thrips, seedling disease and herbicide injury would be additive. Using herbicides at recommended rates and according to label restriction should reduce the risk of compounding the effects of thrips or seedling disease with herbicide injury.

Table of Contents

List of Tables

Appendix I.

Appendix II.

Appendix III.

Appendix IV.

List of Figures

Appendix II.

Introduction

Cotton History and Production

Cotton is the world's most important natural fiber, accounting for almost 40% of total worldwide production with upland cotton being the most common species cultivated throughout the world (Naranjo and Luttrell 2009). Albers (1993) states, Cotton is a semi-tropical, perennial plant that has been bred and cultivated for production as an annual plant under a wide range of temperate climates. The harvestable fiber comes from the fruit or bolls of the plant and the seeds are used as animal feed or in the production of oil found in food products (Naranjo and Luttrell 2009).

Upland cotton, *Gossypium hirsutum L.*, known in the United States as American Upland, is a member of the *Malvaceae* L. or Mallow family. This species is native to Mexico and Central America and has been developed for extensive use in the United States, accounting for more than ninety-five percent of U.S. production (Cotton Incorporated 2014). According to the National Cotton Council (1995), cotton is grown in seventeen states covering more than twelve million acres in the United States with an average annual harvest of about 15 million bales or 3,311,000 metric tons. In the United States, 75% of the fiber produced goes into apparel, 18% goes into home furnishings and 7% goes into industrial products each year (National Cotton Council 2015). Annual cottonseed production in the United States averages about 4,717,000 metric tons with more than 2,720,000metric tons of cottonseed and cottonseed meal used in feed for livestock, dairy cattle and poultry while over 340,000,000 liters of cottonseed oil are used in food products (National Cotton Council 2015).

The production of cotton ranks third in terms of cash receipts for Tennessee farmers. Cotton is grown in 23 counties in Tennessee with most of the production in the western part of the state. Production varies considerably with an historical average around 222,500 to 263,000 hectares planted annually and average lint yields of 656 to 983 kilograms per hectare (Main

2012). Tennessee is on the northern edge of the Cotton Belt with production being hampered by a short growing season with frequent cool, wet weather during the spring and fall months (Shelby 1996). This means that early season management is key in order to reach maximum maturity and growing potential before harvest.

Thrips

During the early growing season, thrips (Thysanoptera: Thripidae) are one of the most important cotton insect pests in the United States (Zhang et al. 2013) and are consistently among the top three economically most important pests of cotton grown in Tennessee, with economic damage being inflicted to some fields on an annual basis (Stewart and Lentz 2010). Thrips belong to the order Thysanoptera which is divided into two suborders, Tubulifera and Terebrantia. All species that are pests of cotton belong to the suborder, Terebrantia, which insert their eggs into plant tissue (Reed et al. 2006). Species of thrips that commonly infest cotton seedlings in the United States include tobacco thrips, *Frankliniella fusca* (Hinds); flower thrips, *Frankliniella tritici* (Fitch); western flower thrips, *Frankliniella occidentalis* (Pergande); onion thrips, *Thrips tabaci* (Linderman); and soybean thrips, *Neohydatothrips variabilis* (Beach) (Leigh et al. 1996; Albeldano et al. 2008). In Tennessee, the genus *Frankliniella* is the predominant genus of thrips that feed on cotton (Stewart and Lentz 2010). Tobacco thrips, *Frankliniella fusca* (Hinds) made up 39.3% and 69.8% of the species composition in a two year study during 2009 and 2010 in Tennessee, respectively (Stewart et al. 2013).

Thrips are small, slender insects with adults being 1.5 to 2.0 mm long. Adults can be distinguished from immature thrips by the presence of two pairs of wings that are folded behind the back when not being used for flight. Adult color can vary with species but range from a yellowish to black in color while immature thrips found in cotton are usually pale yellow to straw-colored (Stewart and Lentz 2010). Thrips have a wide host range and can be found in all parts of Tennessee with many wild and cultivated hosts being potential sources of thrips that infest cotton, especially ones that are maturing or “drying down” (Stewart and Lentz 2010). Thrips overwinter as larvae on winter plants, pupae in the soil, or as hibernating adults (Layton and Reed 2002). Adult females laying between 30–300 eggs. Mated female thrips lay eggs that produce both males and females but a very high percentage of thrips that feed on seedling cotton are female (Layton and Reed 2002). Immature thrips can be more numerous than adults due to the fact that female adults will leave the field, and immature thrips will remain on the plants for long periods of time before pupating. Therefore, much of the damage to the seedling cotton may result from immature thrips (Layton and Reed 2002).

The thrips’ life cycle consists of six stages including the egg, two larval stages, prepupa, pupa and adult stages with the prepupa and pupa stages being non-feeding and usually found in the soil (Layton and Reed 2002). Development from egg to adult can be from 15 days to several weeks, depending on temperature with development taking longer during cooler temperatures (Layton and Reed 2002). Most thrips species have multiple generations per year with the first generation occurring on early spring hosts and dispersing into cotton fields as soon as seedlings emerge (Layton and Reed 2002, Stewart and Lentz 2010). Thrips are capable of flight but due to their small size tend to move with the prevailing winds. This may lead to heavy thrips infestations in cotton during windy planting seasons because the wind is continuously blowing new series of thrips into the field (Layton and Reed 2002).

Thrips have rasping-sucking mouthparts which allow them to rupture the epidermal cells of the cotton plant and insert their maxillary stylet to extract the cellular fluids (Cook et al. 2011, Layton and Reed 2002). Air partially fills the damaged cells which results in a silvery or white sheen where feeding has occurred (Layton and Reed 2002, Stewart and Lentz 2010). The removal of cellular fluids cause the cells to appear wrinkled or depressed (Cook et al. 2013). This type of injury causes little harm to developed, expanded leaves, but when thrips feeding occurs on undeveloped leaves in the terminal bud, the damage is magnified as the tissue fails to grow properly while undamaged tissue continues to grow (Layton and Reed 2002). Prolonged feeding or feeding by numerous thrips results in a ragged appearance and reduction in leaf area while heavily injured leaves often curl upwards at the margins resulting in "possum-eared cotton" (Layton and Reed 2002). Heavy thrips populations can result in stunted growth, "crazy cotton" or loss of apical dominance, delayed maturity or fruiting at higher positions and if left unchecked, seedling death and a reduction in crop stand (Herbert 2013, Layton and Reed 2002, Stewart and Lentz 2010). Thrips injury may result in boll development and maturation periods extending into the latter part of the growing season, thus delaying crop maturity and timely harvest (Cook et al. 2013). This damage can result in a delay in fruiting and an average yield reduction of over 100 kilograms of lint per hectare with extreme infestations causing death of seedling plants (Stewart et al. 2007). Cotton is most susceptible to thrips injury from emergence until it reaches the three to four leaf stage (Layton and Reed 2002). Thrips injury can be magnified when cotton seedlings are subject to adverse growing conditions such as cool weather or drought, which retard plant growth and result in a larger "window of susceptibility" (Herbert 2013, Layton and Reed 2002)

Thrips Control

Generally, thrips are controlled in Tennessee by using at-planting systemic insecticides. This includes the use of in-furrow granular applications of aldicarb (Temik; Bayer CropScience, Raleigh, NC), liquid in-furrow sprays of acephate (e.g., Orthene; AMVAC–Chemical, Los

Angeles, CA) or neonicotinoid seed treatments such as imidacloprid (Gaucho; Bayer CropScience) or thiamethoxam (Cruiser; Syngenta, Greensboro, NC) (Stewart et al. 2010). However, Temik is no longer being manufactured, and its registration has been voluntarily cancelled.

In the last 15 years, neonicotinoid seed treatments have been widely adopted by growers in many areas of the Cotton Belt (Stewart et al. 2013) and currently represent the first line of defense for control of thrips in Tennessee. The insecticide market was dominated by three classes of insecticides the organophosphates, the carbamates and the synthetic pyrethroids until the introduction of imidacloprid by Bayer CropScience in 1991 (Maienfisch et al. 2001). Neonicotinoids have been the fastest-growing class of insecticides in modern crop protection since the commercialization of pyrethroids (Jeschke and Nauen 2008) and represent the most effective chemical class for the control of sucking insect pests such as thrips (Elbert et al. 2008).

Neonicotinoids are systemic insecticides that are modeled after the natural insecticide, nicotine (Fishel 2013). Neonicotinoids act on the central nervous system of insects as agonists of the post-synaptic nicotinic acetylcholine receptors, nAChRs (Nauen and Denholm 2005) which are ligand-gated ion channels that mediate fast synaptic transmission in the insect nervous system (Jones and Sattelle 2010). This mimicry action results in excitation of the nerves, paralysis and eventually death (Fishel 2013). Due to the fact that the neonicotinoids only bind to this specific binding site, they are not cross-resistant to other classes of insecticides such as the carbamates, organophosphates, or the synthetic pyrethroids (Fishel 2013).

Plant Diseases

Seedling diseases are the number one disease problem in Tennessee cotton (Kelly 2015, Newman 1996). According to Newman (1996), from 1989 to 1996, the average estimated loss was 9.3 percent annually, with a range from 5 – 18% while the average for the rest of the Cotton Belt averaged about 3%, and from 1996 to 2011, the average estimated loss was 7.53% ranging from 3 – 22% while the average for the rest of the Cotton Belt was 3% (Kelly 2015). This prevalence of seedling diseases in Tennessee is probably due to the state's positon on the northern edge of the cotton production in the U.S. where cool, wet weather can be common at planting (Shelby 1996). Losses from seedling diseases can be severe in cool, wet planting seasons, when planting into no-till or stale seed beds, or when earlier than normal planting occurs (Newman 1996).

Rhizoctonia solani (Cantharellales: Ceratobasidiaceae) is the fungus most commonly associated with seedling diseases in Tennessee but, during cool wet planting seasons, *Pythium* spp. (Pythiales: Pythiaceae) may be more prevalent (Newman 1996). *Thielaviopsis basicola* (Microascales: Ceratocystidaceae) or black root rot can also be found but is not as prevalent in Tennessee (Kelly 2015). *Rhizoctonia solani* and *Thielaviopsis basicola* are true fungi, and do not produce oospores and have cell walls composed of chitin. *Pythium* spp. are oomycotes which produce oospores and have cell walls composed of beta glucans. When isolated, *Rhizoctonia solani* can be identified by the characteristic right-angle branching of hyphae.

The pathogen, *Rhizoctonia solani*, causes seed rot, pre-emergence damping-off, and to a greater extent, post-emergence dampening off in cotton (Fulton and Bollenbacher 1959, Neal 1942). Post-emergence dampening off is the death of seedlings shortly after emergence from the soil (Kelly 2015; Newman 1996). This is known as "sore shin" when only stem girdling occurs (Kelly 2015, Newman 1996). Symptoms of shore shin include lesions on the hypocotyl near or below the soil line and can be brown to reddish brown in color (Rothrock 2001). In contrast to *Pythium* spp. or *Thielaviopsis basicola*, the role of the environment is less stringent to the formation of *Rhizoctonia solani* with seedling disease occurring over a wide range of temperatures (18º- 33º C) (Rothrock 2001).

Rhizoctonia solani has a wide host range, parasitizing crops and weed species and survives in the absence of the host as dormant mycelial fragments and sclerotia (Rothrock 2001). Infection occurs from the pathogen forming a dome shaped infection cushion from the accumulation of axial hyphe. The tips of the hyphe on the underside of the cushion then become swollen and produce infection pegs. These infection pegs penetrate the cuticle and epidermal cell walls. (Rothrock 2001). The pathogen grows intercellularly and intracellularly with eventual colonization of all of the cells, except the xylem (Rothrock 2001). Rothrock states, “Diseased plants have reduced ability to compensate for other stresses, and plant maturity may be delayed, resulting in in yield reductions” (2001).

Seedling disease control is accomplished by the use of seed treatments and/or as fungicide soil treatments which include adding fungicides in the planter and in-furrow spray or granular applications (Kelly 2015). Popular fungicides include: strobilurins, which are Qol inhibitors that inhibit the respiratory chain in cellular respiration; phenylpyrroles, which inhibit the transport-associated phosphorylation of glucose; Phenylamides, which inhibit RNA synthesis and Triazoles, which inhibit cell membrane ergosterol synthesis. Cultural practices such as planting seed with 80% or higher warm-germination rates, planting during the recommended time frame, which in Tennessee is April 20th through May 10th, planting into a well prepared seed bed, turning over crop residues and crop rotation can help in reducing seedling disease (Kelly 2015; Newman 1996). Furthermore, infection is more likely if planting when soil temperatures are below 20º C (68º F) because of slow germination rates.

Weed Management

Weed control is another important aspect of early season management in Tennessee cotton. There are five general categories for controlling weeds in cotton: cultural, mechanical, biological, radiant energy or flaming and chemical (Buchanan 1992). Control of weeds with synthetic herbicides began in the 1940s with the introduction of 2, 4-D (Duke 2008). The increasing scarcity and high cost of labor along with the necessity for a clean field contributed to rapid acceptance of herbicides by cotton farmers (Buchanan 1992). During the 1950s development of selective herbicides continued slowly but steadily with more selective post-emergence herbicides being introduced in the later 1960s (Buchanan 1992).

Glyphosate (Roundup; Monsanto, St. Louis, MO) was introduced in the 1970s but was highly phytotoxic to cotton (Buchanan 1992) and was used for removing weeds before planting or in situations where the chemical could not come into contact with crop foliage for the first twenty years after commercialization (Duke and Powles 2008). Glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway. This inhibition of EPSPS leads to reduced feedback inhibition in the pathway, resulting in carbon flow to shikimate-3-phosphate that is converted to high levels of shikimate. (Duke 1988). The exact way that inhibition of the shikimate pathway kills the plant is not understood (Duke and Powles 2008).

Glyphosate-resistant crops were first introduced in the United States soybeans in 1996 (Monsanto 2015a). Tolerance to glyphosate in crops was accomplished by inserting glyphosate-resistant clone CP4-EPSPS into plants, allowing the shikimate pathway to still function in the presence of glyphosate (Dill et al. 2008). In 1997, Roundup Ready Cotton, was introduced by Monsanto, providing cotton farmers with herbicide tolerance to Roundup and other glyphosate-based herbicides (Monsanto 2015b). By 2001, seventy percent of cotton acreage in the United States was planted with glyphosate-resistant varieties (Gianessi 2005). In 2015, 98.8% of the upland cotton planted in the United States were glyphosate-resistant varieties (USDA 2015). This rapid adoption of glyphosate resistant crops and glyphosate herbicide was due to cost savings, better weed management, and simplicity of use (Duke and Powles 2009). Growers soon began to simplify their production systems and weed management regime. A reduction in tillage practices along with reliance on a reduced number of herbicides to manage weeds led to higher selection pressure, which led to weed shifts and ultimately, resistance (Owen 2008). The first glyphosate-resistant weed was discovered in Australia in 1996, and to date, there are thirty two glyphosate-resistant weed species worldwide (Heap 2015).

The first glyphosate–resistant weed was confirmed in Tennessee in 2001, and to date, there are seven glyphosate-resistant weed species in TN including: horseweed, *Conyza Canadensis*; palmer amaranth, *Amaranthus palmeri*; giant ragweed, *Ambrosia trifida*; tall waterhemp, *Amaranthus tuberculatus* (=*A. rudis*); annual bluegrass, *Poa annua*; goosegrass, *Eleusine indica*; and Italian ryegrass, *Lolium perenne* ssp. *Multiflorum* (Heap 2015). One of the most troublesome of these resistant weeds in Tennessee is palmer amaranth due to a high photosynthetic rate, high germination rate and ability to develop herbicide-resistant biotypes to commonly used herbicides in row crops (Steckel 2007). Glyphosate-resistant palmer amaranth was confirmed in 2006 in Tennessee (Steckel et al. 2008). In response to the necessity to control glyphosate-resistant weeds, farmers re-adopted weed management regimes that included the application of pre-emergence residual herbicides and post-emergence contact herbicides (Merchant et al. 2014; Prince et al. 2012; Sosnoskie and Culpepper 2014; Whitaker et al. 2011a; Whitaker et al. 2011b). Some of the post-emergence herbicides that are being used to combat glyphosate-resistant weeds, particularly glufosinate (Liberty) mixed with S-metolachlor (Dual Magnum) have been known to effect crop maturity and yield (Steckel et al. 2012).

Beginning around 2011 in the Mid-South, thrips control failures with neonicotinoid seed treatments, particularly thiamethoxam (Cruiser), became more commonly observed (Stewart 2013). In 2012, anecdotal observations indicated that the use of pre-emergence herbicides was negatively affecting the performance of insecticide seed treatments (Stewart, personal comm.). The objectives of this research was to determine if and how commonly used, pre-emergence and post-emergence herbicides are impacting the performance of standard insecticide and fungicide seed treatments in seedling cotton.

Literature Cited

Albeldano, W.A., J.E. Slosser, and M.N. Parajulee. 2008. Identification of thrips species on cotton on the Texas rolling plains. Southwest Entomol. 33:43-51.

Albers, D.W. 1993. Cotton plant development and plant mapping. p. 1. Publ. G4268 University of Missouri Ext., Portageville, MO.

Buchanan, G.A. 1992. Trends in weed control methods. p. 47-84 *In* C.G. McWhorter and J.R. Abernathy (ed.) Weeds of Cotton: Characterization and Control. The Cotton Foundation, Memphis, TN.

Cook, D., A. Herbert, D.S. Akin, and J. Reed. 2011. Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. J. Integ. Pest Mngmt. 2(2).

Cotton Incorporated. 2014. Fiber, quality, classification of cotton, overview [Online]. Available at http://www.cottoninc.com/fiber/quality/Classification-Of-Cotton/Overview/ (verified 15 March 2015).

Dill, G.M., C.A. CaJacob, and S.R. Padgette. 2008. Glyphosate-resistant crops: adoption, use and future considerations. Pest Manag. Sci. 64:326-331.

Duke, S.O. 1988. Glyphosate. p. 1-70 *In* P.C. Kearney and D.D. Kaufman (ed.) Herbicides: Chemistry, Degradation, and Mode of Action. Vol. 3. Marcel Dekker. New York, New York.

Duke, S.O., and S.B. Powles. 2008. Glyphosate: A once-in-a-century herbicide. Pest Manag. Sci. 64:319-325.

Duke, S.O., and S.B. Powles. 2009. Glyphosate-resistant crops and weeds: now and in the future. AgBioForum. 12(3&4):346-357.

Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 64:1099-1105.

Fishel, F.M. 2013. Pesticide toxicity profile: neonicotinoid pesticides. Publ. PI–80. Florida Coop. Ext. Serv., Gainesville, FL.

Fulton, N.D., and K. BollenBacher. 1959. Pathogenicity of fungi isolated from diseased cotton seedlings. Phytopathology. 49:684-689.

Gianessi, L.P. 2005. Economic and herbicide use impacts of glyphosate-resistant crops. Pest Manag. Sci. 61:241-245.

Heap, I. 2015. The International survey of herbicide resistant weeds, herbicide resistant weeds in Tennessee [Online]. Available at http://weedscience.org/details/usstate.aspx?State Abbr=TN (verified 4 May 2015)

Herbert, D.A. 2013. Field crops: cotton: insects: thrips. Publ. 4-105. Virginia Coop. Ext., Tidewater AREC, VA.

Jeschke, P., and R. Nauen. 2008. Neonicotinoids-from zero to hero in insecticide chemistry. Pest Manag. Sci. 64:108 -1098

Jones, A.K., and D.B. Sattelle. 2010. Diversity of insect nicotinic acetylcholine receptor subunits. Adv. Exp. Med. Biol. 683:25-43

Layton, B., and J.T. Reed. 2002. Biology and Control of Thrips in Seedling Cotton. Publ. 2302. Mississippi State Ext. Serv., Starkville, MS.

Leigh, T.F., S.H. Roach, and T.F. Watson. 1996. Biology and ecology of important insect and mite pests of cotton. p. 17–86 *In* E. King et al. (ed.) Cotton insect and mites: characterization and management. The Cotton Foundation, Memphis, TN.

Kelly, H.M. 2015. Cotton disease and nematode control 2015. University of Tennessee Extension, Knoxville, TN.

Maienfisch, P., M. Angst, F. Brandl, W. Fischer, D. Hofer, H. Kayser, W. Kobel, A. Rindlisbacher, R. Senn, A. Steinemann, and H. Widmer. 2001. Chemistry and biology of thiamethoxam: a second generation neonicotinoid. Pest Manag. Sci. 57:906-913.

Main, C.L. 2012. Cotton production in Tennessee. Publ. W288. University of Tennessee Ext., Knoxville, TN.

Merchant, R.M., A.S. Culpepper, P.M. Eure, J.S. Richburg, and L.B. Braxton. 2014. Controlling glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in cotton with resistance to glyphosate, 2, 4-D, and glufosinate. Weed Technology 28:291-297.

Monsanto. 2015a. Who we are, company history, original Monsanto company, 1996 [Online]. Available at http://www.monsanto.com/whoweare/pages/monsanto-history.aspx (verified 23 April 2015).

Monsanto. 2015b. Who we are, company history, original Monsanto company, 1997 [Online]. Available at http://www.monsanto.com/whoweare/pages/monsanto-history.aspx (verified 23 April 2015).

Naranjo, S.E., and R.G. Luttrell. 2009. Cotton arthropod IPM. p. 324-340 *In* E.B. Radcliffe et al. (ed.) Integrated Pest Management. Cambridge University Press, Cambridge, United Kingdom.

National Cotton Council. 2015. Economics, world of cotton [Online]. Available at http://www.cotton.org/econ/world/ (verified 15 March 2015).

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. Physiol. 58:200-215

Neal, D.C. 1942. *Rhizoctonia* infection of cotton and symptoms accompanying the disease in plants beyond the seedling stage. Phytopathology 32:641-642.

Newman, M.A. 1996. Cotton diseases. p. 6 *In* cotton production in Tennessee. Publ. 1514. University of Tennessee Ext., Knoxville, TN.

Newman, M.A. 1996. Cotton Diseases. p. 16-18. *In* cotton production in Tennessee. Publ. 1514. University of Tennessee Ext., Knoxville, TN.

Owen, M.D.K. 2008. Weed species shifts in glyphosate-resistant crops. Pest Manag. Sci. 64:377-387.

Prince, J.M., D.R. Shaw, W.A. Givens, M.E. Newman, M.D.K. Owen, S.C. Weller, B.G. Young, R.G. Wilson, and D.L. Jordan. 2012. Benchmark Study: III. Survey on changing herbicide use patterns in glyphosate-Resistant cropping systems. Weed Technology 26:536-542.

Reed, J.T., C. Allen, R. Bagwell, D. Cook, E. Burris, B. Freeman, R. Leonard, and G. Lentz. 2006. A key to the thrips on seedling cotton in the Midsouthern United States. Bulletin 1156. Mississippi State Ext. Serv., Starkville, MS.

Rothrock, C.S. 2001. Diseases caused by *Rhizoctonia solani*. p. 15-16 *In* T.L. Kirkpatrick and C.S. Rothrock (ed.) Compendium of cotton diseases second edition. The American Pathological Society. St. Paul, MN.

Shelby, P.P. 1996. Cotton production in Tennessee. p. 1-2. *In* Cotton production in Tennessee. Publ. 1514. University of Tennessee Extension, Knoxville, TN.

Sosnoskie, L.M., and A.S. Culpepper. 2014. Glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. Weed Science 62:393-402.

Steckel, L.E. 2007. The Dioecious *Amaranthus* spp.: here to stay. Weed Technology 21:567-570.

Steckel, L.E., C.L. Main, A.T. Ellis, and T.C. Mueller. 2008. Palmer Amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technology 22:119-123.

Steckel, L.E., D. Stephenson, J. Bond, S.D. Stewart, and K.A. Barnett. 2012. Evaluation of WideStrike® flex cotton response to over-the-top glufosinate tank mixtures. J. Cotton Sci. 16:88-95.

Stewart, S.D. 2012. Annual report: cotton insect pest management. Agreement 10–648TN. [Online]. Available at http://www.utcrops.com/MultiState/Trials/2012/CI%2010-648TN-Final-2012-Stewart.pdf (verified 1 Aug. 2015).

Stewart, S.D. 2013. Annual report. Cotton insect pest management. Agreement 13–589TN. [Online]. Available at http://www.utcrops.com/MultiState/Trials/2013/CI%2013-589TN-Final-2013-Stewart.pdf (verified 1 Aug. 2015).

Stewart, S.D., D.S. Akin, J. Reed, J. Bacheler, A. Catchot, D. Cook, J. Gore, J. Greene, A. Herbert, R.E. Jackson, D.L. Kerns, B.R. Leonard, G.M. Lorenz, S. Micinski, D. Reisig, P. Roberts, G. Studebaker, K. Tindall, and M. Toews. 2013. Arthropod management & applied ecology: survey of thrips species infesting cotton across the Southern U.S. Cotton Belt. J. Cotton Sci. 17:263-269.

Stewart, S.D., and G.L. Lentz. 2010. Cotton insects: thrips. Publ. W026. University of Tennessee Extension, Knoxville, TN.

Stewart S.D., G.M. Lorenz, K.L. Willis, B.A. Hanks, S.J. Steckel and C.K. Colwell. 2007. Thrips control in seedling cotton. p. 1654-1658. *In* Cotton insect research and control. Proc. Beltwide Cotton Conference. New Orleans, Louisiana. 9-12 Jan. 2007.

Stewart, S.D., R. Patrick, and A. McClure. 2010. Cotton insect control recommendations. p. 3-16. *In* 2010 Insect control Recommendations for field crops, cotton, soybeans, field corn, sorghum, wheat, and pasture. Publ. 1768. University of Tennessee Extension, Knoxville, TN.

United States Department of Agriculture. 2015. Estimated percentage of Upland cotton planted to leading specified varieties, by growth area, 2015 Crop. p. 2. *In* Cotton varieties planted 2015 crop. United States Department of Agriculture, Agricultural Marketing Service – Cotton and Tobacco Program, Memphis, Tennessee.

Whitaker, J.R., A.C. York, D.L. Jordan, and A.S. Culpepper. 2011a. Weed management with glyphosate- and glufosinate-based systems in PHY 485 WRF cotton. Weed Technology 25:183-191.

Whitaker, J.R., A.C. York AC, and D.L. Jordan, A.S. Culpepper, and L.M. Sosnoskie. 2011b. Residual herbicides for palmer amaranth control. J. Cotton Sci. 15:89-99.

Zhang, J., H. Fang, H. Zhou, S.E. Hughs, and D.C. Jones. 2013. Inheritance and transfer of thrips resistance from Pima cotton to upland cotton. J. Cotton Sci. 17:163-169.

Part I

Potential Interactions of Pre-emergence Herbicides and At-planting Insecticides on Thrips Control in Cotton

Abstract

The increasing occurrence of glyphosate-tolerant weeds has dramatically increased the use of pre-emergence herbicides in cotton, *Gossypium hirsutum* L., grown in Tennessee. These herbicides may have negative effects on seedling growth and potentially affect the control of thrips (Thysanoptera: Thripidae) with at-planting insecticides, including seed treatments. This increase in the use of pre-emergence herbicides has coincided with a reduction in efficacy of insecticide seed treatments used for thrips control. Field studies were done in 2013 and 2014 to investigate the impact and interaction of selected pre-emergence herbicides and at-planting insecticides used for thrips control. Non-treated plots and plots treated with a thiamethoxam seed treatment (Cruiser) generally had higher numbers of thrips, more thrips injury, less vigor, delayed maturity, and lower yields than those treated with either aldicarb (Temik) or an imidacloprid seed treatment (Gaucho). This data suggested and reinforced observations about the declining efficacy of thiamethoxam in controlling tobacco thrips infestations in cotton. There was a tendency for higher thrips populations where pre-emergence herbicides were used. Combination treatments of Cotoran plus Dual Magnum or Cotoran plus Reflex tended to reduced plant vigor ratings and seedling biomass. While there was clear evidence that thrips and pre-emergence herbicides could both affect plant health, there was little evidence of interactions between these two factors. Also, there was no indication that pre-emergence herbicides reduced the concentrations of neonicotinoids present in the leaves of seedling plants. In these tests, the efficacy of thrips treatments was the primary factor affecting final yield. Pre-emergence herbicides may compound injury caused by thrips, but my data suggests that these effects on plant health would be additive in nature.

Introduction

Thrips are a common pest of cotton that routinely rank among the top three insects reducing yield (Williams 2013). The primary injury occurs during the seedling stage where feeding on emerging leaves and terminal buds can delay maturity and cause plant death in extreme circumstances (Layton and Reed 2002). Because Tennessee is located on the northern edge of the Cotton Belt, maturity delays can be especially important. Several species of thrips may attack seedling cotton, but tobacco thrips (*Frankliniella fusca*, Thysanoptera: Thripidae) typically composes the vast majority of thrips found in the Mid-South (Stewart et al. 2013). Preventative at-planting treatments, either in-furrow granular or liquid insecticides or seed treatments, are almost always recommended to control thrips infestations in seedling cotton (Stewart et al. 2014). In the last 10 years, insecticide seed treatments such as Gaucho (Imidacloprid; Bayer CropScience, Raleigh, NC) or Cruiser (Thiamethoxam; Syngenta, Greensboro, NC) have been used almost exclusively in Tennessee for thrips control.

The use of pre-emergence herbicides has increased dramatically in Tennessee and many other areas of the Cotton Belt during the last 5-7 years in response to weed resistance to glyphosate (e.g., Roundup, Monsanto Co., St Louis, MO), especially Palmer pigweed, *Amaranthus palmeri* (Merchant et al. 2014; Prince et al. 2012; Sosnoskie and Culpepper 2014; Whitaker et al. 2011a; Whitaker et al. 2011b).

These pre-emergence herbicides may cause crop injury that can also result in maturity delays (Main et al. 2012). Concurrent with the increased use of these herbicides has been an apparent reduction in the efficacy of insecticide seed treatments, especially thiamethoxam-based seed treatments, and in some cases thrips injury appeared worse where pre-emergence herbicides were used (Stewart, personal communication). Consequently, this study was done to elucidate the effects and possible interactions commonly-used pre-emergence herbicides on thrips control provided by at-planting insecticides.

Materials and Methods

Field Study

Two identical experiments were done in 2013 and 2014 at the Milan Research and Education Center in Milan, TN. The tests were conducted as a factorial of four insecticide treatments: non-treated, Cruiser 5F® (thiamethoxam; Syngenta, Greensboro, NC), Gaucho 600® (imidacloprid; Bayer Crop Science, Raleigh, NC), and Temik® (aldicarb; Bayer Crop Science) and four pre-emergence herbicide treatments: non-treated, Cotoran 4L® (fluometuron; DuPont, Eleutherian Mills, DE), Cotoran 4L® (fluometuron) plus Reflex® (fomesafen; Syngenta), and Cotoran 4L® plus Dual Magnum® (S-metolachlor; Syngenta). Phytogen 375 WRF® was planted on May 14, 2013 and Phytogen 333 WRF® was planted on May 13, 2014 at a seeding rate of 13.2 seed per meter and at a depth of 1.9 cm. Plots were four rows wide (102-cm spacing) and 10.7 m long with four replications for each treatment arranged in a randomized complete block design. Each plot received a standard fungicide seed treatment. Insecticide seed treatments of imidacloprid (Gaucho) and thiamethoxam (Cruiser) were applied at a rate of 0.375 mg ai/seed and in-furrow insecticide applications of aldicarb (Temik) were applied at a rate of 820 g ai/hectare. Pre-emergence herbicide treatments were applied at the following rates: fluometuron (Cotoran) (2.84 L/Ha), fluometuron (Cototran) (2.84 L/Ha) fomesafen (Reflex) (1.13 L/Ha), and fomesafen (Reflex) (2.84 L /Ha) s-metolachlor (Dual Magnum) (1.42 L/Ha) using a pressurized CO_2 backpack sprayer calibrated to deliver 151 L per hectare using XR 8002 flat fan nozzles at 275 kPa on May 15 in 2013 and 2014. Either Roundup® WeatherMAX®

(glyphosate; Monsanto Company, St. Louis, MO) or Gramoxone® (paraquat; Syngenta, Greensboro, NC Syngenta) was used for a burn down application before planting.

Thrips Counts and Plant Biomass. Thrips were collected at the first (18 days after planting, DAP) and second true leaf stage (22 DAP) of cotton growth in 2013 and at the first (16 DAP) and second true leaf stage (23 DAP) of cotton growth in 2014 to estimate the numbers of thrips present. Two plants from each of the four rows for a total of eight plants per plot, per growth stage at the ground level and placing them in plastic bags with the exception of the first thrips count in 2013 where only five plants were collected. Each plant was taken out of the bag and rinsed with ethyl alcohol over a glass container topped with a sieve to collect the thrips. The plastic bag was then rinsed with ethyl alcohol over the sieve to collect any remaining thrips left inside. The sieve was then rinsed with ethyl alcohol into a gridded petri dish and the thrips counted underneath a microscope. The thrips were counted and categorized as either adult or immature and the number recorded. The same plants collected for the thrips counts were used to record above ground biomass for each plot. The fresh weight of each sample was recorded and the samples were put in a refrigerator to await thrips counts.

Thrips Injury and Vigor Ratings. Thrips injury ratings were taken at the third (22 DAP) and fourth true leaf (27 DAP) stage of cotton growth in 2013 and at the second (20 DAP) and fourth true leaf (26 DAP) stage of cotton growth in 2014 to convey the amount of thrips damage as a whole, per plot. These ratings were based on a 0 – 5 scale where 0 is no injury in the plot and 5 is 100% plant death in the plot.

Vigor ratings were taken at the fourth true leaf stage (27 DAP) of cotton growth in 2013 and at the fourth true leaf stage (26 DAP) of cotton growth in 2014 to convey the health of the cotton as a whole, per plot. These ratings were based on a 0 – 5 scale where 0 is no vigor in the plot and 5 is complete stands with highly vigorous plants.

Plant, Plant Mapping, and Yield Data. Various plant mapping techniques were conducted to convey the effects of treatments on the maturity of the cotton. Stand counts were taken at the cotyledon and first true leaf stage of cotton growth in 2013 and at the cotyledon and tenth node stage of cotton growth in 2014. Bloom counts of every white to pinkish bloom in the middle two rows of each plot were taken at the fifteenth node (72 DAP) in 2014. Average node above white flower counts of ten random plants per plot were taken at the seventeenth node (74 DAP) in 2013 and seventeenth node (86 DAP) in 2014. Seed cotton yield was collected at 187 DAP in 2013 and at 178 DAP in 2014 from the center two rows of each plot with a small-plot cotton picker designed for research plots.

Leaf Samples for Neonicotinoid Insecticide Concentration. In 2013, to determine how herbicide treatment affected the uptake of insecticides by the plant, 15 terminal leaves were collected from each Cruiser treated plot. Expanded leaves were collected at the second-leaf stage, and placed into self-sealing plastic bags. In the laboratory, the leaves were rinsed and dried before being placed in a freezer (-20ºC) until shipped to the USDA-AMS Laboratory in Gastonia, NC for analysis of neonicotinoid concentration levels.

Samples were analyzed to determine the levels of neonicotinoid residues by the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories' Gastonia Lab in Gastonia, NC. This laboratory is accredited to ISO/IEC 17025:2005 for specific tests in the fields of chemistry and microbiology, including testing for pesticide residues. The samples were extracted for analysis of agrochemicals using a refined methodology for the determination of neonicotinoid pesticides and their metabolites using an approach of the official pesticide extraction method (AOAC 2007.01), also known as the QuEChERS method, and analyzed by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS) (Kamel 2010, Lehotay et al. 2005, Zhang et al. 2011). Samples were analyzed for the presence of 17 insecticides or their metabolites. Quantification was performed using external calibration standards prepared from certified standard reference material. The analytical limit of detection (LOD) for each neonicotinoid insecticide and its metabolites are shown in Table 1.

Data Analyses. All data were analyzed using the Proc GLIMMIX procedure of SAS (ver. SAS 9.4; SAS Institute; Cary, NC). When significant interactions across years occurred for at least one main effect, means and mean separation are shown both across and within years. Main effects of insecticide or herbicide treatment were considered to be fixed model effects, and replicates (nested within years) was considered to be a random effects as suggested by Carmer et al. (1989). Fisher's protected LSD (LS Means) at $\alpha = 0.05$ was used as the criteria to separate individual treatment means. Within year mean separation was based on the LS Means for each main effect by year component of the across-year model.

Greenhouse Study

A greenhouse test was also done with potted plants at the West Tennessee Research and Education Center in Jackson, TN during 2013. The experiment was a factorial design of two insecticide treatments, Cruiser 5F (thiamethoxam) and Gaucho 600 (imidacloprid), and four herbicide treatments including a non-treated check, Cotoran 4L (fluometuron), Reflex (fomesafen), and Dual Magnum (s-metolachlor). Each treatment combination was replicated four times in 9.46 L plastic pots. Insecticide seed treatments of Gaucho 600 and Cruiser 5F were applied at a rate of 0.375 mg ai/seed. Twelve seed were planted per pot on July 1, 2013. Pre-emergence herbicide treatments were applied the day of planting at the following rates: Cotoran 4L (2.84 L/ Ha, Reflex (1.13 L/Ha), and Dual Magnum (1.42 L /Ha) using a pressurized CO_2 backpack sprayer calibrated to deliver 151 L per hectare using XR 8002 flat fan nozzles at 275 kPa. The pots were then arranged in a randomized complete block fashion in the greenhouse and were watered as needed on multiple occasions during the experiment. The experiment was terminated at the third leaf stage of cotton growth.

Herbicide Injury, Plant Biomass, and Neonicotinoid Concentrations in Leaves. At 7 DAP, a visual inspection of plants was made to quantify the number of plants in ach plot showing evidence of herbicide injury based on the presence of “leaf burn” (spots of necrosis and discoloration). Above ground biomass weights were collected at the third true leaf stage (21 DAP) of cotton growth. All plants in each pot were cut at the soil level and put into labeled, self-sealing plastic bags. The fresh weight of each sample was recorded. The leaves of plants from each pot were removed from the stem of the cotton plant, rinsed with tap water, and allowed to air dry. These leaf samples were stored at -20ºC in labeled plastic bags until shipped for analysis of neonicotinoid concentration levels in the leaf tissue as previously described to determine how herbicides affected insecticide levels within the leaves. Statistical analyses were done similarly to the field study.

Data Analyses. All data were analyzed using the Proc GLIMMIX procedure of SAS (ver. SAS 9.4; SAS Institute; Cary, NC). When significant interactions across years, where relevant, occurred for at least one main effect, means and mean separation are shown both across and within years. Main effects of insecticide or herbicide treatment were considered to be fixed model effects, and replicates (nested within years) was considered to be a random effects as suggested by Carmer et al. (1989). Fisher’s protected LSD (LS Means) at $\alpha = 0.05$ was used as the criteria to separate individual treatment means. Within year mean separation was based on the LS Means for each main effect by year component of the across-year model.

Results

There were many examples of year by main effect interactions. Unless specifically indicated, interactions between the main effects of herbicide and insecticide treatments were not significant (P > 0.05), and thus, data for these main effects are presented separately.

Field Study

Thrips Counts and Plant Biomass. The first thrips counts were taken 18 days (2013 or 16 days (2014) after planting (DAP). For immature and total thrips, there was a significant impact of insecticide treatment and an interaction between years and insecticide for immature (F = 3.21; df = 3, 93; P = 0.0265) and total thrips (F = 6.02; df = 3, 93; P = 0.0009) (Table 2). In both years, any insecticide treatment decreased immature and total thrips numbers compared with the non-treated check. Temik provided the greatest reduction in immature thrips numbers, and Gaucho reduced immature thrips numbers more so than Cruiser in 2013. Temik reduced total thrips numbers compared with Gaucho and Cruiser in 2013. In 2014, Temik and Gaucho reduced immature and adult thrips numbers compared with Cruiser. No significant herbicide effects were observed in either year on numbers of immature thrips (P = 0.5911) or total thrips (P = 0.3974) (Table 2).

Thrips were counted a second time 22 (2013) or 23 (2014) DAP. Across both years, both insecticide and herbicide treatment affected immature thrips numbers (Table 3). There were no treatment by year interactions although the interaction of year and herbicide approached significance (F = 2.63; df = 3, 93; P = 0.055). Insecticide and herbicide treatment affected total thrips numbers across years, and there was a significant interaction of year and herbicide (F =

3.82; *df* = 3, 93; *P* = 0.0125). Similar to the previous thrips sample, Temik and Gaucho provided the best thrips control. Cruiser did not reduce the number of thrips. Herbicide treatments tended to increase the numbers of thrips, and plots treated with only Cotoran or Cotoran plus Reflex had significantly more thrips than non-treated plots in 2013 (Table 3).

Biomass weights were taken 22 (2013) and 23 (2014) DAP. Both insecticide and herbicide treatment had significant impacts on plant biomass (Table 4). There was a significant interaction between years and insecticide treatments (*F* = 7.78; *df* = 3, 93; *P* = < 0.0001) but not for years and herbicide treatments (*F* = 0.22; *df* = 3, 93; *P* = 0.8825). In 2013, there was a trend towards increased biomass in plots treated with insecticide, but only plots treated with Temik had more biomass than non-treated plots (Table 3). In 2014, all insecticide treatments increased biomass compared with the non-treated check. Plots treated with Temik had the most biomass followed by Gaucho and then Cruiser. Across both years, biomass was greater in plots treated with only Cotoran compared with treatments receiving Cotoran plus Dual Magnum or Cotoran plus Reflex but was not higher than plots not receiving a pre-emergence herbicide (Table 4).

Thrips Injury and Vigor Ratings. Thrips injury ratings were taken 22 (2013) or 23 (2014) DAP. There was a significant impact of insecticide treatment and an interaction (*F* = 9.50; *df* = 3, 93; *P* < 0.0001) between years and insecticide (Table 5). In both years, thrips injury was numerically highest in plots not treated with insecticide. Temik provided the greatest reduction in thrips injury followed by Gaucho. Cruiser did not reduce thrips injury in 2014 compared with plots not treated with insecticide. There was a significant impact of herbicide treatment and an interaction (*F* = 3.68; *df* = 3, 93; *P* = 0.0148) between years and herbicide (Table 4). In 2013, Plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex had higher thrips injury scores. In 2014, there was no significant impact of herbicide application on thrips injury scores (Table 5).

Thrips injury was rated a second time at 27 (2013) or 26 (2014) DAP. There was a significant impact of insecticide treatment and an interaction ($F = 3.54$; $df = 3, 93$; $P = 0.0178$) between years and insecticide (Table 5). In 2013, Temik provided the greatest reduction in thrips injury and Gaucho reduced thrips injury more so than Cruiser. Cruiser did not reduce thrips injury compared with the non-treated check. In 2014, thrips injury was highest in the non-treated check compared with the use of any insecticide. Again, Temik provided the greatest reduction in thrips injury and Gaucho reduced thrips injury more so than Cruiser (Table 4). There was no interaction between herbicide treatment and year ($F = 2.39$; $df = 3, 93$; $P = 0.0739$). When analyzed across years, plots treated with pre-mergence herbicides tended to have higher thrips injury scores ($F = 4.84$; $df = 3, 93$; $P = 0.0036$). Specifically, plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex had higher thrips injury scores than non-treated plots or plots only treated with Cotoran (Table 5).

Vigor ratings were taken at 27 (2013) or 26 (2014) DAP. There were significant impacts of both insecticide and herbicide treatment but no interaction ($P > 0.05$) between insecticide or herbicide and year. Across years, vigor was lowest in the non-treated check compared with the use of an insecticide. Temik and Gaucho increased plant vigor more so than Cruiser. Herbicide treatments tended to reduce vigor, and plots treated with Cotoran plus Dual Magnum and Cotoran plus Reflex had less vigor than plots not treated with pre-emergence herbicides (Table 4). Other vigor ratings showed similar results (data not shown).

Plant, Plant Mapping, and Yield Data. In 2013, there was no significant impact on stand from either the insecticide or herbicide treatments. In 2014, a final stand count was taken

49 days after pre-emergence application. Gaucho and Temik increased stand (*F* = 15.13; *df* = 3, 45; *P* = < 0.0001) but there was no significant impact from herbicide application.

Bloom counts were taken 72 DAP in 2014. Insecticide and herbicide treatments had significant impacts on bloom counts (Table 6). Temik and Gaucho increased the number of blooms compared with Cruiser, which did not increase bloom counts more so than the non-treated check. Plots treated with Cotoran had more blooms than plots treated with Cotoran plus Dual Magnum and Cotoran plus Reflex but did not increase blooms more so than plots not receiving a pre-emergence herbicide.

Node above white flower counts were taken 74 (2013) and 86 (2014) DAP. Insecticide treatments had a significant impact on node above white flower ($P < 0.0001$) and there was an interaction (*F = 3.73; df = 3, 93; P* = 0.0139) between insecticide treatment and year. Although Temik and Gaucho decreased the number of node above white flower counts in 2013, there was no significant insecticide effects. In 2014, Temik and Gaucho decreased the number of node above white flower counts compared with the Cruiser treatment and plots not receiving an insecticide treatment (Table 6). Herbicide treatment had no significant impact across years on node above white flower (Table 6).

Insecticide treatment affected seed cotton yield, and there was a year by insecticide interaction (*F* = 5.51; *df* = 3, 93; *P* = 0.0016). In both years, Temik increased seed cotton yields compared with plots not treated with insecticide (Table 5). Gaucho also increased yield in both years but only significantly in 2014. Cruiser did not significantly increase yield in either year. There was not a year by herbicide interaction (*F* = 0.29; *df* = 3, 93; *P* = 0.8316). There was a trend of less yield where Cotoran plus Dual was applied compared with other herbicide treatments, but this difference was not significant when analyzed across years (F = 2.42; df = 3, 93; P = 0.0713)

Neonicotinoid Insecticide Concentrations in Leaves. In 2013, insecticide concentrations were taken at the third true leaf stage (20 DAP). Depending upon the treatment, the total concentration of thiamethoxam plus its metabolites ranged from 4,026 - 7,142 PPB in the leaf tissue. Thiamethoxam and its primary metabolite, clothianidin, composed approximately 75.8% and 19.6%, respectively, of the total neonicotinoid concentration found in the leaf tissue. Plots treated with Cotoran plus Dual Magnum had statically higher concentrations of thiamethoxam and clothianidin compared with those not treated with Dual Magnum, including those treated with Reflex or Cotoran (Table 7).

Greenhouse Study

Herbicide Injury, Plant Biomass, and Neonicotinoid Concentrations in Leaves. In the greenhouse study done in 2013, there was no significant impact from the insecticide or herbicide treatments on plant emergence. There were more injured plants in pots treated with Gaucho compared with Cruiser (F = 11.43; df = 1, 20; P = 0.0030), although the reasons for this are not clear. Plant injury was generally higher for in that received an herbicide application. Application of Reflex resulted in the highest number of injured plants followed by Dual Magnum. Plant injury in the Cotoran treatment was not different from the non-treated check (Table 8).

There was no significant impact of insecticide treatment on above ground biomass, but herbicide treatment did impact biomass (F = 4.04; df = 3, 20; P = 0.0213). There was a significant reduction in biomass for Cotoran and Dual Magnum. The treatment of Reflex did not reduce biomass compared with plants in pots not treated with herbicide (Table 8).

Analyses of leaves collected at the third leaf stage showed much higher concentrations of imidacloprid in plants treated with a Gaucho seed treatment relative to thiamethoxam concentrations in plants treated with Cruiser (Table 8). This includes the parent compounds and its metabolites listed in Table 6. Herbicides tended to increase total neonicotinoid concentrations in leaf tissue, but this effect was not significant ($F = 1.87$; $df = 3, 20$; $P = 0.1668$).

Discussion

The research was conducted to further investigate the apparent reduction in crop protection provided by insecticide seed treatments during the past several years. Observations in test plots and production fields indicated diminished performance of neonicotinoid seed treatments, and especially thiamethoxam (Cruiser), in reducing thrips populations and thrips injury. One hypothesis for this decreased performance might be the use and interactions of insecticide seed treatments with pre-emergence herbicides that are now being used more widely at a relatively high rates in response to glyphosate-resistant weeds.

These data indicated that aldicarb (Temik) provided the greatest reduction in thrips populations and injury compared with insecticide seed treatments. This is consistent with most previous research (Burris et al. 1989). Temik has been the standard preventative thrips treatment in years past, but due to environmental concerns, the product has been taken off the market (Cone 2010). However, even before Temik's removal from the market, most growers in the Mid-South had already transitioned to using insecticide seed treatments because of convenience, generally satisfactory protection against thrips, and safety considerations. Both imidacloprid (Gaucho) and thiamethoxam (Cruiser) reduced thrips numbers compared with seed not treated with an insecticide. However, Cruiser was not as effective. The relative performance of these thrips treatments was reflected in other measures of plant health such as vigor, plant biomass, maturity, and yield. Plots not treated with insecticide or those treated with Cruiser had generally less vigor, delayed maturity, and lower yields. Cruiser did not provide adequate protection against thrips in this study.

The pre-emergence herbicides used in this test are commonly used in Tennessee. Maximum labeled use rates were used to maximize the likelihood of inflicting crop injury and observing interactions with insecticide treatments used for thrips control. Also, Reflex was used post-planting which is not recommended on silt loam soil types because of the threat of crop injury. There was a tendency for high thrips populations where pre-emergence herbicides were used, particularly during 2013. It is unclear why this occurred. In 2013, generally higher thrips injury was observed in plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex. However, it is possible that ratings of thrips injury was confounded with injury caused by herbicides. Herbicides, especially Cotoran plus Dual Magnum or Cotoran plus Reflex reduced plant health as evidenced by vigor ratings, plant biomass measurements and bloom counts.

There was clear evidence that thrips treatments and pre-emergence herbicides could both affect plant health. However, there was little evidence of interactions between these two factors, Thus, pre-emergence herbicides may compound injury caused by thrips, but these data suggests that effects on plant health would be additive in nature. These data also reinforces concerns about thiamethoxam (Cruiser) and its declining efficacy against thrips populations (Stewart 2013). The assays of neonicotinoid insecticide concentrations from the field and greenhouse study suggested, if anything, concentrations tended to be higher in seedling plants where pre-emergence herbicides were used. This may be because the herbicides slowed growth and reduced plant biomass. Thus, insecticide concentrations were less diluted in plants stunted with herbicides. Regardless, it does not support that pre-emergence herbicides were somehow impending the uptake of insecticides. In 2013, Syngenta confirmed cases of resistance to thiamethoxam in tobacco thrips populations in the Mid-South.

Although pre-emergence herbicides are an aggravating factor, results in these tests indicate that the efficacy of insecticide seed treatments in controlling thrips control was the primary factor affecting plant health and yield in this experiment. The use of pre-emergence herbicides is needed in Tennessee because of glyphosate-resistant Palmer amaranth and other herbicide resistant weeds. Using pre-emergence herbicides at recommended rates and according to label restriction should help minimize compounding thrips injury with herbicide injury. Of greater concern would be declining performance of imidacloprid, following suite of thiamethoxam, in controlling infestations of tobacco thrips. There are no currently-labeled alternative treatments for thrips control that provide the consistent level of protection as did imidacloprid or thiamethoxam seed treatments.

Literature Cited

Burris, E., K.J. Ratchford, A.M. Pavloff, D.J. Boquet, B.R. Williams, and R.L. Rogers. 1989. Thrips on seedling cotton: related problems and control. Bulletin 811. Louisiana State University, Baton Rouge, LA.

Carmer, S.G., W.E. Nyquist, and W.M. Walker. 1989. Least significant differences for combined analyses of experiments with two- or three-factor treatment designs. Agronomy Journal 81(4):665-672.

Cone, M. 2010. Environmental health news: toxic pesticide banned after decades of use [Online]. Available at http://www.scientificamerican.com/article/toxic-pesticide-banned-after-decades-of-use/ (verified 1 Aug. 2015).

Kamel, A. 2010. Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography–tandem mass spectrometry (LC-MS/MS). J. Agric. Food Chem. 58:5926-5931.

Layton B., and J.T. Reed. 2002. Biology and control of thrips in seedling cotton. Publ 2302. Mississippi State Ext. Serv., Starkville, MS.

Lehotay, S. J., A. DeKok, M. Hiemstra, and P. van Bodegraven. 2005. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. J. of AOAC Inter. 88:595-614.

Main, C.L., J.C. Faircloth, L.E. Steckel, A.S. Culpepper, and A.C. York. 2012. Cotton tolerance to fomesafen Applied preemergence. J Cotton Sci 16:80-87.

Merchant, R.M., A.S. Culpepper, P.M. Eure, J.S. Richburg, and L.B. Braxton. 2014. Controlling glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in cotton with Resistance to glyphosate, 2,4-D, and glufosinate. Weed Technology 28:291-297.

Prince, J.M., D.R. Shaw, W.A. Givens, M.E. Newman, M.D.K. Owen, S.C. Weller, B.G. Young, R.G. Wilson, and D.L. Jordan. 2012. Benchmark study: III. Survey on changing herbicide use patterns in glyphosate-resistant cropping systems. Weed Technology 26:536-542.

Sosnoskie, L.M., and A.S. Culpepper. 2014. Glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. Weed Science 62:393-402.

Stewart, S.D., R. Patrick, and A. McClure. 2014. Cotton insect control recommendations. p. 3-16. *In* 2014 insect control recommendations for field crops, cotton, soybeans, field corn, sorghum, wheat, and pasture. Publ. 1768. University of Tennessee Extension, Knoxville, TN.

Whitaker, J.R., A.C. York, D.L. Jordan, and A.S Culpepper. 2011a. Weed management with glyphosate- and glufosinate-based systems in PHY 485 WRF cotton. Weed Technology 25:183- 191.

Whitaker, J.R., A.C. York, D.L. Jordan, A.S. Culpepper, and L.M. Sosnoskie. 2011b. Residual herbicides for palmer amaranth control. J. Cotton Sci. 15:89-99.

Williams, M.R. 2013. Resources, extension, crop losses. Cotton insect losses – 2013. Table 8, summary of all states [Online]. Available at http://www.entomology.msstate.edu/ resources/croplosses/2013loss.asp (verified 5 June 2015).

Zhang, K., J. W. Wong, P. Yang, K. Tech, A.L. DiBenedetto, N.S. Lee, D.G. Hayward, C.M. Makovi, A.J. Krynitsky, K. Banaerjee, L. Jao, S. Dasgupta, M.S. Smoker, R. Simonds, and

A. Schreiber. 2011. Multiresidue pesticide analysis of agricultural commodities using acetonitrile salt-out extraction, dispersive solid-phase sample clean-up, and high-performance liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 59:7636-7646.

Appendix I

Table 1. Neonicotinoid residues of parent compounds and their metabolites and the analytical limit of detection (LOD) that were screened for during analyses.

Pesticide Residue	LOD (ng/g)	Pesticide Residue	LOD (ng/g)
Thiamethoxam	1.0	Imidacloprid	1.0
Thiamethoxam metabolites		Imidacloprid metabolites	
Clothianidin	1.0	6-Chloronicotinic acid	30.0
Clothianidin MNG	50.0	Imidacloprid 5-hydroxy	1.0
Clothianidin TMG	50.0	Imidacloprid des nitro hcl	2.0
Clothianidin TZMU	50.0	Imidacloprid olefin	10.0
Clothianidin TZNG	50.0	Imidacloprid olefin des nitro	16.0
		Imidacloprid urea	1.0

Table 2. The effects of insecticide and herbicide treatments on the average number of immature and total thrips per plant. Analyses are shown across and within years.

Treatment	Immature Thrips			Total Thrips		
	16-18 DAP	16 DAP	18 DAP	16-18 DAP	16 DAP	18 DAP
Insecticide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	18.6 a	17.9 a	19.4 a	22.6 a	22.1 a	23.1 a
Cruiser	9.70 b	10.4 b	8.97 bc	14.2 b	14.6 b	13.8 b
Gaucho	3.94 c	6.70 c	1.19 d	8.51 c	13.0 b	4.02 c
Temik	1.14 d	1.11 d	1.16 d	2.42 d	2.83 c	2.02 c
F-value	*84.41*	*3.21*		*88.45*	*6.02*	
df	*3,93*	*2,93*		*3,93*	*3,93*	
P-value	*< 0.0001*	*0.0265*		*< 0.0001*	*0.0009*	
Herbicide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	7.44 a	8.44 a	6.45 a	10.6 a	11.9 a	9.29 a
Cotoran	8.25 a	8.83 a	7.67 a	12.2 a	13.1 a	11.3 a
Cotoran + Dual Mag.	8.91 a	9.10 a	8.71 a	12.6 a	13.4 a	11.8 a
Cotoran + Reflex	8.81 a	9.78 a	7.84 a	12.3 a	14.1 a	10.5 a
F-value	*0.64*	*0.20*		*1.00*	*0.22*	
df	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*0.5911*	0.8947		*0.3974*	0.8836	

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Table 3. The effects of insecticide and herbicide treatments on average numbers of immature and total thrips per plant. Analyses are shown across and within years.

Treatment	Immature Thrips			Total Thrips		
	22-23 DAP	22 DAP	23 DAP	22-23 DAP	22 DAP	23 DAP
Insecticide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	14.5 a	16.16 a	12.93 b	19.7 a	21.4 ab	17.9 b
Cruiser	13.3 a	13.63 ab	12.95 b	20.4 a	21.8 a	19.0 ab
Gaucho	6.51 b	6.93 c	6.09 c	11.6 b	12.6 c	10.6 c
Temik	2.31 c	1.69 d	2.93 d	5.96 c	5.61 d	6.3 d
F-value	*61.80*	*1.56*		*55.17*	*0.98*	
df	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*<0.0001*	*0.2049*		*< 0.001*	*0.4043*	
Herbicide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	7.21 b	6.40 c	8.02 bc	11.5 c	10.4 d	12.7 cd
Cotoran	9.91 a	10.6 ab	9.23 bc	15.6 ab	17.4 ab	13.7 cd
Cotoran + Dual Mag.	8.95 ab	8.86 bc	9.03 bc	14.0 bc	14.1 bc	13.8 bcd
Cotoran + Reflex	10.6 a	12.6 a	8.63 bc	16.6 a	19.6 a	13.7 cd
F-value	*4.01*	*2.63*		*5.74*	*3.82*	
df	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*0.0098*	*0.0550*		*0.0012*	*0.0125*	

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Table 4. The effects of insecticide and herbicide treatments on average plant biomass and the average vigor score per plot. Analyses are shown across and within years.

Treatment	Biomass (g)			Vigor*
	22-23 DAP	22 DAP	23 DAP	26-27 DAP
Insecticide	2013-2014	2013	2014	2013-2014
Non-treated	1.68 d	1.35 f	2.01 d	2.18 d
Cruiser	1.84 c	1.41 ef	2.28 c	2.45 c
Gaucho	2.03 b	1.48 ef	2.58 b	3.11 b
Temik	2.33 a	1.63 e	3.02 a	3.41 a
F-value	*24.42*	*7.78*		*75.24*
df	*3,93*	*3,93*		*3,93*
P-value	*< 0.0001*	*< 0.0001*		*< 0.0001*
Herbicide	2013-2014	2013	2014	2013-2014
Non-treated	1.99 ab	1.49 ab	2.48 a	2.93 a
Cotoran	2.11 a	1.63 a	2.59 a	2.84 ab
Cotoran + Dual Mag.	1.86 b	1.32 b	2.41 a	2.66 c
Cotoran + Reflex	1.91 b	1.42 ab	2.41 a	2.71 bc
F-value	*3.67*	*0.22*		*3.68*
df	*3,93*	*3,93*		*3.93*
P-value	*0.0151*	*0.8825*		*0.0149*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

*Vigor per plot rated on a (0-5) scale with 5 being most vigorous.

Table 5. The effects of insecticide and herbicide treatments on thrips injury per plot. Analyses are shown across and within years.

Treatment	Thrips Injury*					
	20-22 DAP	22 DAP	20 DAP	26-27 DAP	27 DAP	26 DAP
Insecticide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	3.66 a	3.89 a	3.44 b	4.27 a	4.11 b	4.43 a
Cruiser	3.21 b	3.34 b	3.07 c	4.01 b	3.93 b	4.09 b
Gaucho	2.01 c	2.11 d	1.92 de	2.56 c	2.56 c	2.56 c
Temik	1.53 d	1.31 f	1.74 e	1.94 d	1.64 e	2.24 d
F-value	*254.20*	*9.50*		*280.72*	*3.54*	
df	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*< 0.0001*	*< 0.0001*		*< 0.0001*	*0.0178*	
Herbicide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	2.43 c	2.38 c	2.48 bc	3.04 b	2.76 b	3.33 a
Cotoran	2.53 bc	2.51 bc	2.55 bc	3.10 b	2.96 b	3.23 a
Cotoran + Dual Mag.	2.68 ab	2.89 a	2.47 bc	3.30 a	3.26 a	3.34 a
Cotoran + Reflex	2.77 a	2.86 a	2.67 ab	3.34 a	3.25 a	3.43 a
F-value	*5.85*	*3.68*		*4.84*	*2.39*	
df	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*0.0011*	*0.0148*		*0.0036*	*0.0739*	

Means not followed by a common letter are significantly different.

DAP = Days after planting.

*Thrips injury rated per plot on a (0-5) scale with 5 being the most injury.

Table 6. The effects of insecticide and herbicide treatments on the number of blooms per hectare, the number of nodes above a first position white flower (NAWF) per 10 plants and final seed cotton yield in (Kg/Ha). Analyses are shown across and within years for NAWF and yield.

Treatment	Blooms	NAWF			Seed Cotton		
	72 DAP	74-86 DAP	74 DAP	86 DAP	178-187 DAP	187 DAP	178 DAP
Insecticide	2014	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	13,377 b	6.93 a	7.51 a	6.35 b	2,957 b	3,263 b	2,651 c
Cruiser	17,903 b	6.86 a	7.54 a	6.19 b	2,969 b	3,296 b	2,641 c
Gaucho	26,494 a	6.55 b	7.43 a	5.68 c	3,349 a	3,409 ab	3,290 b
Temik	26,609 a	6.53 b	7.39 a	5.68 c	3,469 a	3,556 a	3,381 ab
F-value	*14.49*	*7.55*	*3.73*		*19.02*	*5.51*	
df	*3,45*	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*< 0.0001*	*0.0001*	*0.0139*		*< 0.0001*	*0.0016*	
Herbicide	2014	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	21,189 ab	6.77 a	7.47 a	6.07 b	3,267 a	3,445 a	3,089 bc
Cotoran	25,543 a	6.63 a	7.38 a	5.89 b	3,261 a	3,425 a	3,097 bc
Cotoran + Dual Mag.	19,315 b	6.79 a	7.55 a	6.04 b	3,077 b	3,276 ab	2,878 c
Cotoran + Reflex	18,335 b	6.68 a	7.47 a	5.89 b	3,139 ab	3,377 a	2,900 c
F-value	*3.43*	*1.02*	*0.23*		*2.42*	*0.29*	
df	*3,45*	*3,93*	*3,93*		*3,93*	*3,93*	
P-value	*0.0249*	*0.3872*	*0.8723*		*0.0713*	*0.8316*	

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Table 7. The effects of herbicide treatments on the concentration (PPB) of thiamethoxam and its metabolites in the third true leaf of cotton, field study, 2013.

Herbicide Treatment	Cloth.	Clot. TMG	Clot. TZMU	Clot. TZNG	Thiam.	Total
Non-treated	891.3 b	75.8 a	60.9 b	297.5 a	2,700 b	4,026 b
Cotoran	921.8 b	28.3 a	85.9 ab	0.0 b	3,920 b	4,956 b
Cotoran + Dual Mag.	1370 a	69.2 a	108.6 a	125.0 ab	5,470 a	7,143 a
Cotoran + Reflex	847.0 b	32.8 a	65.8 b	0.0 b	3,550 b	4,496 b
F-value	*6.38*	*1.69*	*4.30*	*5.54*	*5.81*	*5.14*
df	*3, 9*	*3, 9*	*3, 9*	*3, 9*	*3, 9*	*3, 9*
P-value	*0.0131*	*0.2380*	*0.0384*	*0.0197*	*0.0172*	*0.0243*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Table 8. The effects of insecticide and herbicide treatments on plant emergence, plant injury, average above ground biomass per plant, and total neonicotinoid concentrations (PPB) found in leaves of plants from the greenhouse study, 2013.

Treatment	Emerged Plants* 7 DAP	Injured Plants* 7 DAP	Biomass (g) 21 DAP	Total Insecticide Concentrations 21 DAP
Insecticide	2013	2013	2013	2013
Cruiser	9.06 a	2.25 b	1.80 a	17,563 b
Gaucho	9.00 a	3.36 a	1.81 a	51,694 a
F-value	*0.02*	*11.43*	*0.01*	*21.85*
df	*1,20*	*1,20*	*1,20*	*1,20*
P-value	*0.8837*	*0.0030*	*0.9151*	*0.0001*
Herbicide				
Non-treated	9.25 a	1.13 c	2.19 a	25,988 a
Cotoran	9.13 a	2.10 bc	1.52 b	48,961 a
Dual Magnum	9.13 a	2.38 b	1.68 b	28,500 a
Reflex	8.63 a	5.63 a	1.82 ab	35,065 a
F-value	*0.46*	*36.80*	*4.04*	*1.87*
df	*3,20*	*3,20*	*3,20*	*3,20*
P-value	*0.7166*	*< 0.0001*	*0.0213*	*0.1668*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

* Number of plants out of a total of 12 per pot.

Part II

Potential Interactions of Pre-emergence Herbicides and Fungicide Seed Treatments on Seedling Disease Control in Cotton

Abstract

Seed and seedling pathogens are the most important diseases affecting cotton in Tennessee. Commercial cotton seed varieties planted in Tennessee come with a base fungicide treatment, and producers often elect to add additional fungicide seed treatments. Herbicides may injure seedling plants, providing entry routes for plant pathogens or delay the growth of seedlings, and extend the window of susceptibility to seedling diseases. Thus, the increasing use of pre-emergence herbicides to combat glyphosate-tolerant weeds, especially Palmer amaranth (*Amaranthus palmeri*), may affect the performance of fungicide seed treatments. Field studies were done in 2013 and 2014 to investigate how commonly used pre-emergence herbicides might impact the occurrence of seedling disease or the performance of selected fungicide seed treatments in cotton. Although there was minimal effects of herbicide treatments on plant stands, some herbicide treatments were negatively affecting plant health, especially combinations with Reflex or Dual Magnum. Fungicide seed treatment improved stand density in all experiments, and data indicated at least some seed treatments increased the vigor and biomass of seedling plants. Generally, Trilex Advanced and Dynasty CST treatments provided the best level of protection against seedling disease, primarily *Rhizoctonia*. Under adverse weather conditions and when a high incidence of seedling disease was observed, Dynasty CST provided better protection from seedling diseases than Trilex Advanced, which was reflected in stand counts, mid-season bloom counts, and yield. While there was clear evidence that fungicide seed treatments and pre-emergence herbicides could both affect plant health, there was little evidence of interactions between these two factors. Pre-emergence herbicides may compound the negative effects of seedling disease, but my data suggests that effects on plant health would be additive in nature and that the efficacy of seedling disease control was the primary factor affecting final yield. This data reinforces the need of

fungicide seed treatments in order to produce a viable plant stand, especially in cool, wet planting conditions conducive to poor seedling emergence and seed and seedling pathogens.

Introduction

Seed and seedling pathogens are the most important diseases affecting cotton in Tennessee (Kelly 2015; Newman 1996). These diseases cause annual yield losses in Tennessee that have been estimated from 3-22% from 1996 to 2011 (Kelly 2015). All commercial cotton seed planted in the US comes treated with base fungicide seed treatments including preventative formulations such as strobilurins and curative formulations such as triazoles. Additional fungicide treatments are often made to further enhance the control of seed and seedling disease. Historically, these treatments may include in-furrow spray or granular applications. More recently, additional fungicide seed treatments have all but replaced the use of in-furrow treatments (Kelly 2015). These treatments may be applied at the wholesale or retail level. Seed treatments typically provide adequate protection from seedling diseases (Newman 1996). Common seedling diseases include *Rhizoctonia solani*, *Pythium spp*., and *Thielaviopsis basicola* (Kelly 2015; Newman 1996). Seedling diseases often reduce the general health and vigor of plants and potentially make the plants more susceptible to other biotic or abiotic stressors (Wrather and Sweets 2009).

Pre-emergence herbicides, which are being more commonly used in Tennessee during recent years because of the development of glyphosate-resistant weeds, (Merchant et al. 2014; Prince et al. 2012; Sosnoskie and Culpepper 2014; Whitaker et al. 2011a; Whitaker et al. 2011b) are one factor that may impact seedling diseases or the performance of fungicide treatments. These herbicides can negatively affect plant health and vigor (Main 2012). Herbicide injury may provide routes of disease entry into plants or delay the growth of seedlings, thus extending the window of susceptibility. This study was done to investigate how commonly used pre-emergence herbicides might impact the occurrence of seedling disease or the performance of selected fungicide seed treatments in cotton.

Materials and Methods

Experimental Design

Three identical experiments were done in 2013 and 2014 at the Milan Research and Education Center in Milan, TN. The tests were conducted as a factorial of four fungicide treatments: non-treated, Apron Maxx® (mefenoxam, fludioxonil; Syngenta, Greensboro, NC), Dynasty CST® (mefenoxam, fludioxonil, azoxystobin; Syngenta), and Trilex Advanced® (trifloxystrobin, triadimenol, metalaxyl; Bayer Crop Science, Raleigh, NC) and four pre-emergence herbicide treatments: non-treated, Cotoran 4L® (fluometuron; DuPont, Eleutherian Mills, DE), Cotoran 4L® (fluometuron) plus Reflex® (fomesafen; Syngenta), and Cotoran 4L® plus Dual Magnum® (S-metolachlor; Syngenta). Phytogen 375 WRF® was planted on May 14, 2013 and Phytogen 333 WRF® was planted on May 13, 2014 and May 28, 2014 at a seeding rate of 13.2 seed per meter and at a depth of 1.9 cm. Plots were four rows wide (102-cm spacing) and 10.7 m long with four replications for each treatment arranged in a randomized complete block design. A strain of *Rhizoctonia* AG2-2 IIIB was grown on millet seed and inoculated at 1-2 gm/0.3 m of row during planting in 2013 and the first planting in 2014. Each plot received a standard insecticide seed treatment of Cruiser 5F® (thiamethoxam at 0.375 mg ai/seed; Syngenta) in 2013 or Gaucho 600® (imidacloprid at 0.375 mg ai/seed; Bayer Crop Science) in 2014. Fungicide seed treatments were applied at the following rates: Apron Maxx (88.7 ml /45.4 kg of seed), Dynasty CST (103.5 ml/45.4 kg of seed) and Trilex Advanced (47.3 ml/45.4 kg of seed). Pre-emergence herbicide treatments were applied at the following rates: fluometuron (Cotoran) (2.84 L/ha), fluometuron (Cotoran) (2.84 L/ha) fomesafen (Reflex) (1.13 L/ha), and fluometuron (Cotoran) (2.84 L /ha) s-metolachlor (Dual Magnum) (1.42 L/ha) using a CO_2 backpack sprayer calibrated to deliver 151 L per hectare using XR 8002 flat fan nozzles at 275 kPa on May 15 in 2013. Herbicides were applied similarly in 2014 on May 15 for the first planting and May 29 for the second planting. Either Roundup® WeatherMAX® (glyphosate; Monsanto Company, St. Louis, MO) or Gramoxone® (paraquat; Syngenta, Greensboro, NC Syngenta) was used for a burn down application before planting.

Thrips Counts and Plant Biomass

Thrips were collected at the second true leaf stage (20 days after planting, DAP) of cotton growth in 2013, the first true leaf stage (20 DAP) in the early planting of 2014 and third true leaf stage (21 DAP) in the late planting of 2014 to estimate treatment effects on the numbers of thrips present. Five plants per plot were cut at the ground level and placed in plastic bags with the exception of the thrips count in the late planting in 2014 where two plants from each of the four rows for a total of eight plants per plot were collected. Each plant was taken out of the bag and rinsed with ethyl alcohol over a glass container topped with a sieve to collect the thrips. The plastic bag was then rinsed with ethyl alcohol over the sieve to collect any remaining thrips left inside. The sieve was then rinsed with ethyl alcohol into a gridded petri dish and the thrips counted underneath a microscope. The thrips were counted and categorized as either adult or immature and the number recorded. The same plants collected for the thrips counts were used to record above ground biomass for each plot. The fresh weight of each sample was recorded and the samples were put in a refrigerator to await thrips counts.

Thrips Injury, Herbicide Injury and Vigor Ratings

Visual ratings of thrips injury were taken at the third true leaf stage (24 DAP) in 2013, at the second (24 DAP) and fourth true leaf stage (30 DAP) in the early planting of 2014, and at the first (15 DAP) and third true leaf stage (21 DAP) in the late planting of 2014 to estimate the amount of thrips damage on a whole-plot basis. These ratings were based on a 0 – 5 scale where 0 is no injury in the plot and 5 is 100% plant death in the plot.

Because some herbicide injury was apparent in 2013, a visual estimate of percent injury (i.e., leaf burn) on leaf tissue was taken 9 days after application. Plant vigor ratings were taken at the third (24 DAP) and fourth true leaf stage (29 DAP) in 2013, at the second (24 DAP) and fourth true leaf stage (30 DAP) in the early planting of 2014, and at the first (15 DAP) and third true leaf stage (21 DAP) in the late planting of 2014 to evaluate treatment effects on plant health. These ratings were based on a 0 – 5 scale where 0 is no vigor in the plot and 5 is complete stands with highly vigorous plants.

Plant Mapping, Weather and Yield Data

Various plant mapping techniques were conducted to determine treatment effects on plant stands, plant maturity, and yield. Stand counts were taken 15 DAP in 2013, 15 and 30 DAP in the early planting of 2014, and at 9 and 16 DAP in the late planting of 2014 on a per hectare basis. Bloom counts of every white to pinkish bloom in the middle two rows of each plot were taken at the seventeenth node (76 DAP) in the early planting of 2014. The number of nodes above an uppermost first-position white flower was counted for ten random plants per plot at 90 DAP in the early planting of 2014. Weather data was obtained from the NOAA weather station at the West Tennessee Research and Education Center in Milan. DD60s were calculated using the following equation: (((daily maximum temperature + daily minimum temperature) / 2) – 60). Yield data were collected at 189 DAP in 2013, at 182 DAP in the early planting of 2014, and at 166 DAP in the late planting of 2014. Seed cotton weights were collected from the center two rows of each plot with a small-plot cotton picker designed for research plots.

Data Analyses

All data were analyzed using the Proc GLIMMIX procedure of SAS (ver. SAS 9.4; SAS Institute; Cary, NC). When significant interactions across years occurred for at least one main effect, means and mean separation are shown both across and within years. Main effects of insecticide or herbicide treatment were considered to be fixed model effects, and replicates (nested within years) was considered to be a random effects as suggested by Carmer et al. (1989). Fisher's protected LSD (LS Means) at $\alpha = 0.05$ was used as the criteria to separate individual treatment means. Within year mean separation was based on the LS Means for each main effect by year component of the across-year model.

Results

There were many examples of year by main effect interactions. Unless specifically indicated, interactions between the main effects of herbicide and insecticide treatments were not significant ($P > 0.05$), and thus, data for these main effects are presented separately.

Thrips Counts and Plant Biomass

Across years, there was no significant impact from either fungicide treatment ($F = 0.71$; $df = 3, 45$; $P = 0.5504$) or herbicide treatment ($F = 0.64$; $df = 3, 45$; $P = 0.5920$) on the number of thrips found in seedling cotton. However, there was a significant interaction between fungicide and herbicide treatments in the early planting of 2014 ($P = 0.0489$). Numbers ranged from 1.20 – 4.15 immature thrips per plant, depending upon the treatment. Thrips numbers were not particularly high, and the pattern of this interaction is difficult to interpret (Table 1).

Above-ground plant biomass data were collected at 20 or 21 DAP in all three years. There was no significant impact of fungicide seed treatment on plant biomass across years (Table 2), but herbicide treatment had a significant impact where Cotoran plus Dual Magnum reduced biomass compared with all other treatments (Table 2).

Thrips Injury, Herbicide Injury and Vigor Ratings

When analyzed across years, fungicide seed treatments had no significant impact on visual estimates of thrips injury (Table 2). When analyzed across years thrips injury was higher in plots treated with Cotoran plus Dual Magnum and Cotoran plus Reflex compared with plots not treated with pre-emergence herbicides. Fungicide treatment impacted thrips injury ratings take at 30 DAP in 2014, but herbicide treatment had no impact (Table 2). Thrips injury scores tended to be lower in all plots receiving a fungicide seed treatment, but injury was lower in plots treated with Dynasty CST compared with all other treatments (Table 2). Fungicide treatment did not impact thrips injury ratings taken at the first true leaf stage of 2014, but there was an impact from herbicide treatment (Table 2). Higher thrips injury scores were generally given to plots treated with pre-emergence herbicides, and both Cotoran plus Dual Magnum and Cotoran plus Reflex treatments had higher injury scores than plots not treated with pre-emergence herbicides. Thrips injury was higher where Cotoran plus Dual Magnum were applied than where just Cotoran was applied.

A visual estimate of herbicide injury (i.e., % leaf burn) was taken in 2013 because some herbicide injury was apparent. Fungicide treatment had no significant impact on injury ratings taken 9 DAP (Table 2). However, herbicide treatment did impact herbicide injury scores, where Cotoran plus Reflex caused more leaf burn than all other treatments.

Across years, fungicide seed treatments and pre-emergence herbicides impacted vigor ratings taken 21-24 DAP (Table 3). There was also a significance interaction of year and fungicide treatment ($F = 25.65$; $df = 6, 141$; $P < 0.0001$) and year and herbicide treatment ($F = 2.43$; $df = 6, 141$; $P = 0.0287$). In 2013, there was no difference in vigor scores between fungicide treatments, but plots treated with Cotoran plus Dual Magnum had less vigor than other herbicide treatments. Vigor scores were generally low in the early planting of 2014, with relatively large differences among fungicide seed treatments. In the early planting of 2014, the highest vigor scores were in plots treated with Dynasty CST followed by Trilex Advanced, then Apron Maxx. Vigor scores were significantly higher in all plots that received a fungicide treatment compared with the non-treated check (Table 3). Plots treated with Cotoran plus Reflex had more vigor than other treatments. Vigor scores of all treatment combinations were relatively high in the late planting of 2014. There was no difference between fungicide treatments, but plots treated with Cotoran plus Dual Magnum had less vigor than those treated with Cotoran or those not treated with pre-emergence herbicides.

Vigor ratings were taken again at the fourth true leaf stage (29 DAP) in 2013. Fungicide and herbicide treatments impacted vigor (Table 3). Differences in vigor ratings among fungicide seed treatments were relatively small, but non-treated plots and those treated with Trilex Advanced had lower vigor than those treated with Apron Maxx. There was also a significant impact from fungicide treatment in vigor ratings taken at the fourth true leaf stage in the early planting of 2014, but there was no impact from herbicide treatment (Table 3). All fungicide treatments increased vigor scores compared with the non-treated check. Vigor ratings were highest in plots treated with Dynasty CST, followed by Trilex Advanced and Apron Maxx (Table 3). Similar to the subsequent rating, fungicide treatment did not impact vigor scores taken at the first true leaf stage in the late planting of 2014 (Table 3). Similar to vigor ratings taken a week later, there was a significant decrease in vigor scores in the plots treated with Cotoran plus Dual Magnum compared with other herbicide treatments (Table 3).

Plant Mapping, Weather and Yield Data

Stand counts were taken 15 DAP in 2013, 15 DAP in the early planting of 2014 and 16 DAP in the late planting of 2014. Across years, fungicide seed treatment affected plant stand counts taken 15 or 16 DAP, but herbicide treatment had no significant impact (Table 4). There was also a significant year by fungicide interaction ($F = 16.06$; $df = 3, 141$; $P < 0.0001$). In 2013, plots treated with Dynasty CST and Trilex Advanced had more plants per acre than plots not treated with a fungicide. The Trilex Advanced treatment also had more plants per acre than plots treated with Apron Maxx. Seed and seedling diseases were very prevalent in the early planting of 2014. All fungicide seed treatments increased plant stands. The highest stand counts were observed in plots treated with Dynasty CST followed by Trilex Advanced and Apron Maxx (Table 4). Fungicide seed treatments also tended to increase plant stands in the late planting of 2014, and stand counts in plots treated with Trilex Advanced were higher than plots not treated with fungicide (Table 4).

A final stand count was taken 30 DAP in the early planting of 2014 because treatment effects were so evident. As with the previous count, all fungicide treatments increased the number of plants per hectare (Table 4). The Dynasty CST treatment was superior to Trilex Advanced and Apron Maxx. Again, herbicide treatments had no effect on final plant populations.

Differences were observed between years and plantings in precipitation and temperature. In 2013, 18.3 mm of rain occurred during the first five days after planting. In 2014, the early and late planting accumulated 82.8 and 6.4 mm of rainfall, respectively (Figure 1). A total of 63.5 DD60s accumulated in the five days after planting during 2013, while 12 and 82.5 DD60s were accumulated in this same time frame in 2014 for the early and late planting, respectively.

Fungicide treatment impacted the number of blooms present at 76 DAP in the early planting of 2014, but there was no significant impact from herbicide treatment (Table 5). The numbers of blooms per hectare were highest in plots treated with Dynasty CST, followed by Trilex Advanced and Apron Maxx (Table 5).

Fungicide seed treatment impacted the number of nodes of white flower (NAWF) on counts collected 90 DAP in the early planting of 2014 (data not shown; $F = 8.90$; $df = 3, 24$; $P = 0.0004$). There was a significant decrease in NAWF in plots treated with Dynasty CST compared with Trilex Advanced and Apron Maxx. Herbicide treatment had no significant effect ($F = 1.43$; $df = 3, 24$; $P = 0.2577$). However, NAWF could not be estimated in plots not treated with a fungicide seed treatment or plots that received an application of Cotoran plus Dual Magnum because plots were so delayed that white flowers were not present at the time of rating (data not shown).

Fungicide treatment significantly affected seed cotton yields, but there was no significant impact of herbicides (Table 5). However, there was a significant interaction between fungicide treatment and year ($F = 13.13$; $df = 6, 135$; $P < 0.0001$). In 2013 and the late planting of 2014 there was no significant effect of fungicide seed treatment on yield. In contrast, fungicide seed treatments dramatically improved yield in the early planting of 2014 where the incidence of disease was substantially higher. All fungicide treatments increased yield. The highest yield was in the plots that were treated with Dynasty CST (3,008 Kg/Ha), followed by Trilex Advanced (2,673 Kg/Ha), and Apron Maxx (2,001 Kg/Ha).

Discussion

Seedling diseases are the number one disease problem of cotton in Tennessee (Kelly 2015, Newman 1996). Therefore, commercial cotton seed varieties planted in Tennessee come with a base fungicide treatment, and producers often elect to add additional fungicide seed treatments. Due to the presence of glyphosate-resistant weeds, pre-emergence herbicides are being applied more commonly and at higher rates on Tennessee cotton acres and in much of the Mid-South and Southeast (Merchant et al. 2014; Prince et al. 2012; Sosnoskie and Culpepper 2014; Whitaker et al. 2011a; Whitaker et al. 2011b). This component of the research investigated potential negative effects and interactions of selected pre-emergence herbicides on the performance of fungicide seed treatments.

Although there was minimal effects of herbicide treatments on plant stands (Table 4), it was apparent that some herbicide treatments were negatively affecting plant health. Pre-emergence herbicides, particularly the treatment containing fomesafen (Reflex), caused leaf chlorosis of seedling leaf tissue in 2013, but this was not apparent in the other tests. However, the Cotoran plus Dual Magnum treatment consistently reduced seedling biomass and vigor (Tables 2 and 3), similar to that observed in Chapter II.

The main effect of fungicide seed treatment affected stand density in all experiments, and my data indicated at least some fungicide seed treatments increased vigor and biomass of seedling plants. Generally, Trilex Advanced and Dynasty CST treatments provided the best level of protection against seedling disease, primarily *Rhizoctonia solani*, in these tests. However, in the early planting of 2014 when a very high incidence of seedling disease was observed, Dynasty CST provided better protection from seedling disease than Trilex Advanced.

Besides stand and vigor ratings, this increased level of protection was reflected in bloom counts and seed cotton yield (Table 5).

Apron Maxx is not labeled or recommended for use on cotton, in part because it lacks a strobilurin component. It was included to provide an intermediate level of seedling protection, which it did. It should be mentioned that Trilex Advanced and Dynasty CST are common, additional treatments made to cotton seed, but all commercial cotton seed comes with a base fungicide seed treatment. Base treatments were not used in these tests to increase the likelihood of seeing variable levels of plant protection, hopefully improving the possibility of detecting potential interactions between pre-emergence herbicides and fungicide seed treatments. Despite having significant effects of both herbicide and fungicide seed treatments, very little indication of interactions between these factors was evident. Thus, it appears these two factors acted independently in this experiment.

The main effect of fungicide seed treatments was much more pronounced in the early planting of 2014. Indeed, the late planting was made over concerns that the first planting would fail to establish. The variable response of fungicide seed treatments across years was likely due to environmental conditions that occurred immediately after planting. During the early planting of 2014, a rainfall event (2.20 inches) occurred the day after planting (Figure 1). Temperature cooled substantially, and there was minimal accumulation of DD60s in the 5 days after planting (Figure 2). These are poor emergence conditions and optimal for the development of seedling diseases in cotton (Newman 1996). The results of my tests, where there was increased response to fungicide seed treatments under conditions of poor seedling emergence, is consistent with most previous research (Rothrock et al. 2012).

In this study, a base insecticide seed treatment was applied to all seed prior to treating with fungicide. I used thiamethoxam (Cruiser) in 2013 but elected to use imidacloprid (Gaucho) in 2014 because Cruiser was consistently showing diminishing levels of thrips control in these and other experiments. Across years and in the early planting of 2014 in particular, I observed less thrips injury in plots treated with Dynasty CST. This probably resulted from these plants growing more vigorously, and thus being less susceptible to thrips injury. As seen in Chapter II, there was a trend of increased thrips injury where Dual Magnum or Reflex was applied pre-emergence. It is unclear whether this represents an actually reduction in thrips injury or whether symptoms herbicide injury was confused with thrips injury during the rating process. Regardless, these differences were relatively modest, in part because an insecticide seed treatment was used in all treatments.

The pre-emergence herbicides used in this test are commonly used in Tennessee. I used near maximum labeled rates to maximize the likelihood of inflicting crop injury and observing interactions with fungicide treatments used for seedling disease control. Also, Reflex was used post-planting which is not recommended in Tennessee because of the threat to crop injury. A small trend of decreased stand density was noted, especially with the Cotoran treatment, but these differences were relatively minor and difficult to explain. In summary, there was clear evidence that fungicide seed treatments and pre-emergence herbicides could both affect plant health. However, there was little evidence of interactions between these two factors. Pre-emergence herbicides may compound the negative effects of seedling disease, but my data suggests that effects on plant health would be additive in nature. Although pre-emergence herbicides are an aggravating factor, results in these tests indicate that the efficacy of seedling disease control was the primary factor affecting the yield of cotton. This is similar to the conclusions summarized in Chapter II regarding insecticide treatments for thrips control. My data reinforces the need of fungicide seed treatments in order to produce a viable plant stand, especially in cool, wet planting conditions.

Literature Cited

Carmer, S.G., W.E. Nyquist, and W.M. Walker. 1989. Least significant differences for combined analyses of experiments with two- or three-factor treatment designs. Agronomy Journal 81(4):665-672.

Kelly, H.M. 2015. Cotton disease and nematode control 2015. University of Tennessee Extension, Knoxville, TN.

Main, C.L., J.C. Faircloth, L.E. Steckel, A.S. Culpepper, and A.C. York. 2012. Cotton tolerance to fomesafen applied preemergence. J Cotton Sci 16:80-87.

Merchant, R.M., A.S. Culpepper, P.M. Eure, J.S. Richburg, and L.B. Braxton. 2014. Controlling glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in cotton with resistance to glyphosate, 2, 4-D, and glufosinate. Weed Technology 28:291-297.

Newman, M.A. 1996. Cotton Diseases. p. 16-18. *In* cotton production in Tennessee. Publ. 1514. University of Tennessee Extension, Knoxville, TN.

Prince, J.M., D.R. Shaw, W.A. Givens, M.E. Newman, M.D.K. Owen, S.C. Weller, B.G. Young, R.G. Wilson, and D.L. Jordan. 2012. Benchmark Study: III. Survey on changing herbicide use patterns in glyphosate-Resistant cropping systems. Weed Technology 26:536-542.

Rothrock, C.S., S.A. Winters, P.K. Miller, E. Gbur, L.M Verhalen, B.E. Greenhagen, T.S. Isakeit, W.E. Batson Jr., F.M. Bourland, P.D. Colyer, T.A. Wheeler, H.W. Kaufman, G.L. Sciumbato, P.M. Thaxton, K.S. Lawrence, W.S. Gazaway, A.Y. Chambers, M.A. Newman, T.L. Kirkpatrick, J.D. Barham, P.M. Phipps, F.M. Shokes, L.J. Littlefield, G.B. Padgett, R.B. Hutmacher, R.M. Davis, R.C. Kemerait, D.R. Sumner, K.W. Seebold Jr., J.D. Mueller, and R.H. Garber. 2012. Importance of fungicide seed treatment and environment on seedling diseases of cotton. Plant Disease 96(12):1805-1817.

Sosnoskie, L.M., and A.S. Culpepper. 2014. Glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. Weed Science 62:393-402.

Wrather A., and L. Sweets. 2009. Cotton seedling diseases: answers to frequently asked questions. Publ. MP 734. University of Missouri Extension, Columbia, MO.

Whitaker, J.R., A.C. York, D.L. Jordan, and A.S. Culpepper. 2011a. Weed management with glyphosate- and glufosinate-based systems in PHY 485 WRF cotton. Weed Technology 25:183-191.

Whitaker, J.R., A.C. York AC, and D.L. Jordan, A.S. Culpepper, and L.M. Sosnoskie. 2011b. Residual herbicides for palmer amaranth control. J. Cotton Sci. 15:89-99.

Appendix II

Table 1. Effects of fungicide seed treatments and pre-emergence herbicides on numbers of immature thrips in the early planting of 2014 at 20 days after planting.

Treatment	No. of Immature Thrips per Plant			
	Herbicide			
Fungicide	Non-treated	Cotoran	Cotoran + Dual Mag.	Cotoran + Reflex
Non-treated	1.90 bc	4.15 a	1.10 c	2.50 abc
Apron Maxx	2.15 bc	2.55 abc	2.15 bc	2.70 abc
Dynasty CST	1.20 c	1.65 c	2.85 abc	1.45 c
Trilex Advanced	3.75 ab	2.00 bc	1.80 bc	1.50 c
F-value	*2.11*			
df	*9, 45*			
P-value	*0.0489*			

Means not followed by a common letter are significantly different.

Table 2. The effects of fungicide seed treatments and pre-emergence herbicides on above ground biomass per plant, thrips injury per plot, and herbicide injury ratings (i.e., leaf burn) as a percentage. Analyses are shown across tests for biomass, across and within tests for thrips and with the test at 9 DAP in 2013 for herbicide injury.

Treatment	Biomass (g)	Thrips Injury*			Herbicide Injury
	20-21 DAP	21-24 DAP	30 DAP	15 DAP	9 DAP
<u>Fungicide</u>	<u>2013-2014</u>	<u>2013-2014</u>	<u>2014 E</u>	<u>2014 L</u>	<u>2013</u>
Non-treated	1.20 a	2.63 a	2.51 a	1.48 a	7.06 a
Apron Maxx	1.21 a	2.60 ab	2.44 a	1.46 a	5.88 a
Dynasty CST	1.27 a	2.54 b	2.24 b	1.48 a	5.81 a
Trilex Advanced	1.27 a	2.57 ab	2.46 a	1.42 a	8.75 a
F-value	*1.55*	*1.47*	*5.55*	*1.92*	*1.34*
df	*3,141*	*3,141*	*3,45*	*3,45*	*3,45*
P-value	*0.2050*	*0.2240*	*0.0025*	*0.1398*	*0.2726*
<u>Herbicide</u>	<u>2013-2014</u>	<u>2013-2014</u>	<u>2014 E</u>	<u>2014 L</u>	<u>2013</u>
Non-treated	1.25 a	2.51 b	2.40 a	1.41 c	2.63 b
Cotoran	1.29 a	2.56 ab	2.37 a	1.45 bc	2.50 b
Cotoran + Dual Mag.	1.14 b	2.64 a	2.41 a	1.52 a	5.56 b
Cotoran + Reflex	1.27 a	2.62 a	2.46 a	1.47 ab	16.8 a
F-value	*5.36*	*3.37*	*0.61*	*4.80*	*32.54*
df	*3,141*	*3,141*	*3,45*	*3,45*	*3,45*
P-value	*0.0016*	*0.0204*	*0.6107*	*0.0055*	*< 0.0001*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

*Thrips injury rated per plot on a (0-5) scale with 5 being the most injury.

Table 3. The effects of fungicide seed treatments and pre-emergence herbicides on the average vigor score per plot. Analyses are shown across and within tests at 21-24 DAP and within tests for remaining DAP.

Treatment	Vigor*						
	21-24 DAP	21-24 DAP			29 DAP	30 DAP	15 DAP
Fungicide	2013-2014	2013	2014 E	2014 L	2013	2014 E	2014 L
Non-treated	2.38 d	2.45 a	0.59 f	4.09 a	2.42 b	0.71 c	2.62 a
Apron Maxx	2.56 c	2.57 a	1.03 e	4.09 a	2.56 a	1.34 b	2.61 a
Dynasty CST	2.96 a	2.57 a	2.18 c	4.13 a	2.51 ab	2.60 a	2.77 a
Trilex Advanced	2.70 b	2.62 a	1.34 d	4.13 a	2.44 b	1.56 b	2.71 a
F-value	*32.98*		*25.65*		*3.74*	*61.57*	*0.73*
df	*3, 141*		*6, 141*		*3, 45*	*3, 45*	*3, 45*
P-value	*< 0.0001*		*< 0.0001*		*0.0175*	*< 0.0001*	*0.5407*
Herbicide	2013-2014	2013	2014 E	2014 L	2013	2014 E	2014 L
Non-treated	2.72 a	2.71 c	1.24 f	4.22 a	2.55 a	1.59 a	2.80 a
Cotoran	2.66 a	2.63 c	1.19 f	4.16 a	2.54 a	1.44 a	2.83 a
Cotoran + Dual Mag.	2.50 b	2.32 d	1.24 f	3.94 b	2.36 b	1.62 a	2.36 b
Cotoran + Reflex	2.71 a	2.55 c	1.46 e	4.13 ab	2.48 a	1.57 a	2.71 a
F-value	*5.93*		*2.43*		*6.37*	*0.63*	*5.66*
df	*3, 141*		*6, 141*		*3, 45*	*3, 45*	*3, 45*
P-value	*0.0008*		*0.0287*		*0.0011*	*0.5974*	*0.0022*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

*Vigor per plot rated on a (0-5) scale with 5 being the most vigorous.

Table 4. The effects of fungicide seed treatments and pre-emergence herbicides on number of plants per hectare. Analyses are shown across and within tests at 15-16 DAP and within the test at 30 DAP in the early planting of 2014.

Treatment	No. of Plants per Hectare				
	15-16 DAP	15 DAP	16 DAP	16 DAP	30 DAP
Fungicide	2013-2014	2013	2014 E	2014 L	2014 E
Non-treated	72,986 d	103,525 d	12,598 h	102,833 d	9,456 c
Apron Maxx	78,799 c	106,956 bcd	24,505 g	104,938 cd	19,460 b
Dynasty CST	91,234 a	111,424 ab	53,651 e	108,628 abcd	49,500 a
Trilex Advanced	85,190 b	113,039 a	32,317 f	110,214 abc	24,966 b
F-value	*42.41*		*16.06*		*66.36*
df	*3, 141*		*6, 141*		*3, 45*
P-value	*< 0.0001*		*< 0.0001*		*< 0.0001*
Herbicide	2013-2014	2013	2014 E	2014 L	2014 E
Non-treated	83,662 a	109,637 a	31,971 b	109,378 a	26,696 a
Cotoran	80,231 b	107,302 a	28,368 b	105,024 a	24,793 a
Cotoran + Dual Mag.	82,259 ab	108,109 a	31,943 b	106,725 a	25,687 a
Cotoran + Reflex	82,057 ab	109,896 a	30,789 b	105,486 a	26,206 a
F-value	*1.35*		*0.26*		*0.15*
df	*3, 141*		*6, 141*		*3, 45*
P-value	*0.2608*		*0.9559*		*0.9282*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Table 5. The effects of fungicide and herbicide treatments on the number of blooms per hectare at 76 DAP in the early planting of 2014 and seed cotton yield (Kg/Ha) across and within tests.

Treatment	Blooms 76 DAP	Seed Cotton 166-189 DAP	Seed Cotton 189 DAP	182 DAP	166 DAP
Fungicide	2014 E	2013-2014	2013	2014 E	2014 L
Non-treated	4,440 d	2,379 c	3,523 a	1,378 f	2,237 de
Apron Maxx	8,678 c	2,610 b	3,561 a	2,001 e	2,267 de
Dynasty CST	22,746 a	2,971 a	3,678 a	3,008 b	2,227 de
Trilex Advanced	13,117 b	2,892 a	3,622 a	2,673 c	2,382 cd
F-value	*29.01*	*17.77*		*13.13*	
df	*3, 45*	*3, 135*		*6, 135*	
P-value	*< 0.0001*	*< 0.0001*		*< 0.0001*	
Herbicide	2014 E	2013-2014	2013	2014 E	2014 L
Non-treated	12,368 a	2,722 a	3,715 a	2,133 b	2,317 b
Cotoran	10,465 a	2,726 a	3,645 a	2,134 b	2,401 b
Cotoran + Dual Mag.	12,541 a	2,668 a	3,449 a	2,402 b	2,152 b
Cotoran + Reflex	13,607 a	2,736 a	3,575 a	2,391 b	2,243 b
F-value	*0.81*	*0.23*		*1.83*	
df	*3, 45*	*3, 135*		*6, 135*	
P-value	*0.4975*	*0.8744*		*0.0977*	

Means not followed by a common letter are significantly different.

DAP = Days after planting.

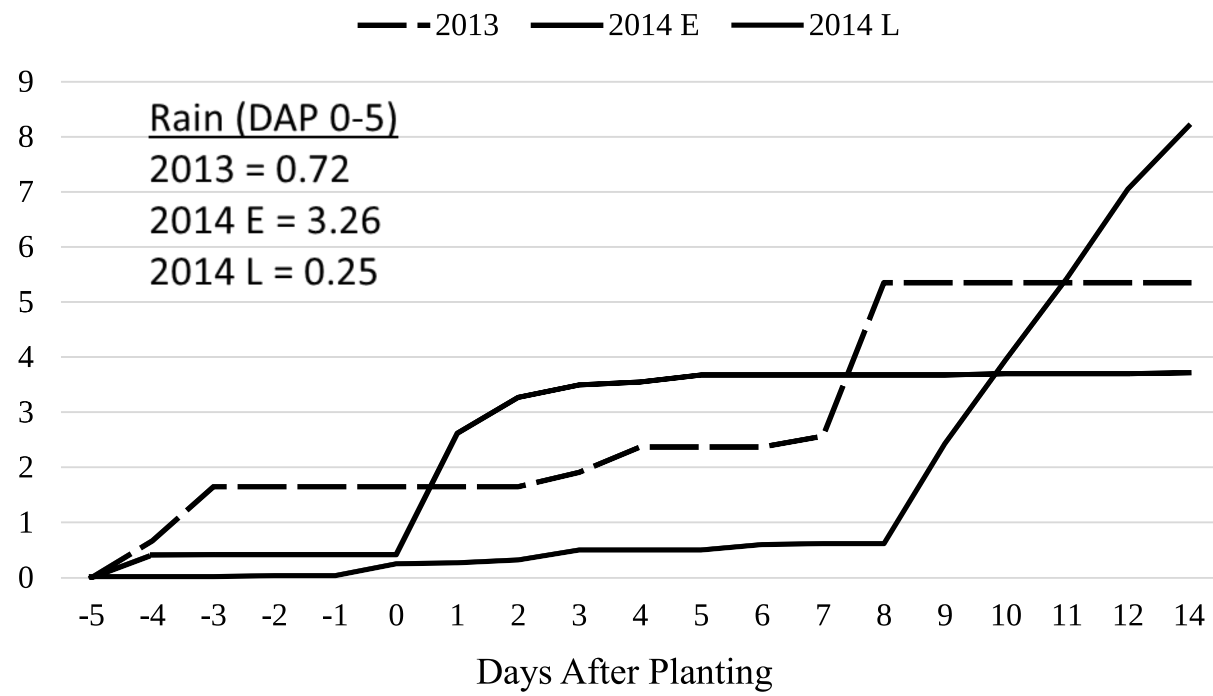

Figure 1. Cumulative precipitation (in.) for the planting dates in 2013, the 2014 early planting date, and the 2014 late planting of the fungicide study.

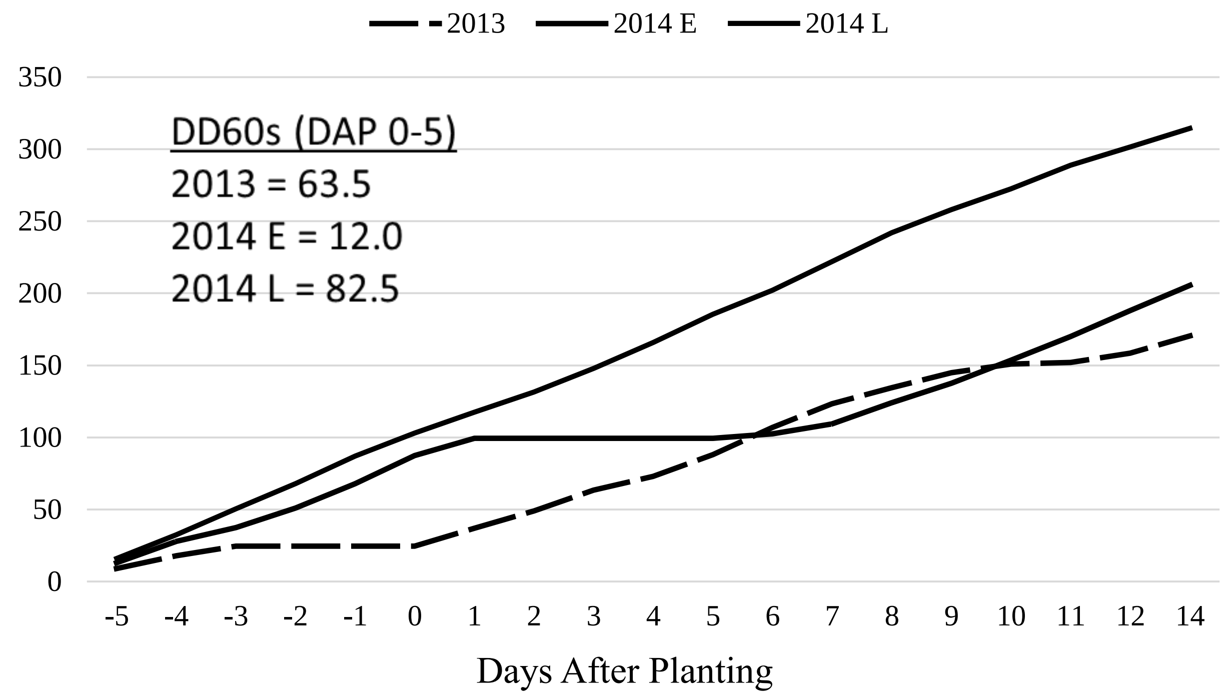

Figure 2. Cumulative DD60s for the planting dates in 2013, the 2014 early planting date, and the 2014 late planting of the fungicide study.

Part III

Potential Interactions of Post-emergence Herbicides and Insecticide Seed Treatments on Thrips Control in Cotton

Abstract

The use of post-emergence herbicides has increased dramatically in Tennessee and many other areas of the Cotton Belt during the last 5-7 years in response to weed resistance to glyphosate. Specifically, s-metolachlor (Dual Magnum) and glufosinate (Liberty) are now commonly applied post-emergence to seedling cotton. Both glufosinate, specifically when applied to WideStrike cotton varieties, and s-metolachlor can cause injury to seedling plants. Field studies were done in 2013 and 2014 on WideStrike cotton to investigate possible interactions between these herbicides and insecticide seed treatments used for thrips control. Although a thiamethoxam insecticide seed treatment (Cruiser) did not provide satisfactory control of thrips in the 2013 experiment, and imidacloprid (Gaucho) was used in 2014, the use of an insecticide seed treatments generally improved seedling health as measured by plant vigor and biomass. In 2013, applications of Liberty or Liberty plus Dual Magnum caused much more plant injury as evidenced by low vigor ratings, decreased plant biomass and height, and yield. Plots treated with Liberty or Liberty plus Dual Magnum tended to have had higher thrips injury scores, although these ratings may have been confounded by herbicide injury. In 2014, the results from treating plots with Liberty or Liberty plus Dual Magnum were much less pronounced. While there was clear evidence that thrips and post-emergence herbicides, especially glufosinate, could negatively affect plant health, there was little evidence of interactions between these two factors. These data reinforce concerns about incomplete tolerance of WideStrike cotton to glufosinate, indicating that herbicide injury may result in yield loss. They also indicate, as in Part I of this thesis, that thiamethoxam (Cruiser) is not providing adequate control of thrips.

Introduction

Thrips are a common pest of cotton and are consistently among the top three economically most important pests of cotton grown in Tennessee, with economic damage being inflicted to some fields on an annual basis (Stewart and Lentz 2010). Thrips primarily injure cotton during the seedling stage where feeding on emerging leaves and terminal buds can delay maturity and cause plant death in extreme circumstances (Layton and Reed 2002). Because Tennessee is located on the northern edge of the Cotton Belt, maturity delays can be especially important. Several species of thrips may attack seedling cotton, but tobacco thrips (*Frankliniella fusca*, Thysanoptera: Thripidae) typically composes the vast majority of thrips found in the Mid-South (Stewart et al. 2013). Preventative at-planting treatments, either in-furrow granular or liquid insecticides or seed treatments, are almost always recommended to control thrips infestations in seedling cotton (Stewart et al. 2014). In the last 10 years, insecticide seed treatments such as Gaucho (imidacloprid; Bayer CropScience, Raleigh, NC) or Cruiser (thiamethoxam; Syngenta, Greensboro, NC) have been used almost exclusively in Tennessee for control of thrips.

The use of pre-emergence herbicides as increased dramatically in Tennessee and many other areas of the Cotton Belt during the last 5-7 years in response to weed resistance to glyphosate (e.g., Roundup, Monsanto Co., St Louis, MO), especially Palmer pigweed, *Amaranthus palmeri* (Merchant et al. 2014; Prince et al. 2012; Sosnoskie and Culpepper 2014; Whitaker et al. 2011a; Whitaker et al. 2011b). There has been an increased use of post-emergence herbicides for the same reason. Specifically, Dual Magnum (s-metolachlor; Syngenta, Greensboro, NC) is frequently used. Liberty (glufosinate; Bayer CropScience, Raleigh, NC) is also commonly used on glufosinate tolerant cotton varieties. In both cases, these herbicides are sometimes tank mixed with Roundup (glyphosate; Monsanto Co., St Louis, MO). Cotton cultivars with tolerance to both glufosinate and glyphosate are commonly grown in Tennessee (USDA 2015). Specifically, Phytogen Cottonseed (Dow AgroSciences, Indianapolis, IN) containing the WideStrike® trait have been widely used in Tennessee for the past five years (USDA 2015). The Bt cotton trait in WideStrike® varieties is paired with a phosphinothricin-N-acetyltransferase (PAT) gene. The PAT gene provides partial tolerance to glufosinate applications, but significant crop injury sometimes occurs when applications are made to WideStrike® cottons (Steckel et al. 2012, Stewart et al. 2013). Glufosinate and s-metolachlor are often applied during the seedling stage at the same time that thrips may also be causing injury. Thus, there is a possibility that injury resulting from post-emergence herbicide applications and thrips have additive or interactive effects on plant health and the subsequent yield of cotton. This study was done to evaluate the impacts of commonly used post-emergence herbicides on thrips control provided by insecticide seed treatments.

Materials and Methods

Experimental Design

Two similar experiments were done at the West Tennessee Research and Education Center in Jackson, TN (2013) and the Milan Research and Education Center in Milan, TN (2014) to evaluate how commonly used post-emergence herbicides may interact with the performance of insecticide seed treatments used for thrips control. These tests were designed as a factorial of two insecticide treatments and four herbicide treatments. Insecticide treatments were a non-treated and Cruiser 5F® (thiamethoxam; Syngenta, Greensboro, NC) in 2013 or Gaucho 600® (imidacloprid; Bayer Crop Science, Raleigh, NC) in 2014. The insecticide seed treatment of either Cruiser or Gaucho was applied at a rate of 0.375 mg ai/seed. The four herbicide treatments consisted of a non-treated, Liberty® (glufosinate; Bayer Crop Science), Dual Magnum® (s-metolachlor; Syngenta), and Liberty® (glufosinate) plus Dual Magnum® (s-metolachlor). In all treatments, Liberty and Dual Magnum were applied at a rate of 2.34 L/ha and 1.17 L/ha.

Phytogen 375 WRF® was planted on May 16, 2013 and Phytogen 333 WRF® was planted on May 13, 2014 at a seeding rate of 13.2 seed per meter and at a depth of 1.9 cm. A pre-emergence herbicide treatment of Cotoran® (fluometuron; Makhteshim Agan of North America, Raleigh, NC) and Caporal® (prometryn, Syngenta) was applied as a tank mix to the test area on the day of planting, both at a rate of 1.46 L/ha. Plots were of four rows wide (102-cm spacing) and 10.7 m long with four replications for each treatment arranged in a randomized complete block design. Herbicide treatments were applied with a CO2 backpack sprayer calibrated to deliver 151 L/ha using XR 8002 flat fan nozzles at 275 kPa on June 6, 2013 and June 2, 2014. Broadcast applications of Roundup® WeatherMax® (1.61 L/ha) and hand weeding were subsequently done as needed to maintain a weed-free environment.

Thrips Counts and Plant Biomass

Thrips counts were collected at the third true leaf (27 days after planting, DAP) in 2013 and at the second true leaf (27 DAP) in 2014 to estimate the density of thrips. This was done by clipping five (2013) or eight (2014) plants at random from each plot at the ground level and placing them into 3.79-1 self-sealing plastic bags. Prior to making the above thrips counts, the same plants collected for the thrips counts were used to record above ground biomass for each plot. The weight of each sample was recorded, and the samples were put in a refrigerator to await thrips counts. For each sample, plants were taken out of the bag and rinsed with ethyl alcohol over a glass container topped with a sieve to collect the thrips. The plastic bag was then rinsed with ethyl alcohol over the sieve to collect any remaining thrips. The sieve was then rinsed with ethyl alcohol into a gridded petri dish and the thrips counted underneath a microscope. Numbers of adult and immature thrips were recorded.

Thrips Injury, Herbicide Injury and Vigor Ratings

Thrips injury ratings were taken at the third true leaf (25 DAP) in 2013 and at the second true leaf (24 DAP) in 2014 to convey the amount of thrips damage as a whole, per plot. These ratings were based on a 0 – 5 scale where 0 is no injury in the plot and 5 is 100% plant death in the plot.

A visual estimate of herbicide injury (i.e., % leaf burn) was taken on a per plot basis at the third true leaf (25 DAP, 4 DAA) in 2013 and at the fourth leaf (28 DAP, 8 DAA) in 2014. Vigor ratings of each plot were also taken at the fifth leaf (36 DAP) in 2014 to estimate the relative health of the plot. These ratings were based on a 0 – 5 scale where 0 is no vigor in the plot (essentially no surviving plants) and 5 is complete stands with highly vigorous plants.

Stand Counts, Plant Mapping and Yield

Some plant monitoring was done to document treatment effects on plant health. A final stand count was taken 51 DAP in 2013. The average height of ten random plants per plot were taken at the sixth true leaf (39 DAP) in 2013. Square counts on 1 meter of row were taken at the tenth node (53 DAP) in each plot during 2013. Bloom counts of every white to pinkish bloom in the middle two rows of each plot were also taken at the fifteenth node (83 DAP) in 2013 and (76 DAP) in 2014. Counts of the number of nodes above a first position white flower (NAWF) were made for ten randomly selected plants per plot at 90 DAP in 2014. Seed cotton yield was collected at 151 DAP in 2013 and at 182 DAP in 2014 from the center two rows of each plot with a cotton picker designed for harvesting small, research plots.

Data Analyses

All data were analyzed using the Proc GLIMMIX procedure of SAS (ver. SAS 9.4; SAS Institute; Cary, NC). When significant interactions across years occurred for at least one main effect, means and mean separation are shown both across and within years. Main effects of insecticide or herbicide treatment were considered to be fixed model effects, and replicates (nested within years) was considered to be a random effects as suggested by Carmer et al. (1989). Fisher's protected LSD (LS Means) at $\alpha = 0.05$ was used as the criteria to separate individual treatment means. Within year mean separation was based on the LS Means for each main effect by year component of the across-year model.

Results

There were many examples of year by main effect interactions. Unless specifically indicated, interactions between the main effects of herbicide and insecticide treatments were not significant ($P > 0.05$), and thus, data for these main effects are presented separately.

Thrips Counts and Plant Biomass

For immature and total thrips numbers collected 27 DAP, which was approximately the third true leaf stage, there was a significant impact of insecticide treatment (Table 1) and an interaction between years and insecticide for immature ($F = 27.16$; $df = 1, 45$; $P < 0.0001$) and total thrips ($F = 25.07$; $df = 1, 45$; $P < 0.0001$). In 2013, immature and total thrips numbers were higher in plots treated with an insecticide seed treatment (i.e., Cruiser) compared with the non-treated check. Thrips density was considerably lower during 2014. Thrips numbers were lower in Gaucho-treated plots in 2014, but these differences were not significant. Across both years herbicide treatment did not impact immature thrips numbers (Table 1), there were significant interactions between years and herbicide treatments for both immature (*F* = 3.24; *df* = 3, 45; *P* = 0.0307) and total thrips (*F* = 3.62; *df* = 3, 45; *P* = 0.0200). In 2013, plots treated with Dual Magnum had higher numbers of thrips than other herbicide treatments. Herbicide treatments did not affect thrips density in 2014.

Both insecticide and herbicide treatment had significant impacts on plant biomass (Table 4), and there was no interaction between years and insecticide treatments (*F* = 3.16; *df* = 1, 45; *P* = 0.0821) or herbicide treatments (*F* = 1.52; *df* = 3, 45; *P* = 0.2219). Across years, biomass was higher in plots receiving an insecticide seed treatment. Biomass was reduced in plots that received applications of Liberty and Liberty plus Dual Magnum (Table 4). The biomass of plots only treated with Dual Magnum was not different than the non-treated plots.

Thrips Injury, Herbicide Injury and Vigor Ratings

For thrips injury rated 25 (2013) or 28 (2014) DAP after planting, there was a significant impact of insecticide treatment across years but also an interaction (*F* = 83.84; *df* = 1, 45; *P* < 0.0001) between years and insecticide treatment. In 2013, thrips injury did not differ between Cruiser treated and non-treated plots. In 2014, thrips injury was lower in plots treated with Gaucho than those no having an insecticide seed treatment (Table 2). There was no interaction between herbicide treatment and years (*F* = 0.65; *df* = 3, 45; *P* = 0.5900). When analyzed across years, plots treated with post-emergence herbicides tended to have higher thrips injury scores (*F* = 8.94; *df* = 3, 45; *P* < 0.0001). Specifically, plots treated with Liberty and Liberty plus Dual Magnum were rated as having higher thrips injury scores than those only treated with Dual Magnum (Table 2).

Much more injury (i.e., leaf burn) resulted from application of foliar herbicides in 2013 compared with 2014 (F = 792.6; *df* = 1, 45; P < 0.0001). There was a significant impact of herbicide treatment (Table 2) and a strong interaction between years and herbicide (F = 236.8; *df* = 3, 45; P < 0.0001). In 2013, herbicide injury was 39% where Liberty was applied alone and even higher (46%) when it was tank mixed with Dual Magnum. Visual injury in non-treated plots and those treated only with Dual Magnum was approximately 2 and 9%, respectively. In contrast, there was no significant impact of herbicide treatment on the amount of leaf burn observed during 2014 (Table 2).

Across years, there was no significant impact of insecticide treatment on herbicide injury ratings taken 25 (2013) and 28 (2014) days after planting (Table 2) nor was there an interaction between insecticide treatment and years (P > 0.26). However, there was an interaction between insecticide and herbicide main effects when data were analyzed across years (F = 3.26; *df* = 3, 45; P = 0.0302). When analyzed within year, the interactions between insecticide and herbicide treatments on herbicide injury were not significant or readily apparent (Table 3) (F = 2.56; *df* = 3, 21; P = 0.0826 and (F = 2.53; *df* = 3, 21; P = 0.0846), respectively for 2013 and 2014.

Both insecticide (F = 169.2; *df* = 1, 21; P < 0.0001) and herbicide (F = 8.94; *df* = 3, 21; P = 0.0005) treatment had a significant impact on vigor ratings taken at 36 DAP in 2013. Across years, vigor ratings were higher where an insecticide treatment was applied compared with seed not treated with insecticide. Vigor ratings were statistically similar and lower in plots treated with a foliar-applied herbicide compared with non-treated plots (Table 2). Vigor ratings were not taken in 2013, but herbicide applications containing Liberty were clearly less vigorous during early season as evidenced by biomass measurements (Table 4), reduced plant height, and a reduction in the numbers of squares and blooms (see below).

Stand Counts, Plant Mapping and Yield

Final stand counts were taken 49 (2013) and 51 (2014) DAP. Although there was a trend of increased stand in plots with an insecticide treatment and a reduction in stand in plots where Liberty was applied, across years, there was no significant impact of insecticide (F = 2.02; df = 1, 45; P = 0.1624) or herbicide (F = 0.93; df = 3, 45; P = 0.4323) (data not shown).

There was no also impact of insecticide treatment on plant height measurements take 39 DAP in 2013. There was a significant impact of herbicide treatment on average plant height (F = 9.15; df = 3, 21; P = 0.0005). Plants were shorter in plots that received applications of Liberty (13.7 cm) and Liberty plus Dual Magnum (13.4 cm) compared with plots that received only Dual Magnum (17.8 cm) and the non-treated check (17.1 cm).

There was no significant impact of insecticide treatment on square counts taken at 53 DAP in 2013, but there was a significant impact of herbicide on the number of squares (F = 6.31; df = 3, 21; P = 0.0032). The average number of squares per meter was lower in plots that received applications of Liberty (21.3) and Liberty plus Dual Magnum (19.6) compared with plots that received only Dual Magnum (47.0) and the non-treated check (37.8).

There was no significant interactions among years and treatment factors (P > 0.27 for all). Across both years, there was a 32% increase in the number of blooms for plots treated with an insecticide seed treatment compared with those not receiving insecticide (Table 4). Application of Liberty plus Dual Magnum reduced the number of blooms compared with plots only treated Dual Magnum and the non-treated check. Plots treated only with Liberty also had lower numbers of blooms than plots treated with Dual Magnum and those not treated with herbicide, but this difference was not significant.

Average yield across treatments were about 33% lower in 2013 compared with 2014 (F = 38.0; df = 1, 45; P < 0.0001). There were no interactions between main effects or between years and main effects (P > 0.22 for all). Across years, insecticide seed treatment increased yield by 10.5%, but this difference was not significant (Table 4). When years were analyzed independently, the application of Liberty plus Dual Magnum reduced yield compared with plots not treated with herbicide (F = 4.12; df = 3, 21; P = 0.0191), but when averaged across years, herbicide effects were not significant.

Discussion

The research was done to further investigate the apparent reduction in crop protection provided by insecticide seed treatments during the past several years. Observations in test plots and production fields indicated diminished performance of neonicotinoid seed treatments, and especially thiamethoxam (Cruiser), in reducing thrips populations and thrips injury. One hypothesis for this decreased performance might be the use and interactions of insecticide seed treatments with post-emergence herbicides that are now being used more widely at a relatively high rates in response to glyphosate-resistant weeds. S-metolachlor (Dual Magnum) is known to cause injury often described as leaf spotting or burn when applied to cotton. Glufosinate (Liberty) is known to cause sometimes substantial injury when applied on WideStrike cotton (Steckel et al. 2012; Stewart et al. 2013). Thus, there is a possibility that injury resulting from both post-emergence herbicides and thrips have additive or interactive effects on the health and subsequent yield of cotton. Consequently, this study was done to evaluate the effects and possible impact of commonly used post-emergence herbicides on thrips control provided by insecticide seed treatments.

Plant protection provided by insecticide seed treatments was reflected in other measures of plant health such as vigor, plant biomass, and square and bloom counts. Because of the concern with the apparent declining efficacy of thiamethoxam on thrips, Cruiser was used in my experiment during 2013. However, it did not provide adequate protection against thrips in this study, regardless of herbicide treatment. In fact, more thrips were found in plots treated with Cruiser compared with those not treated with insecticide. The reason for this is uncertain, but there was some initial protection of seedling plants from the use of Cruiser as evidenced by biomass ratings and other plant health parameters. These 'healthier' seedlings may have been more attractive or a better hosts for thrips. Ultimately, there may have been a low chance of observing interactions between the insecticide and herbicide treatments in 2013 considering the generally poor performance of Cruiser in controlling thrips. Imidacloprid (Gaucho) was used as the insecticide seed treatment in 2014 to create a more obvious difference between treated and non-treated plots. Thrips numbers in this test were relatively low, but substantially less thrips injury was observed in insecticide treated plots (Table 2).

The post-emergence herbicides applied in these tests are commonly used in Tennessee (Steckel et. al 2015). I used relatively high rates to increase the likelihood of inflicting crop injury and observing interactions with insecticide treatments used for thrips control. In 2013, significant herbicide injury resulted from applications of Liberty and Liberty plus Dual Magnum. In contrast, little herbicide injury was observed in 2014. Although application timings and methods were similar, the wet and extremely humid conditions at the time of application likely explains why more injury was observed in 2013. Applications of Liberty and Liberty plus Dual Magnum generally reduced plant health as evidenced by vigor ratings, plant biomass measurements and bloom counts as well as reduced yield. In 2013, plots treated with Liberty and Liberty plus Dual Magnum had higher thrips injury scores. It is possible, even likely, that ratings of thrips injury was confused with injury caused by herbicides. In 2013, there was a trend of slightly higher thrips populations in the non-treated plots and a higher thrips population in plots treated with only Dual Magnum compared with those where Liberty was applied. This may be at least partially the results of thrips being more attractive to plants with less herbicide injury.

There was clear evidence that thrips treatments and post-emergence herbicides could both affect plant health. However, I saw little evidence of substantial interactions between these two factors. Thus, post-emergence herbicides may compound injury caused by thrips, but my data suggests that effects on plant health would be additive in nature. My data also reinforces concerns about thiamethoxam (Cruiser) and its declining efficacy against thrips populations (Stewart 2013). In 2013, Syngenta acknowledge that some tobacco thrips populations have developed resistance to thiamethoxam in the Mid-South and Southeast.

Thrips have a well-known potential to reduce cotton yields, as evidenced in Part I of this thesis. It is equally apparent that some foliar-applied herbicide application may also cause injury, potentially aggravating the negative impact of thrips. These data are not definitive but suggest the effects of thrips and herbicide injury would be more additive in nature. The use of post-emergence herbicides is needed in Tennessee because of glyphosate-resistant Palmer amaranth and other herbicide resistant weeds. Using post-emergence herbicides at recommended rates and according to label instructions should to minimize negative effects on plant health. Also, the use of varieties that have more tolerance to these herbicides could also help reduce the negative impacts of both herbicide and thrips injury. Of greater concern is the possibility that tobacco thrips may also develop resistance to imidacloprid.

Literature Cited

Carmer, S.G., W.E. Nyquist, and W.M. Walker. 1989. Least significant differences for combined analyses of experiments with two- or three-factor treatment designs. Agronomy Journal 81(4):665-672.

Layton, B., and J.T. Reed. 2002. Biology and control of thrips in seedling cotton. Publ. 2302. Mississippi State Ext. Serv., Starkville, MS.

Merchant, R.M., A.S. Culpepper, P.M. Eure, J.S. Richburg, and L.B. Braxton. 2014. Controlling glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in cotton with resistance to glyphosate, 2, 4-D, and glufosinate. Weed Technology 28:291-297.

Prince, J.M., D.R. Shaw, W.A. Givens, M.E. Newman, M.D.K. Owen, S.C. Weller, B.G. Young, R.G. Wilson, and D.L. Jordan. 2012. Benchmark Study: III. Survey on changing herbicide use patterns in glyphosate-resistant cropping systems. Weed Technology 26:536-542.

Sosnoskie, L.M., and A.S. Culpepper. 2014. Glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. Weed Science 62 393-402.

Steckel, L. E., D. Stephenson, J. Bond, S. D. Stewart and K. A. Barnett. 2012. Tolerance of WideStrike® Flex cotton response to over-the-top glufosinate tank mixtures. J. Cotton Sci. 16:88-95.

Steckel, L. E., G.N. Rhodes Jr., B.D. Sims, R.M. Hayes, A. McClure, T.C. Mueller, B. Brown, T. Raper, S. Senseman, E. Walker, J. Brosnan. 2015. Overtop herbicides for cotton. p. 31. *In* 2015 Weed Control Manual for Tennessee. Publ. 1580. University of Tennessee Extension, Knoxville, TN.

Stewart, S.D. 2013. Annual report. Cotton insect pest management. Agreement 13–589TN. [Online]. Available at http://www.utcrops.com/MultiState/Trials/2013/CI%2013-589TN-Final-2013-Stewart.pdf (verified 1 Aug. 2015).

Stewart, S.D., and G.L. Lentz. 2010. Cotton insects: thrips. Publ. W026. University of Tennessee Extension, Knoxville, TN.

Stewart, S. D., L. E. Steckel and S. J. Steckel. 2013. Evaluation of WideStrike® cotton (*Gossypium hirsutum* L.) injury from early season herbicide and insecticide tank mixes. J. Cotton Sci. 17:219-226.

Stewart, S.D., R. Patrick, and A. McClure. 2014. Cotton insect control recommendations. p. 3-16. *In* 2014 Insect control recommendations for field crops, cotton, soybeans, field corn, sorghum, wheat, and pasture. Publ. 1768. University of Tennessee Extension, Knoxville, TN.

United States Department of Agriculture. 2015. Estimated percentage of Upland cotton planted to leading specified varieties, by growth area, 2015 Crop. p. 2. *In* Cotton varieties planted 2015 crop. United States Department of Agriculture, Agricultural Marketing Service – Cotton and Tobacco Program, Memphis, Tennessee

Whitaker, J.R., A.C. York, D.L. Jordan, and A.S. Culpepper. 2011a. Weed management with glyphosate- and glufosinate-based systems in PHY 485 WRF cotton. Weed Technology 25:183-191.

Whitaker, J.R., A.C. York AC, and D.L. Jordan, A.S. Culpepper, and L.M. Sosnoskie. 2011b. Residual herbicides for palmer amaranth control. J. Cotton Sci. 15:89-99.

Appendix III

Table 1. The effects of insecticide and herbicide treatments on the average number of immature and total thrips per plant. Analyses are shown across and within years.

Treatment	Immature Thrips			Total Thrips		
	27 DAP	27 DAP	27 DAP	27 DAP	27 DAP	27 DAP
Insecticide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	16.4 b	23.3 b	9.44 c	19.7 b	25.5 b	13.9 c
IST	21.4 a	38.2 a	4.65 c	26.3 a	42.8 a	9.9 c
F-value	*7.16*	*27.16*		*9.65*	*25.07*	
df	*1,45*	*1,45*		*1,45*	*1,45*	
P-value	*0.0104*	*< 0.0001*		*0.0033*	*< 0.0001*	
Herbicide	2013-2014	2013	2014	2013-2014	2013	2014
Non-treated	18.1 a	29.5 b	6.73 c	22.2 a	33.0 b	11.5 c
Dual Magnum	23.3 a	40.1 a	6.55 c	27.7 a	44.6 a	10.8 c
Liberty	16.4 a	25.4 b	7.41 c	20.4 a	27.9 b	12.8 c
Liberty + Dual Mag.	17.8 a	28.1 b	7.48 c	21.8 a	31.1 b	12.5 c
F-value	*2.55*	*3.24*		*2.28*	*3.62*	
df	*3,45*	*3,45*		*3,45*	*3,45*	
P-value	*0.0672*	*0.0307*		*0.0921*	*0.0200*	

Mean not followed by a common letter are significantly different.

DAP = Days after planting.

Table 2. The effects of insecticide and herbicide treatments on thrips injury scores, percent herbicide injury, and the average vigor score. Analyses are shown across and within years.

Treatment	Thrips Injury*			Herbicide Injury*			Vigor*
	25-28 DAP	25 DAP	28 DAP	25-28 DAP	25 DAP	28 DAP	36 DAP
<u>Insecticide</u>	<u>2013-2014</u>	<u>2013</u>	<u>2014</u>	<u>2013-2014</u>	<u>2013</u>	<u>2014</u>	<u>2013</u>
Non-treated	3.78 a	4.11 a	3.44 b	14.3 a	23.5 a	5.00 b	2.49 b
IST	3.32 b	4.10 a	2.54 c	14.3 a	24.3 a	4.25 b	3.81 a
F-value	*86.18*	*83.84*		*0.002*	*1.30*		*169.22*
df	*1,45*	*1,45*		*1, 45*	*1, 45*		*1, 21*
P-value	*< 0.0001*	*< 0.0001*		*0.9638*	*0.2600*		*< 0.0001*
<u>Herbicide</u>	<u>2013-2014</u>	<u>2013</u>	<u>2014</u>	<u>2013-2014</u>	<u>2013</u>	<u>2014</u>	<u>2013</u>
Non-treated	3.36 c	3.96 c	2.75 e	3.19 d	2.25 e	4.13 de	3.58 a
Dual Magnum	3.51 b	4.03 bc	2.99 d	6.75 c	9.00 c	4.50 de	3.10 b
Liberty	3.66 a	4.23 a	3.09 d	21.8 b	38.8 b	4.75 de	3.05 b
Liberty + Dual Mag.	3.67 a	4.20 ab	3.14 d	25.4 a	45.6 a	5.13 d	2.86 b
F-value	*8.94*	*0.65*		*254.9*	*236.8*		*8.94*
df	*3,45*	*3,45*		*3, 45*	*3, 45*		*3, 21*
P-value	*< 0.0001*	*0.5900*		*< 0.0001*	*< 0.0001*		*0.0005*

Means not followed by a common letter are significantly different.

*Thrips injury rated per plot on a (0-5) scale with 5 being the most injury; herbicide injury rated per plot and shown as a percentage of leaf burn; vigor per plot rated on a (0-5) scale with 5 being most vigorous.

Table 3. Average herbicide injury (% leaf burn) observed after treatment with foliar-applied herbicides to seedling cotton in 2013 and 2014.

Treatment	Herbicide Injury (%)			
		Herbicides (2013, 25 Days After Planting)		
Insecticide	Non-treated	Dual Magnum	Liberty	Liberty + Dual
Non-treated	2.50 f	11.0 d	38.0 c	42.5 b
IST	2.00 f	7.00 e	39.5 bc	48.8 a
		Herbicides (2014, 28 Days After Planting)		
Insecticide	Non-treated	Dual Magnum	Liberty	Liberty + Dual
Non-treated	5.00 ef	5.00 ef	5.00 ef	5.00 ef
IST	3.25 ef	4.00 ef	4.50 ef	5.25 ef

Means not followed by a common letter are significantly different.

Table 4. The effects of insecticide and herbicide treatments on average plant biomass, the number of blooms per hectare, and final seed cotton yield in (Kg/Ha). Analyses are shown across years.

Treatment	Biomass (g) 27 DAP	Blooms 76-83 DAP	Yield 151-182 DAP
Insecticide	2013-2014	2013-2014	2013-2014
Non-treated	1.96 b	22,305 b	2,631 a
IST	2.41 a	29,482 a	2,906 a
F-value	*11.32*	*8.20*	*2.45*
df	*1,45*	*1,45*	*1,45*
P-value	*0.0016*	*0.0063*	*0.1245*
Herbicide	2013-2014	2013-2014	2013-2014
Non-treated	2.46 a	30,559 a	2,775 a
Dual Magnum	2.47 a	29,042 a	3,014 a
Liberty	1.93 b	24,369 ab	2,698 a
Liberty + Dual Mag.	1.88 b	19,604 b	2,588 a
F-value	*5.67*	*3.91*	*1.06*
df	*3,45*	*3,45*	*3,45*
P-value	*0.0022*	*0.0146*	*0.3771*

Means not followed by a common letter are significantly different.

DAP = Days after planting.

Part IV

Microbial Degradation of Neonicotinoid Insecticides in the Soil

Abstract

A study was performed in 2013 to determine the extent that soil microbes might degrade neonicotinoid insecticides commonly used as insecticide seed treatments into secondary metabolites. Soil was collected from a production field where neonicotinoid seed treatments had been used for many consecutive years and where performance problems against thrips were observed in cotton during 2013. At the same time, soil was also collected from a mowed grassy area with no previous exposure to insecticides. Part of the soil from each location was sterilized by autoclaving. Both sterilized and unsterilized soil were treated with an identical dilution of either Gaucho 600® (imidacloprid) or Cruiser 5F® (thiamethoxam). After 15 days, a soil sample was sent to the USDA AMS National Science Laboratory in Gastonia, NC for analytical testing of neonicotinoid concentrations, including known metabolites. Thiamethoxam and two of its metabolites (clothianidin and clothianidin TZMU) were detected in soil treated with the Cruiser dilution. Imidacloprid and three of its metabolites were detected in soil treated with Gaucho. Sterilizing the soil significantly reduced the concentrations of the imidacloprid and thiamethoxam metabolites. This suggests that soil microbes can degrade both insecticides. However, the amounts of degradation to secondary metabolites were relatively low, approximately 10-12% and less than 2% for imidacloprid and thiamethoxam, respectively. Previous soil exposure to neonicotinoid insecticides had negligible effects on the concentration of secondary metabolites. Considering the low levels of degradation, it seems unlikely that microbial metabolism of either insecticide would appreciably impact their performance as seed treatments.

Introduction

Thrips are a common pest of cotton that routinely rank among the top three insects reducing yield in cotton (Williams 2013). Preventative at-planting treatments, either in-furrow granular or liquid insecticides or seed treatments, are almost always recommended to control thrips infestations in seedling cotton (Stewart et al. 2014). In the last 10 years, insecticide seed treatments such as Gaucho (Imidacloprid; Bayer CropScience, Raleigh, NC) or Cruiser (Thiamethoxam; Syngenta, Greensboro, NC) have been used almost exclusively in Tennessee for thrips control.

Beginning around 2011 in the Mid-South, thrips control failures with neonicotinoid seed treatments, particularly thiamethoxam (Cruiser), became more commonly observed (Stewart 2013). Continuous use of some pesticides has been known to speed up microbial degradation of certain herbicides, such as atrazine (Muller et al. 2010) and insecticides, such as aldicarb (Suett and Jukes 1988). Therefore, a study was performed in 2013 to determine the extent that soil microbes might degrade thiamethoxam and imidacloprid into secondary metabolites.

Materials and Methods

Soil Collection and Preparation

A study was performed in 2013 to determine the extent that soil microbes might degrade neonicotinoid insecticides into secondary metabolites. In early July, soil was collected from a cotton field at the Milan Research and Education Center in Milan, TN where neonicotinoid seed treatments had been used for many consecutive years. At the same time, soil was collected from a mowed grassy area at the West Tennessee Research and Education Center in Jackson, TN, and this soil had no previous exposure to insecticides. Approximately two 19.4-liter buckets (5 gal) of soil were collected at each location from the top 5 – 7.5 cm of the surface. The soil was thoroughly mixed, and about 50% of the soil from each location was sterilized by autoclaving for 60 minutes at 121°C and allowed to cool to ambient temperature.

Soil Insecticide Treatment

Both sterilized and unsterilized soil from each locations were treated with an identical dilution of either Gaucho 600 (imidacloprid, Bayer CropScience) or Cruiser 5F (thiamethoxam, Syngenta). Because drying might affect the viability of soil microbes, there was no attempt to standardize differences in the moisture of soil collected from the two locations or resulting from autoclaving. A dilution was prepared of 0.5 ml of formulated product per 1,000 ml of water. 10 ml of this solution was added to 341 g (12 oz) of soil. This was replicated four times for each combination of insecticide, autoclaving treatment, and soil collection location.

After treating, the soil was mixed within self-sealing plastic bags, transferred to 250 ml plastic beakers, and stored in an open shed for 15 days at shaded, ambient outside temperatures. Distilled water (30 ml) was added to each beaker twice during the 15-day storage period to prevent desiccation. After 15 days, the beakers were transferred to a temperature-controlled room and held for 10 days at 20-23°C to allow for additional drying. It was again mixed, and a 57 g (2 oz) subsample was sent for testing of neonicotinoid concentrations, including known metabolites.

Chemical Analyses

Samples were analyzed to determine the levels of neonicotinoid residues by the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories' Gastonia Lab in Gastonia, NC. This laboratory is accredited to ISO/IEC 17025:2005 for specific tests in the fields of chemistry and microbiology, including testing for pesticide residues. The samples were extracted for analysis of agrochemicals using a refined methodology for the determination of neonicotinoid pesticides and their metabolites using an approach of the official pesticide extraction method (AOAC 2007.01), also known as the QuEChERS method, and analyzed by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS) (Kamel 2010; Lehotay et al. 2005; Zhang et al. 2011). Quantification was performed using external calibration standards prepared from certified standard reference material. The analytical limit of detection (LOD) for each neonicotinoid insecticide and its metabolites were the same as previously presented (Part I, Table 1).

Statistical Analyses

Within an insecticide treatment, data were analyzed as a 2 X 2 factorial of soil location and autoclaving treatment. The relative amount of total metabolites present as a percentage of the total neonicotinoid concentration was calculated for each sample, and these data were subjected to an arcsine transformation prior to analysis. Proc GLIMMIX procedure of SAS (ver. SAS 9.4; SAS Institute; Cary, NC) was used to determine main effects of location, autoclaving treatment, and their interaction on the concentration of neonicotinoid metabolites ($\alpha = 0.05$, LSMEANS, DDFM=SATTERTHWAITE). The relative concentrations of individual metabolites was evaluated similarly.

Results

Thiamethoxam and two of its metabolites, clothianidin and clothianidin TZMU, were detected in soil treated with Cruiser. Clothianidin composed the vast majority of metabolites detected, but only represent < 1% of the total neonicotinoid concentration in soil treated with thiamethoxam. Imidacloprid and three of its metabolites were detected in soil treated with Gaucho. Imidacloprid metabolites found included imidacloprid olefin, imidacloprid olefin des nitro, and imidacloprid urea. Across both locations and autoclaving treatments, these metabolites represented 6.9, 1.1, and 0.6% of the total neonicotinoid concentration, respectively. Average concentration levels (ng/g or ppb) for parent neonicotinoids and total neonicotinoids, including metabolites, are presented in Table 1 by insecticide treatment, location, and autoclaving treatment.

For soil treated with imidacloprid, there was a significant main effect of autoclaving (F = 237; df = 1, 12; $P < 0.0001$). There was approximately a 10-12% reduction in metabolites of imidacloprid when the soil was autoclaved (Table 2). The source of the soil did not affect this result (P = 0.5633), nor was there a significant interaction of soil source and the sterilization treatment (P = 0.0977). Much lower percentages of metabolites were found in soil treated with thiamethoxam, and soil source, the autoclaving treatment, and the interaction of these two factors were highly significant (F = 66.7, 46.3 and 33.2 respectively; df = 1, 12; $P < 0.0001$). In general, a higher percentage of thiamethoxam metabolites was found in soil collected from the agricultural field in Milan compared with the Jackson location (Table 3). For thiamethoxam-treated soil collected from Milan, metabolites as a percentage of total neonicotinoid concentrations were about 5-fold higher in unsterilized soil compared with sterilized soil. In the non-agricultural field (Jackson), total thiamethoxam metabolites were reduced only 2.2-fold by sterilizing the soil.

When analyzed across both locations, within the insecticide treatment, all individual metabolite concentrations were reduced by autoclaving the soil (data not shown, $F > 60$; df =1, 12; $P <$ 0.0001 for all).

Discussion

One possible reason for diminished performance of insecticide seed treatments, and especially thiamethoxam, could be microbial decay of parent compounds into less active metabolites within the soil. In this study, we only allowed 15 days for any degradation of insecticides within the soil before processing the samples for analysis. However, any decay of the parent compounds would have to occur quickly in order to substantially affect performance of the insecticides because cotton plants are primarily susceptible to thrips injury during the first few weeks after emergence. Sterilizing the soil reduced the concentrations of imidacloprid and thiamethoxam metabolites, suggesting that soil microbes can degrade both insecticides. However, the amounts of degradation to secondary metabolites were relatively low, approximately 10-12% and less than 2% for imidacloprid and thiamethoxam, respectively. There was some indication of more degradation in soil previously exposed to neonicotinoid insecticides, but these data were not conclusive and the amount of degradation were relatively minor. Considering the low levels of degradation and that several of the metabolites detected retain at least some insecticidal activity, it seems unlikely that microbial metabolism of either insecticide would appreciably impact their performance as seed treatments.

Literature Cited

Kamel, A. 2010. Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography–tandem mass spectrometry (LC-MS/MS). J. Agric. Food Chem. 58:5926-5931.

Lehotay, S.J., A. DeKok, M. Hiemstra, and P. van Bodegraven. 2005. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Journal of AOAC International 88:595-614.

Mueller, T.C., L.E. Steckel, and M. Radosevich. 2010. Effect of soil pH and previous atrazine use history on atrazine degradation in a Tennessee field soil. Weed Science 58:478-483.

Stewart, S.D. 2013. Annual report cotton insect pest management. Agreement 13–589TN. University of Tennessee Extension, Knoxville, TN.

Stewart, S.D., R. Patrick, and A. McClure. 2014. Cotton insect control recommendations. p. 3-16. *In* 2014 insect control recommendations for field crops, cotton, soybeans, field corn, sorghum, wheat, and pasture. Publ. 1768. University of Tennessee Extension, Knoxville, TN.

Suett, D.L., A.A. Jukes. 1988. Accelerated degradation of aldicarb and its oxidation products in previously treated soils. Crop Protection 7:147-152.

Williams, M.R. 2013. Resources, extension, crop losses. Cotton insect losses – 2013. Table 8, summary of all states [Online]. Available at http://www.entomology.msstate.edu/resources/croplosses/ 2013loss.asp (verified 5 June 2015)

Zhang, K., J.W. Wong, P. Yang, K. Tech, A.L. DiBenedetto, N.S. Lee, D.G. Hayward, C.M. Makovi, A.J. Krynitsky, K. Banaerjee, L. Jao, S. Dasgupta, M.S. Smoker, R. Simonds, and

A. Schreiber. 2011. Multiresidue pesticide analysis of agricultural commodities using acetonitrile salt-out extraction, dispersive solid-phase sample clean-up, and high-performance liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 59:7636-7646.

Appendix IV

Table 1. Mean ± SE concentration levels (ng/g) of parent neonicotinoid insecticides and total neonicotinoids, including metabolites, in soil treated with thiamethoxam (Cruiser) or imidacloprid (Gaucho).

Soil Treatment	Location	Autoclaved	Concentration (ng/g)	
			Thiamethoxam	Including metabolites
Thiamethoxam	Jackson	Yes	6070 ± 347	6076 ± 348
(Cruiser)	Jackson	No	5878 ± 472	5895 ± 486
	Milan	Yes	6480 ± 218	6508 ± 217
	Milan	No	7828 ± 164	7999 ± 165
			Imidacloprid	Including metabolites
Imidacloprid	Jackson	Yes	4660 ± 283	4773 ± 326
(Gaucho)	Jackson	No	3900 ± 330	4544 ± 373
	Milan	Yes	5703 ± 83	5933 ± 90
	Milan	No	5860 ± 489	6760 ± 534

Table 2. Percent of metabolites relative to total neonicotinoid concentrations for unsterilized and sterilized (autoclaved) soil from two locations that was treated with either thiamethoxam of imidacloprid.

Location	Treatment	% Metabolites	
		Unsterilized	Autoclaved
Jackson	Thiamethoxam	0.24	0.10
Milan	Thiamethoxam	2.14	0.43
Jackson	Imidacloprid	14.2	2.2
Milan	Imidacloprid	13.4	3.9

Conclusions

In recent years, a reduction in the performance of neonicotinoid seed treatments in controlling thrips injury, especially in cotton, has been observed. Thiamethoxam (Cruiser) appeared more affected than imidacloprid (Gaucho). There were several possible hypothesis to explain the diminished performance of insecticide seed treatments in controlling thrips and reducing thrips injury. Because this has coincided with the increased use of both pre- and post-emergence herbicides used to control glyphosate-resistance weeds, it was possible that commonly used herbicides were interacting to negatively affect the performance of insecticide seed treatments, perhaps even by inhibiting the uptake of these systemic insecticides into seedling plants. Another possibility was that the complex of soil microbes in agricultural fields where neonicotinoids have been used extensively may have evolved to feed upon and degrade some neonicotinoid insecticides into less active metabolites. My research investigated these hypothesis. I also studied possible interactions between pre-emergence herbicides and the performance of fungicide seed treatments. A final possibility not directly addressed by this research, and which seemed unlikely given the broad host range of thrips species found in cotton, was that thrips had developed resistance to neonicotinoid insecticides, and particularly thiamethoxam.

My goal was to determine whether injury caused by thrips and herbicides interacted to negatively affect the performance of insecticide or fungicide seed treatments used in cotton. I used commonly used pre-emergence herbicides including Reflex, Dual Magnum, and Cotoran. I also did studies with Liberty and Dual Magnum applied post-emergence to WideStrike® cotton varieties. Neonicotinoid seed treatments included in my evaluations included Gaucho and Cruiser. In some tests, an in-furrow treatment of aldicarb (Temik) was also included as an alternative insecticide class known to provide excellent protection of cotton seedling from thrips.

Finally, the performance of several fungicide seed treatments including Apron Maxx, Dynasty CST, and Trilex Advanced was studied in other tests.

I was generally successful in creating different levels of thrips injury or seedling disease in my various experiments, and I was also successful in causing differing levels of plant injury through the use or either pre- or post-emergence herbicides. Although I observed negative effects of thrips, disease and herbicides on plant health, these factors generally acted independently and additively. Thus, control of thrips or seedling disease with seed treatments would be similarly affected by any negative effects of herbicides, regardless of the efficacy of the seed treatment. My studies did not suggest that herbicides reduced the uptake of neonicotinoid insecticides into plants, as might occur if they negatively affected the roots of seedling plants. Although some metabolism of neonicotinoids by soil microbes was observed, the levels of degradation did not seem enough to appreciably affect the efficacy of insecticide seed treatments.

Concurrent to my research, Syngenta, the manufacturer and distributer of Cruiser, confirmed the presence of populations of tobacco thrips that were resistant to thiamethoxam. Tobacco thrips populations from my tests which were sent to Syngenta and an independent laboratory at Mississippi State University (F. Musser) were confirmed as resistant to thiamethoxam (unpublished data). This largely explains the results of my tests which showed the poor performance of Cruiser seed treatments compared with Gaucho or Temik. Ultimately, it was the efficacy of the insecticide or fungicide seed treatments used in these studies that primarily affected plant health. However, any additional negative impacts of herbicides on top of poor performing seed treatments could result in a 'double whammy' to seedling development, plant stands, plant health, and yield. Although there are necessities of maintaining adequate weed control, cotton growers should strive to use a combination of effective thrips treatments and the least injurious herbicide program. For example, my data indicate that Cruiser should not be used for thrips control in cotton. It also suggests that s-metolachlor (e.g., Dual Magnum) should not be used as a pre-mergence herbicide. The use of pre- and post-emergence herbicides is needed in Tennessee because of glyphosate-resistant resistant weeds. Using pre- and post-emergence herbicides at recommended rates and according to label restriction should reduce the risk of compounding the effects of thrips and seedling disease with herbicide injury.

Vita

Cory Joseph Vineyard was born on January 29, 1990 in Knoxville, TN. He is the son of Michael and Sheila Vineyard of Friendsville, TN and the eldest of their two children. He graduated from William Blount High School in May of 2008, and then he attended the University of Tennessee. Between his junior and senior years of undergrad, he conducted a summer internship with the cotton specialist, Dr. Darrin Dodds, at Mississippi State University. In May of 2013, he received his Bachelor of Science in Food and Agricultural Business. The summer after graduation, he worked for Dr. Scott Stewart, the UT extension IPM specialist. In August, he accepted a position at the University of Tennessee as a Graduate Research Assistant in the Department of Entomology and Plant Pathology with Dr. Stewart. He achieved his Master of Science degree in Entomology and Plant Pathology with a concentration in Entomology in December of 2015. Upon graduation, Cory will continue working in agriculture with AgCentral Farmer's Co-op.