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Evaluation of the rainfastness of selected insecticides in cotton

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Rainfastness of insecticides is an understudied aspect of agricultural research. Little is known about the residual of commonly used products for key pests of cotton, as well as their residual after a rainfall event. This project was designed to evaluate the impact of rainfall on the performance of commonly used insecticides for tobacco thrips, *Frankliniella fusca* (Hinds); tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); and bollworm, *Helicoverpa zea* (Boddie), management in cotton. Laboratory and field experiments suggest that rainfall occurring within 16 hours after application had a negative impact on the performance of most insecticides. Chemical analyses of leaf tissue using a mass spectrometer confirmed what was observed with field and laboratory experiments. Although direct comparisons between insecticides cannot be made, results from this study suggest that spinosyns and insect growth regulators appeared to have the longest residual after a rainfall event.

DEDICATION

I dedicate this research to my two loving parents, Ray and Sherry. They instilled the value of a good education in me from a young age. Throughout my undergraduate and graduate school experience, you two have been a constant for me. If there was anything I needed, I could come to you two and I would receive help and unconditional love without hesitation. You taught me what it means to work hard, and I am a better person today because of that. Thank you for providing me with everything I needed to succeed, and all the life lessons.

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CHAPTER I

INTRODUCTION

Cotton

Cotton, *Gossypium hirsutum* L., is an important agricultural commodity in the Mid-Southern United States, and has been the subject of much research, especially from an entomological standpoint. The genus *Gossypium* contains almost 40 different species, both cultivated and wild, *G. hirsutum* L., being the cultivated species in the Mid-south (Fryxell 1986). Cotton is the most industrialized crop in the world, and it is used for a variety of human goods such as clothing, livestock feed, cooking oil, and many more. The majority of the world's cotton, approximately 60%, is grown in China, India, and the United States (Maiti et al. 2011). The amount of cotton acreage in Mississippi went up 13% in 2019 from 2018, however, actual acreage of cotton went down 17% in 2020 from 2019 (USDA NASS) (https://www.nass.usda.gov/).

Cotton Growth and Development

Cotton is a woody perennial that is grown seasonally for the harvest of its mature fruiting structures, or bolls. After planting, the seed germinates and develops a root system before emergence of the true leaves (Mauney 1986, Ritchie et al. 2004). The cotton plant emerges 7-14 days after planting. The first structures to develop on the cotton plant are the true leaves, which marks the beginning of the vegetative stage. As the cotton plant grows, it develops reproductive branches, which are the primary producers of the fruiting structures (Mauney 1986, Ritchie et al. 2004). Cotton fruiting structures go through several stages before reaching maturity and being harvested: squaring (fruiting bud), flowering, and boll development. Depending on environmental conditions, the first squares should emerge around 35 days after planting (Ritchie et al. 2004). Excessively high or low temperatures could affect the rate of development of squares (Mauney 2012).

Flowering occurs as the squares open, which usually happens around 21 days after square emergence (Mauney 2012). The flowering progress can be determined by looking at the color of the petals; flowers begin with white petals that turn pink as it matures (Ritchie et al. 2004). Petals of the pink flowers will begin to dry out and fall off the plant, revealing a small boll. It will take approximately 50 days after pollination for the boll to develop completely. As the boll develops it will elongate its primary fibers and develop secondary wall fibers, before it can produce mature fiber and seed (Ritchie et al. 2004, Mauney 2012). Cotton growth and development is governed by the accumulation of heat units. Heat units are a measurement of the effect of daily temperatures on cotton growth, by taking the average of the minimum and maximum temperatures in Fahrenheit and subtracting 60, which is the lowest temperature at which cotton grows. Colder temperatures cause cotton to grow more slowly (Ritchie et al. 2004).

Tobacco Thrips

Tobacco thrips, *Frankliniella fusca* (Hinds), is a common early season pest of seedling stage cotton in the Mid-south and southeast. This pest has a wide distribution of all cotton growing states across the Mid-south and Southeastern United States (Cook et al. 2011). Thrips oviposit eggs into the plant tissue and emerge as larvae that undergo two larval stages and feed on the surrounding tissue (Cook et al. 2011, D'Ambrosio et al 2018). The larval stage lasts around 2 to 13 days until the pre-pupal and pupal stage begins (Lowry et al. 1992, Cook et al. 2011). Thrips drop from the plant and pupate under the soil surface and emerge as an adult within 1-10 days (Lowry et al. 1992, Cook et al. 2011). Adult thrips are slender in appearance with two pairs of fringed wings, which may be reduced in some adult thrips. The "fringe" on thrips wings are long hairs on the lower end of the wing (Riley et al. 2011).

Thrips adults and immatures feed on plant sap with their rasping and sucking mouthparts (Lewis 1973, Riley et al. 2011). Thrips injury on seedling stage cotton gives the leaves a silvery appearance, caused by air replacing the cellular fluids (Telford and Hopkins 1957, Reed and Reinecke 1990, Cook et al. 2011). This injury also causes the leaves to curl and plant height may be stunted. In cases of severe infestation, plants may show signs of delayed maturity, necrosis, and plant death (Gaines 1934, Dunham and Clark 1937, Davidson et al. 1979, Carter et al. 1989, Bourland et al. 1992, Van Tol and Lentz 1999, Lohmeyer et al. 2003, Cook et al. 2011). Thrips may cause severe damage from emergence through the fourth leaf stage. Thrips may overwinter locally on numerous hosts, such as volunteer peanuts, wheat, or various weeds (Groves et al. 2001). Thrips emerge from overwintering hosts in spring and move into seedling stage cotton.

Tarnished Plant Bug

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton in the Mid-south, including other parts of the eastern and southwestern cotton growing areas. Tarnished plant bug is a polyphagous species, with over 700 known host plants, both wild and cultivated (Young 1986, Parys 2014). This pest can be found in agricultural areas across the contiguous 48 states and into Canada (Young 1986). Tarnished plant bug eggs are inserted into various parts of the cotton plant, where they develop for five to seven days (Fleischer and Gaylor 1988, Leigh et al. 1996). Adult sizes range from 3.9mm to 4.78mm in length, and color may be pale green, straw yellow, or reddish brown. The scutellum of a tarnished plant bug forms a yellow-brown triangle. First and second instar tarnished plant bug nymphs are most commonly a pale green, with the older instars having five black spots on their dorsum (Leigh et al. 1996).

Tarnished plant bugs are capable of causing a wide array of damage to cotton by feeding on developing squares, growing points, and small bolls, especially in the terminal (Strong 1968, Wilson and George 1984, Leigh et al. 1988). Tarnished plant bugs have piercing and sucking mouthparts, with digestive enzymes in their saliva that causes damage to those fruiting structures (Layton 1995). Small squares, bracts, or bolls that are fed on usually desiccate and abscise from the plant. Additional signs of plant bug damage include darkened anther filaments, distorted petals and stigmas, and darkened areas on bolls. The darkened areas of the bolls are caused by necrotic tissue. The most vulnerable time for tarnished plant bug damage is before square formation and just after square formation has started (Ewing 1929, Wene and Sheets 1964, Hanny et al. 1977. Leigh et al. 1996). Overall, cotton can withstand small infestations from tarnished plant bug, but fruiting may be delayed. In contrast, more severe infestations may cause the removal of all squares (Leigh et al. 1996)

Adult stage tarnished plant bugs overwinter in groundcover and move into blooming plants in the spring (Crosby and Leonard 1914, Snodgrass et al. 1984, Leigh et al. 1996). In the United States, each growing season may see up to three generations of tarnished plant bugs on cotton.

Bollworm

Bollworm, *Helicoverpa zea* (Boddie), feeds on numerous cultivated and wild hosts. This species can be found in the tropics, from the West Indies to South America, and in subtropics such as the southeastern portion of North America (Hardwick 1965, Neunzig 1969, Leigh et al. 1996). Out of 106 known hosts, corn is the most preferred (Tietz 1972, Lincoln 1972, King and Coleman 1989, Leigh et al. 1996). Eggs are laid individually on cotton plants and are pearlywhite in color. The eggs take three to four days to hatch, and the larvae go through five to six instars before entering the pupal stage. Pupae take nine to ten days to emerge as moths (King and Coleman 1989). Bollworms can complete a life cycle in twenty-five days and may produce six to eight generations in one season in the Mid-south (Werner et al. 1979). Early instars can range in color from yellowish to reddish with black bumps along its body. Later instars may turn pale green or dark brown color. The bollworm moth is a pale buff color all over, with smaller dark spots on the front wings and pale crescent pattern in the middle of the wing (Werner et al. 1979, Leigh et al. 1996).

Younger larvae feed on structures closest to the oviposition site and prefer smaller fruiting structures. Earlier instars of bollworm larvae are known to tunnel through young terminal leaf buds and smaller squares. As a result, the squares turn brown and are often mistaken for tarnished plant bug damage (Brazzel et al. 1953, Leigh et al. 1996). More severe damage may cause fruiting structures to abscise (Gore et al. 2000). As larvae mature, feeding preferences change from smaller fruiting structures to larger ones (Wilson and Gutierrez 1980). Bollworm size and age directly correlates with the amount of fruit it can consume, therefore, it increases with age (Kincade et al. 1967, Baldwin et al. 1974, Wilson and Gutierrez 1980).

The final generation of bollworm larvae will drop to the soil, pupate, and enter diapause for overwintering. Surviving moths will emerge in March to April depending on weather conditions (Schneider 2003). Before agronomic crops are available, bollworms feed and oviposit on various wild hosts, such as crimson clover, *Trifolium incarnatum* L. As corn, *Zea mays* (L.), emerges, it becomes the preferred host of the bollworm, but bollworms will move into cotton later in the season as corn matures (Stadelbacher 1980).

Insect Control in Cotton

Chemical control has been the primary method used to control insect pests of cotton for many years, although a more dynamic approach has been utilized in recent years. Resistance issues have been observed in populations of tobacco thrips (Huseth et al. 2016, Darnell-Crumpton et al. 2018, Stewart et al. 2020), tarnished plant bug (Snodgrass 1996, Zhu and Snodgrass 2003, Zhu et al. 2004, Dorman et al. 2020), and bollworm (Graves et al. 1963, Graves et al. 1967, Plapp 1971, Sparks 1981, Stadelbacher et al. 1990, Brickle et al. 2001, Jacobson et al. 2009).

Tobacco thrips are an early season pest of cotton; therefore, control has previously been achieved using an at-planting insecticide treatment (Cook et al. 2011). In-furrow granular or liquid insecticides such as aldicarb were widely used for thrips control before the release of neonicotinoid seed treatments. Due to high toxicity and leaching within certain soil types, aldicarb use was discontinued and replaced with neonicotinoid treated seeds. Seed treatments were also a more efficient option than applying an in-furrow insecticide. Neonicotinoid seed treatments such as imidacloprid (Bayer CropScience, Raleigh, NC) and thiamethoxam (Syngenta Crop Protection, Greensboro, NC) were released in the 1990's as a more convenient alternative to aldicarb. However, foliar applications for thrips control has increased in recent years and is likely due to resistance development to neonicotinoids (Huseth et al. 2016, Darnell-Crumpton et al. 2018). Foliar insecticides are still needed for thrips management in cases of resistant populations or weather conditions that delay plant growth and prolong vulnerability to thrips damage.

Tarnished plant bugs are an important pest of cotton that may infest at any point in plant development (Layton 2000). Foliar applied insecticides are the primary method for controlling tarnished plant bugs. Populations of tarnished plant bugs resistant to the pyrethroid and organophosphate classes of insecticides have been observed (Snodgrass 1996, Zhu and Snodgrass 2003, Zhu et al. 2004, Dorman et al. 2020). The Mississippi State University Insect Control Guide recommends a rotation of different classes of insecticide throughout the season to help prevent further resistance issues (Catchot et al. 2020). Additional measures include destruction of alternative hosts such as weeds that may help support higher tarnished plant bug numbers (Snodgrass et al. 2006). Early maturing cotton planted early season may also mitigate damage from tarnished plant bug (Adams 2012).

Bollworm control also requires a multitactical approach, that is largely dependent upon foliar applied insecticides. Previously, bollworm management was achieved with just foliar insecticides, until resistant populations were reported. There is documented resistance to pyrethroids (Stadelbacher et al. 1990; Brickle et al. 2001, Jacobson et al. 2009), chlorinated hydrocarbons (Graves et al. 1963; Graves et al. 1967; Sparks 1981), and organophosphates (Plapp 1971). In an effort to manage heliothine pests with more than just foliar insecticides, Bollgard (Bollgard®, Monsanto, St. Louis, MO), Bollgard II (Bollgard II®, Monsanto, St. Louis, MO), and Bollgard III (Bollgard III®, Monsanto, St. Louis, MO) cottons were commercialized using an insecticidal protein from a naturally occurring bacterium, *Bacillus thuringiensis* Berliner (Perlak et al. 1990; Gore et al. 2001). Some earlier Bt varieties may require supplemental control due to resistance issues, and a foliar application may be needed for bollworm control.

Rainfastness

Previous research conducted on the rainfastness of insecticides used in cotton is very limited. Studies have been performed on a few insecticides to evaluate the rate of wash off of actual product but have not incorporated bioassays. Therefore, little is known about the limitations of these insecticides and how efficacious they are against insect pests after a rainfall event.

Studies were conducted to determine the effect of simulated rainfall on permethrin wash off on cotton plants (Willis 1986). The focus of this study was to determine whether rainfall amount or intensity had a greater effect on insecticide wash off. A multiple intensity rainfall simulator was used to simulate different durations and intensities. The results showed that rainfall amount had more of an impact on permethrin concentrations than rainfall intensity.

Similar studies were conducted to determine the effects of simulated rainfall on the wash off rate of methyl parathion and EPN (O-ethyl O-(p-nitrophenyl)) (McDowell et al. 1984). This was done using a multiple intensity rainfall simulator to determine if intensity had an effect on wash off rate. Results showed that rainfall amount, rather than intensity, affected the wash off rate of these two insecticides.

Research was conducted in cotton to evaluate the effect of wash off on azinphosmethyl, fenvalerate, permethrin, and sulprofos with emphasis on the time of insecticide application and the time of initial rainfall (Willis et al. 1994a, b). Results from both studies showed that wash off rates decreased with an increase in time between application and rainfall. Willis et al. (1994a) suggests that increasing resistance to wash off with increased time between application and rainfall could mean that the insecticide was better able to be absorbed by the plant and those with less time between application and initial rainfall were much easier to wash off.

Mulrooney and Elmore (2000) evaluated the rainfastness of bifenthrin to cotton when using selected adjuvants. Types of adjuvants used included crop oil concentrate, spreader/sticker/penetrant, spreader/extender/deposition agent, nonionic spreader, wetter/spreader/penetrant, nonionic buffer, vegetable oil adjuvant, and a spreader/activator. Adjuvants were added to bifenthrin mixtures based on manufacturer’s recommendations. Similar to Willis et al. (1994a, b), wash off of the insecticide decreased with an increase in time between application and initial rainfall, but it is suggested that this was because there was more time allotted for absorption rather than a function of the adjuvants.

Justification

Rainfastness of insecticides is an understudied facet of agricultural research, therefore, further studies are needed to determine the efficacy of insecticides following rainfall. Transgenic Bt cotton along with foliar applied insecticides are the primary modes of defense against damage from cotton pests (Catchot et al. 2020). With the sporadic control that some Bt cotton varieties are showing, control with foliar applied insecticides will likely be more important in seasons to come. Unfortunately, most insecticide labels do not specify a precise measure of rainfastness for their product if it is mentioned at all. Adjuvants are being marketed as a way to improve the ability of the product to be taken up by the plant, and in some cases, improving rainfastness.

Some detrimental cotton pests include the tarnished plant bug, tobacco thrips and bollworm. These pests are responsible for lowering yields and injuring plants by feeding on various structures, including squares, bolls, and other growing points. Understanding the wash off rates and efficacy of insecticides following various rainfall situations can be very helpful in anticipating pesticide runoff, developing strategies that will minimally harm the environment, learning the effect of wash off on pest populations, and proper planning for respraying fields after a wash off event (Willis et al. 1994a).

The purpose of this research was to evaluate the impact of simulated rainfall on the performance of selected insecticides against insect pests of cotton. Chemical analyses were also performed on selected insecticides to measure exact residual remaining after a rainfall event.

Objective 1: Evaluate the rainfastness of selected insecticides for *Frankliniella fusca* control in cotton.

Objective 2: Evaluate the rainfastness of selected insecticides for *Lygus lineolaris* control in cotton.

Objective 3: Evaluate the rainfastness of selected insecticides for *Helicoverpa zea* control in cotton.

Literature Cited

Adams, B. 2012. The biology and management of tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois), in cotton, *Gossypium hirsutum* (L.), in the Mississippi Delta. M. S. Thesis, Mississippi State University, Mississippi State, MS.

Baldwin, J. L., J. K. Walker, J. R. Gannaway, and G. A. Niles. 1974. Semi dwarf cottons and bollworm attack. J. Econ. Entomol. 67: 779-782.

Bourland, F. M., D. M. Oosterhuis, N. P. Tugwell. 1992. Concept for monitoring the growth and development of cotton plants using main- stem node counts. J. Prod. Agric. 5: 532-538.

Brazzel, J. R., C. Lincoln, L. D. Newsom, F. J. Williams, J. S. Roussel, and G. Barnes. 1953. Bollworm and tobacco budworm as cotton pests in Louisiana and Arkansas. La. Tech. Bull. 482.

Brickle, D. S., S. G. Turnipseed, and M. J. Sullivan. 2001. Efficacy of insecticides of different chemistries against *Helicoverpa zea* (Lepidoptera: Noctuidae) in Transgenic *Bacillus thuringiensis* and Conventional Cotton. J. Econ. Entomol. 94: 86–92.

Carter, F. L., N. P. Tugwell, and J. R. Phillips. 1989. Thrips control strategy: effects on crop growth, yield, maturity, and quality, pp. 295-297. *In* Proc. 1989 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Catchot, A., W. Crow, D. Dodds, J. Gore, F. Musser, T. Irby, D. Cook, B. Layton, E. Larson. 2020. Insect control guide for agronomic crops. Publication 2471. Mississippi State University Extension. Mississippi State, MS.

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. Integr. Pest Manag. 2: B1-B9.

Crosby, C. R. and M. D. Leonard. 1914. The tarnished plant bug, *Lygus pratensis* L. N. Y. Agr. Exp. Sta. Bull. 346: 463-525.

D'Ambrosio, D. A., A. S. Huseth, and G. G. Kennedy. 2018. Determining *Frankliniella fusca* (Thysanoptera: Thripidae) egg distribution in neonicotinoid seed-treated cotton. J. Econ. Entomol. 112: 827-834.

Darnell-Crumpton C., A. L. Catchot, D. R. Cook, J. Gore, D. M. Dodds, S. C. Morsello, F. R. Musser. 2018. Neonicotinoid insecticide resistance in tobacco thrips (Thysanoptera: Thripidae) of Mississippi. J. Econ. Entomol. 111: 2824-2830.

Davidson, R. H., L. M. Peairs, and W. F. Lyon. 1979. Insect pests of farm, garden, and orchard, 7th ed. Wiley, New York.

Dorman, S. J., A. D. Gross, F. R. Musser, B. D. Catchot, R. H. Smith, D. D. Reisig, F. P. F. Reay-Jones, J. K. Greene, P. M. Roberts, and S. V. Taylor. 2020. Resistance monitoring to four insecticides and mechanisms of resistance in *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) populations of southeastern USA cotton. Pest Manag. Sci. 76: 3935-3944.

Dunham, E. W. and J.C. Clark. 1937. Thrips damage to cotton. J. Econ. Entomol. 30: 855-857.

Ewing, K. P. 1929. Effects on the cotton plant of the feeding of certain Hemiptera of the family Miridae. J. Econ. Entomol. 22: 761-765.

Fleischer, S. J. and M. J. Gaylor 1988. *Lygus lineolaris* (Hemiptera: Miridae) population dynamics: nymphal development, life tables, and leslie matrices on selected weeds and cotton. Environ. Entomol. 17: 246-253.

Fryxell, P. A. 1986. Ecological adaptations of *Gossypium* species, pp. 1-7. *In* J.R. Mauney & J. McD. Stewart [ed.], Cotton Physiology. Memphis, TN.

Gaines, J. C. 1934. A preliminary study of thrips on seedling cotton with special reference to the population, migration, and injury. J. Econ. Entomol. 27: 740-743.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to non-transgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Gore, J., B. R. Leonard, J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on ‘Bollgard’ and ‘Bollgard II’ cotton flower bud and flower components. J. Econ. Entomol. 94: 1445–1451.

Graves, J. B., J. S. Roussel, and J. R. Phillips. 1963. Resistance to some chlorinated hydrocarbon insecticides in the bollworm, *Heliothis zea*. J. Econ. Entomol. 56: 442-444.

Graves, J. B., D. F. Clower, and J. R. Bradley, Jr. 1967. Resistance of the tobacco budworm to several insecticides in Louisiana. J. Econ. Entomol. 60: 887-888.

Groves, R. L., Walgenbach J. F., Moyer J. W., Kennedy G. G. 2001. Overwintering of *Frankliniella fusca* (Thysanoptera: Thripidae) on winter annual weeds infected with Tomato spotted wilt virus and patterns of virus movement between susceptible weed hosts. Phytopathology 91: 891-899.

Hanny, B. W., T. C. Cleveland, and W. R. Meredith Jr. 1977. Effects of tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) infestation on presquaring cotton (*Gossypium hirsutum*). Environ. Entomol. 6: 460-462.

Hardwick, D. F. 1965. The corn earworm complex. Memoirs Entomol. Soc. Can. 40: 1-248.

Huseth, A. S., T. M. Chappell, K. Langdon, S. C. Morsello, S. Martin, J. K. Greene, A. Herbert, A. L. Jacobson, F. Reay-Jones, T. Reed, D. D. Reisig, P. M. Roberts, R. Smith, and G. G. Kennedy. 2016. *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. Pest Manag. Sci. 72: 1934-1945.

Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, R. Weinzierl. 2009. Resistance to pyrethroid insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois, J. Econ. Entomol. 102: 2289–2295.

Kincade, R. T., M. L. Laster, and J. R. Brazzel. 1967. Damage to cotton by tobacco budworm. J. Econ. Entomol. 60: 1163-1164.

King, E. G., and R. J. Coleman. 1989. Potential for biological control of *Heliothis* species. Ann. Rev. Entomol. 34: 53–76.

Layton, M. B. 1995. Tarnished plant bug: biology, thresholds, sampling, and status of resistance. pp. 131-133. *In* Proc. 1995 Beltwide Cotton Conf., National Cotton Council.

Layton, M. B. 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. Southwest. Entomol. Suppl. 23: 7-20.

Leigh, T. F., T. A. Kerby, and P. F. Wynholds. 1988. Cotton square damage by the plant bug *Lygus hesperus* (Hemiptera: Heteroptera: Miridae) and abscission rates. J. Econ. Entomol. 81: 1328-1337.

Leigh, T. F., Roach, S. H., and Watson, T. F. 1996. Biology and ecology of important insect and mite pests of cotton. Cotton Insects and Mites: Characterization and Management, The Cotton Foundation Reference Book series. pp. 17-85.

Lewis, T. 1973. Thrips: their biology, ecology, and economic importance. Academic Press, London, United Kingdom.

Lincoln, C. 1972. Seasonal abundance, pp. 2-7. In distribution, abundance, and control of *Heliothis* species in cotton and other host plants. S. Coop. Ser. Bull. 169.

Lohmeyer, K. H., J. N. All, P. M. Roberts, and P. Bush. 2003. Precision application of aldicarb to enhance efficiency of thrips (Thysanoptera: Thripidae) management in cotton. J. Econ. Entomol. 96: 748-754.

Lowry, V. K., J. W. Smith, Jr., and F. L. Mitchell. 1992. Life-fertility tables for *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande) (Thysanoptera: Thripidae). Ann. Entomol. Soc. Am. 85: 744–754.

Maiti, R. K., Pawar, R. V., Misra, S. K., Rajkumar, D., Ramaswamy, A., and Vidyasagar, P. 2011. Comparative anatomy of cotton and its applications. International J. of Bio-resource Stress Manag. 2: 257-262.

Mauney, J. R. 1986. Vegetative growth and development of fruiting sites, pp. 11-28. *In* J.R. Mauney & J. McD. Stewart [ed.], Cotton Physiology. The Cotton Foundation, Memphis, TN.

Mauney, J. R. 2012. Anatomy and morphology of fruiting forms, pp. 1-11. *In* D.M. Oosterhuis & J.T. Cothren [ed.], Flowering and Fruiting in Cotton. William C. Robertson, Cordova, TN.

McDowell, L. L., Willis, G. H., Southwick, L. M., and Smith, S. 1984. Methyl parathion and EPN wash off from cotton plants by simulated rainfall. Environ. Sci. Technol. 18: 423-427.

Mulrooney, J. E. and Elmore, C. D. 2000. Rainfastening of bifenthrin to cotton leaves with selected adjuvants. J. Envron. Qual. 29: 1863-1866.

Neunzig, H. H. 1969. The biology of the tobacco budworm and the corn earworm in North Carolina with particular reference to tobacco as a host. NC Agric. Exp. Sta. Tech. Bull. 196. 76 pp.

Parys, K. A., and G. L. Snodgrass. 2014. Host plants of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). pp. 765. *In* Proc. Beltwide Cotton Res. Conf., National Cotton Council, New Orleans, LA.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuch, S. R. Sims, J. T. Greenplate, and D. A. Fischcoff. 1990. Insect resistant cotton plants. Biotechnol. 8: 939–943.

Plapp, Jr., F. W. 1971. Insecticide resistance in *Heliothis:* tolerance in larvae of *H. virescens* as compared with *H. zea* to organophosphate insecticides. J. Econ. Entomol. 64: 999-1002.

Reed, J. T. and J. Reinecke. 1990. Western flower thrips on cotton: plant damage and mite predation – preliminary observations, pp. 309-310. *In* Proc. 1990 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011. Thrips vectors of tospoviruses. J. Integr. Pest Manag. 1: 1-10.

Ritchie, G., C. Bednarz, P. Jost, and S. Brown. 2004. Cotton growth and development. The University of Georgia College of Agricultural and Environmental Sciences. Bulletin 1252.

Schneider J.C. 2003. Overwintering of *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in cotton fields of northeast Mississippi. J. Econ. Entomol. 96: 1433–1447.

Snodgrass, G. L., W. P. Scott and J. W. Smith. 1984. Host plants and seasonal distribution of the tarnished plant bug (Hemiptera: Miridae) in the delta of Arkansas, Louisiana, and Mississippi. Environ. Entomol. 13: 110-116.

Snodgrass, G. L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783-790.

Snodgrass, G. L. 2006. Status of insecticide resistance for the tarnished plant bug, pp. 1633-1638. *In* Proc. Cotton Beltwide Res. Conf. National Cotton Council, Memphis, TN.

Sparks, T. C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. of the Entomol. Soc. Amer. 27: 186–192.

Stadelbacher, E. 1980. Oviposition preference of the bollworm for species of early-season host plants in the delta of Mississippi. Environ. Entomol. 9: 542-545.

Stadelbacher, E. A., G. L. Snodgrass, G. W. Elzen. 1990. Resistance to cypermethrin in first generation adult bollworm and tobacco budworm (Lepidoptera: Noctuidae) populations collected as larvae on wild geranium, and in the second and third larval generations. J. Econ. Entomol. 83: 1207–1210.

Stewart, S. D., S. Steckel, M. Williams, D. Kerns, C. Perkins, S. Graham, B. Catchot, A. Catchot, F. Musser, J. Gore, G. Lorenz, S. Brown, D. Kerns, G. Kennedy, A. Huseth, D. Reisig, and S. Taylor. 2020. Performance of acephate against tobacco thrips and evidence of possible resistance. pp. 543-547. *In* Proc. 2020 Beltwide Cotton Conf., National Cotton Council, Austin, TX.

Strong, F. E. 1968. The selective advantage accruing to *Lygus* bugs that cause blasting of floral parts. J. Econ. Entomol. 61: 315-316.

Telford, A.D. and L. Hopkins. 1957. Arizona cotton insects. Arizona Agric. Exper. Station Bull. 286. University of Arizona, Tucson, AZ.

Tietz, H. M. 1972. An index to the described life histories, early stages, and hosts of the *Macrolepidoptera* of the continental United States and Canada. Vol. I and II. A. C. Allyn, Allyn Museum of Entomology, Sarasota.

Van Tol, N. B. and G.L. Lentz. 1999. Evaluation of Adage 5FS™ for early season insect control, pp. 1098-1101. *In* Proc. 1999 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Wene, G. P. and L. W. Sheets. 1964. *Lygus* bug injury to presquaring cotton. Ariz. Ag. Exp. Stn. Tech. Bull. 166.

Werner, F. G., L. Moore, and T. F. Watson. 1979. Arizona cotton insects. Univ. of Ariz. Coop. Ext. Serv. Bull. A23R.

Willis, G. H. 1986. Permethrin washoff from cotton plants by simulated rainfall. J. Environ. Quality. 15: 116-120.

Willis, G. H., McDowell, L. L., Southwick, L. M., and Smith, S. 1994a. Azinphosmethyl and fenvalerate washoff from cotton plants as a function of time between application and initial rainfall. Archives Environ. Contam. and Tox. 27: 115-120.

Willis, G. H., McDowell, L. L, Southwick, L. M., and Smith, S. 1994b. Permethrin and sulprofos washoff from cotton plants as a function of time between application and initial rainfall. J. Environ. Qual. 23: 96-100.

Wilson, F. D. and B. W. George. 1984. Pink bollworm (Lepidoptera: Gelechiidae): selecting for antibiosis in artificially and naturally infested cotton plants. J. Econ. Entomol. 77: 720-724.

Wilson, L., and A. Gutierrez. 1980. Fruit predation submodel: *Heliothis* larvae feeding upon cotton fruiting structures. Hilgardia. 48: 24-36.

Young, O. P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79: 747-762.

Zhu, Y. C. and G. L. Snodgrass. 2003. Cytochrome P450 CYP6X1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticides. Insect Molec. Biol. 12: 39-49.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2004. Enhanced esterase gene expression and activity in a malathion-resistant strain of the tarnished plant bug, *Lygus lineolaris*. Insect Biochem. and Molec. Biol. 34: 1175-1186.

CHAPTER II

EVALUATION OF THE RAINFASTNESS OF SELECTED INSECTICIDES FOR FRANKLINIELLA FUSCA CONTROL

Abstract

Laboratory bioassays were conducted in 2019 and 2020 in Stoneville, MS to evaluate the effect of rainfall on insecticides used for tobacco thrips. Bioassays were performed by dipping leaf disks into the insecticide and rinsing at designated timings. Rainfall had a negative effect on the performance of dicrotophos, but not spinetoram in both years. Results from acephate bioassays were inconclusive due to poor control overall. Field experiments were conducted in 2020 in Stoneville, MS to determine the impact of simulated rainfall on the efficacy of various insecticides for use on tobacco thrips, *Frankliniella fusca* (Hinds), in cotton. Dicrotophos or methoxyfenozide + spinetoram were sprayed on seedling cotton with a natural infestation of thrips. Rainfall was simulated using a square pattern sprinkler that delivered 2.54-cm within a 10-minute period. Simulated rainfall impact the performance of either insecticide. Additional field experiments were conducted to determine the impact of adjuvants paired with acephate when subjected to simulated rainfall. Acephate was applied alone and with five different adjuvants, as well as one of the adjuvants being used alone. Acephate, when paired with PrevAm, controlled adult thrips better than acephate alone. However, no differences from acephate alone were observed with other adjuvants. Adjuvants had no impact on acephate performance on immature thrips with or without a rainfall event.

Introduction

Tobacco thrips, *Frankliniella fusca* (Hinds), is an important pest of early season cotton throughout the United States (Cook et al. 2011, Cook et al. 2016, Kerns et al. 2018). This species is widespread, with all cotton growing states reporting infestations. Tobacco thrips can cause significant yield reductions during the seedling stage, so at-planting insecticides became a primary method of control (Cook et al. 2011, Kerns et al. 2018). Aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) was used as a granular in-furrow insecticide to manage thrips prior to the introduction of neonicotinoid seed treatments (Cook et al. 2016). Aldicarb provides effective thrips control (Hayes 1982; Lohmeyer et al. 2003), but is hazardous to human health, can leach into groundwater, and is less convenient to use compared to neonicotinoid seed treatments (Lohmeyer et al. 2003). The neonicotinoid seed treatments imidacloprid (Bayer CropScience, Raleigh, NC), and thiamethoxam (Syngenta Crop Protection, Greensboro, NC) were introduced in 1991 and 1998, respectively (Elbert et al. 2008). Initially, the neonicotinoid seed treatments provided similar levels of thrips control to that of aldicarb based on the frequency of sprays from the early 1990’s to the mid 2000’s (Table 2.1). However, the frequency of sprays has increased since 2005 (Table 2.1). The increased number of insecticide applications is likely due to resistance development to neonicotinoids by tobacco thrips (Huseth et al. 2016, Darnell-Crumpton et al. 2018). Recent field research indicates that the performance of thiamethoxam as a seed treatment was less than that observed with imidacloprid, but imidacloprid did not perform as good as it has in previous years (Cook et al. 2016). Despite the occurrence of resistance, tobacco thrips are still typically managed using in-furrow insecticides or insecticide seed treatments (Stewart et al. 2013). At-planting insecticides are recommended because thrips infestations occur on almost all fields and build quickly after cotton emergence (Cook et al. 2011). Foliar insecticide applications may be needed to control thrips even if an at-planting insecticide was used, especially if temperatures are unfavorable for cotton growth (Cook et al. 2011).

Temperatures that are less than adequate for cotton seedling development or use of ineffective seed treatments could result in the need for a foliar insecticide spray, but little is known about the performance of these insecticides if a rainfall event occurs after application. Adjuvants are marketed as a way to improve efficacy, and in some cases, rainfastness of insecticides. However, research on adjuvants as a means of improving rainfastness is limited. Studies have shown that the more time elapsed between insecticide application and rainfall, the more efficacious bifenthrin, azinphosmethyl, fenvalerate, permethrin, and sulprofos were (Mulrooney and Elmore 2000; Willis et al. 1994a; Willis et al. 1994b). Additionally, it was observed that amount of rainfall, rather than intensity, had a more detrimental effect on the performance of permethrin, methyl parathion, and EPN (O-ethyl O-(p-nitrophenyl)) on cotton leaves (McDowell et al. 1984; Willis et al. 1986). It has also been found that simulated rainfall could be used as a means of controlling thrips populations (Ibrahim and Adesiyum 2010; Rueda et al. 2007; Workman and Martin 2002).

The Mississippi State Insect Control guide recommends four different foliar insecticides for the control of thrips: acephate (Orthene 90SP, Amvac Chemical Corp., Los Angeles, CA), dicrotophos (Bidrin 8E, Amvac Chemical Corp., Newport Beach, CA), dimethoate (Dimethoate 4EC, Drexel Chemical Co., Memphis, TN), methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN) and spinetoram (Radiant SC, Dow Agrosciences, Indianapolis, IN) (Catchot et al. 2020). More research is needed to determine the impact of rainfall on the efficacy of commonly used insecticides for thrips management. Field and laboratory experiments were conducted to better understand the rainfastness and performance of these products to help improve management strategies.

Materials and Methods

Laboratory Bioassay

Bioassays were conducted in 2019 and 2020 at the Delta Research and Extension Center in Stoneville, Mississippi to evaluate the impact of wash off on the performance of spinetoram (Radiant® SC, Corteva AgriScience, Indianapolis, IN), dicrotophos (Bidrin® 8E, Amvac Chemical Corporation, Newport Beach, CA), acephate (Orthene® 90 SP, Amvac, Los Angeles, CA), and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN). Tobacco thrips were collected from blocks of infested peanut and cotton plants grown at the Delta Research and Extension Center without the use of at-planting insecticides. Individual plants were cut at the soil surface, placed in brown paper bags, and transported to the laboratory to collect adult thrips 24 hours before being used in bioassays. Plants were shaken onto a white plastic tray and adult tobacco thrips were aspirated into a vial and transferred into 3.79-L cardboard buckets with washed cabbage leaves. Dark brown to black colored thrips with wings were selected to be used in bioassays. Typically, 67-78% of all thrips found in cotton in the Mid-southern United States are *F. fusca* (Stewart et al. 2013). By selecting darker colored thrips, we can conservatively assume that greater than 90% of thrips used were *F. fusca* females.

Cotton leaves were removed from the upper three nodes of field grown cotton plants that received no prior insecticide treatment to be used in bioassays. Leaf disks were cut from these leaves using a 13-mm diameter cork borer (GSC International, Inc., Nixa, MO). Each disk was dipped into the designated insecticide solution for 3 seconds and placed on a tray lined with paper towels until the designated rinse time with the exception of an untreated control and no rinse treatment. The no rinse treatment was dipped into insecticide but not rinsed with water. Insecticide solutions were field rates of dicrotophos (0.22kg ai/ha), spinetoram (0.0224kg ai/ha), and acephate (0.22kg ai/ha) equivalent to an application volume of 94L/ha. These field rates were scaled down and mixed in 946.3mL of water + dicrotophos, spinetoram, and acephate at 2.37mL, 2.22mL, and 12.47g, respectively. To simulate wash off, leaf disks were dipped for 3 seconds into clean 59.2mL cups (Solo, Dart Container Corp., Mason MI) filled with water and placed onto a white plastic tray lined with paper towels. All leaves were dipped into separate cups with clean water to ensure no contamination. Treatments included an untreated control, insecticide with no wash off, and wash off after 0, 15, 30, 60, 120, 240, or 480 minutes.

After drying for an hour, each leaf disk was placed into the center of an individual self-sealing 50 x 9mm petri dish (Falcon®, Corning, Inc., Corning, NY) which was labelled by treatment. Two adult thrips collected from the field were placed into each dish using a fine bristle artist's paint brush. Each bioassay was replicated four times, with each treatment containing ten dishes. All dishes were observed 24 hours after infestation. Mortality of tobacco thrips was determined by checking for movement after probing the thrips with a fine bristled paintbrush. Percent mortality was calculated by dividing number of dead thrips over total thrips and multiplying by 100.

Thrips bioassay mortality data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Percent mortality was transformed using the arcsine function to convert the percent into radians. Time between application and rainfall was considered a fixed effect in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at $\alpha=0.05$. Insecticides were evaluated in separate bioassays on different days, so no statistical comparisons were made between insecticides.

Field Experiment

Field experiments were conducted in 2020 at the Delta Research and Extension Center in Stoneville, Mississippi to evaluate the impact of simulated rainfall on the performance of foliar insecticides for thrips management in cotton. Separate experiments were conducted for dicrotophos (Bidrin® 8E, Amvac Chemical Corporation, Newport Beach, CA), and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN). Black cotton seed (Deltapine 1822XF, Bayer CropScience, St. Louis, MO) without an insecticide or fungicide seed treatments were planted in a large block on 4 May 2020. Plots consisted of four 102cm rows that were 3.14m long with 1.39m alleys. Each test was arranged as a randomized complete block design with four replications and eight treatments. Treatments included an untreated control, sprayed with no rain, sprayed with simulated rainfall at 0, 15, 30, 60, 120, or 240 minutes after application. Each plot was sprayed with dicrotophos at 0.22kg/ha or methoxyfenozide + spinetoram at 0.0224kg/ha at 94 L/ha. Square pattern sprinklers (#7800, Melnor Inc., Winchester, VA) were placed at the center of each plot at the designated timings. The sprinkler evenly distributed water approximately 1.8-2.1 meters on all sides using rotating blades. Water was delivered to the sprinkler from a standard 1.59 cm garden hose that was attached to a 7,500L nurse tank with a motorized pump. Each plot received 2.54 cm of simulated rainfall within ten minutes. The amount of rainfall was determined by placing a rain gauge under one of the sprinklers at each of the timings. A wire flag was placed at the base of each sprinkler before simulating rainfall, and samples were taken within one meter of either side of the flag to ensure all samples pulled had received rainfall. Plots were allowed to dry for approximately one hour before leaf samples were taken.

Tests were sampled at one and two days after treatment by cutting five random plants from each plot close to the soil surface and placing them in a 946.3mL self-sealed plastic bag (Ziploc, S. C. Johnson & Son, Inc., Racine, WI). A whole plant washing procedure was used to dislodge thrips from plants (Burris et al. 1990). Each bag received a solution of soap, bleach, and water. The contents were then poured into a 2-mm sieve placed on top of a funnel with a 45-µm sieve underneath. Plants were rinsed with water on the large sieve to dislodge small insects into the smaller sieve. Contents of the smaller sieve were rinsed with a 70% ethanol solution onto a 9-cm diam. lined filter paper over a Buchner funnel attached to a vacuum pump. The filter paper was examined under a stereomicroscope at 100x magnification to count number of adult and immature thrips.

Thrips field data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Time (between application and rainfall) was considered a fixed effect in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at α=0.05.

Impact of Adjuvants

Field experiments were conducted in 2020 at the Delta Research and Extension Center to evaluate the impact of rainfall on the performance of acephate (Orthene 90 SP, Amvac, Los Angeles, CA) when combined with selected adjuvants. Cotton seed that did not have insecticide or fungicide seed treatments was planted on 4 May 2020. Plots consisted of four 102cm rows that were 3.14m long with 1.39m non-planted alleys. Each test was arranged as a randomized complete block design with four replications and four treatments. Three separate tests were run, which included five different adjuvants. An organosilicone (DyneAmic, Helena Agri-Enterprises, LLC, Collierville, TN) at 0.25% v/v with acephate, a water conditioner (AgMix, Earth Sciences Laboratories, Rogers, AR) at 0.5% v/v with acephate, untreated control, and acephate with no adjuvant was evaluated in test one. A non-specific insecticide (PrevAm, Oro Agri, Fresno, CA) at 0.78% v/v with acephate, a non-specific insecticide (PrevAm, Oro Agri, Fresno, CA) at 0.78% v/v without acephate, untreated control, and acephate with no adjuvant were evaluated in test two. A nonionic surfactant (Scanner, Loveland Products, Loveland, CO) at 0.25% v/v with acephate, a nonionic wetter-spreader (Induce, Helena Agri-Enterprises, LLC, Collierville, TN) at 0.5% v/v with acephate, untreated control and acephate with no adjuvant were evaluated in test three. Each plot was sprayed with acephate at 0.22kg/ha and an application volume equivalent to 94L/ha, except for the PrevAm only treatment and the untreated control which received no acephate. Rainfall simulations and thrips counts were performed identical to the previous experiment.

Thrips field data were analyzed by test with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Rainfall wash off timings, adjuvants, and the interaction between the two were considered fixed effects in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey’s protected HSD at $\alpha=0.05$.

Results

2019 Laboratory Bioassays

Treatment with dicrotophos had an effect on thrips mortality ($F=5.33$; $df=6, 21$; $P<0.01$), and the performance of dicrotophos was impacted by wash off timing. Mortality of tobacco thrips was similar among wash off timings for dicrotophos treatments (Table 2.2). The 120 and 240-minute timings were the only treatments that resulted in greater mortality of thrips than the untreated control. The 120-minute timing was the only treatment that resulted in greater mortality than the 0-minute timing. Treatment with spinetoram had an effect on thrips mortality ($F=272.32$; $df=7, 20$; $P<0.01$), but wash off did not affect the performance of spinetoram. Mortality of tobacco thrips was the same among wash off timings for spinetoram treatments (Table 2.2). The treatments that received spinetoram and wash off at 0, 15, 30, 60, 120, and 240-minutes after application resulted in greater mortality of thrips than the untreated control. Treatment with acephate did not have an effect on tobacco thrips mortality ($F=2.41$; $df=7, 19.32$; $P=0.06$). The impact of wash off on the efficacy of acephate could not be determined, because overall performance of acephate was poor (<53.0% mortality) (Table 2.2).

2020 Laboratory Bioassays

Treatment with dicrotophos had an effect on thrips mortality ($F=5.09$; $df=7, 21$; $P<0.01$). Mortality was similar between wash off timings for dicrotophos treatments (Table 2.3). The no wash off, 60, 120, and 240-minute timings were the only treatments that resulted in greater mortality of tobacco thrips than the untreated control. Treatment with spinetoram had an effect on thrips mortality ($F=15.81$; $df=7, 21$; $P<0.01$), but wash off did not affect the performance of spinetoram (Table 2.3). All timings resulted in greater mortality of tobacco thrips than the untreated control, with all timings showing similar levels of mortality to the no rain treatment.

Regardless of wash off timing, acephate did not result in greater thrips mortality compared to the untreated control (F=1.74; df=7, 21; P=0.15). The impact of wash off could not be determined due to overall poor control with acephate (Table 2.3).

2020 Field Experiments

Regardless of simulated rainfall timing, dicrotophos reduced adult thrips compared to the untreated control (F=15.54; df=7, 53; *P*<0.01). Timing of simulated rainfall did not affect the efficacy of dicrotophos. (Table 2.4). All simulated rainfall timings resulted in similar numbers of adult tobacco thrips to the no simulated rainfall treatment. There was also an effect of dicrotophos treatment on numbers of immature thrips (F=5.23; df=7, 53; *P*<0.01). All plots that received dicrotophos and simulated rainfall, except the 30-minute simulated rainfall timing, had fewer immature tobacco thrips than the untreated control. All plots that received dicrotophos and simulated rainfall timings had similar numbers of immature tobacco thrips to dicrotophos with no simulated rain (Table 2.4).

Treatment with methoxyfenozide + spinetoram had an effect on the control of adult tobacco thrips (F=15.39; df=7, 53; *P*<0.01), but simulated rainfall did not have an effect. All treatments that were sprayed with methoxyfenozide + spinetoram had fewer adult tobacco thrips than the untreated control (Table 2.5). All simulated rainfall timings resulted in similar numbers of adult tobacco thrips to the no simulated rainfall treatment. There was also an effect of methoxyfenozide + spinetoram on numbers of immature thrips (F=10.83; df=7, 53; *P*<0.01), but simulated rainfall did not have an effect. All treatments that were sprayed with methoxyfenozide + spinetoram had fewer immature tobacco thrips than the untreated control. Immature counts for all simulated rainfall timings were also similar to the no simulated rainfall treatment. (Table 2.5)

Impact of Adjuvants

In test one, there was an effect of simulated rainfall (F=5.10; df=1, 24; *P*=0.03) on adult thrips numbers at one day after treatment, but no effect of insecticide (F=0.29; df=3, 24; *P*=0.83) or no simulated rainfall by treatment interaction (F=0.45; df=3, 24; P=0.72). Mean (SEM) numbers of adult thrips from all plots that received treatment and simulated rainfall were 4.2(0.5), compared to 2.6(0.5) for the plots that received treatment but did not receive simulated rainfall. There was an effect of treatment on numbers of immature thrips (F=4.5; df=3, 24; *P*=0.01), but not simulated rainfall (F=0.03; df=1, 24; *P*=0.86) and there was no interaction between the two (F=0.75; df=3, 24; *P*=0.53) at one day after treatment. All plots that received acephate or acephate plus an adjuvant had fewer immature thrips than the untreated control (Table 2.6)

In test one at two days after treatment, there was an effect of treatment (F=3.02; df=3, 24; *P*=0.04), but not simulated rainfall (F=0.46; df=3, 24; P=0.50) and there was no interaction between the two (F=0.26; df=3, 24; P=0.85) for adult thrips. Treatments that received acephate or acephate plus AgMix had fewer adult thrips than the untreated control (Table 2.6). In test one at two days after treatment, there was an effect of simulated rainfall (F=4.66; df=1, 24; *P*=0.04), but not treatment (F=1.90; df=3, 24; *P*=0.16) and there was no interaction between the two (F=0.45; df=3, 24; *P*=0.72) for immature thrips. Averaged across all treatments, there was a mean (SEM) of 37.56 (3.9) immature thrips with simulated rain compared to 25.7 (3.9) with no simulated rain.

For the second adjuvant test at one day after treatment, there was no effect of simulated rainfall (F=0.00; df=1, 24; *P*=1.00) or treatment (F=1.73; df=3, 24; *P*=0.19) and there was no interaction between the two (F=2.16; df=3, 24; *P*=0.12) on adult thrips. There was also no effect of simulated rainfall (F=0.03; df=1, 21; *P*=0.87) or treatment (F=2.64; df=3, 21; P=0.08) and no interaction between the two (F=1.09; df=3, 21; *P*=0.37) on immature thrips. At two days after treatment, there was an effect of treatment for adult thrips (F=3.08; df=3, 24; *P*=0.04), acephate + PrevAm provided the best control, but no effect of simulated rainfall (F=0.06; df=1, 24; *P*=0.81) and there was no interaction between the two (F=0.45; df=3, 24; *P*=0.72). At two days after treatment, there was an effect of treatment on immature thrips (F=3.56; df=3, 21; P=0.03), but no effect of simulated rainfall (F=1.56; df=1, 21; *P*=0.22) and there was no interaction (F=0.66; df=3, 21; *P*=0.58) between the two. The acephate + PrevAm treatment was the only treatment that reduced immature thrips numbers below the untreated control (Table 2.6).

In test three at one day after treatment, there was an effect of rainfall (F=7.84; df=1, 23; *P*=0.01) on adult thrips counts, but no effect of treatment (F=2.47; df=3, 23; P=0.09) and there was no interaction between the two (F=1.46; df=3, 23; *P*=0.25). Plots that did not receive simulated rainfall had mean (SEM) counts of 4.3 (0.6) adults and plots that did receive simulated rainfall had mean (SEM) counts of 1.9 (0.6) adults. There was an effect of treatment on immature thrips (F=3.14; df=3, 20.02; *P*=0.04), but no effect of simulated rainfall (F=3.81; df=1, 20.02; *P*=0.07) and there was no interaction between the two (F=0.71; df=3, 20.02; *P*=0.60) at one day after treatment. The acephate + NIS and acephate + Induce treatments were the only treatments that reduced numbers of immature thrips below number in the untreated control (Table 2.6).

At two days after treatment, there was no effect of simulated rainfall (F=0.20; df=1, 21; *P*=0.70) or treatment (F=2.04; df=3, 21; *P*=0.14) on numbers of adult thrips, and there was no interaction between the two (F=0.93; df=3, 21; *P*=0.44). There was no effect of treatment (F=2.07; df=3, 21; *P*=0.14) or rainfall (F=0.06; df=1, 21; *P*=0.81) on numbers of immature thrips at two days after treatment, but there was an interaction between the two (F=3.60; df=3, 21;

P=0.03). For cotton sprayed with acephate that received simulated rainfall, numbers of immature thrips were similar to the untreated cotton that received simulated rainfall (Table 2.7). In contrast, cotton sprayed with acephate that did not receive rainfall had fewer immature thrips than untreated cotton that did not receive simulated rainfall.

Discussion

The impact of simulated rainfall on insecticides commonly used for thrips control in cotton was observed and results varied between insecticides. In general, dicrotophos provided moderate control of tobacco thrips in both laboratory and field experiments. Based on laboratory bioassays, wash off timings at <120 minutes and 60 minutes after insecticide application resulted in lower mortality in 2019 and 2020, respectively. This was not observed in field experiments, where simulated rainfall did not affect efficacy with one exception, simulated rainfall at 30 minutes after application was similar to the untreated control. In the field experiment where no simulated rainfall was applied, dicrotophos provided 80.8% control of immature thrips. This is similar to a previous experiment where 77.0% control was observed for a complex of thrips species in cotton (Siebert et al. 2016). In the current experiment, control of tobacco thrips with dicrotophos ranged from 45.7% to 71.3% depending on the simulated rainfall timing. Thrips species from field experiments were not identified, so it is likely that a small percentage of the population was other species based on a previous survey (Stewart et al. 2013).

Methoxyfenozide + spinetoram provided good control of adult and immature thrips in field experiments. Control of adults ranged from 67% to 83%, while immature control ranged from 72% to 84%. All insecticide treatments reduced thrips compared to the untreated control. Similarly, spinetoram provided excellent control of tobacco thrips in laboratory bioassays. All timings from the 2019 laboratory experiment resulted in 100% mortality, while the 2020 laboratory bioassays ranged from 86.0% to 98.8% mortality across all timings. Previous research documented 68% control in field experiments with spinetoram (Siebert et al. 2016). This difference may be the result of inherent differences between field and laboratory experiments.

There was no clear effect of rainfall on acephate in bioassays either year, however, these results could be from poor control with acephate rather than wash off. Acephate field tests showed that the use of an adjuvant with acephate did not improve performance. In the case of test three at two days after treatment, there were fewer thrips in the untreated control that received rain than the untreated control that did not receive rain. This suggests rainfall could lower thrips populations as shown in previous research (Workman and Martin 2002, Rueda et al. 2007, Ibrahim and Adesiyum 2010).

Although no direct comparisons could be made between insecticides, insecticides with spinetoram appeared to provide the most consistent control of thrips in the presence of simulated rainfall. At least 60 to 120 minutes between application of dicrotophos and a rainfall event is needed to see any effects on thrips populations. No conclusions can be drawn about acephate because overall control was poor. Acephate resistance has been observed in Tennessee (Stewart et al. 2020), with percent control decreasing significantly since 2005. It is likely that the poor control from acephate seen in these experiments is due to resistance issues. Results from these experiments will be important for improving recommendations for thrips control in cotton when rainfall occurs or is expected. Foliar insecticides comprise a significant percentage of overall production budgets in cotton, so ensuring their maximum performance is important for maintaining profitability.

Table 2.1 Average number of insecticide applications made per ha for thrips management from 1990 to 2019 in 5 year increments. States represented are Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Virginia.

	Insecticide applications per ha					
State	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2019
AL	0.18	0.48	0.19	0.45	0.35	0.38
AR	0.76	0.24	0.97	0.93	0.96	0.31
GA	0.84	0.10	0.10	0.15	0.48	0.35
LA	0.62	0.58	0.47	0.48	0.67	0.23
MS	1.30	0.45	0.43	0.44	1.04	0.49
NC	0.66	0.42	0.77	1.05	1.00	0.81
SC	0.66	0.23	0.16	0.56	0.82	0.72
TN	0.62	0.30	0.74	0.65	0.91	0.96
TX	0.28	0.15	0.10	0.46	0.29	0.20
VA	0.94	0.70	1.16	1.02	0.91	1.16

Table 2.2 Mean (SEM) percent mortality of tobacco thrips adults in laboratory bioassays conducted in 2019 evaluating the impact of wash off on the residual efficacy of dicrotophos, spinetoram, and acephate in cotton.

	Percent Mortality (SEM)		
Time (min. after spray)	Dicrotophos	Spinetoram	Acephate
Untreated	7.8(4.8) C	17.5(7.5) B	5.0(5.0) A
0	22.5(2.5) BC	100.0(0) A	28.3(8.6) A
15	38.8(5.9) ABC	100.0(0) A	40.0(5.8) A
30	40.0(8.9) ABC	100.0(0) A	40.0(9.1) A
60	37.8(13.5) ABC	100.0(0) A	22.5(7.5) A
120	65.0(6.5) A	100.0(0) A	47.5(17.0) A
240	54.3(8.9) A	100.0(0) A	45.0(8.7) A
480	---	---	53.0(17.0) A
F	5.33	272.32	2.41
d.f.	6, 21	7, 20	7, 19.32
P>F	<0.01	<0.01	0.06

Means within a column followed by the same letter are not significantly different according to Tukey’s HSD (α=0.05).

Table 2.3 Mean (SEM) percent mortality of tobacco thrips adults in laboratory bioassays conducted in 2020 evaluating the impact of wash off on the efficacy of dicrotophos, spinetoram, and acephate in cotton.

	Percent Mortality (SEM)		
Time (min. after spray)	Dicrotophos	Spinetoram	Acephate
Untreated	1.5(1.5) B	1.5(1.5) B	5.0(5.0) A
0	32.3(11.2) AB	86.0(7.6) A	1.5(1.5) A
15	27.5(4.9) AB	91.8(3.7) A	12.0(2.5) A
30	36.8(7.0) AB	87.5(2.9) A	18.3(9.0) A
60	48.5(8.4) A	98.8(1.3) A	28.8(7.2) A
120	53.0(15.9) A	90.3(4.1) A	21.0(7.8) A
240	41.0(8.7) A	90.8(4.0) A	22.3(12.6) A
No Rain	56.3(6.3) A	95.0(3.5) A	17.5(6.3) A
F	5.09	15.81	1.74
d.f.	7, 21	7, 21	7, 21
P>F	<0.01	<0.01	0.153

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

Table 2.4 Mean (SEM) number of tobacco thrips adults and immatures in field experiments conducted in 2020 evaluating the impact of simulated rainfall on the efficacy of dicrotophos in cotton.

	Mean No. Thrips per 5 Plants (SEM)	
Time (min. after spray)	Adults	Immatures
Untreated	20.1(3.9) A	41.1(11.3) A
0	5.1(0.8) B	12.8(1.5) B
15	5.9(1.2) B	16.9(6.2) B
30	6.0(1.4) B	22.3(8.3) AB
60	3.4(1.2) B	14.9(3.9) B
120	4.9(1.6) B	16.0(3.5) B
240	2.8(0.9) B	11.8(2.5) B
No Rain	2.5(0.8) B	7.9(1.6) B
F	15.54	5.23
d.f.	7, 53	7, 53
P>F	<0.01	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey’s HSD (α=0.05).

Table 2.5 Mean (SEM) number of tobacco thrips adults and immatures in field experiments conducted in 2020 evaluating the impact of simulated rainfall on the efficacy of methoxyfenozide + spinetoram in cotton.

	Mean No. Thrips per 5 Plants (SEM)	
Time (min. after spray)	Adults	Immatures
Untreated	18.0(2.1) A	43.5(8.1) A
0	6.0(1.3) B	11.9(2.6) B
15	5.4(1.3) B	9.5(2.0) B
30	5.1(1.5) B	10.6(2.2) B
60	3.0(0.9) B	9.0(1.5) B
120	4.0(1.1) B	11.4(2.9) B
240	4.0(1.4) B	7.1(1.8) B
No Rain	3.4(1.3) B	12.4(3.9) B
F	15.39	10.83
d.f.	7, 53	7, 53
P>F	<0.01	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey’s HSD (α=0.05).

Table 2.6 Mean (SEM) number of tobacco thrips from three separate tests in field experiments conducted in 2020 evaluating the impact of rainfall on the efficacy of acephate and the use of adjuvants in cotton.

Test 1				
	1DAT		2 DAT	
Trt	Adult	Immature	Adult	Immature
UTC	3.6(0.7) A	55.6(6.2) A	5.9(1.1) A	40.3(5.5) A
Acephate	3.7(0.7) A	28.8(6.2) B	1.9(1.1) B	27.0(5.5) A
Ace. + AgMix	2.9(0.7) A	30.3(6.2) B	1.8(1.1) B	23.8(5.5) A
Ace. + Dyne-Amic	3.5(0.7) A	29.4(6.2) B	3.5(1.1) AB	35.5(5.5) A
Test 2				
	1DAT		2 DAT	
Trt	Adult	Immature	Adult	Immature
UTC	4.6(0.9) A	49.4(7.3) A	5.0(1.3) AB	56.1(7.6) A
Acephate	3.0(0.9) A	37.5(7.3) A	7.5(1.3) A	44.9(7.6) AB
Ace. + PrevAm	2.8(0.9) A	35.6(7.3) A	2.1(1.3) B	27.1(7.6) B
PrevAm	5.4(0.9) A	59.8(7.3) A	5.5(1.3) AB	51.8(7.6) A
Test 3				
	1DAT		2 DAT	
Trt	Adult	Immature	Adult	Immature
UTC	4.6(0.9) A	50.5(9.4) A	4.8(1.1) A	46.8(10.2) A
Acephate	4.0(0.9) A	37.8(9.4) AB	4.4(1.1) A	33.9(10.2) A
Ace. + NIS	2.1(0.9) A	25.1(9.8) B	3.4(1.1) A	26.6(10.2) A
Ace. + Induce	1.9(0.9) A	27.8(9.4) B	1.6(1.1) A	24.6(10.2) A

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

Table 2.7 Mean (SEM) number of immature tobacco thrips from test three at 2 DAT in field experiments conducted in 2020 evaluating the interaction between the use of select adjuvants and rainfall in cotton.

	Mean No. Thrips per 5 Plants (SEM)	
Treatment	Rain	No Rain
Untreated	28.3(12.4) B	65.3(12.4) A
Acephate	44.0(12.4) AB	23.8(12.4) B
Acephate + Induce	33.0(12.4) B	16.3(12.4) B
Acephate + NIS	23.3(12.4) B	30.0(12.4) B
F		3.60
d.f.		3, 21
P>F		0.03

Means within the table followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

Literature Cited

Burris, E. A. M. Pavloff, B. R. Leonard, J. B. Graves, and G. Church. 1990. Evaluation of Two Procedures for Monitoring Populations of Early Season Insect Pests (Thysanoptera: Thripidae and Homoptera: Aphididae) in Cotton Under Selected Management Strategies. J. Econ. Entomol. 83: 1064-1068.

Catchot, A., C. Allen, J. Bibb, D. Cook, W. Crow, J. Dean, D. Fleming, J. Gore, B. Layton, N. Little, J. MacGown, F. Musser, S. Winter, D. Dodds, T. Irby, E. Larson, and S. Meyers. 2020. 2020 Insect Control Guide for Agronomic Crops. *In* A. Catchot [ed.]. Mississippi State University Extension.

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. Integr. Pest Manag. 2: B1-B9.

Cook, D. R., J. Gore, S. D. Stewart, D. L. Kerns, G. M. Lorenz, A. L. Catchot, F. R. Musser, G. Studebaker, N. Seiter, and S. Brown. 2016. At-planting management of thrips in mid-south cotton, pp. 526-530. *In* Proc. 2016 Beltwide Cotton Conf., National Cotton Council, New Orleans, LA.

Darnell-Crumpton C., A. L. Catchot, D. R. Cook, J. Gore, D. M. Dodds, S. C. Morsello, F. R. Musser. 2018. Neonicotinoid insecticide resistance in tobacco thrips (Thysanoptera:Thripidae) of Mississippi. J. Econ. Entomol. 111: 2824-2830.

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.

Hayes, W. J., Jr. 1982. Pesticides studied in man. Waverly Press, Baltimore, MD.

Huseth, A. S., T. M. Chappell, K. Langdon, S. C. Morsello, S. Martin, J. K. Greene, A. Herbert, A. L. Jacobson, F. Reay-Jones, T. Reed, D. D. Reisig, P. M. Roberts, R. Smith, and G. G. Kennedy. 2016. *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. Pest Manag. Sci. 72: 1934-1945.

Ibrahim, N. D. and A. A. Adesiyun. 2010. Effect of rainfall in the control of onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) in Sokoto, Nigeria. Agric. Bio. J. N. America. 2151-7517.

Kerns, C. D., J. K. Greene, F. P. F. Reay-Jones, and W. C. Bridges, Jr. 2018. Effects of planting date on thrips (Thysanoptera: Thripidae) in cotton. J. Econ. Entomol. 112: 699-707.

Lohmeyer, K.H., J. N. All, P.M. Roberts, and P. Bush. 2003. Precision application of aldicarb to enhance efficiency of thrips (Thysanoptera: Thripidae) management in cotton. J. Econ. Entomol. 96: 748-754.

McDowell, L. L., Willis, G. H., Southwick, L. M., and Smith, S. 1984. Methyl parathion and EPN washoff from cotton plants by simulated rainfall. Environ. Sci. Technol. 18: 423-427.

Mulrooney, J. E. and Elmore, C. D. 2000. Rainfastening of bifenthrin to cotton leaves with selected adjuvants. J. Environ. Qual. 29: 1863-1866.

Rueda, A., F. R. Badenes-Perez, A. M. Shelton. 2007. Developing economic thresholds for onion thrips in Honduras. Crop Prot. 26: 1099-1107.

Siebert, M. W., S. Nolting, J. E. Dripps, L. C. Walton, D. R. Cook, S. Stewart, J. Gore, A. L. Catchot, G. Lorenz, B. R. Leonard, A. Herbert. 2016. Efficacy of spinetoram against thrips (Thysanoptera: Thripidae) in seedling cotton, *Gossypium hirsutum* L. J. Cotton Sci. 20: 309-319.

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. Rogers Leonard, G. M. Lorenz, S. Micinski, D. Reisig, P. Roberts, G. Studebaker, K. Tindall, and M. Toews. 2013. Survey of thrips species infesting cotton across the southern U.S. cotton belt. J. Cotton Sci. 17: 263-269.

Stewart, S. D., S. Steckel, M. Williams, D. Kerns, C. Perkins, S. Graham, B. Catchot, A. Catchot, F. Musser, J. Gore, G. Lorenz, S. Brown, D. Kerns, G. Kennedy, A. Huseth, D. Reisig, and S. Taylor. 2020. Performance of acephate against tobacco thrips and evidence of possible resistance. pp. 543-547. *In* Proc. 2020 Beltwide Cotton Conf., National Cotton Council, Austin, TX.

Willis, G. H. 1986. Permethrin washoff from cotton plants by simulated rainfall. J. Environ. Quality. 15: 116-120.

Willis, G. H., McDowell, L. L., Southwick, L. M., and Smith, S. 1994a. Azinphosmethyl and fenvalerate washoff from cotton plants as a function of time between application and initial rainfall. Archives of Environ. Contam. and Tox. 27: 115-120.

Willis, G. H., McDowell, L. L, Southwick, L. M., and Smith, S. 1994b. Permethrin and sulprofos washoff from cotton plants as a function of time between application and initial rainfall. J. Environ. Qual. 2: 96-100.

Workman, P. J. and N. A. Martin. 2002. Towards integrated pest management of *Thrips tabaci* in onions. New Zealand Plant Protection. 55: 188-192.

CHAPTER III

EVALULATION OF THE RAINFASTNESS OF SELECTED INSECTICIDES FOR LYGUS LINEOLARIS CONTROL

Abstract

Field and laboratory experiments were conducted in 2019 and 2020 at the Delta Research and Extension Center in Stoneville, MS to evaluate the effect of rainfall on the efficacy of insecticides commonly used for tarnished plant bug management in cotton. Additional studies were conducted to evaluate the impact of adjuvants on rainfastness of insecticides. Water-pick bioassays were conducted by infesting insecticide treated cotton terminals with adult tarnished plant bugs and using a rainfall simulator to impose 2.54cm of rainfall. Rainfall had a significant effect on the performance of dicrotophos, thiamethoxam, acephate, sulfoxaflor, and novaluron. Novaluron was the least impacted by rainfall in these bioassays. Adjuvants did not improve the rainfastness of sulfoxaflor. Field experiments were conducted by spraying plots with insecticide and simulating rainfall with a square-pattern sprinkler. Leaves were pulled from each plot and analyzed using a mass spectrometer to determine residual insecticide concentrations on leaves. All products had very low concentrations after a rainfall event relative to the no simulated rain treatments, except for one product. Novaluron had one simulated rainfall timing, 8 hours after application, with a similar level to the no simulated rainfall, making it the least affected by simulated rainfall.

Introduction

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton in the southern U.S. This is a polyphagous species with at least 700 wild and cultivated host plants (Young 1986, Parys 2014). The earliest generations occur mostly on wild hosts before moving into agronomic crops, starting with corn when it is in the tasseling and green silk stage. Tarnished plant bugs move from corn to cotton as the corn silks turn brown, which generally coincides with the late squaring and early flowering stages of cotton (Abel et al. 2010). Tarnished plant bugs can damage cotton throughout the growing season, though most damage by this pest occurs from first flower bud (square) to early bloom (Layton 1995). Oviposition by tarnished plant bug typically occurs in terminal or flower bud tissue (Fleischer and Gaylor 1988). There are five nymphal stages of tarnished plant bug prior to the adult stage. Tarnished plant bug prefers to feed on immature squares and release digestive enzymes while feeding (Layton 1995). This feeding typically causes the squares to abscise, leading to direct yield losses. If the square does not abscise, the feeding injury usually results in malformed flowers that do not pollinate properly, and the structures may abscise as small bolls (Tugwell et al. 1976).

In a two-year study in multiple states in the Mid-south, tarnished plant bug made up 94% of plant bugs collected in flowering cotton (Musser et al. 2007). Tarnished plant bug thresholds are based on the economic injury level (EIL) at that point in the growing season (Musser et al. 2009). Because thresholds are based on EILs, insecticides must be as efficacious as possible, and rainfall may impact that. In addition, sampling methods vary based on the developmental stage of cotton. Adults are more common during pre-bloom, so a sweep net is the best sampling method (Musser et al. 2009). Nymphs are more common during bloom, so using a drop cloth is the more accurate sampling method. The use of foliar insecticides is the primary method of control for tarnished plant bug in cotton. Recommendations given in the Mississippi State University Insect Control Guide provide options from various insecticide classes such as organophosphates, neonicotinoids, one sulfoxamine, one carbamate, and one insect growth regulator (Catchot et al. 2020). Tarnished plant bug foliar applications have increased across the Mid-south compared to previous years (Table 3.1). Applications went from a low of 1.38/Ha from 1990-1994, to a high of 5.08/Ha from 2010-2014. Other states in the Mid-south have seen similar trends. Number of sprays have been increasing in states such as Alabama, Arkansas, Georgia, Louisiana, North Carolina, South Carolina, Tennessee, and Texas.

Tarnished plant bug has developed resistance to insecticides in the pyrethroid and organophosphate classes (Snodgrass and Scott 1988, Snodgrass 1996, Zhu and Snodgrass 2003, Zhu et al. 2004, Snodgrass and Gore 2007, Dorman et al. 2020) which has made effective and economical management more difficult. The development of resistance to most of the insecticides labeled for tarnished plant bug is likely contributing to the increased number of sprays in most areas. Increased number of insecticide applications have led to increased cost for tarnished plant bug management. Additionally, residual control from most foliar insecticides is short even without the presence of rainfall. This combined with the abundance of alternate hosts, allows new populations to reinfest and survive in cotton throughout the season (Layton 1995).

In general, years with more precipitation in Mississippi are conducive to larger plant bug populations because alternative hosts remain in bloom for a longer period of time (Layton 1995). Tarnished plant bugs will remain on these hosts and build up populations, which allows for a more extended migration time into cotton. A higher population from a wet season, combined with short residual of commonly used insecticides in the presence of rain could result in difficulty managing this pest. The objective of this study was to investigate the impact of rainfall on the performance of selected insecticides for tarnished plant bug control in cotton.

Materials and Methods

Laboratory Bioassays

Bioassays were conducted in 2019 and 2020 in Stoneville, MS to evaluate the impact of simulated rainfall on the performance of acephate (Orthene 90 SP, Amvac Chemical Corporation, Los Angeles, CA), dicrotophos (Bidrin® 8E, Amvac Chemical Corporation, Newport Beach, CA), thiamethoxam (Centric 40WG, Syngenta Crop Protection, Greensboro, NC), sulfoxaflor (Transform WG, Dow AgroScience, Indianapolis, IN), and novaluron (Diamond 0.83EC, ADAMA USA, Raleigh, NC). Tarnished plant bug adults were collected from marestail, *Conyza canadensis* (L.) Cronq., in Sunflower County, MS at least 24 h before each assay. Nymphs from a laboratory colony in Starkville, MS were used in the novaluron bioassay. The collected population was given a sugar/water mixture overnight as a temporary food source. Terminals of unsprayed cotton plants were clipped in Stoneville, MS and placed into 15mL centrifuge tubes (Globe Scientific Inc., Mahwah, NJ) which were filled with water. Tubes were sealed with a rubber cap that had a small opening for the stems. The tubes were inserted into the lids of 355mL clear plastic cups (ULINE, Pleasant Prairie, WI, #S-22278) which held the tubes upright in a wooden rack. Each wooden rack held four plant terminals in water picks. Each terminal was sprayed with insecticide and set aside until the designated rainfall timing. Insecticide solutions were applied using a CO_2 charged backpack sprayer and hand boom. The boom was equipped with Teejet 80° flat fan nozzles. Field rates of acephate (0.756kg ai/ha), dicrotophos (0.56kg ai/ha), thiamethoxam (0.07kg ai/ha), sulfoxaflor (0.053kg ai/ha), or novaluron (0.045kg ai/ha) were scaled down and mixed in 3,785mL of water based on a total application volume of 93.54L/ha. Timings for simulated rainfall were 1, 2, 4, 8, and 16 hours after insecticide application. An untreated control and an insecticide with no rainfall treatment were also included. Terminals were placed under a rainfall simulator (Conservation Demonstrations, Salina, KS) at designated timings and received 2.54cm of rainfall within approximately 5-8 minutes. The simulator received water from a standard garden hose and has an oscillating head that creates a fan distribution to allow for even rainfall. A rain gauge was placed under the simulator at each timing to measure rainfall amount.

After drying for approximately one hour, each cotton terminal was infested with two tarnished plant bug adults. Clear plastic 354.9mL cups which were fitted to the lids (ULINE, Pleasant Prairie, WI, #S-22278) were placed over each terminal to contain the infested tarnished plant bugs. Each water pick bioassay was replicated four times, with each treatment containing ten water picks. All water picks were observed 48 hours after infestation, except for novaluron which was checked at 72 hours after infestation. Mortality of tarnished plant bugs was determined by observing for movement and probing to see if specimens were lethargic and able to right themselves after being turned onto their back. Percent mortality was calculated by dividing number of dead tarnished plant bugs by the total tarnished plant bugs and multiplying by 100.

Tarnished plant bug water pick assay mortality data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Percent mortality was transformed using the arcsine function to put the percent into radians. Time between application and rainfall was considered a fixed effect in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at α=0.05.

Impact of Adjuvants

Field experiments were conducted in 2020 at the Delta Research and Extension Center to evaluate the impact of rainfall on the performance of sulfoxaflor (Transform WG, Dow AgroScience, Indianapolis, IN) when combined with selected adjuvants. Tarnished plant bug collections were made from the same site in Sunflower County as the previous experiment. Cotton terminals were collected from the same block of cotton and placed into water picks identical to the previous experiment. Each test contained four replications and seven treatments. Insecticide solutions were applied using a CO_2 charged backpack sprayer and hand boom. The boom was equipped with Teejet 80° flat fan nozzles. Water picks received an insecticide solution of a field rate of sulfoxaflor (0.053kg ai/ha) except for the untreated control. Two treatments received sulfoxaflor with no adjuvant: sulfoxaflor with no simulated rainfall and sulfoxaflor with simulated rainfall and no adjuvant. The remaining treatments received an adjuvant as well as sulfoxaflor which included an organosilicone (DyneAmic, Helena Agri-Enterprises, LLC, Collierville, TN) at 0.25% v/v, a water conditioner (AgMix, Earth Sciences Laboratories, Rogers, AR) at 0.5% v/v, a nonionic surfactant (Scanner, Loveland Products, Loveland, CO) at 0.25% v/v, and a nonionic wetter-spreader (Induce, Helena Agri-Enterprises, LLC, Collierville, TN) at 0.5% v/v. Rainfall simulation and tarnished plant bug mortality were performed and determined identical to the previous experiment.

Tarnished plant bug adjuvant assay mortality data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at $\alpha=0.05$.

Chemical Analyses

Chemical analyses were conducted in 2019 at the Mississippi State University Chemical Laboratory in Starkville, MS to evaluate the impact of rainfall on the residual concentration of foliar insecticides for tarnished plant bug management on cotton leaves. Separate experiments were conducted for acephate (Orthene 90 SP, Amvac Chemical Corporation, Los Angeles, CA), dicrotophos (Bidrin® 8E, Amvac Chemical Corporation, Newport Beach, CA), thiamethoxam (Centric 40WG, Syngenta Crop Protection, Greensboro, NC), sulfoxaflor (Transform WG, Dow AgroScience, Indianapolis, IN), and novaluron (Diamond 0.83EC, ADAMA USA, Raleigh, NC).

Blocks of cotton seed (Deltapine 1822XF, Bayer CropScience, St. Louis, MO) without any insecticide or fungicide seed treatment were planted at a rate of 12.5 seeds/m at the Delta Research and Extension Center in Stoneville, MS on 5 May 2019. These plots also did not receive any foliar insecticide treatments. Herbicides were applied prior to emergence of cotton to minimize weeds, and cotton was fertilized according to Mississippi State University Extension Service recommendations. The block was separated into plots that were four rows wide with 1.02m row spacing and 3.6m long with 1.39m long alleys. Each test was arranged as a randomized complete block design with four replications and eight treatments: an untreated control, treated with insecticide and no rain control, treated with insecticide and simulated rainfall at 0, 15, 30, 60, 120, 240, 480, and 960 minutes after insecticide application. Plots were sprayed with a John Deere 6000 Hi clearance sprayer (John Deere, Moline, IL) using field rates of acephate (0.756kg ai/ha), dicrotophos (0.56kg ai/ha), thiamethoxam (0.07kg ai/ha), sulfoxaflor (0.053kg ai/ha), and novaluron (0.045kg ai/ha).

Square pattern sprinklers (#7800, Melnor Inc., Winchester, VA) were placed at the center of each plot at the designated timings, with one sprinkler used per replication. The sprinkler evenly distributed water approximately 1.8-2.1 meters on all sides using its rotating blades. The height of the tripod was set to approximately 0.6m above the plant canopy. Water was delivered to the sprinkler from a standard 1.59 cm garden hose that was attached to a tank with a motorized pump. Each plot received 2.54 cm of simulated rainfall within ten minutes, which was determined by placing a rain gauge under one of the sprinklers at each of the timings. A wire flag was placed at the base of each sprinkler before simulating rainfall, within one meter of either side of the flag to ensure all samples pulled had received rainfall. Samples were taken one hour after all plots received rainfall to allow time to dry.

Samples were taken by pulling 20 leaves from the upper one third of plants within 1 m of the wire flag and placing them in 946mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI). Latex gloves were worn and changed between plots to ensure no cross contamination. Samples were then placed in a freezer until analysis was conducted. Analyses were done using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure developed by Anastassiades and Lehotay (2003). Each bag of leaf sample was ground into a powder and 5g of the sample was placed into a 50mL polypropylene tube. Two tubes of clean, lab grown samples were also measured with 5 grams placed into 50mL polypropylene tubes for a “blank” and a “spike” sample. The spike was given an appropriate amount of insecticide to be tested for to ensure a clear reading and the blank was left clean. Each tube received a ceramic bead for homogenizing the samples when centrifuging, and 10mL of high-performance liquid chromatography water. All samples were centrifuged in a GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer for five minutes at 1000 RPM. Each sample received

10mL of acetonitrile (ACN), which allows extraction of the pesticide. Samples were placed in the GenoGrind for an additional five minutes. Afterward, samples appeared more uniform and $MgSO_4$ (anhydrous magnesium sulfate) was added to separate the pesticide from the plant material. Samples were again placed in the GenoGrind for five minutes to drive the water and ACN apart. Samples were removed from the GenoGrind and placed into a centrifuge at 4000 RPM for ten minutes to fully separate the water and ACN. Complete separation of the mixture was achieved with a top liquid layer containing the residual insecticide. This liquid from each sample was extracted and put into a new 15mL polypropylene tube. Approximately 1mL of extracted liquid was placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual ranged between 85-101% (mostly >95%) (Anastassiades and Lehotay 2003).

Chemical analyses data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at $\alpha=0.05$.

Results

Laboratory Bioassays

Simulated rainfall reduced the efficacy of dicrotophos compared to dicrotophos without simulated rainfall ($F=9.5$; $df=6, 21$; $P<0.01$). Mortality of tarnished plant bugs from dicrotophos was similar among wash off timings (Table 3.2), Only dicrotophos without simulated rainfall and dicrotophos with simulated rainfall at 8 hours after treatment resulted in greater mortality of tarnished plant bugs than the untreated control. All dicrotophos treatments that received rainfall at any timing resulted in lower mortality than the dicrotophos treatment with no rainfall.

Simulated rainfall at any timing reduced thiamethoxam efficacy compared to thiamethoxam without simulated rainfall (F=43.27; df=6, 18; *P*<0.01). When simulated rainfall was applied, thiamethoxam did not result in greater mortality compared to the untreated control (Table 3.2).

Simulated rainfall had an effect on the efficacy of acephate (F=79.8; df=6, 21; *P*<0.01). None of the treatments that were sprayed with acephate and received simulated rainfall provided greater mortality than the untreated control. The treatment that was sprayed with acephate and received no simulated rainfall provided 98.7% mortality and was the only treatment that was not similar to the untreated control (Table 3.2).

Simulated rainfall had an effect on the efficacy of sulfoxaflor (F=47.28; df=6, 21; *P*<0.01). The treatment that was sprayed with sulfoxaflor and received rainfall at 4 hours after treatment was the only rainfall timing that resulted in greater mortality than the untreated control. None of the treatments that were sprayed with sulfoxaflor and received simulated rainfall provided greater mortality than the untreated control. The treatment sprayed with sulfoxaflor that did not receive simulated rainfall (No Rain Control), resulted in greater mortality than all other treatments. (Table 3.2).

The efficacy of novaluron was impacted by simulated rainfall (F=10.82; df=5, 15, *P*<0.01). The treatment that was sprayed with novaluron and received rainfall at 8 hours after treatment was the only rainfall timing that resulted in similar mortality to the treatment sprayed with novaluron that did not receive simulated rainfall. All other treatments that were sprayed with novaluron and received simulated rainfall provided greater mortality than the untreated control. Tarnished plant bug mortality where novaluron was sprayed and did not receive simulated rainfall averaged 56.7 percent (Table 3.3).

Impact of Adjuvants

Simulated rainfall had an effect on the performance of sulfoxaflor ($F=57.10$; df=6, 30; $P<0.01$). The addition of adjuvants did not improve the performance of sulfoxaflor (Table 3.4). All sulfoxaflor treatments mixed with an adjuvant resulted in similar mortality of tarnished plant bugs to the sulfoxaflor treatment alone. Sulfoxaflor with and without adjuvants after simulated rainfall also provided similar mortality to the untreated control. Sulfoxaflor with no simulated rainfall provided 85.9 percent mortality.

Chemical Analyses

Simulated rainfall had an effect on the concentration of thiamethoxam ($F=26.09$; df=6, 18; $P<0.01$) on or in cotton leaves. All thiamethoxam treatments that received rainfall at any timing resulted in lower concentrations than the thiamethoxam treatment with no simulated rainfall. Additionally, no difference from the 0-minute control was seen until the rainfall simulated at 240 minutes after application treatment (Fig. 3.1).

Concentrations of acephate were also affected by simulated rainfall ($F=17.86$; df=4, 10; $P<0.01$). Simulated rainfall, regardless of timing, reduced acephate concentrations compared to acephate without simulated rainfall (Fig. 3.2). All acephate treatments that received simulated rainfall had similar concentrations to each other.

Simulated rainfall had an effect on the concentration of sulfoxaflor in or on cotton leaves ($F=66.68$; df=6, 12; $P<0.01$). All sulfoxaflor treatments that received rainfall at any timing resulted in lower concentrations than the sulfoxaflor treatment without simulated rainfall (Fig. 3.3). Treatments that received sulfoxaflor and simulated rainfall at 15, 30, 60, and 120-minutes after application had similar concentrations to each other. The treatment that received simulated rainfall 240-minutes after sulfoxaflor application was the only simulated rainfall timing treatment with a higher concentration of sulfoxaflor than the rainfall at 0-minute after application treatment.

Simulated rainfall also had an effect on novaluron concentrations on or in cotton leaves ($F=18.74$; $df=7, 21$; $P<0.01$). The treatment that received novaluron and simulated rainfall at 8 hours after application was the only simulated rainfall treatment with concentrations similar to the novaluron treatment with no simulated rainfall (Fig. 3.4). Additionally, the treatment that received novaluron and simulated rainfall at 60, 120, and 240-minutes after treatment had higher concentrations of novaluron than the treatment that received novaluron and simulated rainfall at 0-minutes after treatment.

Discussion

The impact of simulated rainfall on insecticides is an understudied aspect of pest management, but a critical factor that could influence the performance of insecticides in agriculture. The current study was designed to evaluate the impact of simulated rainfall on insecticides commonly used for tarnished plant bug control in cotton. Overall, simulated rainfall had a negative impact on the performance of all the insecticides evaluated, but results varied between insecticides. All of the insecticides used in this study were also evaluated in a test conducted across four states in the Mid-south (Steckel et al. 2018, 2019). In general, dicrotophos provided moderate control following a single application, and good control when two sequential applications were made in the Mid-south test. In the rainfastness tests, dicrotophos provided between 33.3-50.7% mortality depending on time elapsed between insecticide application and rainfall compared to 82.5% with no rainfall. The greatest mortality for treatments that received simulated rainfall was seen when rainfall was simulated at eight hours after application (50.7%), which was the only timing with greater mortality than the untreated control. Chemical analyses were not performed on this insecticide due to its high mammalian toxicity.

Thiamethoxam provided poor control of tarnished plant bug adults after a simulated rainfall event in bioassays, with only 9.5-21.3% mortality seen among treatments that received simulated rainfall. Poor control for thiamethoxam was also observed in the Mid-south efficacy tests (Steckel et al. 2018, 2019). None of the thiamethoxam treatments that received rainfall provided greater mortality than the untreated control. Chemical analyses of this product support the bioassay results. The rainfall at 4 hours after thiamethoxam treatment had a higher concentration than the 0-minute timing. Thiamethoxam treatments that received rainfall at less than four hours after application did not statistically separate from the 0-minute timing.

Simulated rainfall caused acephate to perform poorly, although previous research suggests that acephate provides moderate control of tarnished plant bugs (Steckel et al. 2018, 2019). In contrast, resistant plant bug populations have been documented in Mississippi and other areas across the Mid-south (Snodgrass et al. 2009, Dorman et al. 2020), which could account for some of the poor performance observed in these experiments. All acephate and simulated rainfall treatment timings, except for the treatment that received acephate and no simulated rainfall, provided similar mortality to the untreated control. Mortality ranged from 3.8-32.1% for simulated rainfall timings. Chemical analyses of acephate support the results seen in the laboratory bioassay. Concentrations of acephate in all simulated rainfall timings did not differ from the one-hour timing.

Sulfoxaflor performed poorly after simulated rainfall. Simulated rainfall timings provided 11.4-39.0% mortality. However, sulfoxaflor provided the best control of tarnished plant bugs in the Mid-south efficacy trials (Steckel et al. 2018, 2019). More recent research revealed that most tarnished plant bug populations are still susceptible to sulfoxaflor (Dorman et al. 2020), which could mean that the poor control observed in these experiments was caused by a lack of rainfastness. At least four hours are needed between a sulfoxaflor treatment and a rainfall event to provide significantly higher mortality than the untreated control. Chemical analyses of sulfoxaflor support the bioassay findings. All sulfoxaflor and simulated rainfall timings, except for the sulfoxaflor and simulated rainfall at four hours after application timing, provided similar concentrations to the untreated control. No sulfoxaflor with simulated rainfall timing treatments had concentrations similar to the no simulated rainfall treatment.

Simulated rainfall affected novaluron the least. This experiment was the only one in which one of the simulated rainfall timings, novaluron with simulated rainfall at 8 hours after treatment, provided similar mortality to the no simulated rainfall treatment. The no simulated rainfall mortality was low (56.7%). This is not a concern because novaluron is an insect growth regulator and may need several days to achieve full mortality. All remaining treatments that received novaluron and simulated rainfall were not different from the untreated control. Chemical analyses of novaluron after different simulated rainfall timings showed similar results. The eight-hour timing had a similar concentration to the no simulated rainfall treatment. At least one hour is needed between insecticide application and rainfall event to see an improvement from the 0-minute timing.

Simulated rainfall negatively impacted the performance of sulfoxaflor without an adjuvant in laboratory bioassays. Sulfoxaflor without an adjuvant that received simulated rainfall did not perform better than the untreated control. This correlates with the results of the previous sulfoxaflor bioassays, which revealed that sulfoxaflor is not rainfast. Additionally, sulfoxaflor with no simulated rainfall provided similar control to previous assays and is supported by the

Mid-south efficacy trials as well as current research on the status of resistance (Snodgrass et al. 2009, Dorman et al. 2020). After the addition of adjuvants, there was still no improvement in the efficacy of sulfoxaflor after a rainfall event.

Further research is needed to determine the longevity of these products when rainfall is expected. Results may be different in a field setting where insects are already present at the time of the spray. Based on these results, novaluron was the least impacted by rainfall, but it may take longer to see results because it is an insect growth regulator. Dicrotophos provided poor control across simulated rainfall timings relative to the dicrotophos and no rainfall treatment. Thiamethoxam needs at least four hours between application and rainfall event to see any improvement in concentration or mortality from the untreated control. Acephate is not rainfast according to our experiments, but lack of control in some cases could be attributed to resistance. Sulfoxaflor was not rainfast according to our experiments, even when using adjuvants. The insecticides evaluated in this study are commonly used in tarnish plant bug management in cotton, therefore, it is important that we are able to understand the rainfastness of these products.

Table 3.1 Average insecticide applications made per ha for tarnished plant bug management from 1990 to 2019 in 5 year increments. States represented are Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Virginia.

	Insecticide applications per ha					
State	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2019
AL	1.08	0.45	0.56	0.98	1.50	1.38
AR	0.56	0.92	1.63	2.76	4.88	4.76
GA	0.10	0.12	0.09	0.46	0.82	1.04
LA	0.60	1.82	2.90	3.18	3.60	3.80
MS	1.38	1.42	2.44	4.38	5.08	3.72
NC	0	0.02	0.07	0.43	1.20	2.26
SC	0.04	0.03	0.06	0.28	0.22	1.00
TN	0.56	0.52	0.87	1.21	3.36	3.12
TX	0	0.03	0.03	1.02	0.80	0.54

Table 3.2 Mean (SEM) percent mortality of tarnished plant bug adults in laboratory bioassays conducted in 2020 evaluating the impact of rainfall on the residual efficacy of dicrotophos, thiamethoxam, sulfoxaflor, and acephate in cotton.

	Percent Mortality (SEM)			
Time (hours after spray)	Dicrotophos	Thiamethoxam	Acephate	Sulfoxaflor
Untreated	6.7(2.6) C	8.0(1.4) B	6.1(2.7) BC	14.0(1.0) C
1	36.8(13.2) BC	16.3(1.7) B	3.8(2.4) C	11.4(1.2) C
2	37.5(5.9) BC	15.4(2.8) B	3.9(3.9) C	16.3(3.8) BC
4	33.3(2.6) BC	21.3(5.7) B	13.1(5.4) BC	39.0(9.3) B
8	50.7(9.9) B	18.6(5.6) B	7.3(4.2) BC	28.3(6.7) BC
16	43.1(9.4) BC	9.5(3.3) B	32.1(9.2) B	24.5(3.7) BC
No Rain	82.5 (0.91) A	91.3(1.4) A	98.7(1.3) A	92.5(1.4) A
F	9.5	43.27	79.8	47.28
d.f.	6, 21	6, 18	6, 21	6, 21
P>F	<0.01	<0.01	<0.01	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey’s HSD (α=0.05).

Table 3.3 Mean (SEM) percent mortality for tarnished plant bug immatures in laboratory bioassays conducted in 2020 evaluating the impact of rainfall on the residual efficacy of Novaluron in cotton.

	Percent Mortality (SEM)
Time (hours after spray)	Novaluron
Untreated	4.9(2.0) C
1	20.0(4.0) BC
2	17.5(9.2) C
4	17.5(7.2) C
8	46.3(7.2) AB
No Rain	56.7(7.0) A
F	10.82
d.f.	5, 15
P>F	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

Table 3.4 Mean (SEM) percent mortality of tarnished plant bug adults in laboratory bioassays conducted in 2020 evaluating the impact of rainfall on the residual efficacy of sulfoxaflor and the use of adjuvants in cotton.

Treatment	Percent Mortality (SEM)
Untreated	4.1(2.2) B
sulfoxaflor	13.8(4.7) B
sulfoxaflor + AgMix	12.7(2.9) B
sulfoxaflor + Dyne-Amic	13.4(2.5) B
sulfoxaflor + Induce	14.3(2.8) B
sulfoxaflor + Scanner	11.1(3.8) B
sulfoxaflor (no rain)	85.9(5.2) A
F	57.10
d.f.	6, 30
P>F	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

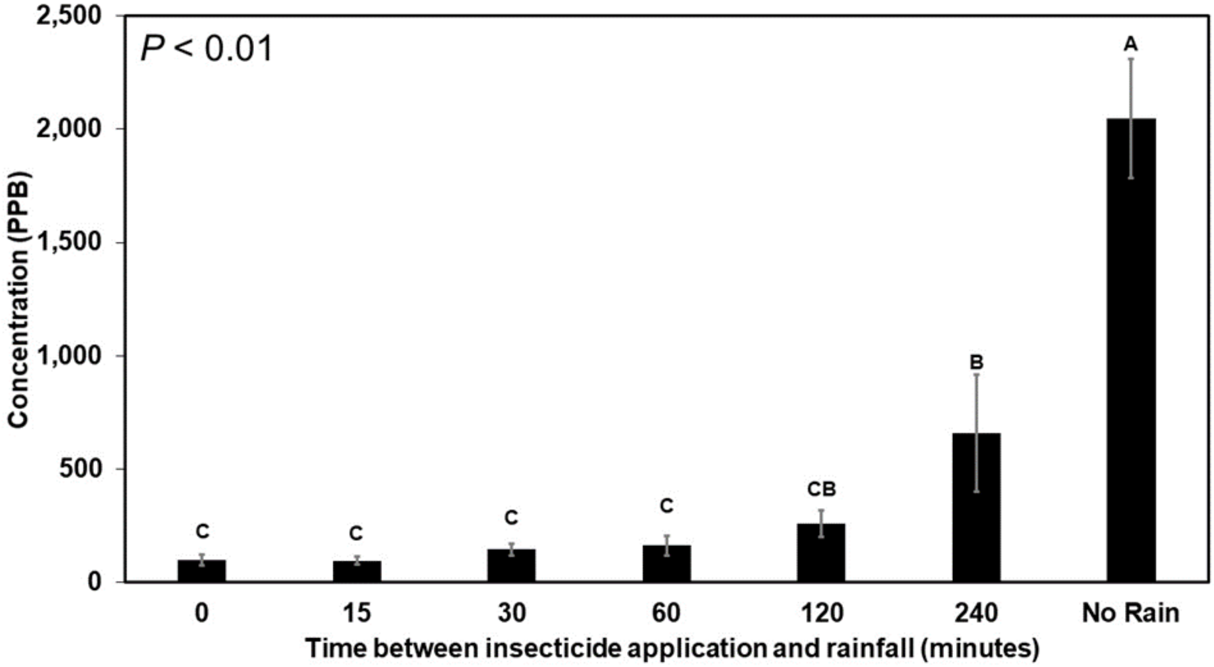

Figure 3.1 Mean (SEM) concentration (PPB) of thiamethoxam after simulated rainfall event evaluating the impact of wash off on the residual of thiamethoxam in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

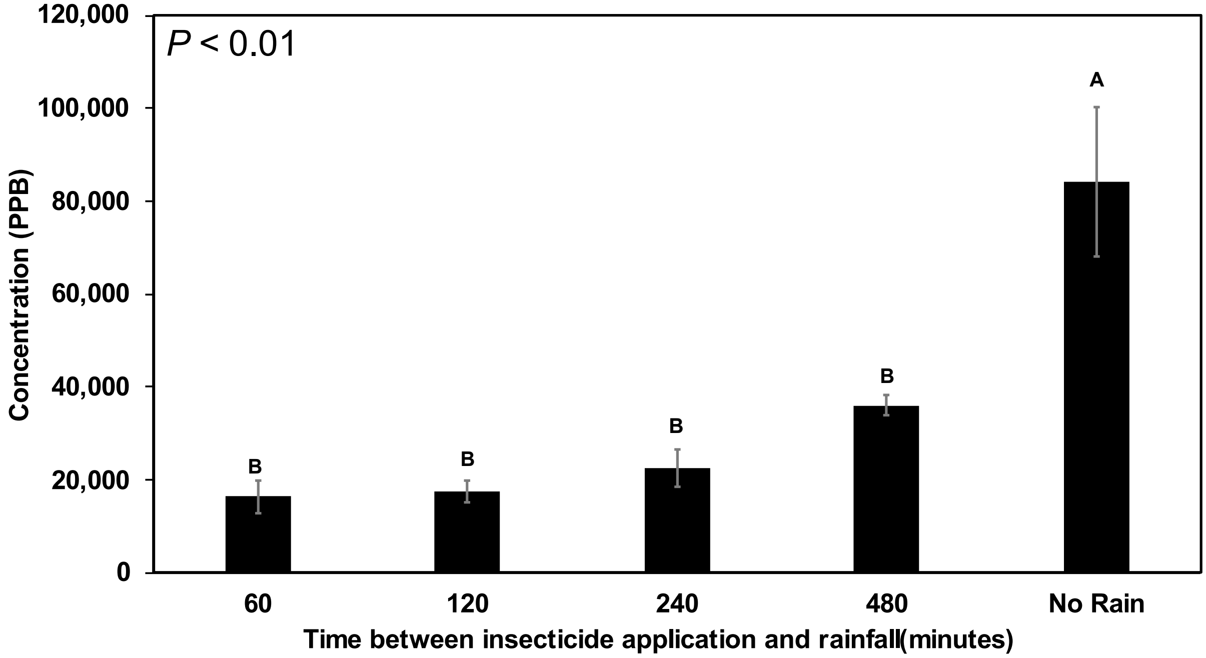

Figure 3.2 Mean (SEM) concentration (PPB) of acephate after simulated rainfall event evaluating the impact of wash off on the residual of acephate in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

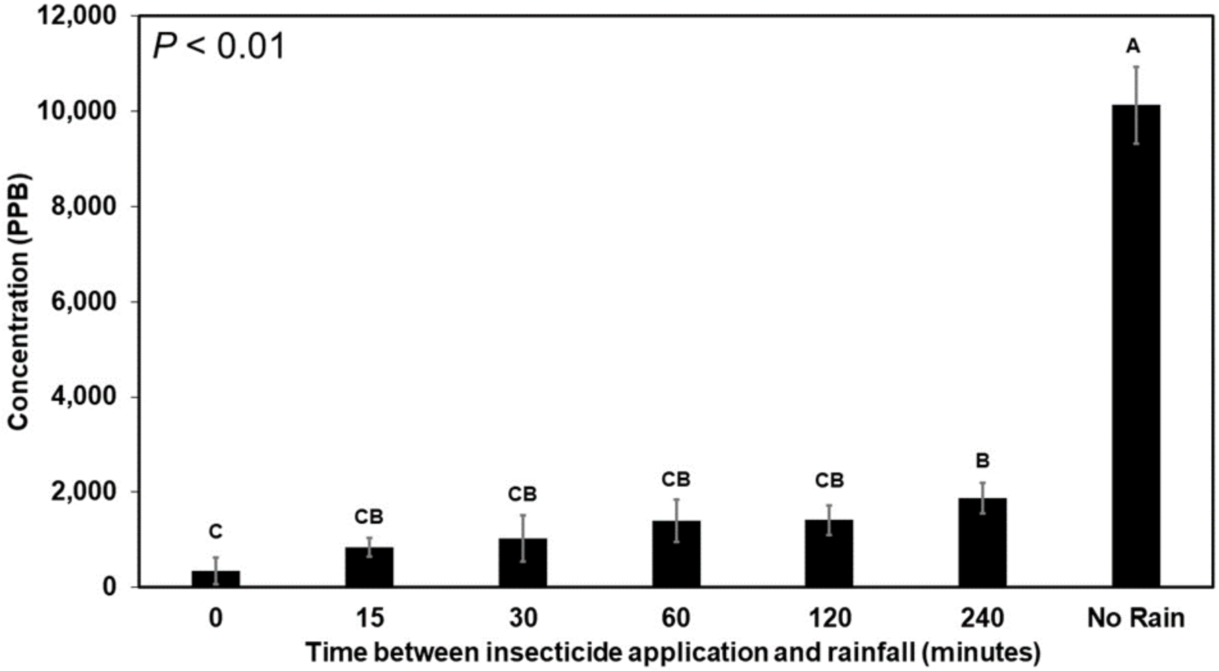

Figure 3.3 Mean (SEM) concentration (PPB) of sulfoxaflor after simulated rainfall event evaluating the impact of wash off on the residual of sulfoxaflor in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

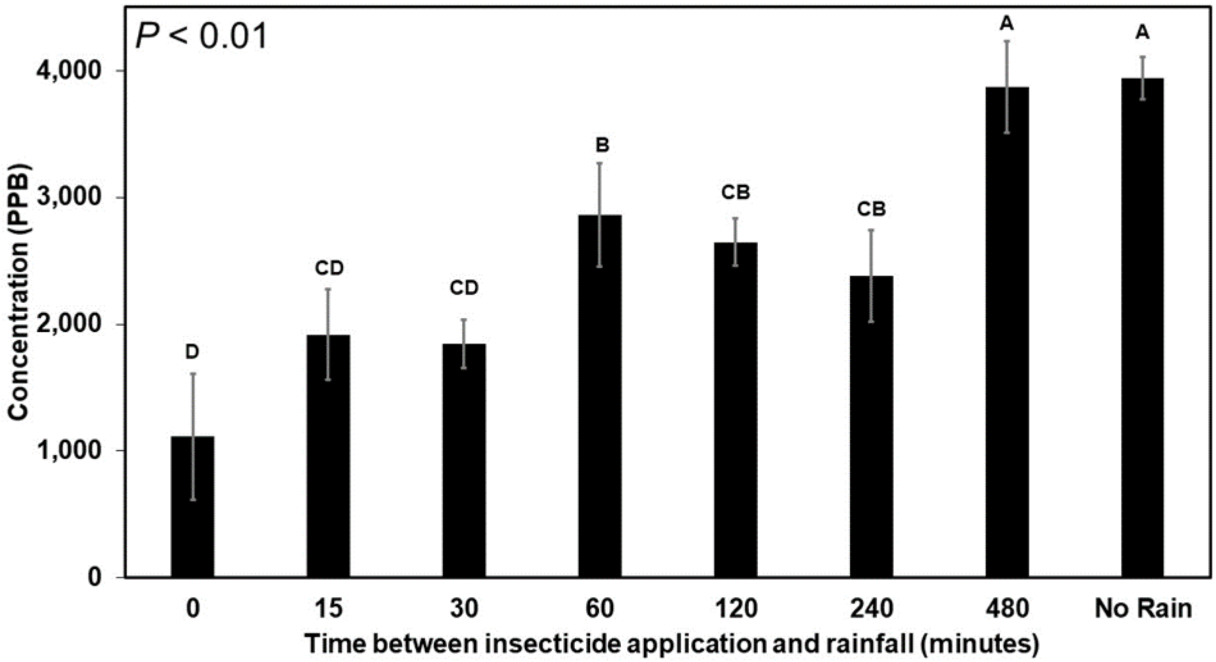

Figure 3.4 Mean (SEM) concentration (PPB) of novaluron after simulated rainfall event evaluating the impact of wash off on the residual of novaluron in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

Literature Cited

Abel, C. A., G. L. Snodgrass, R. Jackson, and C. Allen. 2010. Oviposition and development of the tarnished plant bug (Heteroptera: Miridae) on field maize. Environ. Entomol. 39: 1085-1091.

Anastassiades, M. and S. J. Lehotay. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. J. AOAC Intern. 86: 412-431.

Catchot, A., W. Crow, D. Dodds, J. Gore, F. Musser, T. Irby, D. Cook, B. Layton, E. Larson. 2020. Insect control guide for agronomic crops. Publication 2471. Mississippi State University Extension. Mississippi State, MS.

Dorman, S. J., A. D. Gross, F. R. Musser, B. D. Catchot, R. H. Smith, D. D. Reisig, F. P. F. Reay-Jones, J. K. Greene, P. M. Roberts, and S. V. Taylor. 2020. Resistance monitoring to four insecticides and mechanisms of resistance in *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) populations of southeastern USA cotton. Pest Manag. Sci. 76: 3935-3944.

Fleischer, S.J. and M.J. Gaylor 1988. *Lygus lineolaris* (Hemiptera: Miridae) population dynamics: nymphal development, life tables, and leslie matrices on selected weeds and cotton. Environ. Entomol. 17: 246-253.

Layton, M. B. 1995. Tarnished plant bug: biology, thresholds, sampling, and status of resistance. pp. 131-133. *In* Proc. 1995 Beltwide Cotton Conf., National Cotton Council.

Musser, F., S. Stewart, R. Bagwell, G. Lorenz, A. Catchot, E. Burris, D. Cook, J. Robbins, J. Greene, G. Studebaker and J. Gore 2007. Comparison of direct and indirect sampling methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. J. Econ. Entomol. 100: 1916-1923.

Musser, F., A. L. Catchot, S. D. Stewart, R. D. Bagwell, G. M. Lorenz, K. V. Tindall, G. E. Studebaker, B. R. Leonard, D. S. Akin, D. R. Cook, and C. A. Daves. 2009. Tarnished plant bug (Hemiptera: Miridae) thresholds and sampling comparisons for flowering cotton in the Mid-southern United States. J. Econ. Entomol. 102: 1827-1836.

Parys, K. A., and G. L. Snodgrass. 2014. Host plants of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). pp. 765. *In* Proc. Beltwide Cotton Conf., National Cotton Council, New Orleans, LA.

Snodgrass, G. and W. Scott 1988. Tolerance of the tarnished plant bug to dimethoate and acephate in different areas of the Mississippi delta. pp. 294-295. *In* Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.

Snodgrass, G. L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783-790.

Snodgrass, G., and J. Gore, 2007. Status of insecticide resistance for the tarnished plant bug, pp. 56-59. *In* Proc. Beltwide Cotton Conference. Nation Cotton Council, Memphis, TN.

Snodgrass, G. L., J. Gore, C. A. Abel, and R. Jackson. 2009. Acephate resistance in populations of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River Delta. J. Econ. Entomol. 102: 699-707.

Tugwell, P., S. C. Young, B. A. Dumas and J. R. Phillips 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury, and significance of wild hosts near cotton pp. AR Agric. Exp. Stn. Report University of Arkansas. Fayetteville, AR.

Young, O. P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79: 747-762.

Zhu, Y. C. and G. L. Snodgrass. 2003. Cytochrome P450 CYP6X1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticides. Insect Molec. Bio. 12: 39-49.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2004. Enhanced esterase gene expression and activity in a malathion-resistant strain of the tarnished plant bug, *Lygus lineolaris*. Insect Biochem. and Molec. Biol. 34: 1175-1186.

CHAPTER IV

EVALUATION OF THE RAINFASTNESS OF SELECTED INSECTICIDES FOR HELICOVERPA ZEA CONTROL

Abstract

Field and laboratory experiments were conducted in 2019 and 2020 at the Delta Research and Extension Center in Stoneville, MS to evaluate the effects of simulated rainfall on the efficacy of selected insecticides used for bollworm management in cotton. Leaf dip bioassays were conducted using chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN) and rinsed with simulated rainfall at various intervals after application. Simulated rainfall affected the performance of methoxyfenozide + spinetoram in 2020 but not in 2019, with both years providing moderate to excellent control after a rainfall event. Additionally, simulated rainfall affected the performance of chlorantraniliprole in 2019, providing moderate control but excellent control in 2020 after rainfall. Chemical analyses were performed on chlorantraniliprole and methoxyfenozide + spinetoram by spraying plots with insecticide and simulating rainfall with a square-pattern sprinkler. Leaves were pulled from each plot and analyzed using a mass spectrometer to determine residual concentrations leaf tissue. Both chlorantraniliprole and methoxyfenozide + spinetoram concentrations were impacted by simulated rainfall, with results varying across simulated rainfall timings.

Introduction

The bollworm, *Helicoverpa zea* (Boddie), is a key pest of cotton from Texas to Virginia in the U.S. Bollworm is a polyphagous species with a host range of more than 100 different plant species, at least 30 of which are agronomic crops (King and Coleman 1989, Blanco et al. 2007). Bollworm eggs are individually oviposited onto plant structures and will hatch in three to four days (Barber 1936, Capinera 2000). In cotton, small larvae feed on small fruiting structures around the oviposition site and move onto larger structures as they advance to later instars (Leigh et al. 1996). Bollworm feeding in cotton may cause fruiting structures to abscise, which can result in direct yield losses (Adkisson et al. 1964, Gore et al. 2000). Bollworm larvae undergo six instars before leaving the plant and pupating in the soil (King and Coleman 1989).

The use of foliar insecticides and transgenic Bt plants are the primary management practices used to control bollworm. Resistance to pyrethroids (Stadelbacher et al. 1990, Brickle et al. 2001, Jacobson et al. 2009), chlorinated hydrocarbons (Graves et al. 1963, Graves et al. 1967, Sparks 1981), and organophosphates (Plapp 1971) has been observed in bollworm populations. In an effort to control pests of the heliothine complex, transgenic cotton varieties utilizing insecticidal proteins from the soil microbe, *Bacillus thuringiensis* (Berliner) var. *kurstaki* (Bt), were developed (Perlak et al. 1990; Gore et al. 2001). Bollgard cotton (Bollgard®, Monsanto, St. Louis, MO) was released in 1996, with Bollgard II (Bollgard II®, Monsanto, St. Louis, MO) following in 2003, and most recently Bollgard III (Bollgard III®, Monsanto, St. Louis, MO) in 2018. Other varieties of Bt cotton have been released such as Widestrike, TwinLink, Widestrike 3, and TwinLink Plus. Insecticides are still a primary component of bollworm management in cotton, but now serve as supplemental control in combination with Bt cotton (Catchot et al. 2020).

Growing seasons in which bollworm pressure is high may require foliar insecticide applications even in Bt cotton varieties, however, little is known about the performance of these insecticides after a rainfall event. The Mississippi Delta is a region that may receive many unexpected rainfall events within a growing season. Periods of heavy rainfall often align with periods of heavy pest pressure, and cotton growers may need to make a foliar application when rainfall is eminent. Previous research has shown that time elapsed between insecticide application and rainfall impacted performance more than rainfall intensity in the case of bifenthrin, azinphosmethyl, fenvalerate, permethrin, sulprofos, permethrin, methyl parathion, and EPN (O-ethyl O-(p-nitrophenyl)) on cotton leaves (McDowell et al. 1984, Pick et al. 1984, Willis et al. 1986, Willis et al. 1992, Willis et al. 1994a, b, Mulrooney and Elmore 2000).

The objective of this study was to determine the impact of simulated rainfall on the efficacy of two commonly used insecticides for bollworm management in cotton. The Mississippi State Insect Control Guide recommends seven larvicides for bollworm control (Catchot et al. 2020). In this study, we evaluated two of the recommended insecticides, chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN) in an attempt to determine their rainfastness to help improve management strategies.

Materials and Methods

Laboratory Bioassays

Bioassays were conducted in 2019 and 2020 at the Delta Research and Extension Center in Stoneville, Mississippi to evaluate the impact of rainfall on the performance of chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN). Bollworms were collected in

May 2019 and 2020 from crimson clover, *Trifolium incarnatum* L., and placed into 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI) containing Stonefly *Heliothis* diet (Ward's Science, Rochester, NY). Larvae were maintained in a climate-controlled room set to 26.7°C, 80% humidity, and a light/dark cycle of 16:8 hours. Larvae fed on the stonefly diet until pupation and were removed from the cups. All larvae were rinsed with a solution of 5% sodium hypochlorite and placed into 3.79L cardboard buckets. Each bucket was topped with cheesecloth which acted as a removable oviposition site. Cheesecloth containing eggs were placed into 3.79L self-sealing bags (Ziploc®, S.C. Johnson & Son, Inc., Racine, WI). Once the eggs hatched, neonates were transferred to 59.2 mL cups which contained stonefly diet. Subsequent generations of these collections were used in bioassays.

Blocks of non-Bt cotton (Deltapine 1822XF, Bayer CropScience, St. Louis, MO) were planted on 5 May 2019 and 4 May 2020 and managed according to Mississippi State University Extension Service guidelines, with the exception of no foliar insecticide applications. Whole cotton leaves were removed from the upper three nodes from this block of cotton, which received no insecticide treatment. Leaves were taken to the laboratory and dipped into an insecticide solution of chlorantraniliprole or methoxyfenozide + spinetoram for approximately 3 seconds. After dipping, leaves where placed onto a tray lined with paper towels which were labelled by treatment. Field rates of chlorantraniliprole (1.4kg/ha) or methoxyfenozide + spinetoram (0.56kg/ha) at an application volume equivalent to 94L/ha were used for leaf dip solutions and scaled down to mix with 946.3mL of water. Treatments for bollworm bioassays included an untreated control, insecticide with no simulated rainfall, insecticide with simulated rainfall after 0, 15, 30, 60, 120, and 240 minutes for a total of eight treatments. To simulate rainfall, leaves were sprayed using a 15.9mm diameter hose with a shower nozzle to simulate the pattern of natural rainfall for approximately 10 seconds. Leaves were placed on a rack lined with 6.35mm hardware cloth (YARDGARD, Gemplers, Janesville, WI) while simulating rainfall. The rack was cleaned between each rainfall simulation to avoid contamination. After the simulated rainfall, leaves were placed onto a rack lined with paper towels to allow the leaves to dry.

After drying for one hour, each leaf was placed into a 100x15mm petri dish (Falcon®, Corning, Inc., Corning, NY) which was labelled by treatment and sealed with parafilm. A single third-instar larva from the laboratory colony was placed into each dish using a pair of forceps. Bioassays were replicated four times, with ten dishes of each treatment for each replication. All dishes were observed 48 hours after infestation. Mortality of bollworms was determined by observation or probing specimens to check for lethargic movement. Percent mortality was calculated by dividing number of dead bollworms over total bollworms and multiplying by 100.

Percent mortality data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Percent mortality was transformed using the arcsine function to convert the percent into radians. Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at α=0.05.

Chemical Analyses

Experiments were conducted in 2019 at the Delta Research and Extension Center in Stoneville, Mississippi and at the Mississippi State University Chemical Laboratory in Starkville, MS to evaluate the impact of simulated rainfall on the concentration of foliar insecticides used for bollworm management in cotton. Separate experiments were conducted for chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) and methoxyfenozide + spinetoram (Intrepid Edge®, Dow AgroSciences, Indianapolis, IN).

Black cotton seed that did not receive an insecticide or fungicide seed treatment (Deltapine 1822XF, Bayer CropScience, St. Louis, MO) was planted at 12.5 seeds/m at the Delta Research and Extension Center in Stoneville, MS. This block was divided into plots that were four rows wide with 1.02m row spacing and 3.6m long with 1.39m alleys. Tests were arranged in a randomized complete block design with four replications of eight treatments. The treatments included an untreated control, insecticide with no rain, insecticide with rainfall simulated at 0, 15, 30, 60, 120, and 240 minutes after application. Insecticides were applied with a John Deere 6000 Hi clearance sprayer (John Deere, Moline, IL) with field rates of chlorantraniliprole (1.4kg/ha) and methoxyfenozide + spinetoram (0.56kg/ha) at an application volume of 94L/ha according to the Mississippi State University Insect Control Guide (Catchot et al. 2020).

Rainfall was simulated using square pattern sprinklers (#7800, Melnor Inc., Winchester, VA) mounted on a tripod 0.6m above the plant canopy. The sprinklers were positioned to cover the tops of the plants in an area approximately 1.8-2.1m wide. Sprinklers received water from a standard 1.59cm garden hose which was supplied water from a tank with a motorized pump. Plots received 2.54cm of simulated rainfall, which was collected during each treatment timing by a rain gauge. A wire flag was placed in the ground at the center of each tripod. Leaf samples were taken from the upper three nodes of plants within 1 m of the flag to ensure that leaf samples had received rainfall. Samples were pulled approximately one hour after the latest rainfall timing to ensure the leaves were dry. Samples of 20 leaves from each plot were placed into self-sealing plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and stored in a freezer until analysis. Gloves were worn and disposed of between each treatment to ensure samples were not cross-contaminated.

A modified QuEChERS by LC/MS/MS and GC/MS/MS procedure (Anastassiades and Lehotay 2003) was used to quantify insecticide concentrations. Leaf samples were taken to the Mississippi State Chemical Laboratory and crushed into a fine powder, and 5g of that plant material was transferred into a 50mL polypropylene tube. A lab-grown "blank" and "spike" plant sample were also placed into 50mL polypropylene tubes. The "blank" acted as an untreated control and the "spike" received insecticide to ensure the product was being measured accurately by the mass spectrometer. All sample tubes received 10mL of high-performance liquid chromatography water and a ceramic bead for homogenization while undergoing centrifugation. Samples were capped and placed into a GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer for five minutes at 1000 RPM. Samples were taken out and 10mL of acetonitrile (ACN) was added to extract the insecticide from the leaf material. Samples are placed back in the centrifuge for an additional five minutes. Samples were removed from the centrifuge again and 4g of $MgSO_4$ (anhydrous magnesium sulfate) was added. The sample was placed back into the centrifuge for five minutes to pull water from the ACN. Samples were taken from the GenoGrind and placed into a larger centrifuge for ten minutes at 4000 RPM to fully separate all materials. The sample tubes were removed from the centrifuge with the layer of insecticide at the top. The insecticide layer was extracted and placed into a clean 15mL polypropylene tube. One milliliter was extracted from each sample and placed into auto sampler vials which had a PTFE/PVDF filter. Sample vials were analyzed using an Agilent Technologies LC/MS/MS or GC/MS/MS for GC-amenable pesticides. The mass spectrometer provided a concentration of each insecticide sample in parts per billion.

Chemical analyses data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Degrees of freedom were calculated using the Kenward-Roger method. Means and standard errors were calculated using the PROC MEANS statement. LS MEANS were separated using Tukey's protected HSD at α=0.05.

Results

2019 Laboratory Bioassays

Methoxyfenozide + spinetoram treatment had an effect on bollworm mortality in 2019 (F=41.78; df=7, 21; *P*<0.01), but simulated rainfall did not appear to have an effect. All methoxyfenozide + spinetoram treatments that received simulated rainfall resulted in greater mortality of bollworm than the untreated control, and similar mortality to the methoxyfenozide + spinetoram without simulated rainfall treatment (Table 4.1). Mortality of bollworms was similar among all treatments that received methoxyfenozide + spinetoram including the simulated rainfall timings and the no simulated rainfall treatment.

Treatment with chlorantraniliprole had an effect on bollworm mortality (F=5.50; df=7, 21; *P*<0.01), but simulated rainfall did not have an effect. The 30, 60, and 120-minute timings resulted in mortality similar to the untreated control (Table 4.1). All treatments that received chlorantraniliprole and simulated rainfall resulted in similar mortality of bollworm to the chlorantraniliprole without simulated rainfall treatment. Bollworm mortality was similar among all treatments that received chlorantraniliprole, including those that received simulated rainfall and the no simulated rainfall treatment.

2020 Laboratory Bioassays

Methoxyfenozide + spinetoram treatment had an effect on bollworm mortality (F=49.16; df=6, 21; *P*<0.01), and simulated rainfall impacted performance. The methoxyfenozide + spinetoram with simulated rainfall at 8 and 16-hours after treatment resulted in mortality of bollworm similar to the methoxyfenozide + spinetoram without simulated rainfall treatment (Table 4.2). The methoxyfenozide + spinetoram with simulated rainfall at 4 and 2 hours after treatment resulted in mortality of bollworm similar to the simulated rainfall at 1-hour treatment, but not to each other. All methoxyfenozide + spinetoram with simulated rainfall treatments resulted in greater mortality of bollworm than the untreated control.

Treatment with chlorantraniliprole had an effect on bollworm mortality (F=32.38; df=6, 18; P<0.01), but simulated rainfall did not have an effect. All chlorantraniliprole with simulated rainfall treatments resulted in mortality of bollworm similar to chlorantraniliprole without rainfall (Table 4.2). Additionally, all treatments that received chlorantraniliprole resulted in greater mortality of bollworm than the untreated control.

Chemical Analyses

Simulated rainfall had an effect on the concentration of methoxyfenozide (F=6.12; df=7, 24; P<0.01). The methoxyfenozide + spinetoram treatment with simulated rainfall at 30, 120, and 240-minutes after treatment resulted in similar concentrations of methoxyfenozide as the methoxyfenozide without simulated rainfall (Fig. 4.1). Concentrations of methoxyfenozide for these treatments were also similar to the methoxyfenozide + spinetoram with rainfall simulated at 0 minutes after application. The methoxyfenozide + spinetoram treatment with simulated rainfall at 15 and 60 minutes after treatment resulted in similar concentrations of methoxyfenozide as the methoxyfenozide treatment with simulated rainfall at 0-minutes after application.

There was an effect of simulated rainfall (F=4.49; df=7, 24; P<0.01) on the concentration of spinetoram (Fig. 4.2). The treatments that received spinetoram and simulated rainfall at 15 and 60 minutes after application did not provide similar concentrations to the spinetoram and no simulated rainfall treatment but were similar to the spinetoram and simulated rainfall at 0 minutes after application. All remaining spinetoram and simulated rainfall timings had concentrations similar to the spinetoram and no simulated rainfall treatment.

Chlorantraniliprole concentrations were also affected by simulated rainfall ($F=8.04$; $df=7, 24$; $P<0.01$). All of the chlorantraniliprole and simulated rainfall treatments provided similar concentrations (Fig. 4.3). Treatments that were sprayed with chlorantraniliprole with rainfall simulated at 15, 60, and 240 minutes after application provided similar results to the chlorantraniliprole and no simulated rainfall treatment. No differences in concentration from the untreated control were seen until the chlorantraniliprole and simulated rainfall at 240-minutes after application.

Discussion

Little is known about the effect of rainfall on the performance of insecticides used in cotton. This study was conducted to evaluate the impact of simulated rainfall on two commonly used insecticides to control bollworm in cotton. The impact of simulated rainfall on methoxyfenozide + spinetoram and chlorantraniliprole was observed and results varied over the two years of this study. Overall, methoxyfenozide + spinetoram provided high mortality rates in laboratory bioassays. Bioassays in 2019 evaluated shorter time intervals (wash off at 0, 15, 30, 60, 120, and 240 minutes after application); whereas bioassays in 2020 evaluated longer time intervals (wash off at 1, 2, 4, 8, and 16 hours after application). All treatments with simulated rainfall timings following the application of methoxyfenozide + spinetoram during 2019 laboratory bioassays provided excellent control, with a minimum of 97.5% mortality of bollworm. Each treatment that received methoxyfenozide + spinetoram and simulated rainfall resulted in similar mortality as methoxyfenozide + spinetoram without simulated rainfall.

Methoxyfenozide + spinetoram with simulated rainfall treatments from 2020 provided moderate control up to 4 hours after application. Performance of methoxyfenozide + spinetoram improved when simulated rainfall occurred at ≥8 hours after application, and mortality results were similar to the no simulated wash off treatment. Methoxyfenozide + spinetoram, regardless of simulated rainfall treatment timing, provided greater mortality than the untreated control. Results from a similar study also found that methoxyfenozide and spinetoram performance, which were applied separately, was not impacted by rainfall (Hulbert 2011). Methoxyfenozide and spinetoram were chemically analyzed independently. Methoxyfenozide concentrations varied across timings but retained relatively high concentrations overall after a rainfall event. At least 30 minutes is required between insecticide application and rainfall to have similar results to a methoxyfenozide and no rainfall concentration. A study which evaluated the residue of methoxyfenozide recovered from the surface of blueberry fruit after rainfall found that the majority of the insecticide remained on the fruit despite the rainfall (Hulbert 2011). Spinetoram results were also variable across timings. Concentrations from this test showed similar results between the spinetoram with simulated rainfall at 0 minutes after application and the spinetoram without simulated rainfall treatment. Only two treatments, spinetoram with simulated rainfall at 15 and 60 minutes after application, were not similar to the spinetoram without rainfall treatment. Hulbert (2011) found that more wash-off occurred with spinetoram when compared to methoxyfenozide, but most of the insecticide was still recovered.

Chlorantraniliprole provided moderate control in bioassays performed in 2019, and excellent control in 2020 bioassays. Bioassays conducted in 2019 evaluated the impact of wash off on chlorantraniliprole at 0, 15, 30, 60, 120, and 240 minutes after application. Simulated rainfall at 30, 60, and 120 minutes after chlorantraniliprole application provided similar mortality to the untreated control. However, greater mortality was observed with simulated rainfall at 0, 15, and 240 minutes after chlorantraniliprole application which did provide similar control to the chlorantraniliprole and no simulated wash off treatment. These results are similar to what Pandey et al. (2020) found, where chlorantraniliprole degraded faster when rainfall occurred at less than 4 hours after application. Bioassays performed in 2020 evaluated the effect of wash off on chlorantraniliprole at 1, 2, 4, 8, and 16 hours after application. Wash off had no negative effect on the performance of chlorantraniliprole. Each chlorantraniliprole with simulated wash off treatment timing performed as well as the no simulated wash off treatment. Previous research has also found that rainfall did not affect the performance of chlorantraniliprole in laboratory bioassays (Hulbert 2011). Chlorantraniliprole chemical analyses varied across timings, but results suggest this product is rainfast. Three of the six chlorantraniliprole and simulated rainfall treatment timings, 15, 60, and 240 minutes, yielded concentrations comparable to the chlorantraniliprole and no simulated rainfall concentrations.

Based on these data, we would conclude that methoxyfenozide + spinetoram appears to provide the best overall control after a rainfall event. Although this product was impacted by rainfall in 2020, the mean mortality of all treatment timings from that year was <80%. Chlorantraniliprole provides moderate to excellent control of bollworms after subjected to simulated wash off. Results from these experiments will help with making bollworm control recommendations in the future when rainfall is expected. Insecticide application are costly and knowing the rainfastness of these two commonly used products will be helpful when considering costs or possible loss from rainfall events.

Table 4.1 Mean (SEM) percent mortality of bollworm larvae in laboratory bioassays conducted in 2019 evaluating the impact of rainfall on the residual efficacy of methoxyfenozide + spinetoram and chlorantraniliprole in cotton.

	Percent Mortality (SEM)	
Time (hours after spray)	Methoxyfenozide + spinetoram	Chlorantraniliprole
Untreated	5.0(2.9) B	10.0(4.1) B
0	97.5(2.5) A	80.0(4.1) A
0.25	97.5(2.5) A	80.0(7.1) A
0.50	97.5(2.5) A	57.5(11.8) AB
1	97.5(2.5) A	67.5(6.3) AB
2	97.5(2.5) A	65.0(13.2) AB
4	100.0(0) A	77.5(13.1) A
No Rain	100.0(0) A	95.0(5.0) A
F	41.78	5.50
d.f.	7, 21	7, 21
P>F	<0.01	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

Table 4.2 Mean (SEM) percent mortality of bollworm larvae in laboratory bioassays conducted in 2020 evaluating the impact of rainfall on the residual efficacy of methoxyfenozide + spinetoram and chlorantraniliprole in cotton.

	Percent Mortality (SEM)	
Time (hours after spray)	Methoxyfenozide + spinetoram	Chlorantraniliprole
Untreated	2.5(2.5) D	2.5(2.5) B
1	72.5(2.5) BC	95.0(2.9) A
2	57.5(4.8) C	97.5(2.5) A
4	82.5(4.8) B	100.0(0) A
8	95.0(2.9) A	92.5(4.8) A
16	97.5(2.5) A	100.0(0) A
No Rain	100.0(0) A	92.5(4.8) A
F	49.16	32.38
d.f.	6, 21	6, 18
P>F	<0.01	<0.01

Means within a column followed by the same letter are not significantly different according to Tukey's HSD (α=0.05).

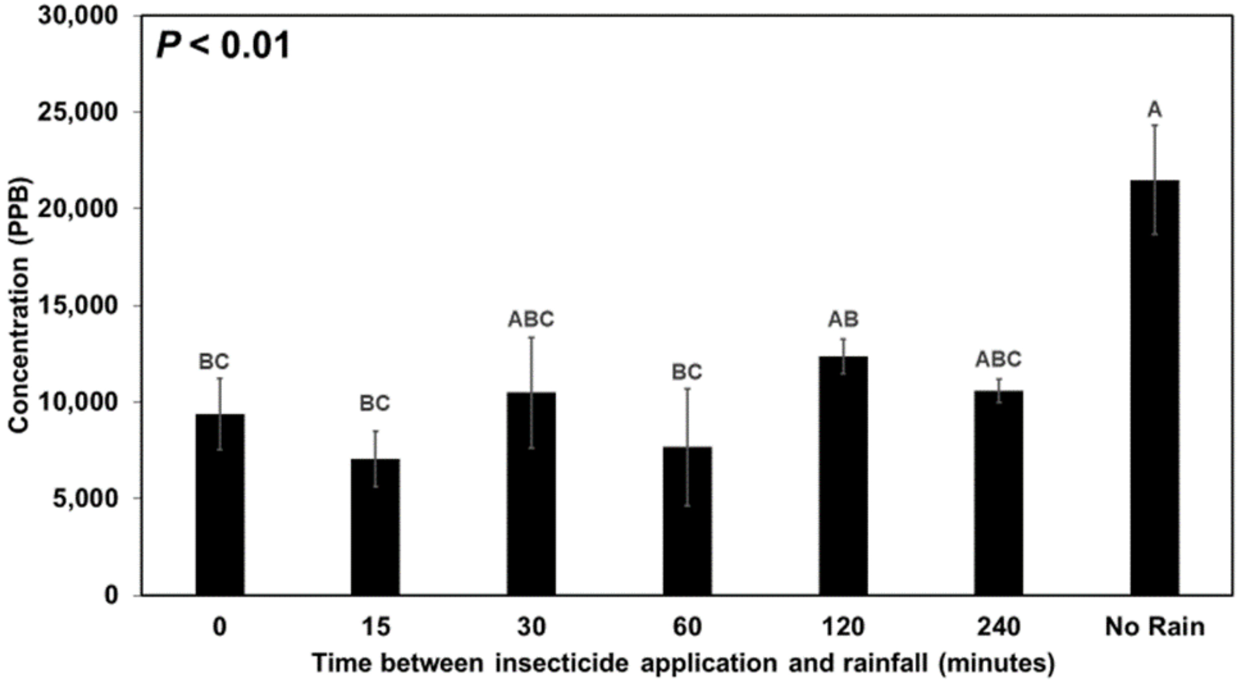

Figure 4.1 Mean (SEM) concentration (PPB) of methoxyfenozide after simulated rainfall event evaluating the impact of wash off on the residual of methoxyfenozide in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

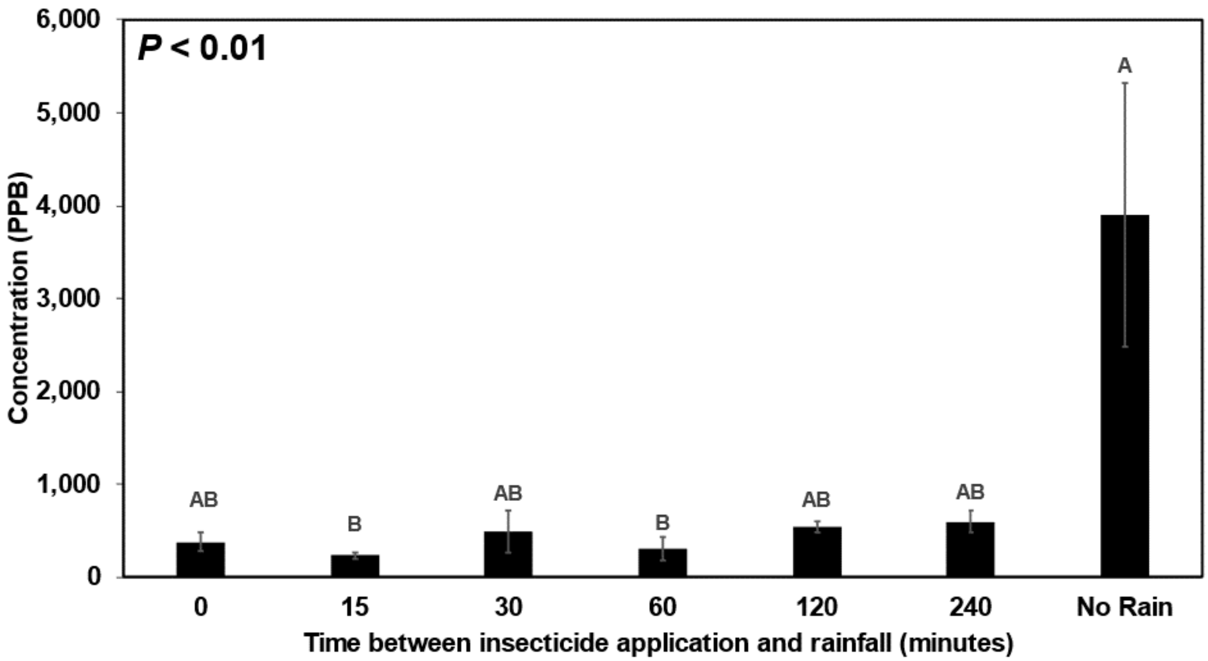

Figure 4.2 Mean (SEM) concentration (PPB) of spinetoram after simulated rainfall event evaluating the impact of wash off on the residual of spinetoram in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

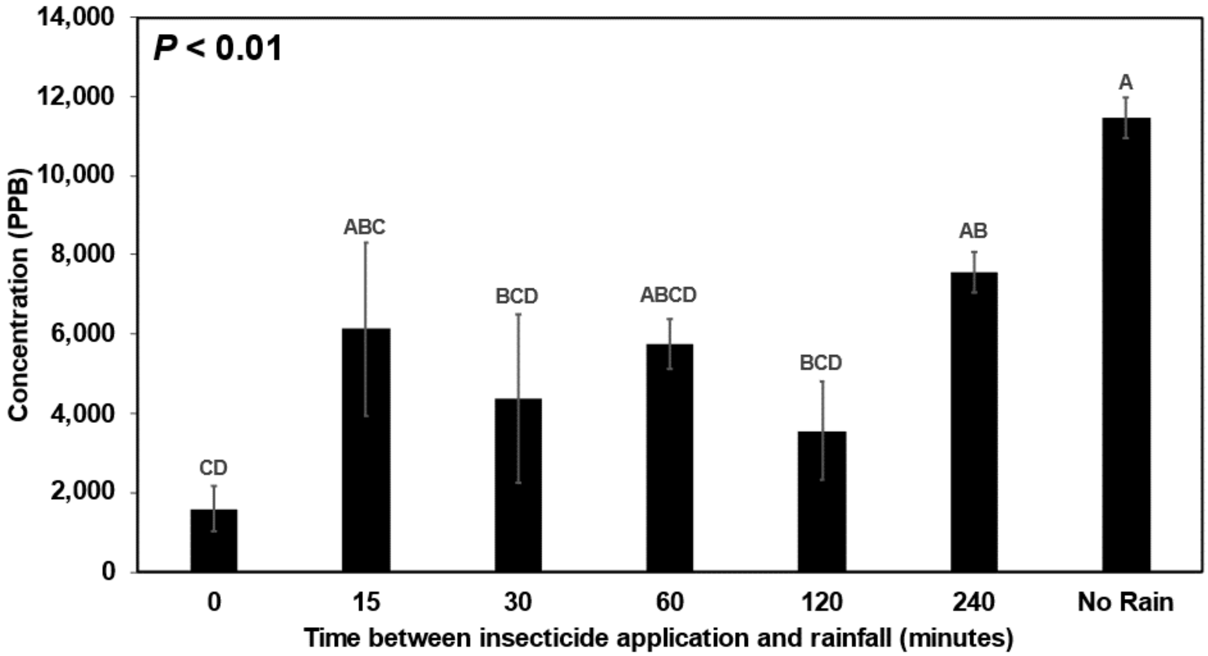

Figure 4.3 Concentration (PPB) of chlorantraniliprole after simulated rainfall event evaluating the impact of wash off on the residual of chlorantraniliprole in cotton. Treatments with the same letter are not significantly different according to Tukey's HSD (α=0.05).

Literature Cited

Adkisson, P. L., C. F. Bailey, R. L. Hanna. 1964. Effect of the bollworm, *Heliothis zea,* on yield and quality of cotton. J. Econ. Entomol. 57: 448–450.

Anastassiades, M. and S. J. Lehotay. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. J. AOAC Internat. 86: 412-431.

Barber, G. W. 1936. The corn ear worm (sic) in southeastern Georgia. Georgia Expt. Sta. Tech. Bull. 192. 18 pp.

Blanco, C. A., A. P. Terán-Vargas, J. D. López Jr., J. V. Kauffman, and X. –K. Wei. 2007. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. Fla. Entomol. 90: 742-750.

Brickle, D. S., S. G. Turnipseed, and M. J. Sullivan. 2001. Efficacy of insecticides of different chemistries against *Helicoverpa zea* (Lepidoptera: Noctuidae) in transgenic *Bacillus thuringiensis* and conventional cotton. J. Econ. Entomol. 94: 86–*92.*

Capinera, J. L. 2000. Corn earworm, *Helicoverpa* (=*Heliothis*) *zea* (Boddie) (Lepidoptera: Noctuidae). Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. EENY-145 (IN302).

Catchot, A., W. Crow, D. Dodds, J. Gore, F. Musser, T. Irby, D. Cook, B. Layton, E. Larson. 2020. Insect control guide for agronomic crops. Publication 2471. Mississippi State University Extension. Mississippi State, MS.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to non-transgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Gore, J., B. R. Leonard, J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on ‘Bollgard’ and ‘Bollgard II’ cotton flower bud and flower components. J. Econ. Entomol. 94: 1445–1451.

Graves, J. B., J. S. Roussel, and J. R. Phillips. 1963. Resistance to some chlorinated hydrocarbon insecticides in the bollworm, *Heliothis zea*. J. Econ. Entomol. 56: 442-444.

Graves, J. B., D. F. Clower, and J. R. Bradley, Jr. 1967. Resistance of the tobacco budworm to several insecticides in Louisiana. J. Econ. Entomol. 60: 887-888.

Hulbert, D. 2011. Rainfastness and leaching of insecticides used in blueberry production. [Unpublished Master's thesis]. Michigan State University, East Lansing, Michigan.

Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, R. Weinzierl. 2009. Resistance to pyrethroid insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois, J. Econ. Entomol. 102: 2289–2295.

King, E. G., and R. J. Coleman. 1989. Potential for biological control of *Heliothis* species. Ann. Rev. Entomol. 34: 53–76.

Leigh, T. F., Roach, S. H., and Watson, T. F. 1996. Biology and ecology of important insect and mite pests of cotton. Cotton Insects and Mites: Characterization and Management, The Cotton Foundation Reference Book series. pp. 17-85.

McDowell, L. L., Willis, G. H., Southwick, L. M., and Smith, S. 1984. Methyl parathion and EPN washoff from cotton plants by simulated rainfall. Environ. Sci. Technol. 18: 423-427.

Mulrooney, J. E. and Elmore, C. D. 2000. Rainfastening of bifenthrin to cotton leaves with selected adjuvants. J. Envron. Qual. 29: 1863-1866.

Pandey, N., D. Rana, G. Chandrakar, G. B. Gowda, N. B. Patil, G. P. Pandi G, M. Annamalai, S. S. Pokhare, P. C. Rath, T. Adak. 2020. Role of climate change variables (standing water and rainfall) on dissipation of chlorantraniliprole from a simulated rice ecosystem. Ecotox. Environ. Safety. 205: 111324.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuch, S. R. Sims, J. T. Greenplate, and D. A. Fischcoff. 1990. Insect resistant cotton plants. Biotechnol. 8: 939–943.

Pick, F. E., L. P. van Dyk, and P. R. de Beer. 1984. The effect of simulated rain on deposits of some cotton pesticides. Pestic. Sci. 15: 616-623.

Plapp, Jr., F. W. 1971. Insecticide resistance in *Heliothis:* tolerance in larvae of *H. virescens* as compared with *H. zea* to organophosphate insecticides. J. Econ. Entomol. 64: 999-1002.

Sparks, T. C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. of the Entomol. Soc. Amer. 27: 186–192.

Stadelbacher, E. A., G. L. Snodgrass, G. W. Elzen. 1990. Resistance to cypermethrin in first generation adult bollworm and tobacco budworm (Lepidoptera: Noctuidae) populations collected as larvae on wild geranium, and in the second and third larval generations. J. Econ. Entomol. 83: 1207–1210.

Willis, G. H. 1986. Permethrin washoff from cotton plants by simulated rainfall. J. Environ. Quality. 15: 116-120.

Willis, G. H., L. L. McDowell, L. M. Southwick, and S. Smith. 1992. Washoff of ultra-low-volume oil-applied insecticides from cotton plants as a function of time between application and rainfall. J. Environ. Qual. 21: 373-377.

Willis, G. H., McDowell, L. L., Southwick, L. M., and Smith, S. 1994a. Azinphosmethyl and fenvalerate washoff from cotton plants as a function of time between application and initial rainfall. Archives Environ. Contam. and Tox. 27: 115-120.

Willis, G. H., McDowell, L. L, Southwick, L. M., and Smith, S. 1994b. Permethrin and sulprofos washoff from cotton plants as a function of time between application and initial rainfall. J. Environ. Qual. 23: 96-100.