ABSTRACT

STAINBACK, CHRISTOPHER BOYD. Fungicide Evaluations for Leaf Spot Disease Control in Flue-Cured Tobacco Production. (Under the direction of Dr. Matthew C. Vann).

Foliar leaf spot diseases, such as target spot (*Rhizoctonia solani*) and brown spot (*Alternaria alternata*), continue to impact flue-cured tobacco (*Nicotiana tabacum* L.) producers in North Carolina. In 2021, cured leaf yield and economic value were estimated to have been reduced by 6.9% and 39 million dollars due to target spot infection. Two fungicides are currently labeled for field applications in tobacco that offer foliar leaf spot suppression, azoxystrobin and mancozeb. Azoxystrobin was approved for use in 2006 and has been the only foliar fungicide applied on tobacco, leading to grower concerns that fungal pathogens may now be resistant or tolerant to the active ingredient. Tobacco buyers have discouraged the use of mancozeb because of high cured leaf residues. Due to the increased frequency of foliar diseases and the limited number of chemical options, commercial tobacco farmers would benefit from new fungicide treatment options. Research was conducted in 2022 and 2023 to evaluate different fungicides for their efficacy against foliar leaf spot diseases. In the first field trial, we evaluated three rates of inpyrfluxam (0.05, 0.10, and 0.25 kg ai ha^{-1}) and compared them to azoxystrobin (0.15-0.16 kg ai ha^{-1}), mancozeb (1.68 kg ai ha^{-1}), and a non-treated control. Treatments were applied sequentially at two, four, and six weeks after transplanting (WAT). Phytoxicity ranged from three to 14 percent and was only observed in treatments of azoxystrobin. Target spot severity was generally reduced by applications of inpyrfluxam, although responses to the higher rates of inpyrfluxam was not consistent. Target spot control from azoxystrobin and mancozeb were more effective than the non-treated control, but were often not as effective as inpyrfluxam. In the second field trial, nine fungicides were evaluated: *Bacillius subtillis* strain AFS032321 (3.36 kg ai ha^{-1}), *Pseudomonas chlororaphis* (1.40 kg ai ha^{-1}), azoxystrobin (0.15-0.16 kg ai ha^{-1}), inpyrfluxam (0.05 kg ai ha^{-1}), difenoconazole + pydiflumetofen (0.08 + 0.04 kg ai ha^{-1}), picoxystrobin + cyproconazole (0.10 + 0.04 kg ai ha^{-1}), mancozeb (1.68 kg ai ha^{-1}), azoxystrobin + flutriafol (0.17 + 0.13 kg ai ha^{-1}), and azoxystrobin + flutriafol + fluindapyr (0.12 + 0.08 + 0.12 kg ai ha^{-1}). A non-treated control was included for comparison. Treatments were applied sequentially at two, four, and six weeks after transplanting (WAT). Similar to the first field trial, phytotoxicity was documented in treatments containing strobilurin fungicides (azoxystrobin and

picoxystrobin) but did not exceed 11% and did not affect yield. In 2022, inpyrfluxam and mancozeb were the most efficacious fungicides for brown spot control. Data for brown spot control is limited to one growing environment; therefore, additional research is needed to confirm these observations. Treatments comprised of inpyrfluxam, picoxystrobin + cyproconazole, azoxystrobin + flutriafol, and azoxystrobin + flutriafol + fluindapyr most consistently reduced target spot severity in 2023. However, cured leaf yield was highest when inpyrfluxam, azoxystrobin + flutriafol, or azoxystrobin + flutriafol + fluindapyr were applied. Target spot control was moderate following applications of azoxystrobin, mancozeb, or difenoconazole + pydiflumetofen. The efficacy of *Bacillus subtillus* and *Psueudomonas chlororaphis* were consistently similar to the non-treated control. Between the two field trials, our collective results demonstrate that fungicides, such as inpyrfluxam, picoxystrobin + cyproconazole, azoxystrobin + flutriafol, and azoxystrobin + flutriafol + fluindapyr may be effective at reducing the impact of foliar leaf spot diseases in North Carolina tobacco production.

Fungicide Evaluations for Leaf Spot Disease Control in Flue-Cured Tobacco Production

by

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DEDICATION

This thesis is dedicated to my parents, Rodney and Kristy Stainback and to my grandparents Ronald and Nancy Stainback and Cynthia Journigan. I also dedicate this thesis to my brother, Cody Stainback and fiancé Lauryn Williams. Each of these individuals have continuously supported my goals and have shaped me into the person I am today. I thank my father for teaching me the value of hard work, how to treat others, for helping me understand the importance of doing things to the absolute best of my ability, and for the endless sacrifices he has made to support my family. I hope to one day be half the father to my children as he has been to me. I thank my mother for teaching me the importance of faith in God, always holding me accountable in school, for believing in me in every situation, and for the ultimate example of how parents should value family and love their children. As I finish this thesis, it’s safe to say that a master’s degree would never be possible without my mothers expectations throughout my educational career. I thank my grandfather, for instilling a passion for tobacco within me at a young age, for being the reason I've always dreamed to have a career in the tobacco industry, for never cutting corners on our beautiful farmland and for establishing my last name to be a respectable one in the local agricultural community. I thank my two grandmothers for always spoiling me, for raising my parents, and for always believing in me. I thank my brother for always being my best friend, for caring about my success just as much or more than his own and for always pushing me to be better in everything I do. I thank my fiancé for her continuous love and support and for always believing that I could accomplish anything.

BIOGRAPHY

Christopher Boyd Stainback, son of Rodney and Kristy Stainback, was born on January 11th, 2000 in Chapel Hill, North Carolina. Raised on a family tobacco farm, established over 100 years ago, he obtained an appreciation of tobacco and established a strong work ethic at an early age. Many of his hobbies revolve around being in the outdoors, which include: hunting, fishing, playing sports, and working on the family farm. He graduated from Northern Vance High School in Henderson, NC, in the spring of 2018 while playing on the varsity baseball team. He began pursuing a degree in Agriculture by attending North Carolina State University in the spring of 2019. He graduated from North Carolina State University in May of 2022 with a Bachelor's of Science degree in Agriculture Science with minors in Crop Science and Agricultural Business Management. Shortly thereafter, he began a Master's of Science degree in Crop Science under the direction of Dr. Mathew C. Vann. Upon completion of his degree, Christopher plans to continue to work in the tobacco industry with Japan Tobacco International.

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CHAPTER 1

INTRODUCTION

Flue-cured tobacco (*Nicotiana tabacum* L.) is one of the most economically important global agricultural commodities, upon which thousands depend on as a significant part of their livelihood (Collins and Hawks, 1993). Despite its known health risks, tobacco continues to be in high demand, due to its addictive nature and cultural significance to a wide diversity of races and social classes. The production and marketing of tobacco and its products has been termed an economic force in many places around the world (Davis and Nielsen, 1999). While its importance is noted around the world, its economic impact is most significant in the southeastern United States. In 2022 and 2023, the total value of all flue-cured tobacco grown in the United States ranged from 636 to 684 million dollars (USDA-NASS). In addition, federal, state and municipal tobacco taxes in the United States totaled approximately 11.26 billion dollars in 2022 (US Congressional Budget Office, 2023). From the first evidence of tobacco being cultured by Native Americans in the "New World," the United States has historically produced high-quality tobacco and has established the "quality standard" worldwide. It has the flavor, color, and aroma that various product manufacturers and consumers desire. United States tobacco producers are equipped with environmental elements that are conducive to high quality production, including suitable soils, appropriate humidity, and temperature, and precipitation (Tobacco Associates n.d.).

Despite the competitive advantages that U.S. tobacco growers may have, its share of the world production, and thus the export trade, has been in a steady decline for many years. At one point, the U.S. produced sixty-four percent of the total world supply (Collins and Hawks, 2013). In 2024, U.S. tobacco production accounted for less than four percent of global production (Universal Leaf Tobacco Company Inc., 2024). This decline is likely associated with the decline in domestic tobacco consumption and increase in foreign production. Despite many advantages, input costs in the US are generally much greater than that of foreign competitors. As a result, industry leaf dealers and product manufacturers purchase low-cost tobacco in foreign markets. Cigarette manufactures now use more foreign tobacco than ever before (Capehart, 2003). As foreign competitors continue to improve production practices and close the gap with US production and its historical quality standards, demand for US tobacco is not nearly what it once was. The result of improved cultivation, higher leaf quality and improved marketing techniques by foreign competitors has prompted U.S. tobacco to lose its global and domestic market share (Capehart, 2003).

Currently there are only five states in the United States that produce flue-cured tobacco. These include Florida, Georgia, North Carolina, South Carolina, and Virginia. North Carolina leads production in the U.S with both area (46,943 hectares) and volume (113,125,000 kg unprocessed leaf in 2022) (USDA, 2023). Since 2014, in which North Carolina tobacco growers experienced record highs of (197,040,364 kg) unprocessed leaf production (Brown and Snell 2015), leaf volume has decreased almost 45 million kg. Unstable market prices, increased investments, and slimmer profit margins resulted in only 105,233,344 kg of unprocessed leaf being produced in 2022 (Brown, 2023). With reduced share in the global market, decreased cigarette consumption, and slimmer profit margins, cognitive decision making to produce a healthy, quality crop by U.S. producers cannot be avoided.

A healthy, successful, and high-quality crop is one that starts fast, is uniform, and produces high yields of acceptable leaf. Successful tobacco requires the recognition, understanding, and management of several different diseases that can occur (Todd, 1981). Tobacco is commonly grown, handled, and managed as an annual plant that is re-established as a seedling each year. Although it is grown as an annual, tobacco is a perennial plant that may survive the above ground foliage undergoes a frost. Although these below freezing temperatures kill the above ground forms of vegetation, the roots of the plant have the potential to live for an additional few weeks, continuing to provide below ground pests with the food supplies they need (Collins and Hawks, 2013).

With increased urgency for quality production, disease management has quickly become one of the biggest concerns. Avoidance of yield and quality reductions that result from disease outbreaks is critical for sustainable flue-cured tobacco production in the U.S.

Disease Infection and Outlook

The tobacco plant is subject to disease and decay from the day the seed is sown until the time it is processed into cigarettes, cigars, pipes, chewing tobacco, or snuff (Lucas, 1965). As such, diseases are a constant threat to successful production. There are many diseases that affect the crop in a variety of ways, and the degree of damage can range from slight to complete destruction. Moreover, some diseases appear infrequently whereas others are a problem each growing season (Collins and Hawks, 1993). The prevalence of diseases and to what extent production practices are influenced depends on a number of factors. Weather, pathogen aggressiveness, varietal susceptibility, and geographical location are all common aspects that influence management practices and loss assessments. The initiation of disease is the result of an interaction between a pathogen, any conducive environmental factor, and a susceptible host (Shew and Lucas, 1991). Causal agents may damage the stalks, roots, leaves, or the entire plant (Todd, 1981). The causal agents for all diseases fall into one of two categories. Most are caused by living microorganisms that are parasites. These obtain their food and nourishment from the plant. The second category, not caused by living organisms, can be broken into two subgroups. The first is related to chemical applications (too much or too little) whereas the second is solely related to weather (Todd, 1981). Of these two groups, living microorganisms are the most problematic. This includes fungi, bacteria, viruses, nematodes, mycoplasmas, and other parasitic plants (Shew and Lucas, 1991). Those resulting in leaf symptomology have a direct effect on yield and visual quality, while others affecting the root or vascular systems often result in premature death and crop loss before the plant is harvested (Shoemaker and Shew, 1999). In some situations, diseases can occur almost simultaneously. Diseases are present on all tobacco farms and the presence of one often affects both the development and severity of another (Todd, 1981).

Tobacco producers have been faced with diseases since the early days of production. Some of the early settlers complained that their land was “tired” of tobacco. By the late 1880s, root-knot nematodes (*Meloidogyne* spp.) were considered a major pest and by 1920, Granville wilt (*Ralstonia solanacearum*) moved tobacco production from central North Carolina to areas further east (Todd, 1981). Some diseases have been prevalent for decades while others have only recently been documented. While infection rates vary, the importance of diseases as a limiting factor in tobacco production has become increasingly evident during the last quarter century. The intensity of disease prevalence and severity of infection has necessitated several radical changes in tobacco culture and successful production (Lucas, 1975). Changes in varietal resistance and lower performance in management efforts have led to economic returns that flue-cured tobacco farmers can no longer afford. Disease prevalence has quickly become another obstacle to quality production. To meet high-quality demands and retain market shares, better understanding of disease and improved performance of management efforts must be a primary focal point.

Disease Management in Tobacco

Disease management measures are numerous and diverse, but there is satisfactory management available for most of the common diseases affecting flue-cured tobacco (Collins and Hawks, 1993). Current management efforts provide control by protection, reduction of pathogen populations, managing spread, and by destroying nutrient sources, such as post-harvest crop debris (Todd, 1981). All control methods are effective but do not eliminate the causal agent and, therefore, the fight against disease is continuous (Todd, 1981). No management practice alone provides full protection and efforts are largely dependent on each crop year. The key to managing flue-cured tobacco diseases over the long-term is to have a thorough understanding of the cause of each disease, its nature, and how it relates to the tobacco plant. Understanding how pathogens spread, infect, overwinter, and ways they may be impacted by management techniques are essential for successful management programs (Collins and Hawks, 1993). As production methods and disease situations continuously change, the need for a well-planned disease management program is imperative for sustainable production. Planning a complete disease management program is a difficult and time-consuming task that involves the consideration of previous and forecasted disease outbreaks, conducive environmental conditions, and potential infestation levels (Todd, 1981). Control methods that complement each other and that work well in combination are necessary for adequate disease protection and higher net profits (Todd, 1981). The most common disease control methods available involve a variety of cultural practices. These include reliance on host resistance, appropriate crop rotation systems, and adjustments of planting, ridging, and cultivation practices (Todd, 1981). While cultural practices are most common, chemical control options are also available. Careful consideration of each chemical and selecting those that best fit within a chosen system is extremely important for establishing a crop that is competitive against disease. Recognizing which efforts complement each other and using effective combinations might be the most important piece of the puzzle. The importance of techniques that work well together are how growers plan effective disease management that will last (Collins and Hawks 2013).

Cultural Disease Management in Tobacco

Most of the important diseases that occur every season are caused by organisms that live in the soil, which can reproduce only on tobacco and a few other plants (Moparthi and Gorny 2023). These include black shank (Phytophthora nicotianae), Granville wilt (Ralstonia solanacearum), root-knot nematode (Meloidogyne incognita), Tobacco mosaic virus, and black root rot (Thielaviopsis basicola). Without tobacco or one of the other host plants, populations of the disease-causing organisms are reduced. Crop rotation must be implemented in planning any disease management program (Moparthi and Gorny 2023). Rotation is considered one of the oldest and most effective methods of disease control for tobacco, and its implementation is often encouraged (Todd, 1981). It involves incorporating a cropping system that allows for tobacco to be rotated with different alternative crops such as wheat (*Triticum aestivum*), soybeans (*Glycine max*), corn (*Zea mays*), peanuts (*Arachis hypogaea*), or anything that does not potentially serve as an alternative host (Todd, 1981). This process denies the causal agent a suitable plant on which to feed and multiply for as long as possible. Proper selection of rotational crops reduces the population of causal agents for several diseases. Rotations are usually the first step in a disease management program that eliminates production risks. A well-planned rotation eliminates production risks and promotes competitive advantages. With rotations, producers have the ability to explore multiple different variety resistance packages and are better fit for a diverse disease management program. The flexibility in control method selection provides an opportunity to design production programs that will produce high yields of acceptable leaf (Todd, 1981). The length of the rotation, or the time-frame between tobacco crops, is the most important factor. In general, the longer the rotation, the greater the reduction of pathogens that live and overwinter in the soil. Thus, four-year rotations (three alternate crops between tobacco crops) are more effective than two- or three-year rotations. If operations are only limited to two-year rotations, the benefits far outweigh the risks associated with not implementing rotations at all (Moparthi and Gorny, 2023). Where tobacco is grown continuously, growers are allowing pest populations to increase every year leading to potentially devastating disease losses (Collins and Hawks, 1993).

The destruction of roots and stalks immediately following harvest is a cultural operation that must be followed, whether disease has been observed or not (Collins and Hawks, 2013). It is a necessity in a good tobacco disease management program to completely destroy the stalks and roots as soon as possible after final harvest. Causal agents which attack tobacco are adapted to survive best in association with tobacco tissues, especially any form of living tissue. Many populations will die if dissociated freely in the soil. Early stalk and root destruction by a combination of plowing and discing two to three times is highly effective at removing any forms of living tissue (Collins and Hawks, 2013). The objective of stalk and root destruction is to promote rapid death and decay of all old plant tissue and debris. If ineffective removal occurs, pests are favored, and growers can ensure survival of pests by allowing portions of plants to remain alive from season to season (Collins and Hawks, 2013).

Other production practices commonly used in flue-cured producing systems promote advantages that favor the success of the tobacco plant. For example, the formation of a high and wide bed row (or row ridge) is important to provide roots with proper conditions for successful development. Soil-borne causal agents, whether affecting root systems or above ground foliage thrive and flourish in soils with poor drainage and high moisture content. The formation of the high, wide bed promotes water drainage, which greatly favors the tobacco plant at the expense of the pathogen (Collins and Hawks, 2013). Plant population and airflow availability can also be associated with disease prevalence and severity. Production systems with dense or abnormal plant populations often suffer disease losses greater than those situations with plants that are spaced further apart. Spacing especially influences diseases such as brown spot (*Alternaria alternata*), target spot *(Rhizoctonia solani*), and blue mold (*Peronospora tabacina*) (Moparthi and Gorny, 2023). These disease causal agents favor humid conditions with minimal airflow and anything else that promotes extended periods of leaf surface moisture. Wider spacing provides more sunlight, better airflow through the canopy, and improved drying conditions for the foliage at the bottom portions of the plant (Moparthi and Gorny, 2023). Collectively, ridging practices, planting density, and adequate airflow are extremely important for disease avoidance.

Disease initiation and progression are also favored by imbalanced nutrient concentrations. A balanced fertilization program, neither excessive nor deficient, promotes a healthy crop that can withstand disease pressure and pathogen competition (Moparthi and Gorny, 2023). Likewise, any conditions that promote premature senescence, abnormal growth, and reduced plant vigor favors opportunistic pathogen development. Understanding the symptoms of plant stress and adjusting accordingly should never be avoided. A healthy crop that can better withstand pathogen competition should be the goal of every tobacco grower.

A management technique commonly overlooked in most situations is the timeliness of post-transplanting cultivation and layby. According to Moparthi and Gorny (2023), if disease appears in only some portions of the field or in isolated areas, it is important to adjust cultivation practices accordingly. Diseased portions of the field must be cultivated last to reduce the chance of spreading the disease organisms to uninfected areas of the field. Proper sanitation of cultivation equipment after completion is also advised (Moparthi and Gorny, 2023). The term “layby” is associated with the last mechanical cultivation (usually around 4-6 weeks after transplanting) that can be made before the crop becomes too tall for subsequent cultivations. As vegetative production intensifies, the plants also develop a dense root system. If the layby cultivation occurs too late, the roots of the tobacco plant can be damaged, which favors pathogen infection. Collins and Hawks (2013) emphasize that some diseases may be reduced by making the last cultivation as early as possible. Soilborne diseases, such as Granville wilt and Fusarium wilt (Fusarium *oxysporum* f. sp. *nicotianae*), require openings or wounds to enter plant roots. Late cultivation practices can cut and slice roots, thus providing an ideal avenue for these organisms to invade the plant. Considering the timeliness and sequence of layby and cultivation practices could be an effective tool at limiting disease prevalence (Collins and Hawks 2013).

The cultural control mechanism that ultimately determines how successful a management program will be is the choice of variety. The choice of variety is one of the most important aspects of any production system. Varietal resistance provides a fulcrum for tobacco disease management (Collins and Hawks, 1993). It can determine how severe disease outbreaks may be, as well as the potential yield and quality of the crop. Todd (1981) emphasized that variety selection is important simply because the variety choice is the most stable and long-lasting method for diseases such as black shank, Granville wilt, tobacco mosaic virus, brown spot, and root-knot nematodes. From a disease management perspective, there are three factors that determine varietal choice: the disease(s) present on the farm; the level of infestation or severity of the disease(s); and the degree of resistance in varieties being considered (Collins and Hawks, 2013).

Further to variety selection, growers should not depend solely on resistant varieties for disease management (Moparthi and Gorny, 2023). Resistant varieties are sometimes severely damaged by disease, especially when rotation, timely stalk and root destruction, and other management tools are not used (Collins and Hawks, 2013). It is important to realize that resistant varieties are not completely immune in most cases (Collins and Hawks, 2013). Instead, the selection of a resistant variety must complement other management efforts and be used in tandem.

Chemical Disease Management

Chemical use practices in both seedling and field production situations offer tobacco growers an additional tool in loss reduction efforts (Collins and Hawks, 2013). The application of chemicals must be done in a cognitive matter and in ways that holistically complement the disease management system. According to Collins and Hawks (2013), chemicals should only be used as part of an integrated disease management program and when non-chemical strategies are not expected to be successful on their own. Unnecessary use and ineffective application methods will contribute to environmental concerns and pathogen resistance (M. Vann, personal communication). Knowing which chemistries to employ and understanding the conditional effects that impact successful control requires extensive knowledge of both the targeted pathogen as well as chemical compound mechanisms. Selecting the proper chemical(s) in relation to the disease situation is mandatory. It is both useless and expensive to expect effective management of a disease problem from a material synthesized for use in other ways that target different microorganisms (Collins and Hawks, 2013).

Throughout the history of production, crop protection agents have most commonly been employed by ways of chemical soil treatment**.** Todd (1981) identified chemical application to the soil, regardless of application mechanisms, to be an important part of producing a successful, healthy, and quality crop. There are several materials available for soil treatment use which include multi-purpose fumigant materials, soil fungicides, and other nematicides that are often incorporated preventatively to avoid disease initiation. Whether before or after the crop has been transplanted, soil treatment is used extensively in most all areas where tobacco is produced. It is estimated that over five million pounds of fumigants are used on tobacco per year in the U.S. (Environmental Protection Agency, 2007).

Fumigants have primarily helped manage Granville wilt and nematodes (Mila and Radcliff, 2015). Although research has shown improved yields and decreased disease infestation levels, fumigant use was much more common 30-40 years ago when compared to modern times (M.Vann, personal communication). As environmental concerns resulting from fumigation have intensified and material cost has reached an all-time high, the application frequency is notably lower. The only multi-purpose fumigants currently registered for use in tobacco are chloropicrin and 1,3-dichlorpropene (Moparthi and Gorny, 2023). Limited control options have reduced the use and need for fumigation practices; however, Granville wilt and pathogenic nematodes still remain a large concern for producers.

Narrow-spectrum chemicals are commonly incorporated in greenhouse float beds, transplant water applications, or directly on top of the soil surface before or during stand initiation. These products are most helpful in controlling black shank. Black shank is among the most destructive and widespread of all tobacco diseases in North Carolina (Ahumada, 2023). Currently, most tobacco breeding efforts revolve around developing plant defense mechanisms against the oomycete that causes black shank; however, fungicides are still used in efforts against the disease. Mefenoxam was used on 67% of tobacco acreage in 2015, primarily for the suppression of black shank (Vann et al., 2015). Fluopicolide was also labeled in 2015 for black shank and was estimated to have been applied on 32 percent of the total acreage (Vann et al., 2015). Historically, black shank has most effectively been managed with a product known as Ridomil® (metalaxyl/mefenoxam) (Collins and Hawks, 2013). More recently, Presidio® (fluopicolide) became available to tobacco growers for control of black shank in 2015, and Orondis Gold 200® (oxathiapiprolin) in 2016 (Moparthi and Gorny, 2023). Each of these products have commonly been applied on top of the soil surface and incorporated before and/or after transplanting and/or in transplant water solutions. Moparthi and Gorny (2023) suggest that fluopicolide (Presidio®) should no longer be applied in transplant water applications due to phytotoxicity risks to young plants. As a result, most present-day applications of narrow-spectrum fungicides applied to the soil consist of mefenoxam, oxathiapiprolin, or tandem applications of each.

The use of chemicals directly onto the foliage of tobacco plants or (over the top) after planting has increased as severity of foliar diseases, such as target spot and brown spot have increased. The logistics of when and how the chemicals are applied doesn’t always align with industry guidelines. As such, foliar fungicide applications are not always encouraged. The opposition to foliage sprays is in large part because the chemical is applied during the harvest period or at timing intervals in which cured leaf residues could be concerning (Todd, 1981). The persistence of the pesticide and cured-leaf residues are always a top concern to tobacco product manufacturers. Certain chemicals used for pest control may leave residues on cured leaves, and some residues have been found in mainstream cigarette smoke (Tso, 1990). As a result, over the top chemical control options are limited and foliar diseases have become a major concern. Foliar disease management efforts preceding the last two decades were largely focused on blue mold prevention. If early soil-treatment mefenoxam applications were not enough, applicators were encouraged to use Forum® (dimethomorph) in combination with Dithane DF Rainshield® (mancozeb) (Moparthi and Gorny, 2023). Blue mold became widespread in 1995, since then it has become less common and only recently been found in one North Carolina County in 2016 and 2017 (Moparthi and Gorny, 2023). As a result, foliar spray efforts have primarily been focused on target spot, brown spot, and frogeye leaf spot (*Cercospora nicotianae*). In 2006, Quadris® (azoxystrobin) was registered for control of target spot (M. Vann, personal communication). Following azoxystrobin registration, it was the only active ingredient producers could use for the control of common leaf spot disease, such as those previously mentioned – blue mold notwithstanding. While the underlying reason as to why there has been a recent increase in foliar leaf spot diseases has not been identified, it has been hypothesized to be the result of limited options and overuse of the same chemical compound (azoxystrobin) (M. Vann, personal communication). Manzate-Prostick® (mancozeb), previously known as Dithane DF Rainshield®, and related compounds such as maneb and dyrene have historically shown suppression of not only blue mold, but to target spot and brown spot as well. However, management efforts involving mancozeb haven't been implemented because of strict residue regulations and lower levels of effectiveness compared to azoxystrobin (M. Vann, personal communication). According to Lucas (1975), research conducted from 1958-1964 in North Carolina showed that maneb and dyrene applied once per week during harvest gave significant brown spot control. It was also emphasized by Shoemaker and Shew (1999) that fungicides such as maneb, mancozeb, anilazine, and iprodione were very effective against brown spot. However, because of residue concerns the use of such fungicides would not be approved.

Foliar fungicide applications are a noticeably important component of the disease management program that must be maintained by conservative, timely and appropriate application techniques. Knowing when to apply foliar chemical mechanisms is the most important factor. Growers who make preventative applications are a lot better off than those who wait for disease onset (Todd, 1981). Starting on time determines the success or failure of chemical management efforts. Care should also be exercised in selecting the time of application in order to prevent high residue content in cured leaves (Todd, 1981). Residue concerns ultimately determine the longevity of products being used. Likewise, having a diversity of options is extremely important for mitigating resistance development. Ultimately, responsible, preventative, and justifiable chemical applications should always be considered, especially with foliar chemical sprays.

Foliar Diseases of Interest

Target Spot (*Rhizoctonia solani)*

Target spot is a potentially devastating leaf spot disease in tobacco that is generally observed when growing conditions are warm and humid. The disease is frequently identified in the tobacco growing areas of South America, South Africa, the southern United States, and Canada and has been a major threat to US tobacco production since it was first observed in the 1980s (Thiessen and Haroldson, 2020). This leaf spot disease is caused by the basidiospores of *Thanatephorus cucumeris* (Shew and Lucas, 1991) but is more commonly known as its asexual stage synonym, *Rhizoctonia solani*. In the United States, the disease was first observed in North Carolina in 1983 and now occurs each year in flue-cured tobacco growing regions of North Carolina and Virginia (Shew and Lucas, 1991). In 1995, it caused the greatest loss of any disease since 1959 (Moparthi and Gorny, 2023). Losses from target spot are usually negligible, but during extended periods of favorable environmental conditions they may exceed 50% in a given field (Shoemaker and Shew, 1999). Plant response mechanisms resulting from infection of *R. solani* reduces the harvestable leaf area, especially in the lower stalk position. The necrotic tissue resulting from infection becomes brittle and often drops out, leaving a shot-hole effect in the leaf (Shew and Lucas, 1991). It has recently become one of the most concerning diseases. In the 2022 production season, Moparthi and Gorny (2023) identify target spot to be one of the diseases responsible for extensive yield reductions.

Pathogen Description

Target spot is caused by an asexual reproductive fungus *Rhizoctonia solani (*Davis and Nielsen, 1999*)*. Each isolate of R. solani are similar in morphology, and all belong to anastomosis group 3 (AG-3) (Shew and Melton, 1995). Rhizoctonia solani is a soil-borne fungal pathogen within the phylum Basidiomycota. The fungus exists primarily as hyphae or sclerotia within the soil and is spread aerially through basidiospores that are formed during extended periods of warm, wet conditions. The hymenium is resupinate, creamy to grayish white in color, and loosely attached to soil or plant material. Optimum temperatures for hymenium formation are between 20-28^{o}C, with growth halting at temperatures above 32^{o}C (Shew, 1991). The pathogen has over two-hundred known hosts with different infection mechanisms, and the degree of infection can vary with each (Thiessen and Haroldson, 2020).

Epidemiology/Disease Cycle

Development and progression of disease occurs when basidiospores are produced and disseminated onto the leaves (Shew and Lucas, 1991). Hymenia and basidiospores are produced readily on soil and plant materials. Following the placement of organic matter several centimeters below the soil surface, the pathogen can initiate colonization (Shew and Lucas, 1991).

Rhizoctonia solani is a polycyclic pathogen, meaning that if environmental conditions are conducive for pathogen development, many cycles of infectious spore production can occur in one season (Thiessen and Haroldson, 2020). The pathogen overwinters as both mycelium and sclerotia within the soil and on crop debris (Thiessen and Haroldson, 2020). Basidia in the hymenium at the soil surface produce basidiospores that are dispersed through the air (Thiessen and Haroldson, 2020). The basidiospores at the soil surface can spread from the soil by splashing from a heavy rainfall event or move with the wind and land on leaf surfaces. After leaf and cell penetration, the pathogen begins to grow and extract nutrients from the leaf (Thiessen and Haroldson, 2020). With appropriate environmental conditions, the pathogen continues to grow outward into neighboring cells (Thiessen and Haroldson, 2020). The previously exploited cells of the leaf die and necrotic lesions become visible on the surface of the tobacco leaf (Thiessen and Haroldson, 2020). Further progression leads to alternating bands of light and dark necrotic tissue giving the disease its target-like appearance (Thiessen and Haroldson, 2020). With extended periods of favorable conditions and continued progression, the pathogen can release secondary inoculum in the form of basidiospores and the spores can be dispersed into leaves further up the stalk of the tobacco plant (Thiessen and Haroldson, 2020). Once environmental conditions are no longer conducive (warm air temperature > 32^{o}C with lower humidity), the necrotic tissue resulting from target spot becomes brittle, falls to the soil surface, and remains there until conditions are appropriate for the pathogen to start its life cycle again (Thiessen and Haroldson, 2020). The longevity of the life cycle varies, but under optimal conditions, sixteen to twenty days are required for completion of the disease cycle (Shew and Main, 1990).

Management

Historically, *R. solani* control options have been limited. Specifically, Shew (1991), reported that "no control measures were recommended for target spot". Rather, it was emphasized that the reliance on sanitation practices and other cultural management techniques that limit the wetting of the tobacco foliage should be utilized (Shew 1991). These strategies included any practices that promote air flow or sun penetration into the canopy, and were largely considered to be the "cure all" approach (Shew, 1991). Management strategies were reassessed with Shoemaker and Shew (1999) when it was reported that sensitivity to fungicides is similar to that of sore shin, which is another disease of tobacco that is caused by *R. solani*. Shoemaker and Shew (1999) further reported the fungicide Rovral® (iprodione) had been used in the US during greenhouse and, in some cases, field production to slow epidemics of the disease. It was further highlighted by Nesmith (2003) that iprodione had been proven to have efficacy against target spot, but was not labeled for in-field use and could not be used commercially. In 2006, Quadris® (azoxystrobin) was registered for control of target spot (Moparthi and Gorny, 2023). Since its registration, preventative actions have solely consisted of foliar applications of azoxystrobin. Moparthi and Gorny (2023) reported that a chemical application of Quadris® at 6 to 12 fluid ounces per acre (8 fl oz/a) has given consistently good results. In opposition, recent reports by Cooperative Extension Agents across the state of North Carolina indicate consistent chemical control failures. Reduced control has been attributed to sequential and curative applications of azoxystrobin to the same pathogen populations over the period of more than a decade. Mancozeb (Manzate Pro Stick®) has a 24c label for use in tobacco but is only moderately effective at reducing target spot (Thiessen and Haroldson, 2020). Historical applications of mancozeb were used for blue mold control, and after continuous aggressive applications in the late 1980s and 1990s, the tobacco industry began identifying cured leaf residues above acceptable levels. As a result, its use has largely been discouraged by tobacco buying entities.

Overall, the lack of diversity with chemical control options and ineffective application methods are believed to have resulted in pathogen tolerance development. In addition, adverse seasonal growing conditions may also be a contributing factor to reduced chemical efficacy. For example, anecdotal observations suggest that prolonged periods of excessive precipitation between layby and flowering often result in above average infestations. Target spot has reestablished itself as a major disease in North Carolina, which is the leading tobacco growing state in the U.S.

Brown Spot (*Alternaria alternata*)

Brown spot can be a serious disease on all tobacco types and has been reported from most countries where the crop is produced (Lucas, 1975). The causal agent of this leaf spot disease is *Alternaria alternata* and it is recognized in many situations as an opportunistic pathogen (Thiessen and Rivera, 2019). While environmental conditions strongly influence the degree of disease outbreak, it is often referred to as a disease of mature or senescent tobacco (Todd, 1981). The disease commonly appears on mature leaves during the later portion of a growing season, or on previously damaged leaf tissue (Moparthi and Gorny, 2023).

It was first reported or became a disease problem in Southern Rhodesia (present day Zimbabwe) in 1931 (Hopkins, 1956) and is recognized as one of the oldest fungal diseases that has devastated many areas around the world. In North Carolina, Ellis and Everhart first reported brown spot on tobacco in 1892 (Lucas, 1965). Although it has occurred commonly for many years in the southeastern United States, it had traditionally been considered a minor disease prior to the mid-1950s (Lucas, 1965). Crop losses in the mid-1950s were estimated at 10 million dollars in North Carolina alone (Lucas, 1965). From 1959-1964, losses resulting from brown spot in North Carolina ranged from five to 20 million dollars annually (Lucas, 1965). Brown spot occurs worldwide but is most severe on tobacco grown in warm climates (Spurr, 1991). Unlike diseases such as black shank, Granville wilt, and target spot, which are observed almost every growing season, brown spot is a disease whose prevalence often goes unnoticed. Loss assessments of brown spot vary over time, giving the impression that severity cycles are to be expected in the future (Spurr, 1991). Not only does this disease cause direct yield reductions by loss of leaves before harvest, but it also has the potential to affect leaf quality by alterations in chemical constituents of the leaf with increased disease severity (Lucas, 1975). Such alterations would include changes in the sugar and alkaloid ratio and others that affect the taste and aroma profile (Lucas, 1975). Each of these characteristics are aspects that can impact cigarette products.

The disease typically affects lower or more mature tobacco leaves and because the causal agent populations increase exponentially during the season, plants that stay in the field longer are the most damaged (Thiessen and Rivera, 2019). Currently, weather conditions ultimately influence the degree of infection. With extended periods of rainfall late in the harvest season, on more senescent leaves, the disease can become very destructive (Moparthi and Gorny, 2023).

Pathogen Description

The brown spot fungus belongs to a species composed of many strains that vary in their ability to attack different hosts (Lucas, 1975). In early reports by Tisdale and Wadkins in 1931, researchers were only able to infect tobacco with the isolates of the brown spot fungus (Lucas, 1975). Further research eventually reported infections and findings in a variety of situations, and brown spot is now recognized as a potential pathogen in a wide range of hosts, such as peppers, pears, apples, wheat, tomatoes, beans, cotton and a variety of others (Thiessen and Rivera, 2019).

Historical research was not always able to definitively identify the causal agent of brown spot. For example, Lucas (1975) highlighted the variability in species identification and described multiple collaborations that would identify the pathogen. In 1892, Ellis and Everhartt describe a "brown spot" of tobacco caused by *Macrosporium longipes* n. sp. (Ellis and Everhart, 1892). In 1931, Tisdale and Wadkins described the brown spot of tobacco to be caused by *Alternaria* (Tisdale and Wadkins, 1931). Differences were not deemed sufficient and a new combination of *Alternaria longipes* was suggested (Lucas, 1975). By the late 1950s, at a time in which brown spot was becoming one of the most important diseases of tobacco in North Carolina, *Alternaria longipes* was the name that was most widely accepted among the scientific community (Lucas, 1975). Simmons (1967) clarified the proper name of the pathogen to be *Alternaria alternata* and that the latter name should be adopted for brown spot fungus as rapidly as feasible.

The *A. alternata* fungus is in the class Hyphomycetes, a group of fungi for which no sexual state has been identified (Shoemaker and Shew, 1999). As such, the pathogen can reproduce by way of asexual spores called conidia (Shoemaker and Shew, 1999). Conidia are produced on short stalks called conidiophores, which arise from the concentric rings that are often observed on the surface of the leaf spot (Shoemaker and Shew, 1999). Although *A. alternata* can colonize a wide number of hosts, wounding is often required for disease to develop (Thiessen and Rivera, 2019).

Epidemiology/Disease Cycle

Fields that are continuously used for tobacco production are at greater risk of having problems from brown spot when compared to fields where crop rotation is practiced (Shoemaker and Shew, 1999). This pathogen can survive, grow, and reproduce by way of conidia on tobacco debris left from the previous season (Shoemaker and Shew, 1999). In the absence of rotation, brown spot may also impact early stages of tobacco growth. Shoemaker and Shew (1999) explained that shortly after transplanting, the conidia produced from leftover crop debris can be splashed up onto the leaves closest to the soil and any form of moisture can initiate germination and further progression.

Conidia of *A. alternata* begin to germinate in the water film on leaf surfaces in less than one hour, as such free water must be readily available for conidia to germinate (Lucas, 1975). If conidia germinate successfully, the resulting germ tubes will penetrate the leaf either directly through the cuticle or through the stomates (Shoemaker and Shew, 1999). Primary infection is usually on the lowermost leaves, particularly where wet or humid conditions persist for longer periods than on upper stalk leaves (Spurr, 1991). Moreover, several disease cycles can occur within a growing season. A new production of conidia can begin to develop in seven to eight days (Shoemaker and Shew, 1999). Early in the season, the fungus is capable of penetrating the leaf in only weakened areas (Lucas, 1975). As the season progresses after several infection cycles, the fungus affects healthy tobacco tissue of any age (Lucas, 1975). Brown spot disease build-up is also progressive. Severity and incidence are less noticeable for the first two months following transplanting and suddenly becomes obvious in the final three to four weeks of the season (Shoemaker and Shew, 1999). Todd (1981) further elaborated that damage is slight on lower and middle stalk leaves but can become more severe on those near the top of the plant during some seasons. This reinforces Shoemaker and Shews (1999) idea of the disease having a progressive build, being the most problematic later in the season. During warm, wet weather with prolonged periods of high humidity or heavy dews, a significant number of lesions can appear, often upwards of two hundred per leaf (Lucas, 1975).

Management

No single, specific practice is adequate for brown spot control. The disease is best controlled by following sound cultural and production practices (Shoemaker and Shew, 1999). Keeping records of field locations in which brown spot was severe, and avoiding those areas in the future is an effective approach. Also, fields known to be infested with root-knot nematodes should be treated or completely avoided (Shoemaker and Shew, 1999). The use of disease-free seed lots and plant bed fumigation with methyl bromide reduces the initial occurrence (Spurr, 1991). Timely and effective sucker control management and any practice that promotes vigorous growth can help avoid infection. Delaying topping and allowing sucker growth increases the susceptibility of the plant to brown spot (Shoemaker and Shew, 1999), as it will prematurely initiate senescence. As previously discussed, when considering the progressive buildup potential, timely harvest is key. Todd (1981) emphasized that increasing priming or harvesting rate can be an effective attempt to stay ahead of the disease progression and avoid severe outbreaks.

Fungicides such as maneb and mancozeb are very effective against brown spot (Shoemaker and Shew, 1999), but because of concerns of fungicide residues, their use is essentially non-existent. Lucas (1975) identified maneb and dyrene applied once per week during harvest to be viable options for brown spot management. However, Lucas (1975) further stated that residue information was needed and that farmer recommendations were being withheld pending residue and smoke tests. Over the last half century, chemical options specifically for brown spot control have not been available.

Variety resistance is not a common control method but some burley and flue-cured varieties are reported to have tolerance to the disease, and it has not been a serious problem on such cultivars (Spurr, 1991). Brown spot infection tends to be very sporadic. As a result, resistance levels and defense mechanisms are still largely unknown. New fungicide chemistries with minimal residue concerns are needed. If a highly active compound for *Alternaria* were found that was safe with negligible residues, fungicidal control of brown spot would become a possibility (Shoemaker and Shew, 1999).

Frogeye Leaf Spot (*Cercospora nicotianae*)

Frogeye leaf spot (FLS) is one of the oldest known tobacco diseases. It was first reported in North Carolina by Ellis and Everhart in 1893 and has been prevalent in the major tobacco-growing areas of the world (Lucas, 1991). Since 1960, Frogeye leaf spot has become a severe leaf disease because of increased tobacco acreage in Central America, particularly shade grown cigar wrapper tobacco. It is prevalent in Honduras, Nicaragua, Jamaica, Panama and is reported as a major disease in cigar tobacco production in the Philippines (Lucas, 1975). Frogeye leaf spot prevalence varies each year but causes losses in most years in the tropical areas that grow flue-cured tobacco (Lucas, 1991). The fungus that causes this disease is commonly known as *Cercospora nicotianae,* first reported by Ellis and Everhart, but historical records indicate *C. apii* Fresen. f. sp. *nicotianae* has also been identified (Lucas, 1991). The disease has historically been an issue for ripe tobacco that is ready for harvest, and has become increasingly evident in recent years (Thiessen and Ernst, 2020). Under favorable weather conditions, FLS can cause severe damage to flue-cured leaves but its effect is most noted in burley and dark tobacco. Frogeye leaf spot is one of the major leaf spot diseases of tobacco grown in Kentucky. It reduces yield potential by up to 30 percent and can affect quality so severely that cured leaves may be rejected at the point of sale (Pfeufer, 2020). Frogeye leaf spot is generally regarded to be an issue on mature leaves. Historically, manufacturers and leaf buyers of flue-cured tobacco have not been opposed to seeing bails or bundles of leaves with slight levels of FLS symptoms. Lucas (1975) emphasized that a small amount of FLS has long been assumed to be a desirable indication that leaves were mature when harvested. Kucharek (1981) reiterated that some people equate the presence of FLS with ripe, high quality tobacco. Typically, it has not been a disease of commercial importance because it occurred so late in the growing season. Symptoms resulting from *C. nicotianae* are different from other leaf spot diseases which usually allows for easy visual identification. The lesions are usually small, 2-15 mm in diameter, brown or tan in color, with parchment-like centers (Lucas, 1975). The pale white coloration in the center of the necrotic lesions resembles a frog-like eye appearance.

<u>Pathogen Description</u>

The *Cercospora nicotianae* fungus is in the class Hyphomycetes, order Moniliales (Lucas, 1975). Like many pathogens, *C. nicotianae* is favored by warm, humid conditions (Thiessen and

Ernst, 2020). According to Lucas (1975), it is generally regarded as a weak parasite that only attacks physiologically declining tissue, but the fungus will also infect rapidly expanding leaves. For most isolates of the fungus, optimum temperature for sporulation is around 18^{o}C and mycelial or hyphae growth is around 27^{o}C (Lucas 1991). Lesions resulting from frogeye leaf spot infection seldom attain a diameter of more than 10 mm (Lucas 1975) but can be consistently distributed across the entirety of the leaf.

Epidemiology/Disease Cycle

Frogeye leaf spot epidemics are solely dependent upon the weather. It spreads rapidly during warm weather, particularly with intermittent rains (Lucas, 1965). Like most tobacco parasites, the fungus is closely linked to tobacco tissue and can survive and reproduce unless proper management strategies are implemented. *Cercospora nicotianae* overwinters on infected tobacco debris or other susceptible host plants. During the era of plant bed use for seedling production, epidemics were started when conidia blew in or splashed onto tobacco leaves and sometimes continued with the transplants as they were taken from the plant bed to the field (Lucas, 1991). The fungus enters the leaf through the stomata and free water on the leaves is necessary for spore germination (Lucas, 1975). After dispersion onto host tissue, asexual spores (conidia) germinate and form lesions that contain hyphae of the fungus (Thiessen and Ernst, 2020). From within the hyphal mats, conidiophores arise from plant tissues by way of asexual reproductive structures known as stroma (Thiessen and Ernst, 2020). Conidia are later produced, and after dispersal to susceptible tissues, further germination results in new lesion development (Thiessen and Ernst 2020).

Management

Currently, FLS is a disease that can only be controlled with effective cultural practices. Historically, the disease was successfully controlled with the fungicide azoxystrobin, similar to target spot. Within the last decade, levels of tolerance and resistance have been identified in strains of *C. nicotianae* isolates. It was reported by Johnson (2021) that insensitivity to azoxystrobin was detected in tobacco leaf spot pathogens in both Kentucky and North Carolina every year since 2017. In another report, Thiessen (2018) explains that samples of FLS lesions were tested for strobilurin fungicide (Group 11/Azoxystrobin) and results confirmed resistance.

In the same study, it was further identified that conventional fungicides labeled for flue-cured tobacco will not be effective for managing FLS (Thiessen, 2018). Vann (2021) in a newsletter discussing the importance of applying pesticides at appropriate timings states that using azoxystrobin for leaf holdingability (an effort to retain or maintain leaf quality) is very likely one of the underlying causes behind the current azoxystrobin resistance we are now finding in FLS. Foliar spray options are limited in tobacco leaf spot disease management and those that are available have little to no control of *C. nicotianae.* Any cultural practices that delays the maturation process makes a tobacco crop more susceptible to FLS infestation. Lucas (1991) explains that overfertilization with nitrogen should be avoided and that flue-cured tobacco should be primed (harvested) before it becomes overripe (past desirable maturity). Stated in other terms, the longer tobacco remains in the field, the more likely it is to be affected by FLS. With no chemical options and no known host resistance, reliance on common cultural practices is the only tool that growers have. Only pathogen free seed should be used, infected residues should be plowed under, and crop rotation should be practiced (Lucas 1991).

Fungicides labeled for leaf spot disease in Flue-cured Tobacco.

Currently, the only systemic fungicide labeled for leaf spot disease control in the field is azoxystrobin (Quadris®) (Thiessen, 2020). Other fungicides such as mancozeb (Manzate Prostick®) or organic products such as Bacillius *subtillis* strain AFS032321 (Theia®) or *Pseudomonas chlororaphis* (Howler® EVO) are not systemic and are only effective at the application site. Azoxystrobin and mancozeb are labeled for blue mold, FLS, and target spot suppression. Theia received registration in 2022 for angular leaf spot (*Pseudomonas spp*.), anthracnose (*Colletotrichum spp.*), FLS, brown spot, blue mold, gray mold (*Botrytis* spp.), collar rot (*Sclerotinia sclerotiorum*), powdery mildew (*Erysiphe cichoracearum*) and target spot. *Pseudomonas chlororaphis* also received registration in 2017 for control of the same diseases. Despite being labeled for use, the suppression potential of both organic products, specifically for target spot, brown spot, and FLS remains unknown. As previously emphasized, modern foliar fungicide applications solely consist of azoxystrobin. Additional fungicides tobacco farmers can depend on for control of these leaf spot diseases are desperately needed.

Review of Inpyrfluxam Use in Other Crops

Inpyrfluxam is a broad-spectrum fungicide registered for control of select diseases in apple, peanut, soybean, and sugar beet systems (Anonymous 2023a). It is recognized as a new SDHI (FRAC group 7) fungicide that was discovered by the Sumitomo Chemical company (Kiguchi et al., 2021). Inpyrfluxam is a part of the pyrazole-carboxamides chemical group with a target site of action at Complex II; succinate-dehydrogenase. As with all group 7 fungicides, inpyrfluxam acts by inhibiting succinate dehydrogenase, a key enzyme in the fungal respiration chain (Anonymous, 2023a). It is a compound that is commonly formulated and sold as Excalia®, which shows strong growth suppression of Basidiomycota and Ascomycota fungi (Kiguchi et al., 2021). After registration by the US Environmental Protection Agency (EPA) in 2020, it has been widely adopted and used to protect a variety of crops against fungal pests, such as *Rhizoctonia* spp. (Environmental Protection Agency 2020).

According to the label specifications, inpyrfluxam is a systemic compound that provides translaminar protection if solutions are properly absorbed (Anonymous, 2023a). Succeeding absorption, the compound moves upward but not downward in the plant, moving from the oldest to most recent growth (Anonymous, 2023a). Inpyrfluxam is identified and sold as a protectant that should be applied prior to disease infection for adequate disease prevention (Anonymous, 2023a).

Like many foliar fungicide applications, spray coverage and successful absorption are extremely important for product efficacy. According to the label, when ground application techniques are used, a minimum carrier volume of 93 L ha^{-1} should be used for field and vegetable crops. Inpyrfluxam is recognized as a long-lasting residual fungicide with a low use rate. It’s highly systemic action and fast movement into plants (Anonymous, 2023c) likely favors the minimal dose requirements. Necessary rates are situational and largely dependent upon forecasted disease pressure. Maximum use rates range from 0.05 to 0.10 kg ai ha^{-1} per application and from 0.05 to 0.19 kg ai ha^{-1} per growing season (Anonymous, 2023a).

<u>Target Organisms</u>

Inpyrfluxam provides protection against *Rhizoctonia* species that cause seed decay, seedling damping-off, and root rot (Environmental Protection Agency, 2020). In addition, it is also registered and used in apple production for control of apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), and cedar apple rust (*Gymnosporangium juniperi-virginianae*) (Anonymous, 2023a). Causal agents of both apple scab and powdery mildew are ascomycete fungi in the dothideomycete and leotiomycete classes, respectively (Kiguchi et al., 2021). These pathogenic organisms differ slightly from *R. solani* and related species. A review of inpyrfluxam, conducted by the Biological and Economic Analysis Division (2020), compared inpyrfluxam with other fungicides (penthiopyrad and kresoxam-methyl) commonly used for powdery mildew control in an attempt to determine its registration potential. Results indicated that inpyrfluxam, penthiopyrad, and kresoxam-methyl reduced powdery mildew from 20 percent in the non-treated control to two, six and, six percent infection, respectively (Anonymous, 2021). Results indicated efficacy in unprecedented use sights for inpyrfluxam, further validating its broad-spectrum potential.

In peanuts, inpyrfluxam is used for suppression of *Rhizoctonia* foliar blight, peg and root rot (*Rhizoctonia solani*), Sclerotinia blight (*Sclerotinia minor*), peanut rust (*Puccinia arachidis*), and both early (*Cercospora arachidicola*) and late (*Cercosporidium personatum)* leaf spot diseases (Anonymous, 2023a). Its use in peanuts is most widely noted for white mold (*Sclerotium rolfsii)* control (Valent USA, 2020). According to an article by Valent USA (2020), the US EPA granted federal registration of Excalia® to offer powerful white mold control, even under heavy pressure situations, and to help growers prevent yield losses often associated with *S. rolfsii* infection. In 2017, white mold was the most problematic disease for Georgia peanut growers, reducing crop value by nearly eight percent (Little, 2017). As a result of the recent registration, peanut growers and industry stakeholders now recognize inpyrfluxam to be a flexible new option that strengthens the soilborne disease control system by attacking a wide range of pathogens, most notably *S. rolfsii* (Little, 2017).

Soybean systems are no different than apple or peanut. Inpyrfluxam targets both rhizoctonia aerial blight (*Rhizoctonia solani*) and asian soybean rust (*Phakopsora pachyrhizi*) (Anonymous, 2023a). In a study reported by Kiguchi et al. (2021), that highlighted the discovery of inpyrfluxam and its effectiveness against various fungal pathogens, results demonstrated that inpyrfluxam strongly suppresses the growth of Basidiomycota and Ascomycota fungi. Median effective concentration numbers were extremely low when tested on each isolate of *R. solani* as well as *V. inaequalis*. This suggests that inpyrfluxam can be used in various crops, owing to its potent activity against a variety of fungal pathogens, including *R. solani* and *V. inaequalis* (Kiguchi et al., 2021). Inpyrfluxam is a chemical option recognized by Kiguchi et al. (2017) to be a new solution for soybean farmers struggling to control Asian Soybean Rust. It's further stated in the article that inpyrfluxam could and should be evaluated in other crop systems because of its activity on a variety of fungal pathogens (Kiguchi et al., 2017).

Outside of California-specific use restrictions, inpyrfluxam is also consistently used in sugar beets for *Rhizoctonia* foliar blight control and both crown and root rot suppression (Anonymous, 2023a). *Rhizoctonia solani* is the causal agent of each disease. Infection resulting from *R. solani* occurs wherever sugar beets are grown and major losses are identified worldwide (Neher and Gallian, 2011). On average, *R. solani* is responsible for two percent yield losses annually (Neher and Gallian, 2011). However, it is not uncommon to observe 30 to 60 percent, or even greater, losses of entire fields (Neher and Gallian, 2011). Field studies were conducted by Valent USA in 2022 (Anonymous 2023c) in Idaho, Michigan, Minnesota, and North Dakota to compare inpyrfluxam to grower standard practices and a non-treated control for management of *Rhizoctonia* foliar blight and crown and root rot in sugar beet systems. Results indicated that post transplanting applications of Excalia® at 0.15 L ha^{-1} reduced root rot incidence relative to the non-treated control by approximately 25 percent (Anonymous, 2023b).

Although current use sites are limited, inpyrfluxam continues to perform quite well. The new generation SDHI inhibitor has not only been effective at suppressing *R. solani* and related species, but has displayed its broad-spectrum capabilities by being of use in a variety of situations.

Environmental Fate and Activity of Inpyrfluxam

As fungal diseases continue to be a major threat to crop production, the application of fungicides to control fungal infestations is considered indispensable for the security of the global food supply (Zubrod et al., 2019). However, fungicides can be highly toxic to a broad-range of organisms and can pose a risk to non-targeted aquatic and terrestrial biota (Zubrod et al., 2019). Approximately 400,000 tons of fungicides are applied globally, which represents 17.5% of global pesticide applications (Gikas et al., 2022). The degree to which they affect the environment and factors that influence risk and exposure concerns are summarized by environmental fate. The environmental fate of fungicides depends on various physiochemical parameters, such as ionization (pk_a), water solubility, volatility, K_{OW}, and half-life in soil and water (DT_{50}) (Gikas et al., 2022). Soil characteristics, such as texture, organic carbon content, pH, clay mineral type, dissolved organic matter, and cation exchange capacity also play an essential role in the environmental fate of fungicides (Gikas et al., 2022). After application, a pesticide can move away from the release site (dissipation) or can be broken down and remain in the environment and exist in chemical forms that may behave differently from the parent compound (degradation) (Buhler and Frank, 2022). Both dissipation and degradation mechanisms along with chemical properties determine the environmental fate of pesticides (Joern et al., 1994). Understanding chemical properties as well as degradation and dissipation mechanisms not only determines the fate, but also allows applicators to select the most effective and environmentally safe product (Joern et al., 1994).

According to the ecological risk assessment conducted by the US EPA, inpyrfluxam is a compound comprised of two ring structures: a phenyl moiety and a pyrazolyl moiety, with most knowledge being from research conducted on the R-isomer (EPA, 2020). As identified in the ecological risk assessments, inpyrfluxam is not likely to volatilize, as it has a low vapor pressure (9.00×10^{-10} mmHg at 25^{o}C) (EPA, 2020). Inpyrfluxam is further identified to be moderately persistent and moderately mobile, with a water solubility value of 16.4 mg L^{-1} and a soil-water distribution coefficient (K_{oc}) of 691 L kg^{-1} (EPA, 2020). In terrestrial environments, inpyrfluxam is resistant to photolysis on top of the soil, with a half-life of 627 days (EPA, 2020). The EPA further identified mechanisms that influence metabolism and how compounds are transformed (degradation) once applied to the environment. In the same ecological significance study, the aerobic soil metabolism half-life ranged from 121 to 1,212 days, meaning the compound is more persistent in some situations than others (EPA, 2020). Organic matter was found to be a contributing factor to the adsorption capability of inpyrfluxam, thus, it is possible that binding mechanisms influence transformation rate (EPA, 2020). The study later identified inpyrfluxam to be moderately mobile in the soil with organic matter concentration as the leading contributing factor (EPA 2020). Other soil-related factors, such as percent clay, cation exchange capacity, and pH were reported to be insignificant (EPA, 2020). In four of the five field dissipation studies, inpyrfluxam was not detected deeper than six inches in the soil, unless excessive rainfall occurred (EPA, 2020). It was concluded that the major routes of inpyrfluxam dissipation in the environment following application may include spray drift, runoff on eroded soil, leaching through the profile, and transformation (EPA, 2020).

Justification of Work

Foliar leaf spot diseases, such as *R. solani* and *C. nicotianae* have increased in severity over the last decade in many tobacco producing regions in North Carolina. A recent survey of county extension agents suggests that yield losses from *R. solani* may exceed 6% across the state and 30% in isolated situations (M Vann, personal communication). Currently, the only two chemistries used in tobacco for such diseases are azoxystrobin and mancozeb. As a result of industry concern for high residue levels associated with mancozeb, most chemical control efforts have consisted of azoxystrobin, since its introduction in 2006. After providing successful results for nearly two decades, recent control failures have sparked tolerance and resistance concerns among commercial farmers and Cooperative Extension employees. As stated by Moparthi and Gorny (2023), difficult-to-control leaf spot diseases continue to be damaging to tobacco in North Carolina, management strategies to maintain chemical efficacy against pathogens and improve control will be the primary focal point of research objectives going forward. In 2018, *C. nicotianae* resistance to azoxystrobin was identified in Kentucky (M Vann, personal communication) and North Carolina (Thiessen, 2018). After reports of FLS control failures, Thiessen (2018) collected leaf spot lesion samples and tested them for strobilurin fungicide resistance. Results indicated that the lesions tested positive for strobilurin resistance and it was further concluded that conventional fungicides labeled for use in tobacco (azoxystrobin) would no longer be effective for managing FLS. Although *R. solani* resistance to azoxystrobin has not been identified, limited control options may have promoted levels of tolerance that many believe have prompted recent yield losses (M Vann, personal communication). As flue-cured tobacco farmers face record high input costs (12,350 USD ha^{-1}) (Dorfman, 2024), while investment returns are at historical lows, yield reductions resulting from *R. solani* and *C. nicotianae* are two of the biggest concerns. Alternative control options must continue to be investigated.

Additional and effective chemical control options for tobacco can benefit producers in many ways that improve economic returns for US growers (Clapp, 2019). The diversity of modes of action is a core component of anti-resistance strategies (Hollomon, 2015). One of the components of fungicide resistance is that pathogens show cross resistance to compounds with the same mode of action, but not to other modes of action (Hollomon, 2015). Control of the major ascomycete and basidiomycete (*R. solani, C. nicotianae, A. alternata*) diseases usually relies on fungicides that inhibit steps in one of the following: sterol biosynthesis, respiration, methionine biosynthesis, tubulin function or signal transduction (Hollomon, 2015). Resistance to each has already been identified in many pathogen populations. This alone significantly limits the scope of durable anti-resistance strategies (Hollomon, 2015). With knowledge of the current situation in tobacco production and the understanding of what adjustments could aid in current yield limitations, the authors believe that inpyrfluxam as well as other fungicides would be useful in flue-cured tobacco disease management systems. Label expansions of multiple products for use in US tobacco production should be considered.

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CHAPTER 2

Quantifying the Effects of Inpyrfluxam on Foliar Leaf Spot Disease Control in Flue-Cured Tobacco (*Nicotiana tabacum L.*)

ABSTRACT

Foliar leaf spot diseases, such as target spot (*Rhizoctonia solani*) and brown spot (*Alternaria alternata*) continue to impact flue-cured tobacco (*Nicotiana tabacum* L.) farmers in North Carolina. In 2021, yield losses from target spot were estimated at 6.9% or more than $39 million. Due to resistance/tolerance concerns relative to azoxystrobin and residue restrictions for mancozeb, additional fungicides are needed for leaf spot disease control. As such, field experiments were conducted in five environments from 2022 to 2023 to evaluate inpyrfluxam for target spot and brown spot control. Foliar fungicide treatments were comprised of three different rates of inpyrfluxam: 0.05, 0.10, and 0.25 kg ai ha^{-1} and were compared to azoxystrobin (0.15, 0.15, 0.16 kg ai ha^{-1}) or mancozeb (1.68 kg ai ha^{-1}). A non-treated control was included for comparison. Treatments were applied at four, six, and eight weeks after transplanting using carrier volumes of 234, 327, and 468 L ha^{-1}. Treatments consisting of azoxystrobin exhibited leaf phytotoxicity following each application, although observations did not exceed 10-15%. Visual injury in treatments comprised of inpyrfluxam or mancozeb were not observed. Disease pressure was limited in 2022; therefore, treatment response was minimal. In contrast, significant target spot infestation was observed at each environment in 2023. Relative to the non-treated control, inpyrfluxam was responsible for a 50-60% reduction in target spot severity and incidence. A dose response among the three rates of inpyrfluxam was not observed. Despite suspicions of resistance, azoxystrobin still produced a 30-40% reduction in target spot infection relative to the non-treated control. Target spot infection was similar between mancozeb and the non-treated control in most environments. In spite of positive disease control responses, differences in yield and quality were insignificant among the treatments evaluated, in four out of five environments. In one environment, yield was similar between inpyrfluxam and azoxystrobin, which were more effective than mancozeb. In another environment, visual quality was higher in inpyrfluxam-treated tobacco compared to azoxystrobin-treated tobacco. Our results suggest that inpyrfluxam would be an effective addition to a tobacco foliar leaf spot disease management program.

NOMENCLATURE

Nicotiana tabacum L., tobacco.

KEY WORDS

Fungicide, target spot, *Rhizoctonia solani*, severity, incidence, phytotoxicity, yield

INTRODUCTION

Flue-cured tobacco (*Nicotiana tabacum* L.) is considered a major agricultural commodity in many areas of the world (Collins and Hawks, 2013). During the twentieth century, tobacco became one of the most economically important consumer goods in over a hundred countries, worldwide. Historically, tobacco produced in the U.S. has been preferred by cigarette manufacturers due to its cleanliness, style, flavor, and aroma (Brown and Snell, 2017). Producers in the U.S. are equipped with soil, climatic, and a variety of production practices that are conducive to high quality production and desirable leaf characteristics (Whaley, 2017) that drive demand. As a result of competitive advantages, at one point in time the U.S. produced sixty-four percent of the total world supply (Collins and Hawks, 2013). Of the tobacco produced in the U.S., North Carolina leads production in both size (46,943 hectares) and volume (113,125,844 kg unprocessed leaf) (USDA, 2023). Despite numerous advantages, tobacco produced in the U.S. comes at a greater cost of production than that of most competitors. As foreign competitors have improved production and closed the gap in leaf quality, cigarette manufacturers now purchase and use more tobacco produced in other origins (Capehart 2003). Recent disease pressure has limited further advancements of yield and quality in tobacco produced in the U.S. Losses resulting from leaf spot diseases such as target spot have exceeded historical records and current chemical control programs need improvising. With increased urgency for quality production, disease management has become a top concern. Improving management efforts and exploring alternative fungicide programs for leaf spot disease control is vital for sustainable tobacco production in the US.

The application of fungicides directly over-the-top (OT) of tobacco in an effort to limit leaf spot diseases has often been discouraged by industry representatives, due to concerns about cured leaf residues (Todd, 1981) and what impact they might have on consumer health. As a result, chemical control options registered for such applications are limited and control of leaf spot diseases has been ineffective.

Despite numerous advancements in production practices such as variety improvements, greenhouse seedling management and mechanization, efforts to chemically manage leaf spot disease have remained stagnant. In 2006, azoxystrobin (Quadris® Flowable) (Syngenta Crop Protection, Greensboro, NC, USA) was labeled for control of target spot (*Rhizoctonia solani)* and frogeye leaf spot (*Cercospora nicotianae*) control. Alternative compounds such as mancozeb (Manzate® Pro-Stick™) (UPL Corporation Limited Group Company, King of Prussia, PA), have been discouraged because of residue concerns. As a result, management efforts directed towards leaf spot diseases have solely consisted of azoxystrobin. While proving to be effective since its registration, many credit continuous exposure and over-application to be concerning. Specifically, resistance to azoxystrobin in *Cercospora nicotianae* has been identified in multiple scenarios. According to Thiessen (2018), conventional fungicides labeled for use should no longer be used to control frogeye leaf spot as lesions have consistently tested positive for strobilurin resistance. Although resistance of *R. solani* hasn't been identified, suspected tolerance has been what many believe to be the cause of recent yield reductions.

Additional and effective chemical control options for tobacco can benefit producers in many ways that improve economic returns for US growers (Clapp, 2019). Having a variety of options and diversifying management strategies is beneficial in every disease management situation. As disease outbreaks become more abundant and production methods continuously change, a well-planned disease management program is imperative for sustainable production. No disease management practice alone provides full protection, control methods that complement each other and work well in combination are necessary for adequate disease protection and higher net profits (Todd 1981). Successful management programs and anti-resistance efforts are greatly influenced by the diversity of chemical control options available. Inability to overcome resistant situations results in lack of alternatives and continuous exposure to the same mode of action. This alone significantly limits the scope of durable anti-resistance strategies (Hollomon, 2015).

Inpyrfluxam is a foliar fungicide option that has been proven effective for control of leaf spot diseases in a variety of crops. Following approval by the EPA in 2020, it has been widely used for variety of fungal pests, most notably, *R. solani* (Environmental Protection Agency

2023). Inpyrfluxam (Excalia®) according to Kiguchi et al., 2021, shows strong suppression of higher fungi (basidiomycetes or ascomycetes) such as *R. solani*. It is used in peanuts, soybeans and other specialty crop systems and has been identified to display broad-spectrum capabilities (Valent U.S.A 2020). While its importance is noted in many agricultural sectors, previous research has not yet evaluated its potential in flue-cured tobacco. The objectives of this study were to evaluate inpyrfluxam for control of *R. solani*, compare it against current control options, identify dose response significance and identify phytotoxicity implications.

MATERIALS AND METHODS

Site and Experimental Design

Field experiments were conducted in 2022 at the Cunningham Research Station (CRS) in Kinston, North Carolina, and the Oxford Tobacco Research Station (OTRS) in Oxford, North Carolina. Research was continued in 2023 at the Cunningham Research Station, the Oxford Tobacco Research Station, and the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount, North Carolina. The varieties planted at each research site were NC 1226 (Goldleaf Seed Company, Hartsville, SC) at the CRS, GL 365 (Goldleaf Seed Company, Hartsville, SC) at the OTRS, and NC 960 (Foley Seed and Service, Ashland, VA) at the UCPRS. The varieties are further identified in Table 1 and possess no known host-resistance to foliar leaf spot diseases that affect tobacco in North Carolina. With the exception of foliar fungicide applications, tobacco was produced using practices recommended by the North Carolina Cooperative Extension Service (Fisher, 2022). Examples of these practices are the use of herbicides for weed control and synthetic suckersides for axillary bud control (Vann 2023). The herbicides clomazone (2-[(2-chlorophenyl) methyl]-4,4-dimethyl-3-isoxazolidinone) (840 g a.i. ha^{-1}) and sulfentrazone (N-{2,4-dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] phenyl] methanesul-fonamide) (1750 g a.i. ha^{-1}) (FMC Corporation, Philadelphia, PA) were applied pre-transplanting, less than seven days before transplanting and incorporated into the soil using a field cultivator. A single systemic insecticide application of imidacloprid (1- [(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) (10-20 g a.i./1000 plants) (Bayer CropScience, St. Louis, MO) was applied in the greenhouse for early season insect and tomato spotted wilt virus protection. Tobacco was plowed approximately three times during the first five weeks it was planted for weed control and row ridge support (Vann, 2023). In addition, synthetic suckersides, including fatty-alcohol(s) (Fair 85) (C_8-C_{10},) (Fair Products Inc, Cary, NC) and flumetralin (Drexalin Plus) (Drexel Chemical Company, Memphis, TN) were applied periodically before a single application of maleic hydrazide (potassium salt of 6-hydroxy-3-(2H)-pyridazinone) (Super Sucker-Stuff) (Drexel Chemical Company, Memphis, TN) for axillary bud suppression.

Fungicide treatments were replicated three or four times within each environment and arranged in a randomized complete block design. Individual plots contained four rows, each measuring 15.24 meters in length. Row width ranged from 1.11 to 1.21 meters across environments (Table 1). The center two rows of each plot were used for treatment application, data collection, and harvest. Harvest dates and data collection intervals varied by location and are listed in Table 2. Planting density was 14,820 plants ha^{-1} in all environments.

Description of Fungicide Application Timing, Application Rates and Carrier Volume

Five different fungicide treatment programs were evaluated for foliar leaf spot disease suppression: inpyrfluxam (0.05 kg ai ha^{-1}), inpyrfluxam (0.10 kg ai ha^{-1}), inpyrfluxam (0.25 kg ai ha^{-1}), azoxystrobin (0.15 + 0.16 + 0.16 kg ai ha^{-1}) and mancozeb (1.68 kg ai ha^{-1}). Three sequential applications of each fungicide active ingredient were evaluated for *R. solani* (target spot) and *A. alternata* (brown spot) control in naturally infested fields of tobacco. A non-treated control was included for comparison at each research site. A summary of active ingredients, trade names, formulation concentrations, manufacturers, application timings, rates, and modes of action are listed in Tables 3 and 4.

All fungicides were applied with a CO_2 pressurized backpack sprayer. The application volumes used to deliver treatments were 234, 327, and 467 L ha^{-1} at four, six, and eight weeks after transplanting (WAT), respectively. Application volumes were based upon the size of the crop canopy and were selected to promote the most effective and practical spray coverage possible. Application spray boom and nozzle arrangement varied with each application. During the first application (4 WAT), a single full-cone TeeJet TG6 (Spraying Systems Co., Wheaton, IL, USA) nozzle was used. Nozzle arrangement, flow pattern and spray coverage at the first application are presented in Figures 1 and 2. The second application was made six WAT with three TeeJet XR 8004-VS flat fan nozzles (Spraying Systems Co., Wheaton, IL, USA) configured on a 51 cm boom (Figure 3). To roughly align with pre-harvest intervals identified by chemical manufacturers and current extension recommendations, the final spray application was made at eight WAT. As suggested by Mila and Radcliff (2013), for locally systemic compounds, such as Quadris® Flowable (azoxystrobin), while accounting for total leaf area, a drop nozzle application was made. To achieve a desired volume of 467 L ha^{-1} within the final application, five TeeJet XR 11003-EVS (Spraying Systems Co., Wheaton, IL, USA) and two TeeJet XR 8003-VS (Spraying Systems Co., Wheaton, IL, USA) flat fan nozzles were utilized on a drop nozzle style boom that was 97 cm in width. A 104 cm drop was included to deliver consistent spray coverage in each stalk position group. Nozzle arrangement, flow pattern and spray coverage at application three are presented in Figures 4 and 5. Application volumes, nozzle configurations and materials used for each application are listed in Table 5. Individual application dates varied across research sites and field seasons (Table 2). Research plots were harvested four times, once per stalk position group. Harvest dates at each location are listed in Table 2.

Field Conditions

Field conditions, such as soil series, transplanting date, soil texture, cumulative rainfall, air temperature, and relative humidity levels varied by location and are presented in Tables 1, 6, 7, and 8. Among all growing environments, soil pH ranged from 5.8 to 6.2 per NC State Extension recommendations (Vann et al., 2023). The tobacco was transplanted on April 11 at the CRS and May 12 at the OTRS in 2022. In 2023, tobacco was transplanted on April 18th at the CRS, April 24th at the UCPRS, and May 24th at the OTRS. From transplanting to onset of floral initiation, fertilization programs, cultivation timings, and pest management practices (insect and weed) were conducted in accordance with recommendations from the North Carolina Cooperative Extension Service (Fisher et al., 2022). Leaf spot disease pressure was variable across field sites and growing seasons. Disease pressure was limited in 2022, when precipitation was less frequent between transplanting and flowering (Table 6), leading to less conducive environmental conditions for target spot (*R. solani*). In 2022, observations mostly consisted of brown spot (*A. alternata*) at both locations. In contrast, research environments were heavily infested with target spot in 2023, where the cumulative rainfall ranged from 76-91 cm between transplanting and flowering (Table 6).

CRS-2022/2023. Tobacco was produced on a Norfolk loamy sand soil (fine-loamy, kaolinitic, thermic Typic Kandiudults) during both years of the study at the CRS (Table 1). Lower precipitation frequency, specifically during the months of April, May, and June, contributed to less disease pressure in 2022 (Table 6). The CRS-2022 environment received 22.25 cm less rainfall relative to the 30-year average (Table 6). In 2023, the average precipitation in April was nearly three times that in 2022 (Table 6). Collectively, rainfall was 13.5 cm greater from April to June 2023 when compared to the same period in 2022. The cumulative rainfall (Table 6), measured from April to September, was 58.4 cm in 2022 and 76.8 cm in 2023, which was nearly a 19 cm difference. The early season average humidity level (Table 8), specifically in the month of April, was nearly 15% higher in 2023 relative to 2022. Leaf spot disease observations were primarily brown spot (*Alternaria alternata*) at the 2022 CRS field site, although infestation was limited. In contrast, the research environment in 2023 was heavily infested with target spot. Early season rainfall (April) was nearly double that of the 30-year average (Table 6). With an increase in both precipitation and humidity, field conditions in 2023 were much more conducive for pathogen development and disease observations were reflected. The CRS in 2023 was considered a high target spot pressure environment in the lower stalk position group at the initial disease rating (Table 10). Alternatively, target spot was not as severe in the middle and upper stalk positions (Table 10), and a treatment response was not recorded at the second rating interval. As a whole, disease pressure at the CRS in 2023 was moderate. It was recognized as the environment with the least amount of disease pressure relative to the other 2023 field sites.

OTRS- 2022/2023. Tobacco was produced on a Helena sandy loam soil (Fine, mixed, semiactive, thermic Aquic Hapludults) during both years of the study at the OTRS (Table 1). Rainfall was more frequent in 2023, particularly during the early stages of crop development. For example, in April 2023, the average rainfall was nearly 18 cm greater than in April 2022 (Table 6). In addition, rainfall was 12.6 cm greater (in April) when compared to the 30-year average (Table 6). From April to June 2023, precipitation was almost double when compared to the same period in 2022 (Table 6). Average precipitation in the month of July was 20.04 cm (Table 6), twice of that measured at each of the other 2023 field sites. As such, target spot was more noticeable at the second disease rating (Tables 12 and 13) when comparing this environment to the other 2023 environments. Overall, total precipitation, from April through September, was 38 cm greater in 2023 when compared to the 2022 growing season and 26 cm greater when compared to the 30-year average (Table 6). Average relative humidity (Table 8) was slightly higher in the month of June in 2023, being 5% higher than the 2022 environment. An increase in both relative humidity (Table 8) and precipitation (Table 6) soon after transplanting (Table 1), likely contributed to higher *R. solani* infestation levels in 2023. The OTRS in 2023 was considered as a high disease (target spot) pressure environment in each stalk position group at both rating intervals (Tables 10-13). It was the environment with the most precipitation (Table 6) across the two field seasons. Moreover, researchers considered this environment to have the second most disease pressure, with a measurable treatment response in multiple stalk position groups at each rating.

UCPRS- 2023. Tobacco was produced on a Goldsboro fine sandy loam soil (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults) at the UCPRS in 2023 (Table 1). Like the other 2023 field environments, rainfall events were much more frequent early in the season, especially in the month of April (Table 6). Precipitation in April was 5.3 cm greater when compared to the 30-year average (Table 6). With the highest average precipitation in the month of June (10.43 cm) (Table 6), target spot severity and incidence were more noticeable at the first disease rating, specifically in the middle stalk position (Tables 10 and 11) when compared to the other locations in 2023. Disease incidence was also more noticeable in the lower stalk position at the first rating (Table 11). Along with high precipitation, the average relative humidity in the month of June was highest at the UCPRS (Table 8). As such, the UCPRS was considered to be the field site with the greatest target spot pressure, especially early in the growing season.

Data Collection

Leaf Phytotoxicity. Visual estimates of tobacco injury resulting from treatment exposure were collected one week after each foliar spray application. Evaluations were conducted five, seven, and nine weeks after transplanting, using a visual injury percentage scale of 0 to 100. Observations of 0% were plots absent of any phytotoxicity, whereas 100% injury would be indicative of plant death. Injury percentages were based upon the size of the crop, total leaf surface area, and total leaf area exhibiting phytotoxicity symptoms. Injury diagnosis consisted of a composite rating of leaf area symptomology relative to the two outermost rows in each plot (non-treated), as well as the non-treated control.

Disease Severity and Incidence. After treatment applications and injury ratings were completed, and immediately prior to lower leaf removal (first harvest), visual evaluations were conducted to quantify leaf spot disease severity. A severity percentage chart (Figure 6, Jadhav and Patil, 2016) was used as a reference for disease severity percentages (5, 15, 30, 40, 60 %) presented in Figures 7, 8, 9, 10, and 11. Five random plants within the two treated rows of each plot were evaluated. Plants at the end of each plot were avoided to ensure treatment exposure consistency. Every leaf on each of the five plants evaluated were assessed for leaf spot infestation. Severity evaluations were divided into three stalk position groups: lower, middle and upper. The average total number of leaves on plants being evaluated after floral elimination was ≈ 21. With n= 21 leaves per plant, the lower-stalk evaluations consisted of the bottom-most seven leaves, with middle and upper stalk evaluations following the same criteria. Each leaf displaying at least one symptom of leaf spot infection (chlorotic and/or necrotic lesions) was assigned a severity percentage. Leaves absent of symptoms were not reported. Severity percentages were quantified based on cumulative leaf area displaying disease symptomology relative to the portions of the leaf absent of disease. The disease incidence was quantified simultaneously with the severity evaluations. This was a measurement of the total number of leaves within each stalk position group exhibiting disease infection symptomology. Disease incidence data were transformed and are presented as a percentage of the total leaves within a stalk position group. Incidence data was collected concurrent to severity evaluations, a few days preceding the first and third harvest.

Yield, quality, and value. Plots were harvested at four different intervals. During the first interval, the lowest portions of the stalk were harvested. During the second interval, the cutter leaves or middle portions of the stalk were harvested. In the remaining harvesting intervals, the leaf and tip positions of the plant were removed. Each of the harvest removal dates are listed in Table 2. Yield, quality, and value data were collected from each research environment. Following harvest, leaves were cured and weighed for yield and assigned a USDA government grade for quality. Each government grade has an associated grade index value ranging from 0-100 which describes the leaf maturity and ripeness (Bowman et al., 1988). Cured leaf value was determined using a combination of leaf yield and quality with value estimates reported by Vann et al. (2022).

Data Analysis

Analysis of variance was conducted using PROC Mixed in SAS version 9.4 (SAS Institute, Cary, NC, USA) to test the effects of fungicide program on visual injury, leaf spot severity and incidence, yield, quality, and value. Within each analysis, fungicide treatment programs were considered fixed factors, while environment and replication were considered random factors. Treatment means were separated using Fisher's Protected LSD test at $P < 0.05$. Due to variation in disease pressure and observations, results are presented for individual growing locations for each parameter that was investigated.

RESULTS & DISCUSSION

Visual Injury

Visual injury ratings were affected by fungicide treatment programs at each location. Leaf phytotoxicity was not apparent in any fungicide program except for those containing azoxystrobin (Table 9). In 2022, leaf phytotoxicity after the first application (1 WAA) (four weeks after transplanting) was minimal (3%) and was only observed at the CRS (Table 9). Following the second application (3 WAA) (weeks after first application) (six weeks after transplanting) phytotoxicity increased slightly (5%) but was still considered to be minimal (Table 9). Observations continued to be limited to the CRS environment (Table 9). Observations following the third and final application (5 WAA) (eight weeks after transplanting) identified slightly more injury (13%) (Table 9). With a 12 % increase in relative humidity (July) (Table 8) leaf phytotoxicity was also observed at the OTRS (7%) (Table 9). Values were still considered as minimal. It is plausible that the slight increase in injury was a result of the changes in azoxystrobin application rates between the first and second treatment intervals (Table 3). Injury observations did not have an impact on cured leaf yield in 2022 (Table 14).

Similar trends were identified in 2023. However, due to the presence of weather fleck (ozone injury) and the difficulty with distinguishing between weather fleck and azoxystrobin injury, only leaf phytotoxicity after the first application (1 WAA) is presented from two growing environments. Visual injury resulting from azoxystrobin, a FRAC (fungicide resistance action committee) group 11 fungicide, is sometimes mistaken for weather fleck. Mila et al (2006) evaluated azoxystrobin phytotoxicity and efficacy in flue-cured tobacco and highlighted that weather flecking was reported with the use of the product. However, weather flecking is a weather-related disorder, as it is caused by ozone (O_3) exposure, which is an air pollutant (Anonymous, 2016). Weather fleck and leaf phytotoxicity resulting from azoxystrobin applications, although similar in visual appearances, are not the same. Injury observations in the present research were conducted while considering the possibility of both issues, and data were excluded from the analyses where the disorder was observed.

In 2023, visual injury was only identified in treatments containing azoxystrobin (Table 9). Collectively, observations following the first application (1WAA) were minimal at both locations reported (5%) (Table 9). As the application rate increased during the second application (six weeks after transplanting) (5 WAA), injury estimates slightly increased at the CRS (14%) but decreased or remained the same at the other locations (Table 9). Injury was less noticeable following the final application 8 WAT (weeks after transplanting) (5 WAA) at each of the three locations. However, observations continued to be the highest at the CRS. The lowest rainfall total was measured at the CRS in the month of June (Table 6) when compared to other environments. It is plausible that the slight increase in precipitation (Table 6) resulted in less injury at the OTRS and UCPRS after the second and third applications. Similar to observations reported in 2022, cured leaf yield was not impacted by visual injury from azoxystrobin treatments in 2023 (Table 14).

Ultimately, the crop safety factor, or the potential for phytotoxicity associated with mancozeb and the differing rates of inpyrfluxam are desirable for tobacco production. In a product review panel initiated by the EPA (environmental protection agency), it is highlighted in a review of the registration submission that inpyrfluxam use rates on labeled crops are generally lower (0.05 – 0.10 kg a.i. ha^{-1} per application) than other fungicides such as azoxystrobin" (EPA 2021). Moreover, the fact that injury was not documented following repeated 10x treatments of inpyrfluxam should ensure that crop safety is acceptable with commercial applications of the fungicide.

Disease Control (Severity/ Incidence)

Severity. Disease severity ratings prior to first harvest were only affected by treatment programs at the Oxford environment in 2022 (Table 10). Collectively, leaf spot disease pressure was minimal at both field sites and within each stalk position group (<5.35 %) as environmental conditions were generally less conducive for infection. As highlighted in Table 6, rainfall was less frequent with cumulative precipitation ranging from 53.51 – 58.44 cm across both research sites. In addition, the majority of precipitation occurred in the later portion of the season (July – September) (Table 6). When compared to a 30-year average, rainfall was 22.25 cm less at the CRS and 11.56 cm less at the OTRS (Table 6). Based on leaf samples submitted to the NC State University Plant Disease and Insect Clinic from both environments in 2022, brown spot (*A. alternata*) was the only foliar leaf spot pathogen identified within our field trials. Treatment differences were only identified at the OTRS (Table 10). Inpyrfluxam is labeled for and believed to be most effective against diseases caused by *Rhizoctonia spp*. However, inpyrfluxam is also described to provide control of alternaria blotch (*Alternaria mali*) (EPA, 2021), a disease in apples (*Malus pumila*) that is caused by an organism within the same genus as tobacco brown spot. Despite the unknown suppression potential for each of the treatments evaluated relative to brown spot, inpyrfluxam reduced severity in the lower stalk position relative to the non-treated control, azoxystrobin and mancozeb at one field site (Table 10). Within the lower stalk position, brown spot severity was similar among the three rates of inpyrfluxam (2.42 to 2.86%) (Table 10). In contrast, azoxystrobin (3.89%) and mancozeb (4.16%) only provided moderate suppression. Relative to the non-treated (5.02%), applications of inpyrfluxam reduced brown spot severity by 2.16 to 2.6%. Treatment response was better in the middle stalk position, where each fungicide program reduced brown spot severity relative to the non-treated control (Table 10). A treatment response was not recorded within the upper stalk position, most likely due to limited disease pressure at the top of the plant. Leaf spot disease was not observed during the initial rating in the upper stalk at the CRS in 2022 (Table 10).

In contrast to 2022, disease severity prior to first harvest was affected by treatment programs in 2023. Target spot (*R. solani*) pressure was consistent across each field site and, in general, similar trends were identified (Table 10). Inpyrfluxam reduced target spot severity in lower and middle stalk positions relative to the non-treated control, azoxystrobin, and mancozeb (Table 10). However, the disease severity was similar among the three rates of inpyrfluxam in each stalk position group. Fungicide treatment response was greatest at the UCPRS in both the lower and middle stalk positions. Within the lower stalk position, inpyrfluxam (1.82 – 2.13%) (Table 10) reduced target spot severity by 5.62 to 5.93% relative to the non-treated control (7.75%) (Table 10). In addition, a 4.3 to 5.25% reduction was also observed at the OTRS in the lower stalk position (Table 10). Similar results were also identified in the middle stalk position at each location. For example, inpyrfluxam reduced target spot severity by 7.2% and 1.8% at the UCPRS and OTRS, respectively, when compared to the non-treated control (Table 10). A treatment response was also recorded following azoxystrobin application. Within the lower stalk position, azoxystrobin reduced target spot severity by 3.5% and 2.58% at the UCPRS and OTRS, respectively, relative to the non-treated control (Table 10). In addition, 4.93% reduction was also observed in the middle stalk position at the UCPRS (Table 10). Within the lower stalk position, mancozeb only provided moderate suppression (Table 10). Alternatively, within the middle stalk position, mancozeb (5.84%) reduced target spot severity by 2.95% when compared to the non-treated control (8.79%) at one location (Table 10). Impactful treatment responses were not recorded within the upper stalk position group, most likely due to limited disease pressure within that stalk position group (Table 10).

A second rating was conducted immediately prior to the third harvest interval (out of four), following the removal of all lower stalk leaves. As previously stated, target spot is most problematic at earlier stages of growth and on leaves that are closest to the soil surface. Brown spot, however, is often referred to as a disease of mature tobacco (Todd, 1981), that commonly appears on mature leaves during the later portion of a growing season (Moparthi and Gorny, 2023). Temperatures in July and August were slightly above average (Table 7) and brown spot was only observed in small amounts in 2022 and not apparent in 2023. Despite an abundance of rainfall in August (Table 6), target spot observations were also less frequent compared to the first rating. Thus, fungicide treatment response was less apparent at the second rating. No treatment response was observed in 2022 at both locations infested with brown spot (Table 12). A middle stalk position rating is only reported at the OTRS in 2023. Leaves at each of the other field environments had been removed prior to data collection. Within the second disease rating, the three rates of inpyrfluxam had a lower disease severity (1.52 to 2.59%) than the non-treated control (7.13%) and mancozeb (4.17%) (Table 12). Inpyrfluxam at the 5x rate (0.25 kg ai ha^{-1}) (1.52%) was also statistically different than azoxystrobin (3.27%) (Table 12). Disease severity percentages among the three rates of inpyrfluxam ranged from (1.52 to 2.59%), with each rate reducing the level of severity by approximately 4.54 to 5.61% when compared to the non-treated control (7.13%) and 1.58 to 2.65% when compared to mancozeb (Table 12). A positive treatment response was also recorded with mancozeb (4.17%) and azoxystrobin (3.27%) when compared to the non-treated control (7.13%). Azoxystrobin was responsible for a 3.86% reduction, while mancozeb reduced severity by 2.96%.

The second disease rating in the upper stalk position group was consistent across each environment; however, treatment responses were only identified at two of the locations (Table 12). Even with limited disease pressure in the upper stalk position and ratings being collected so late in the 2023 growing season, a positive response was recorded with inpyrfluxam. Disease severity within the upper stalk position ranged from 1.17 to 1.71% across the three rates of inpyrfluxam (Table 12). This accounted for a 2.29 to 2.64% reduction at the OTRS and a 1.48 to 1.56% reduction at the UCPRS when compared to the non-treated control (4.00 and 2.73%, respectively). Inpyrfluxam performance was not significantly different from azoxystrobin or mancozeb at the OTRS. Both azoxystrobin and mancozeb reduced severity by 1.72 to 2.25%, respectively when compared to the non-treated control (Table 12). Inpyrfluxam was the only treatment more effective than the non-treated control at the UCPRS, though disease pressure was minimal. Even with disease pressure limitations inpyrfluxam continued to be an effective option, displaying consistent results across each stalk position group.

Incidence. Across environments, fungicide treatments performed similarly to what was previously discussed relative to disease severity. Collectively, disease pressure was minimal across field sites and stalk position groups in 2022 (Table 11). Environmental conditions (rainfall frequency, Table 6) were less conducive for disease development and treatment responses were less apparent. The 2022 incidence ratings were evaluating fungicide programs for suppression of brown spot at each field site (CRS-2022 and OTRS-2022). Incidence ratings were only affected by fungicide treatment programs at the OTRS in 2022 (Table 11). Moreover, inpyrfluxam, when applied at the 5X rate (51%) was less effective than each of the remaining treatments in the lower stalk position at the CRS, including the non-treated control (Table 11). Alternatively, inpyrfluxam when applied at the 1X rate (36%) reduced brown spot incidence in the lower stalk position relative to the non-treated control and mancozeb at the OTRS (Table 11). Brown spot incidence was reduced by 29% when comparing inpyrfluxam (1X) (36%) to the non-treated control (65%) and 10% in comparison to mancozeb (46%) (Table 11). Inpyrfluxam efficacy when applied 2X and 5X was similar to azoxystrobin and mancozeb (Table 11) at the same field site. Brown spot incidence ranged from 43 to 46% among those treatments, accounting for a 19 to 22% reduction when compared to the non-treated control (65%) in the same stalk position group (Table 11). The variability in treatment response across field sites in similar stalk position groups is reflective of limited disease pressure resulting from environmental conditions previously discussed and is identified in Tables 6-8. Similar trends were identified in the middle stalk position. Each fungicide reduced brown spot incidence relative to the non-treated control. Within the middle stalk position group, incidence was similar among the three rates of inpyrfluxam and azoxystrobin (18 to 21%) (Table 11). Relative to the non-treated control (46%), applications of inpyrfluxam and azoxystrobin reduced incidence by 25 to 28%, respectively. Mancozeb (30%) also accounted for a 16% reduction when compared to the non-treated control (46%) (Table 11). A treatment response was not recorded within the upper stalk position across both environments during the initial rating in 2022, most likely due to limited disease pressure.

Disease incidence ratings prior to first harvest were affected by treatment programs across each environment in 2023 but were most apparent at the OTRS and UCPRS (Table 11). Target spot pressure was consistent across each field site and similar trends were identified within each stalk position group. Inpyrfluxam reduced target spot incidence in lower, middle, and upper stalk positions relative to the non-treated control, azoxystrobin, and mancozeb (Table 11). Disease incidence was also similar among the three rates of inpyrfluxam in each stalk position group. In the lower stalk position at the UCPRS, inpyrfluxam (18 to 33%) accounted for a 43 to 58% incidence reduction relative to the non-treated control (76%) (Table 11). In addition, a 15% reduction was also observed at the OTRS. Similar results were also identified in the middle stalk position. Inpyrfluxam (5 to 11%) reduced target spot incidence by 40 to 46% at the UCPRS and 28 to 30% at the OTRS (14 to 16%, respectively) when compared to the non-treated control (44 to 51%) (Table 11). A treatment response was only recorded at one location (OTRS) in the upper stalk position, with inpyrfluxam being the only fungicide program with a positive response. Azoxystrobin also reduced target spot incidence in the lower and middle stalk positions at both locations. In the lower stalk position, azoxystrobin (29 to 56%) accounted for a reduction of 13% at the OTRS and 20% at the UCPRS when compared to the non-treated control (42 to 76%, respectively) (Table 11). Similar trends were identified in the middle stalk position. Azoxystrobin reduced target spot incidence by 24% at the OTRS and 15% at the UCPRS when compared to the non-treated control (44 to 51%, respectively). Treatment response to mancozeb was similar to the non-treated control in the lower and middle stalk position groups with the exception of one location (OTRS) (Table 11). Mancozeb (31%) reduced target spot incidence by 13% in the middle stalk position when compared to the non-treated control (44%) at the OTRS.

A second rating was conducted immediately prior to the third harvest (out of four), following the removal of all lower stalk leaves. Leaf spot diseases were not apparent at the OTRS in 2022 at the time of the second rating (Table 13). A middle stalk position rating is only reported at the OTRS in 2023, as leaves at each of the other field environments had been removed prior to data collection. Within the second rating, the three rates of inpyrfluxam (1x, 2x, 5x) had a lower incidence (29 to 41%) than the non-treated control (86%) and azoxystrobin (56 %) in the middle stalk position (Table 13). Inpyrfluxam at the 1x rate (0.05 kg ai ha^{-1}) was similar to mancozeb (52%). Relative to the non-treated control (86%), inpyrfluxam reduced target spot incidence by 45 to 57% in the middle stalk position. A positive treatment response was also recorded with azoxystrobin (56%) and mancozeb (52%) when compared to the non-treated control (86%). Overall, each fungicide was responsible for an incidence reduction of 30 to 34% in the middle stalk position group (Table 13). Treatment responses were only identified at two locations (OTRS and UCPRS) in the upper stalk position group at the second rating (Table 13). Each fungicide program reduced target spot incidence when compared to the non-treated control at the OTRS (Table 13). Azoxystrobin (10%), Inpyrfluxam (1x) (0.05 kg ai ha^{-1}) (17%), and Inpyrfluxam (5x) (0.25 kg ai ha^{-1}) were the most effective treatments at the UCPRS. Azoxystrobin (10%) and inpyrfluxam (17 to 18%) reduced target spot incidence by 17 to 25% when compared to the non-treated control (35%) at the UCPRS (Table 13). Performance variability across stalk positions is most likely related to limited disease pressure.

Yield, Quality, and Value

Yield. Cured leaf yield was only affected by treatment programs at one location (CRS-2022) in each of the two growing seasons (Table 14). Treatments containing mancozeb (3,752 kg ha^{-1}) had lower yields than inpyrfluxam (3,975 to 4,113 kg ha^{-1}) and the non-treated control (3,972 kg ha^{-1}) and were similar to azoxystrobin (3,923 kg ha^{-1}) (Table 14). Numerically, mancozeb was the least effective treatment for brown spot severity in both the lower (2.87, 5.35%) (Tables 10 and 12) and upper (1.34%) (Table 12) stalk positions across multiple ratings at the same location. Although not statistically different, this could explain why lower yields were observed at the CRS in 2022. Collectively, yields were below average, specifically in 2023.

Disease pressure increase and environmental conditions (Tables 6-8) are most likely why yield was so variable between growing seasons. Despite this observation, researchers believe further research is warranted to further quantify yield impacts of these fungicides.

Quality. A treatment response in cured leaf quality was only observed at the CRS-2023 environment (Table 14), where azoxystrobin (57) had a lower visual quality than inpyrfluxam (64 to 66) and the non-treated control (68) (Table 14). Interestingly, azoxystrobin application resulted in the most chemical injury (5, 14, and 11%) (Table 9) at the same location. This interaction may be reflected in the visual quality estimates.

Value. A treatment response was only recorded relative to crop value when yield impacts were likewise identified. As such, the crop value with mancozeb (\$11,142 ha^{-1}) was lower than azoxystrobin (\$12,750 ha^{-1}) and inpyrfluxam (\$12,813 to 13,767 ha^{-1}) and similar to the non-treated control (\$12,230 ha^{-1}) at the CRS in 2022 (Table 14).

CONCLUSIONS

Results from this study identify the potential use of a new fungicide chemistry for target spot and brown spot control in flue-cured tobacco. Inpyrfluxam was the most effective fungicide treatment for both target spot (2023) and brown spot (2022) suppression in our field trials. Moreover, when compared to azoxystrobin and mancozeb, inpyrfluxam reduced severity and incidence across stalk position groups, in spite of variable disease pressure. In addition, chemical injury from inpyrfluxam was not documented, further highlighting the possible benefits of the fungicide.

Equally as important is that despite suspected resistance to azoxystrobin, Quadris® applications proved to be more effective than mancozeb for target spot control in 2023. Our data suggest that azoxystrobin is still an effective fungicide and that with adjustments to application techniques (such as ensuring appropriate spray coverage) and application timeliness, azoxystrobin could continue to be an effective option for commercial farmers. Azoxystrobin was the only treatment to exhibit leaf phytotoxicity, though injury was limited and was not reflected in yield.

Environmental conditions, specifically timeliness of rainfall and relative humidity appear to have the greatest influence on natural disease infestation levels. Consistent rainfall soon after transplanting (3-4 weeks) in combination with above average humidity significantly influenced what disease was present and how severe infestation levels were across both growing seasons.

Having a variety of fungicide options and incorporating a spray rotation is most beneficial for every foliar disease management system. Effective suppression of these leaf spot diseases could greatly influence commercial tobacco production in the US.

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Table 1. Soil series, soil texture, taxonomic class, transplanting date, variety, and row spacing at each location.[a]

Environment	Soil Series	Soil Texture	Taxonomic Class	Transplanting Date	Variety	Row Spacing (meters)
CRS-2022	Norfolk	Loamy Sand	Fine-loamy, kaolinitic, thermic Typic Kandiudults	April 11, 2022	NC1226	1.11
OTRS-2022	Helena	Sandy Loam	Fine, mixed, semiactive, thermic Aquic Hapludults	May 12, 2022	GL 365	1.21
CRS-2023	Norfolk	Loamy Sand	Fine-loamy, kaolinitic, thermic Typic Kandiudults	April 18, 2023	NC 1226	1.11
OTRS-2023	Helena	Sandy Loam	Fine, mixed, semiactive, thermic Aquic Hapludults	May 24, 2023	GL 365	1.11
UCPRS-2023	Goldsboro	Fine Sandy Loam	Fine-loamy, siliceous, subactive, thermic Aquic Paleudults	April 24, 2023	NC 960	1.21

[a] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

Table 2. Fungicide application, injury evaluations, harvest, and disease rating date at each location

	2022		2023		
	CRS	**OTRS**	**CRS**	**OTRS**	**UCPRS**
Application date(s)[b]	5/10/2022 5/26/2022 6/7/2022	6/8/2022 6/22/2022 7/6/2022	5/16/2023 5/30/2023 6/13/2023	6/27/2023 7/11/2023 7/26/2023	5/22/2023 6/9/2023 6/19/2023
Injury evaluation	5/17/2022 6/2/2022 6/14/2022	6/15/2022 6/29/2022 7/11/2022	5/23/2023 6/6/2023 6/20/2023	7/5/2023 7/18/2023 8/1/2023	5/31/2023 6/15/2023 6/26/2023
1st Harvest	6/28/2022	8/2/2022	7/11/2023	8/8/2023	7/28/2023
2nd Harvest	7/21/2022	8/30/2022	7/31/2023	9/1/2023	8/14/2023
3rd Harvest	8/4/2022	10/20/2022	8/16/2023	9/26/2023	8/22/2023
4th Harvest	8/10/2022	10/21/2022	9/7/2023	9/28/2023	8/23/2023
1ST Disease Rating[c]	6/21/2022	8/1/2022	7/10/2023	8/3/2023	6/26/2023
2ND Disease Rating[d]	7/20/2022	--[e]	8/7/2023	8/23/2023	7/24/2023

[a] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[b] Date refers to the date on which treatments/harvest(s)/evaluations were made

[c] Treatment evaluation prior to first harvest.

[d] Treatment evaluation prior to third harvest.

[e] Foliar leaf spot diseases not observed.

[f] A fourth harvest removal date was not collected due to environmental/disease limitations.

Table 3. List of treatments, application rate, timing and solution volumes used. [b]

Treatments	Application Rate	Application Timing	Solution Volume
	kg ai ha^{-1}	WAT[a]	L ha^{-1}
Non-treated Control	--	--	--
Inpyrfluxam (1x)	0.05	4; 6, 8	234, 327, 467
Inpyrfluxam (2x)	0.10	4, 6, 8	234, 327, 467
Inpyrfluxam (5x)	0.25	4, 6, 8	234, 327, 467
Azoxystrobin	0.15, 0.16, 0.16	4, 6, 8	234, 327, 467
Mancozeb	1.68	4, 6, 8	234, 327, 467

[a] WAT; weeks after transplanting

[b] "," In the application rate column indicates that the rate was changed following the first application.

Table 4. Fungicide active ingredient, trade name, formulation concentration, manufacturer, and mode of action.

Active Ingredient	Trade Name	Formulation Concentration	Manufacturer	Mode of Action
Inpyrfluxam	Excalia®	340 g ai L^{-1}	Valent U.S. A	Inhibition of succinate dehydrogenase (SDHI), Carboxamides, FRAC group 7
Azoxystrobin	Quadris® Flowable	249 g ai L^{-1}	Syngenta	Inhibition of Quinone outside, Strobilurines, FRAC group 11
Mancozeb	Manzate® Pro-Stick™	750 g ai/kg	UPL	Multi-site contact activity, dithiocarbamates, FRAC group M03

Table 5. Application type, volume, nozzle arrangement and speed at each interval.

Timing	Volume	Spray Nozzles	Type	Speed
WAT[a]	**L ha^{-1}**			**kph**
4	234	TG6 (full cone nozzle)	OT[b]	4.82
6	327	XR8004- XR8004- XR8004 (flat fan nozzles)	OT[b]	4.82
8	467	XR8003 + XR11003 (flat fan nozzles)	Drop Nozzle	4.82

[a] WAT; weeks after transplanting

[b]OT, over-the-top application

Table 6. Monthly, cumulative, and 30 year precipitation average in each growing environment.[a]

	2022		2023			30 year average		
Month	**CRS**	**OTRS**	**CRS**	**OTRS**	**UCPRS**	**CRS**	**OTRS**	**UCPRS**
	cm							
April	5.68	4.67	16.02	22.09	14.35	8.96	9.44	8.99
May	9.98	10.82	9.80	8.12	8.17	9.95	8.94	9.22
June	4.52	3.32	7.89	10.31	10.43	14.04	12.72	12.21
July	14.04	17.09	10.94	20.04	10.69	14.70	10.84	12.95
August	11.17	6.42	19.43	10.64	11.55	16.15	12.11	13.41
September	13.03	11.17	12.75	20.09	16.35	16.86	10.99	15.62
Cumulative[b]	58.44	53.51	76.86	91.33	71.57	80.69	65.07	72.41

[a] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[b] Total refers to cumulative precipitation from April through September in each environment.

Table 7. Montly temperature maxiumum, minimum, cumulative average, and 30 year average in each growing environment. [a]

	2022		2023			30 year average		
Month	**CRS**	**OTRS**	**CRS**	**OTRS**	**UCPRS**	**CRS**	**OTRS**	**UCPRS**
	°C							
Maximum[b]								
April	23.4	21.4	23.9	22.0	23.4	24.1	21.4	21.8
May	27.8	25.5	24.2	22.7	23.8	27.7	25.4	25.6
June	31.0	30.1	28.9	27.3	28.3	31.1	29.5	29.6
July	32.1	31.4	32.4	31.4	32.5	32.5	31.7	31.3
August	31.0	29.9	32.5	30.2	32.1	31.5	30.6	29.6
September	28.4	27.0	28.1	26.4	27.4	28.7	26.4	27.4
Minimum[c]								
April	10.4	9.6	11.9	10.1	11.3	10.7	8.7	8.5
May	17.4	15.3	14.2	13.2	13.9	15.2	14.0	13.9
June	19.4	18.9	18.1	17.0	17.7	19.7	18.6	18.6
July	23.0	21.7	22.3	21.6	22.3	21.8	20.8	20.7
August	20.9	20.1	21.8	20.4	21.3	20.8	19.9	19.7
September	16.9	16.4	17.8	16.8	17.5	17.8	16.3	16.8
Cumulative[d]	23.1	21.9	22.7	21.3	22.3	23.5	22.0	21.9

[a] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.
[b] Average maximum air temperature at each location.
[c] Average minimum air temperature at each location.
[d] Cumulative refers to average temperature from April through September in each environment.

Table 8. Average monthly relative humidity level at each growing environment in 2022 and 2023. [a]

	2022		2023		
Month	**CRS**	**OTRS**	**CRS**	**OTRS**	**UCPRS**
	%				
April	61.3	58.5	70.2	68.0	69.5
May	73.9	76.4	70.4	70.7	70.1
June	68.9	68.1	74.4	73.2	74.5
July	81.9	80.6	80.7	78.3	79.1
August	81.1	79.3	76.5	77.7	75.7
September	78.5	74.5	78.6	81.2	82.6

[a] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

Table 9. The effect of fungicide program on visual chemical injury one week after each treatment application in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	1 WAA[d]	3 WAA	5 WAA
CRS 2022		%	
Non-treated Control	0 b	0 b	0 b
Inpyrfluxam (1x)	0 b	0 b	0 b
Inpyrfluxam (2x)	0 b	0 b	0 b
Inpyrfluxam (5x)	0 b	0 b	0 b
Azoxystrobin	3 a	5 a	13 a
Mancozeb	0 b	0 b	0 b
P>F	<0.001	<0.001	<0.001
OTRS 2022			
Non-treated Control	0	0	0 b
Inpyrfluxam (1x)	0	0	0 b
Inpyrfluxam (2x)	0	0	0 b
Inpyrfluxam (5x)	0	0	0 b
Azoxystrobin	0	0	7 a
Mancozeb	0	0	0 b
P>F	ns	ns	<0.001
CRS 2023			
Non-treated Control	0 b	0 b	0 b
Inpyrfluxam (1x)	0 b	0 b	0 b
Inpyrfluxam (2x)	0 b	0 b	0 b
Inpyrfluxam (5x)	0 b	0 b	0 b
Azoxystrobin	5 a	14 a	11 a
Mancozeb	0 b	0 b	0 b
P>F	<0.001	<0.001	<0.001

Table 9. The effect of fungicide program on visual chemical injury one week after treatment application in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	1 WAA[d]	3 WAA	5 WAA
OTRS 2023		%	
Non-treated Control	0 b	0 b	0 b
Inpyrfluxam (1x)	0 b	0 b	0 b
Inpyrfluxam (2x)	0 b	0 b	0 b
Inpyrfluxam (5x)	0 b	0 b	0 b
Azoxystrobin	5 a	3 a	3 a
Mancozeb	0 b	0 b	0 b
P>F	<0.001	<0.001	<0.001
UCPRS 2023			
Non-treated Control	--[e]	0 b	0 b
Inpyrfluxam (1x)	--	0 b	0 b
Inpyrfluxam (2x)	--	0 b	0 b
Inpyrfluxam (5x)	--	0 b	0 b
Azoxystrobin	--	5 a	2 a
Mancozeb	--	0 b	0 b
P>F	--	<0.001	0.002

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant. Injury ratings conducted one week following each treatment application. Ratings assigned on a scale of 0-100, with 100 representing plant death.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] WAA; weeks after first application.

[e] Injury ratings not collected due to ozone damage.

Table 10. The effect of fungicide program on disease severity in lower, middle, and upper stalk leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	Lower Stalk	Middle Stalk	Upper Stalk
CRS 2022		%	
Non-treated Control	4.12	2.68	--[d]
Inpyrfluxam (1x)	4.64	3.25	--
Inpyrfluxam (2x)	3.75	4.84	--
Inpyrfluxam (5x)	4.08	4.56	--
Azoxystrobin	3.73	7.44	--
Mancozeb	5.35	0.63	--
P>F	ns	ns	--
OTRS 2022			
Non-treated Control	5.02 a	2.48 a	1.83
Inpyrfluxam (1x)	2.86 b-d	1.39 b	1.53
Inpyrfluxam (2x)	2.68 cd	1.23 b	1.66
Inpyrfluxam (5x)	2.42 d	1.32 b	1.32
Azoxystrobin	3.89 a-c	1.55 b	1.65
Mancozeb	4.16 ab	1.46 b	1.66
P>F	0.006	0.004	ns
CRS 2023			
Non-treated Control	7.30 a	1.23 bc	2.00
Inpyrfluxam (1x)	4.37 bc	1.87 a	3.00
Inpyrfluxam (2x)	3.74 c	1.30 bc	1.00
Inpyrfluxam (5x)	3.67 c	1.66 ab	1.00
Azoxystrobin	4.52 bc	1.06 c	1.04
Mancozeb	6.04 ab	1.19 bc	1.00
P>F	0.009	0.031	ns

Table 10. The effect of fungicide program on disease severity in lower, middle, and upper stalk leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	**Lower Stalk**	**Middle Stalk**	**Upper Stalk**
OTRS 2023		%	
Non-treated Control	7.21 a	2.08 a	0.37
Inpyrfluxam (1x)	1.96 c	0.21 c	0.32
Inpyrfluxam (2x)	2.91 bc	0.35 c	--
Inpyrfluxam (5x)	2.75 bc	0.26 c	0.25
Azoxystrobin	4.63 b	1.09 b	0.29
Mancozeb	6.83 a	0.99 b	0.47
P>F	0.001	<0.001	ns
UCPRS 2023			
Non-treated Control	7.75 a	8.79 a	1.00 c
Inpyrfluxam (1x)	2.13 c	1.59 c	5.00 b
Inpyrfluxam (2x)	1.84 c	1.54 c	1.00 c
Inpyrfluxam (5x)	1.82 c	1.58 c	--
Azoxystrobin	4.25 b	3.86 b	7.00 a
Mancozeb	6.33 a	5.84 b	1.00 c
P>F	<0.001	<0.001	<0.001

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] Foliar leaf spot diseases not observed.

Table 11. The effect of fungicide program on disease incidence in lower, middle, and upper stalk leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	Lower Stalk	Middle Stalk	Upper Stalk
CRS 2022		%	
Non-treated Control	37 b	4	0
Inpyrfluxam (1x)	41 b	11	0
Inpyrfluxam (2x)	40 b	5	0
Inpyrfluxam (5x)	51 a	9	1
Azoxystrobin	36 b	4	0
Mancozeb	39 b	4	0
P>F	0.014	ns	ns
OTRS 2022			
Non-treated Control	65 a	46 a	25
Inpyrfluxam (1x)	36 c	18 c	16
Inpyrfluxam (2x)	45 b	21 bc	16
Inpyrfluxam (5x)	44 bc	19 c	14
Azoxystrobin	43 bc	21 bc	18
Mancozeb	46 b	30 b	16
P>F	<0.001	0.001	ns
CRS 2023			
Non-treated Control	56	12	3 b
Inpyrfluxam (1x)	55	17	0 c
Inpyrfluxam (2x)	56	9	6 a
Inpyrfluxam (5x)	59	12	3 b
Azoxystrobin	51	8	3 b
Mancozeb	58	11	3 a
P>F	ns	ns	<0.001

Table 11. The effect of fungicide program on disease incidence in lower, middle, and upper stalk leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c,d]

Fungicide Program	Lower Stalk	Middle Stalk	Upper Stalk
OTRS 2023		%	
Non-treated Control	42 a	44 a	17 a
Inpyrfluxam (1x)	28 bc	15 c	4 bc
Inpyrfluxam (2x)	27 c	14 c	0 c
Inpyrfluxam (5x)	26 c	16 c	8 a-c
Azoxystrobin	29 bc	20 c	11ab
Mancozeb	39 ab	31 b	12 ab
P>F	0.031	<0.001	0.043
UCPRS 2023			
Non-treated Control	76 a	51 a	0
Inpyrfluxam (1x)	18 c	11 c	1
Inpyrfluxam (2x)	33 c	9 c	1
Inpyrfluxam (5x)	21 c	5 c	0
Azoxystrobin	56 b	36 b	1
Mancozeb	60 ab	51 a	1
P>F	<0.001	<0.001	ns

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] Incidence was quantified as a percentage, based on the number of leaves with disease infection out of seven.

Table 12. The effect of fungicide program on disease severity in middle and upper stalk leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	Middle Stalk	Upper Stalk
<u>CRS 2022</u>	———%	———
Non-treated Control	2.19	1.23
Inpyrfluxam (1x)	1.74	1.19
Inpyrfluxam (2x)	1.92	1.29
Inpyrfluxam (5x)	2.06	1.33
Azoxystrobin	2.29	1.14
Mancozeb	2.87	1.34
P>F	ns	ns
<u>OTRS 2022</u>		
Non-treated Control	--[d]	--
Inpyrfluxam (1x)	--	--
Inpyrfluxam (2x)	--	--
Inpyrfluxam (5x)	--	--
Azoxystrobin	--	--
Mancozeb	--	--
P>F	--	--
<u>CRS 2023</u>		
Non-treated Control	--	1.96
Inpyrfluxam (1x)	--	1.75
Inpyrfluxam (2x)	--	2.10
Inpyrfluxam (5x)	--	2.00
Azoxystrobin	--	2.08
Mancozeb	--	1.85
P>F	--	ns

Table 12. The effect of fungicide program on disease severity in middle and upper stalk leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	Middle Stalk	Upper Stalk
<u>OTRS 2023</u>	——————%	——————
Non-treated Control	7.13 a	4.00 a
Inpyrfluxam (1x)	2.59 cd	1.71 b
Inpyrfluxam (2x)	2.45 cd	1.36 b
Inpyrfluxam (5x)	1.52 d	1.45 b
Azoxystrobin	3.27 bc	2.28 b
Mancozeb	4.17 b	1.75 b
P>F	<0.001	0.008
<u>UCPRS 2023</u>		
Non-treated Control	--	2.73 b
Inpyrfluxam (1x)	--	1.17 c
Inpyrfluxam (2x)	--	1.25 c
Inpyrfluxam (5x)	--	1.24 c
Azoxystrobin	--	2.08 bc
Mancozeb	--	3.72 a
P>F	--	0.001

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] Stalk position not present at time of data collection.

Table 13. The effect of fungicide program on disease incidence in middle and upper stalk leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	Middle Stalk	Upper Stalk
CRS 2022	%	
Non-treated Control	48	24
Inpyrfluxam (1x)	44	32
Inpyrfluxam (2x)	49	32
Inpyrfluxam (5x)	49	32
Azoxystrobin	52	32
Mancozeb	43	26
P>F	ns	ns
OTRS 2022		
Non-treated Control	--[d]	--
Inpyrfluxam (1x)	--	--
Inpyrfluxam (2x)	--	--
Inpyrfluxam (5x)	--	--
Azoxystrobin	--	--
Mancozeb	--	--
P>F	--	--
CRS 2023		
Non-treated Control	--	49
Inpyrfluxam (1x)	--	43
Inpyrfluxam (2x)	--	50
Inpyrfluxam (5x)	--	36
Azoxystrobin	--	42
Mancozeb	--	41
P>F	--	ns

Table 13. The effect of fungicide program on disease incidence in middle and upper stalk leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	Middle Stalk	Upper Stalk
OTRS 2023	——%	——
Non-treated Control	86 a	24 a
Inpyrfluxam (1x)	41 cd	11 b
Inpyrfluxam (2x)	29 d	10 b
Inpyrfluxam (5x)	32 d	11 b
Azoxystrobin	56 b	8 b
Mancozeb	52 bc	13 b
P>F	<0.001	0.014
UCPRS 2023		
Non-treated Control	--	35 a
Inpyrfluxam (1x)	--	17 bc
Inpyrfluxam (2x)	--	27 ab
Inpyrfluxam (5x)	--	18 bc
Azoxystrobin	--	10 c
Mancozeb	--	33 a
P>F	--	0.016

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] Stalk position not present at time of data collection.

Table 14. The effect of fungicide program on cured leaf yield, visual quality, and economic value in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	Yield	Visual Quality[d]	Value
CRS 2022	**kg ha^{-1}**		**\$US ha^{-1}**
Non-treated Control	3,972 ab	71	12,230 bc
Inpyrfluxam (1x)	3,975 ab	75	12,813 ab
Inpyrfluxam (2x)	4,113 a	78	13,767 a
Inpyrfluxam (5x)	3,832 bc	73	12,178 bc
Azoxystrobin	3,923 a-c	74	12,750 ab
Mancozeb	3,752 c	70	11,142 c
P>F	0.011	ns	0.019
OTRS 2022			
Non-treated Control	4,017	74	12,456
Inpyrfluxam (1x)	4,171	78	13,967
Inpyrfluxam (2x)	3,976	79	13,813
Inpyrfluxam (5x)	3,958	83	14,565
Azoxystrobin	4,078	78	13,941
Mancozeb	4,132	74	13,286
P>F	ns	ns	ns
CRS 2023			
Non-treated Control	2,461	68 a	7,371
Inpyrfluxam (1x)	2,190	64 ab	6,247
Inpyrfluxam (2x)	2,349	66 a	6,683
Inpyrfluxam (5x)	2,471	65 ab	7,118
Azoxystrobin	2,513	57 c	5,779
Mancozeb	2,447	60 bc	6,303
P>F	ns	0.009	ns

Table 14. The effect of fungicide program on cured leaf yield, visual quality, and economic value in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c,]

Fungicide Program	Yield	Visual Quality[d]	Value
OTRS 2023	**kg ha^{-1}**		**\$US ha^{-1}**
Non-treated Control	2,788	59	6,891
Inpyrfluxam (1x)	3,027	60	7,562
Inpyrfluxam (2x)	2,947	61	7,524
Inpyrfluxam (5x)	3,111	60	7,771
Azoxystrobin	2,980	59	7,338
Mancozeb	3,156	60	7,995
P>F	ns	ns	ns
UCPRS 2023			
Non-treated Control	2,899	61	7,826
Inpyrfluxam (1x)	3,054	58	7,798
Inpyrfluxam (2x)	3,093	61	8,328
Inpyrfluxam (5x)	3,096	60	8,178
Azoxystrobin	3,027	63	8,373
Mancozeb	2,996	59	8,290
P>F	ns	ns	ns

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.

[b] CRS; Cunningham Research Station in Kinston, NC, OTRS; Oxford Tobacco Research Station in Oxford, NC, UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[c] Inpyrfluxam (1x); 0.05 kg ai ha^{-1}, Inpyrfluxam (2x); 0.10 kg ai ha^{-1}, Inpyrfluxam (5x); 0.25 kg ai ha^{-1}, Azoxystrobin; 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}.

[d] Cured leaf quality assessed on a scale of 1-100, with 100 being of the highest quality.

Figure 1. Spray pattern of the single solid-cone TG6 nozzle during application one, four weeks after transplanting.

Figure 2. Spray coverage with the single solid-cone TG6 nozzle during application one, four weeks after transplanting.

Figure 3. Spray pattern of the three flat fan nozzle arrangement (XR8004VS) during application two, six weeks after transplanting.

Figure 4. Spray pattern of the seven flat fan nozzles (five XR11003EVS and two XR8003VS) affixed to a dropline arrangement during application three, eight weeks after transplanting.

Figure 5. Spray coverage with the seven flat fan nozzles (five XR11003EVS and two XR8003VS) affixed to a dropline arrangement during application three, eight weeks after transplanting.

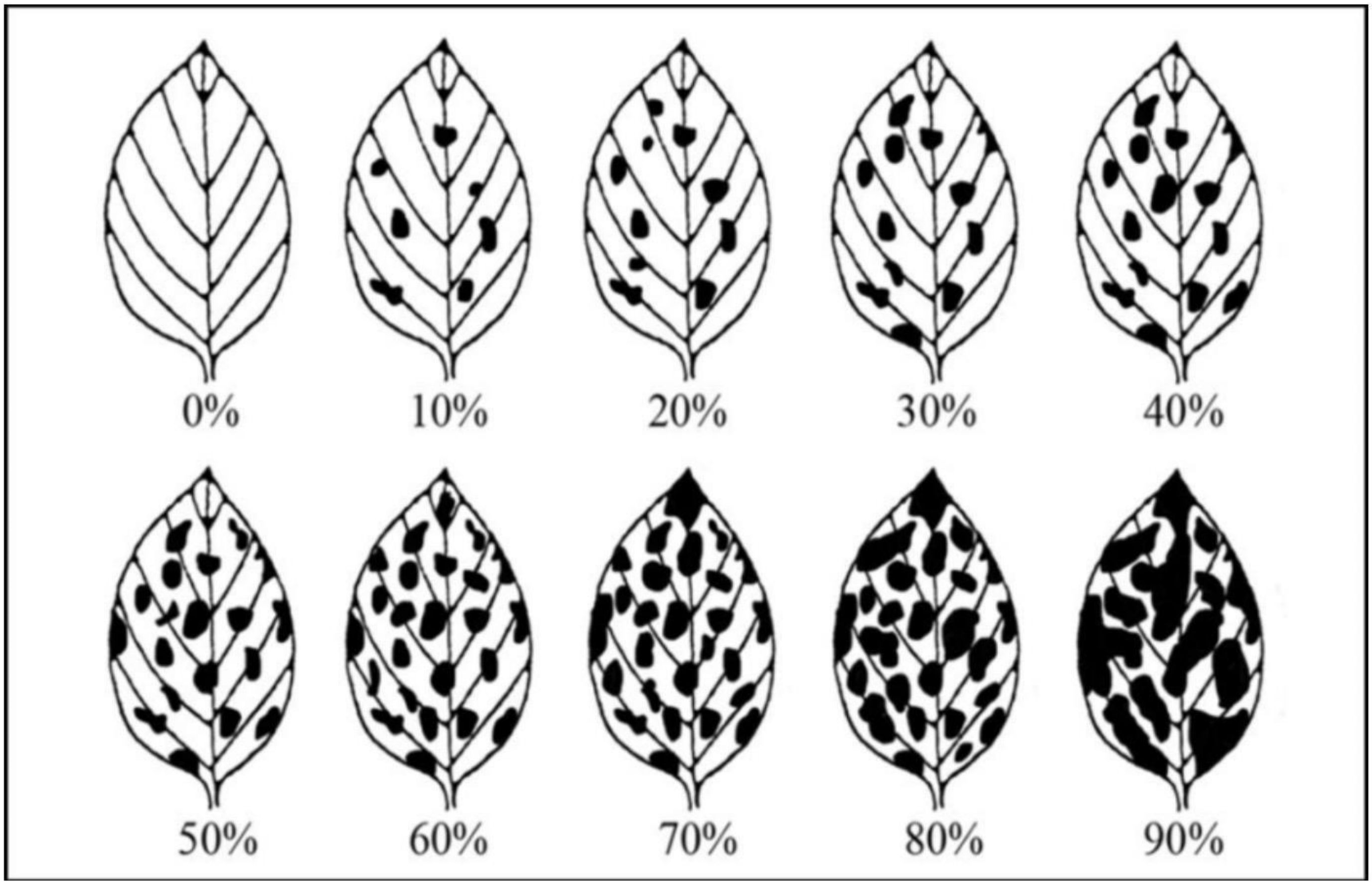

Figure 6. Severity percentage chart used in treatment evaluations (Jadhav and Patil 2016).

Figure 7. Tobacco leaf displaying a 5% disease severity.

Figure 8. Tobacco leaf displaying a 30% disease severity.

Figure 9. Tobacco leaf displaying a 40% disease severity.

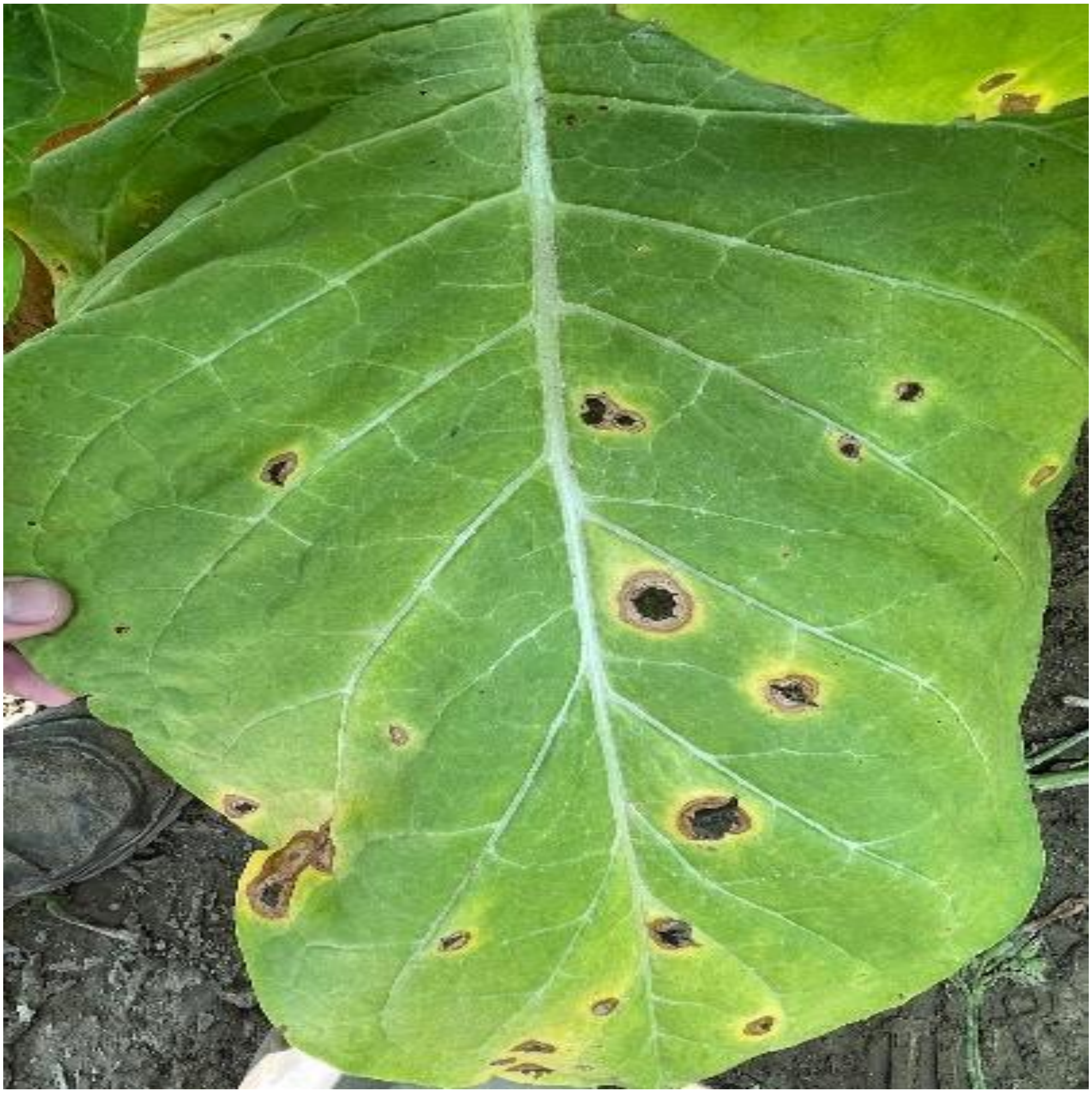

Figure 10. Tobacco leaf displaying a 15% disease severity.

Figure 11. Tobacco leaf displaying a 60% disease severity.

CHAPTER 3

Screening Foliar Fungicides for Leaf Spot Disease Management in Flue-Cured Tobacco (*Nicotiana tabacum L.)*

ABSTRACT

Foliar leaf spot diseases, such as *Rhizoctonia solani*, *Cercospora nicotianae,* and *Alternaria alternata* have increased in severity over the last decade in North Carolina. As a result, producers have experienced lower yields and reduced economic returns. New fungicides for leaf spot disease control are greatly needed. Due to resistance and residue concerns for currently available fungicides (azoxystrobin and mancozeb, respectively), field experiments were conducted in four environments to evaluate *Bacillus subtillis* strain AFS032321, *Pseudomonas chlororaphis* strain AFS009, inpyrfluxam, pydiflumetofen and difenoconazole, picoxystrobin and cyproconazole, azoxystrobin and flutriafol, and azoxystrobin and flutriafol and fluindapyr for target spot, brown spot and frogeye leaf spot control. Conventional treatments of currently labeled fungicides containing azoxystrobin or mancozeb were also evaluated, and a non-treated control was included for comparison. Treatments consisting of azoxystrobin or picoxystrobin, whether applied alone or in combination with other fungicides exhibited leaf phytotoxicity following each application, although observations did not exceed 10 – 15%. Phytotoxicity was not observed in other treatments. Disease infestation was limited to brown spot in 2022, with *Psueudomonas chlororaphis*, mancozeb, and difenoconazole and pydiflumetofen reducing disease incidence. In contrast, relative to the non-treated control, inpyrfluxam reduced target spot severity by more than 90% in 2023. In spite of resistance concerns, azoxystrobin when applied in combination with flutriafol and fluindapyr reduced target spot severity by 85% in similar environments. Cured leaf yield and economic value were improved by treatments of inpyrfluxam, azoxystrobin and flutriafol and fluindapyr, and azoxystrobin and flutriafol when compared to the non-treated control. Mancozeb and the two biological products were similar to the non-treated control. Our results indicate that inpyrfluxam, azoxystrobin and flutriafol and fluindapyr, and azoxystrobin and flutriafol would be advantageous in a tobacco foliar leaf spot disease management program.

NOMENCLATURE

brown spot, *Alternaria alternata*, flue-cured tobacco, *Nicotiana tabacum* L., target spot, *Rhizoctonia solani*

KEY WORDS

Disease, fungicide, incidence, severity, yield

INTRODUCTION

Tobacco is the most economically important non-food crop grown in the world (Tso 1990). It is grown for commercial purposes in at least 97 countries (Shew and Lucas 1991). The People's Republic of China, the United States, Brazil, and Zimbabwe are the world's largest producers (Collins and Hawks, 1993). Despite declines in both cigarette consumption and production volumes, it continues to contribute economically in many places around the world (Davis and Nielsen, 1999), most notably the U.S. It is one of the five crops that is worth more than a billion dollars annually to the U.S. farmer (Lucas, 1965). United States produced leaf has historically been the quality standard for the rest of the world (Anonymous n.d.). Multiple soil, climatic, and management factors allow for the color, flavor, and aroma in U.S. tobacco that are preferred by manufacturers (Brown and Snell, 2017). At one time, the U.S. produced sixty-four percent of the total world supply (Collins and Hawks, 2013). As competitors have improved production practices and manufacturers look for cheaper tobacco, the U.S. share of world production has been in a steady decline. Now only about 10% (Collins and Hawks, 2013) or less is produced in the U.S. Cigarette manufacturers now use more foreign tobacco than ever before (Capehart, 2003). Many believe tobacco produced in the U.S. will always be the quality standard and quality cigarettes cannot be produced without it. However, as the quality gap between the U.S. and tobacco produced elsewhere narrows (Capehart, 2003), competition has led to a decrease in U.S. tobacco producers as farming has become increasingly difficult. To sustain the quality standards and leaf production volumes, losses, specifically those resulting from disease, must be minimized. The current chemical leaf spot disease management programs need improvising and additional fungicides are desperately needed.

Approximately 400,000 tons of fungicides are applied globally, representing 17.5% of global pesticide applications (Gikas et al., 2022). According to Buhler (n.d.), there are 45 different FRAC code group numbers and 3 different letter groups that can be used to distinguish the different fungicides based on their mode of action. Of those used globally, only two FRAC group numbers are labeled for leaf spot disease management in U.S. flue-cured tobacco production. Current available options include a compound with a mode of action (MOA) of inhibition of quinone outside (strobilurins), a FRAC group 11 fungicide, (Quadris® Flowable) and a compound with multisite activity in the dithiocarbamates family, in the FRAC group M03 (Manzate® Pro-Stick™). Historically, mancozeb (Manzate® Pro-Stick™) was used for blue mold control. According to Lucas (1975), dithiocarbamates were also historically believed to show suppression of *R. solani and A. alternata.* However, after mancozeb was overused for blue mold control in the 1990s and the near disappearance of blue mold in the southern U.S., applications of mancozeb has mostly been discouraged and have not been recommended by cooperative extension due to high cured leaf residue concerns (Stainback and Vann 2023). Thiessen and Haroldson (2020) further identify mancozeb to only be moderately effective for reducing leaf spot diseases such as target spot. As a result, azoxystrobin (Quadris® Flowable) is the only chemical option tobacco producers have that offers control of target spot (Vann, 2021). After registration in 2006, foliar disease management practices have been solely composed of applications of azoxystrobin. Similar to palmar amaranth resistance to glyphosate, repeated applications of the same chemical mode of action over time without any rotation selects for and promotes resistant populations (Vann, 2021). This along with ineffective application methods is very likely one of the underlying reasons behind the current azoxystrobin resistance now being found with frogeye leaf spot populations (Vann, 2021). Frogeye leaf spot resistance to azoxystrobin has been identified in areas of Kentucky and in North Carolina (Thiessen, 2018). As a result, no fungicides are currently available for suppression of *Cercospora nicotianae*. Resistance of *R. solani* to azoxystrobin hasn't been identified, yet recent disease outbreaks have led many to desire alternatives. No foliar fungicide has ever been labeled in tobacco solely for *A. alternata* control. Efforts that follow sound cultural production practices (Shoemaker and Shew, 1999) such as varietal tolerance (Spurr, 1991), have historically been what producers relied on for brown spot control. Shoemaker and Shew (1999) highlight the importance of fungicidal control for brown spot explaining, if a highly active compound for *Alternaria* were found that was safe with negligible residues, control of brown spot would be a great possibility. With all things considered, current leaf spot disease management tools aren't achieving acceptable control and alternative chemical control options are desperately needed.

Having a variety of chemical control options and diversifying management strategies is beneficial in every disease management situation. As such, research was conducted in the 2022 growing season and repeated in 2023 to screen seven foliar fungicide products that are new or not currently labeled for tobacco production. Treatments included three FRAC (fungicide resistance action committee) group numbers (BM02, 7, 3) without a current application label in tobacco. *Pseudomonas chlororaphis* strain AFS009 (Howler® EVO) is an OMRI listed compound labeled in apples, blueberries, cucurbits, grapes, leafy vegetables, stone fruit and strawberries with broad spectrum capabilities (AgBiome, 2024). It is a FRAC BM02 fungicide, that targets both *Rhizoctonia* and *Alternaria* species (AgBiome 2024). *Bacillius subtillis* strain AFS032321 (Theia®) is another OMRI listed compound labeled in berries, cucurbits, cole crops, fruiting vegetables, grapes, hops, pome fruit and stone fruit that through multiple modes of action, blocks fungal and bacterial pathogens and activates crops' natural defense mechanisms (AgBiome, 2024). Theia serves as a great complement to Howler Evo, targeting *Alternaria* species in respective systems (AgBiome 2024). Inpyrfluxam (Excalia®) is another foliar fungicide with acceptable control in a variety of crop production systems. It is a FRAC Group 7, a succinate dehydrogenase inhibitor (Anonymous, 2023a) that is consistently used in peanuts, soybeans and other specialty crop situations. Inpyrfluxam targets a variety of fungal pests, most notably, *Rhizoctonia solani* (Environmental Protection Agency, 2020). Pydiflumetofen and difenoconazole (Miravis® Top) is a fungicide formulated with two chemical compounds that have never been evaluated in tobacco. Difenoconazole (FRAC Group 3) is a sterol biosynthesis inhibitor (Chase, 2010) and pydiflumetofen (FRAC Group 7) is a succinate dehydrogenase inhibitor (SDHI) (FRAC n.d.). Collectively, these two compounds, according to the manufacturer, are built tougher to fight diseases like frogeye leaf spot and target spot (Syngenta, 2021). These compounds are labeled in soybeans, sweet potatoes, and a large diversity of other crop production systems. Picoxystrobin and cyproconazole (Aproach® Prima) are two compounds formulated to offer broad spectrum control of foliar plant diseases having preventative, curative and systemic activity (Anonymous, 2022d). Picoxystrobin (FRAC group 11) is a compound like azoxystrobin, a quinone-outside inhibitor (QOI) (Peptech Biosciences (n.d.), commonly recognized as a strobilurin fungicide. Cyproconazole (FRAC Group 3) is another of the sterol biosynthesis inhibitor compounds, similar to difenoconazole. With multiple modes of action, Aproach® Prima is a product that targets brown spot, frogeye leaf spot and target spot (Corteva, 2024) in soybeans, peanuts, and corn (Anonymous, 2022). Flutriafol and azoxystrobin (TopGuard® EQ) was one of treatments being evaluated formulated with a new to tobacco compound in combination with azoxystrobin. Flutriafol is another of the of the sterol biosynthesis inhibitors (FRAC group 3) that rapidly penetrates the waxy layer of the leaf and moves upward through the plant to help prevent disease onset (FMC, 2024). Collectively, these two compounds make up the only registered premix of azoxystrobin and FMC-patented flutriafol (FMC, 2024). It is labeled for use in wheat, soybeans, peanuts, corn and many others for control of *Rhizoctonia*, *Alternaria*, and *Cercospora* initiated leaf spot diseases (Anonymous, 2020). Fluindapyr, flutriafol and azoxystrobin (VJR90, Table 4) was the only treatment that contained a premix of three different compounds, two of which (fluindapyr, flutriafol) not currently registered for use in tobacco. VJR90 (Table 4) is an experimental product manufactured by FMC Corporation. Fluindapyr is a compound that just recently received approval by the EPA (FMC, 2024). It is a novel broad spectrum succinate dehydrogenase inhibitor (SDHI) (FRAC Group 7) that will provide farmers around the world with preventative control of various fungal diseases in row crops (FMC, 2024). Treatment list, active ingredients, product manufacturers and mode of action are identified in Tables 3 and 4. With no prior knowledge on how each of these new to tobacco compounds would work against the three most problematic leaf spot diseases, the objectives of this study were to screen alternative chemical compounds for control of *R. solani*, *A. alternata*, and *C. nicotianae*, evaluate control compared to currently labeled options, and identify any potential tobacco production limitations new fungicide chemistries might impose.

MATERIALS AND METHODS

Site and Experimental Design

Field experiments in 2022 were conducted at the Oxford Tobacco Research Station (OTRS) in Oxford, North Carolina. Research was continued in 2023 at the Oxford Tobacco Research Station (OTRS), the Cunningham Research Station (CRS) in Kinston, North Carolina, and the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount, North Carolina. The varieties planted at each research site were GL 365 (Goldleaf Seed Company, Hartsville, SC) at the OTRS, NC 1226 (Goldleaf Seed Company, Hartsville, SC) at the CRS and NC 960 (Foley Seed and Service, Ashland, VA) at the UCPRS. The varieties are further identified in Table 1 and possess no known host-resistance to foliar leaf spot diseases that affect tobacco in North Carolina.

With the exception of foliar fungicide applications, tobacco was produced using practices recommended by the North Carolina Cooperative Extension Service (Fisher, 2022). Examples of these practices are the use of herbicides for weed control and synthetic suckersides for axillary bud control (Vann, 2023). The herbicides clomazone (840 g a.i. ha^{-1}) and sulfentrazone (1,750 g a.i. ha^{-1}) (FMC Corporation, Philadelphia, PA) were applied pre-plant, preemergence, days before transplanting and incorporated into the soil using a field cultivator. A single systemic insecticide application of imidacloprid (10-20 g a.i./1,000 plants) (Bayer CropScience, St. Louis, MO) was applied in the greenhouse for early season insect protection. Tobacco was plowed approximately three times during the first five weeks it was planted for weed control and row ridge support (Vann, 2023). In addition, synthetic suckersides were used, such as, C_8-C_{10} fatty-alcohol (Fair 85) (4-5% v/v concentration) (Fair Products Inc, Cary, NC), flumetralin (672 g a.i. ha^{-1}) (Drexel Chemical Company, Memphis, TN) and were applied periodically before a single application of maleic hydrazide (252 g a.i. ha^{-1}) (Drexel Chemical Company, Memphis, TN) for axillary bud suppression. In addition, soil pH ranged from 5.8 to 6.2 across all test sites.

Treatments were replicated four times within each environment and arranged in a randomized complete block design. Individual plots contained four rows, each measuring 15 meters in length. Row width ranged from 1.11 to 1.21 meters across environments (Table 1). The center two rows of each plot were used for treatment application, data collection, and harvest. Harvest dates and data collection intervals varied by location and are listed in Table 2. Planting density was 14,820 plant ha^{-1} in all environments. Tobacco was transplanted on May 12 at the OTRS in 2022. In 2023, tobacco was transplanted on April 14, April 19, and May 24 at the UCPRS, CRS, and OTRS, respectively.

Description of Fungicide Application Timing, Rates and Carrier Volumes

Nine fungicide treatments were evaluated for foliar leaf spot suppression: *Bacillius subtillis* strain AFS032321, *Pseudomonas chlororaphis*, azoxystrobin, inpyrfluxam, difenoconazole and pydiflumetofen, picoxystrobin and cyproconazole, mancozeb, azoxystrobin and flutriafol, and azoxystrobin and flutriafol and fluindapyr. Fungicide application rates (kg ai ha^{-1}) are listed in Table 3. Three sequential applications of each fungicide were evaluated for *R. solani* (target spot) and *A. alternata (*brown spot) control in naturally infested fields of tobacco. A non-treated control was included for comparison at each research site. A summary of active ingredients, trade names, formulation concentrations, manufacturers, application timings, rates, and modes of action are listed in Tables 3 and 4.

All fungicides were applied with a CO_2-pressurized backpack sprayer. The application volumes used to deliver treatments were 234, 327, and 467 L ha^{-1} at four, six, and eight weeks after transplanting (WAT), respectively. Application volumes were based upon the size of the crop canopy and were selected to promote the most effective spray coverage. In order to achieve the desired application volumes, spray boom and nozzle arrangements varied within each treatment interval. The first application was made at four WAT using a single full-cone TeeJet TG6 (Spraying Systems Co., Wheaton, IL, USA) nozzle. Nozzle arrangement, flow pattern and spray coverage at the first application are presented in Figures 1 and 2. The second application was made six WAT with three TeeJet XR 8004-VS flat fan nozzles (Spraying Systems Co., Wheaton, IL, USA) configured on a 51 cm boom (Figure 3). To roughly align with pre-harvest intervals identified by chemical manufacturers and current extension recommendations, the final spray application was made at eight WAT. To achieve a desired volume of 467 L ha^{-1} within the final application, five TeeJet XR 11003-EVS (Spraying Systems Co., Wheaton, IL, USA) and two TeeJet XR 8003-VS (Spraying Systems Co., Wheaton, IL, USA) flat fan nozzles were utilized on a drop nozzle style boom that was 97 cm in width. A 104 cm drop was included to deliver consistent spray coverage in each stalk position group. Nozzle arrangement, flow pattern and spray coverage for application three are presented in Figures 4 and 5. Application volumes, nozzle configurations and materials used for each application are listed in Table 5. Individual application dates (Table 2) varied across research sites and field seasons.

Field Conditions

Field conditions (soil series, soil texture, transplanting date, cumulative rainfall, air temperature, and relative humidity) varied by growing environment and are presented in Tables 1, 6, 7, and 8, respectively. All foliar leaf spot disease pressure was variable across field sites and growing seasons. Disease pressure was low in 2022 (<7.5%). Rainfall in the 2022 environments was less frequent and relative humidity was lower from transplanting through final harvest (Tables 6 and 8). As a result, field conditions were less conducive for disease development and the 2022 observations primarily consisted of brown spot (*A. alternata*). In contrast, research environments were moderate (10-15%) to highly (45%) infested with target spot in 2023, where the cumulative seasonal rainfall range was 76-91 cm across the three environments (Table 6). Most importantly, precipitation from April through June 2023 was 14.14 to 21.71 cm higher than during the same period in 2022. In the month of April alone, each of the 2023 environments experienced 5.36 to 12.65 cm more rainfall than the respective 30-year average at each location (Table 6).

OTRS-2022/2023. Tobacco was produced on a Helena sandy loam soil (Fine, mixed, semiactive thermic Aquic Hapludults) within each growing season at the OTRS (Table 1). Precipitation frequency, specifically early in the growing season (April – June) was higher in 2023 (Table 6). In the month of June alone, total rainfall increased by seven cm when comparing 2022 to 2023 (Table 6). Collectively, from April to June, the 2023 environment received 40.52 cm of rainfall, more than double of that which was measured in 2022 (Table 6). In total the 2023 environment received 38 cm more precipitation than the 2022 environment and experienced 26 cm more than the 30-year average (Table 6). Leaf spot diseases were not observed after the first harvest in 2022. As such, a second disease rating was not collected (Table 2). In contrast, with 31 cm of precipitation late in the season (July-August), target spot was prevalent at the 2023 field site. Thus, a treatment response was identified at the second rating. The 2023 environment had the most cumulative precipitation (91.33 cm) (Table 6) when compared to each of the other field sites in 2023. The OTRS-2023 was considered a moderate target spot disease pressure environment (13% severity in the lower stalk position at first rating). In contrast, OTRS-2022 was a low disease pressure environment for brown spot (3% severity in the lower stalk position at first rating).

CRS-2023. Tobacco was produced on a Norfolk loamy sand soil (fine-loamy, kaolinitic, thermic Typic Kandiudults) at the CRS in 2023. Early season precipitation (April-May) was measured at 25.82 cm (Table 6), which was the second most among all environments. Rainfall in April was nearly double that of the 30-year average (Table 6). The CRS was also the hottest environment, with a cumulative temperature average of 22.7^{o}C (Table 7). As such, chemical injury was most apparent, specifically with azoxystrobin plus flutriafol (Table 9). In the month of August, the CRS had the most frequent rainfall, the highest average temperature and was the most humid environment (Tables 6, 7 and 8). With the most rainfall occurring in the month of August (19.43 cm) (Table 6), which was more than each of the other 2023 field sites, research plots were harvested earlier than normal. As such, the third and fourth stalk positions were removed one day apart (Table 2). Disease pressure was favorable for treatment evaluations at the CRS; however, treatment responses were more prominent at other locations. The CRS 2023 was the lowest target spot severity environment (8% severity in the lower stalk position at the first rating).

UCPRS-2023. Tobacco was produced on a Norfolk loamy sand soil (fine-loamy, kaolinitic, thermic Typic Kandiudults) at the UCPRS in 2023. Early season rainfall, relative humidity and temperature were highly conducive for target spot development (Tables 6-8). Moreover, our research plots were contained within a 4 ha tobacco field, which limited airflow throughout the crop canopy. With the highest average precipitation (10.43 cm) (Table 6) in the month of June, disease infestation early in the 2023 season was higher relative to other sites. For reference, a plot absent of foliar fungicide treatment is presented in Figure 7. The UCPRS was considered the highest disease pressure environment in 2023 (45% target spot severity in the lower stalk position at the first rating).

Data Collection

Leaf Phytotoxicity. Visual estimates of tobacco injury resulting from treatment exposure were collected one week after each foliar spray application. Evaluations were conducted five, seven, and nine weeks after transplanting, using an injury percentage scale of 0 to 100%. Observations of 0% were plots absent of any phytotoxicity, whereas 100% injury would be indicative of plant death. Injury percentages were based upon the size of the crop, total leaf surface area, and total leaf area exhibiting phytotoxicity symptoms. Injury diagnosis consisted of a composite rating of leaf area symptomology relative to the two outermost rows in each plot (non-treated), as well as the non-treated control.

Disease Severity and Incidence. After treatment applications and injury ratings, a few days prior to lower leaf removal, visual evaluations using a severity percentage chart identified in Figure 8 (Jadhav and Patil 2016) was used to quantify leaf spot disease severity (5, 15, 30, 40, 60 %) presented in Figures 8 through 12. Five random plants within the two treated rows (center two rows) were evaluated. Plants at the end of each plot were avoided to ensure treatment exposure consistency. Every leaf on each of the five plants evaluated were assessed for leaf spot infestation. Severity evaluations were split up into three stalk position groups. The average total number of leaves remaining on plants being evaluated after floral elimination was about 21-22 leaves. With n = 21 to 22 leaves, the lower-stalk evaluations consisted of the bottom-most seven leaves, with middle and upper stalk evaluations following the same criteria. Each leaf displaying at least one symptom of leaf spot infection (necrotic lesions) was quantified with a severity percentage. Leaves absent of symptomology were not reported. Severity percentages were quantified based on cumulative leaf area displaying disease symptomology relative to the portions of the leaf absent of disease. The disease incidence (count), data was quantified simultaneously with the severity evaluations, a few days preceding the first and third harvest (Table 2). This was a measurement of the total number of leaves within each stalk position group exhibiting disease infection symptomology. Incidence was also quantified and reported as a percentage, on the basis of leaves (out of 7) showing disease infection.

Yield, quality, and value. Plots were harvested at four different intervals. During the first interval, the lowest portions of the stalk were harvested. During the second interval, the cutter leaves or middle portions of the stalk were harvested. In the remaining harvesting intervals, the leaf and tip positions of the plant were removed. Each of the harvest dates are listed in Table 2. Yield, quality, and value data were collected from each research environment. Following harvest, leaves were cured and weighed for yield and assigned a USDA government grade for quality. Each government grade has an associated grade index value ranging from 0-100 which describes the leaf maturity and ripeness (Bowman et al., 1988). Cured leaf value was determined using a combination of leaf yield and quality with value estimates reported by Vann et al. (2022)

Data Analysis

Analysis of variance was conducted using PROC Mixed procedure in SAS version 9.4 (SAS Institute, Cary, NC, USA) to test the effects of fungicide treatment programs on visual injury, leaf spot disease severity and incidence, yield, quality, and value. Within each analysis, fungicide treatment programs were considered fixed factors, while environment and replication were considered random factors. Treatment means were separated using Fisher's Protected LSD test at $P < 0.05$. Data for 2022 and 2023 are reported by environment as a result of variability in disease infestation levels and the disease species being evaluated.

RESULTS & DISCUSSION

Visual Injury

Visual injury ratings were affected by fungicide treatment programs at each location in the 2022 and 2023 growing seasons. Leaf phytotoxicity was apparent in each fungicide program containing FRAC (Fungicide Resistance Action Committee) group 11 (azoxystrobin and picoxystrobin) compounds, whether applied alone (Quadris® Flowable) or in combination with other active ingredients, such as cyproconazole, flutriafol, or flutriafol and fluindapyr. Injury was not observed with any fungicide program that did not include a FRAC group 11 fungicide. More specifically, in 2022, phytotoxicity was only observed after the third application (5 weeks after application (WAA)) (Table 9), which was applied 8 weeks after transplanting. Within the single 2022 environment, the average maximum air temperature was 31.4ºC (Table 7), which was measured in the month of July, when the third application was conducted (Table 2). Collectively, injury observations were minimal in 2022, ranging from three to six percent (Table 9). Leaf phytotoxicity resulting from azoxystrobin (6%), azoxystrobin and flutriafol (6%) and azoxystrobin and flutriafol and fluindapyr (5%) was similar (Table 9). However, injury resulting from picoxystrobin and cyproconazole (3%) was slightly less than azoxystrobin alone (6%) or in combination with flutriafol (6%) (Table 9). Injury observations did not have an impact on yield in 2022 (Table 14).

Similar trends were identified in 2023. However, due to the presence of weather fleck (ozone injury) and the difficulty with distinguishing between weather fleck and chemical injury, leaf phytotoxicity assessments following the first application (1WAA) are not presented from the UCPRS (Table 9). In 2023, observations remained limited to treatments containing a FRAC group 11 fungicide containing azoxystrobin or picoxystrobin (Table 9). Our observations were consistent across environments following each fungicide application. Relative humidity (June) and air temperature (July) were higher in 2023 relative to 2022 (Tables 7 and 8) and are believed to have influenced our observations. Collectively, phytotoxicity observations following the first application (1WAA) were <5% across two locations. Injury was similar (5%) among four treatments at the OTRS: azoxystrobin, picoxystrobin and cyproconazole, azoxystrobin and flutriafol, and azoxystrobin and flutriafol and fluindapyr (Table 9). In contrast, azoxystrobin and flutriafol (0.5%) displayed 2 to 4.5% less injury than azoxystrobin (3%), azoxystrobin and flutriafol and fluindapyr (5%), and picoxystrobin and cyproconazole (4 %) at the CRS (Table 9). Injury estimates slightly increased at both the CRS and UCPRS during the second application (six weeks after transplanting) (5 WAA) but remained the same or slightly less at the OTRS. Across the three environments, phytotoxicity estimates did not exceed 11% (Table 9). Azoxystrobin and flutriafol continued to exhibit the least amount of injury at the OTRS (Table 9). Alternatively, with a 10.5% increase from the first to the second application, injury resulting from azoxystrobin and flutriafol (11%) was the most severe at the CRS; which was 3 to 4% higher than azoxystrobin and picoxystrobin and cyproconazole (Table 9). Injury resulting from azoxystrobin (applied alone) (9%) was the highest after the second application at the UCPRS (Table 9), which was 3% higher than the other fungicides that produced injury. Injury estimates were slightly lower at each environment following application three (8 weeks after transplanting) (5WAA) with a maximum of 9%. Leaf phytotoxicity was the most apparent at the CRS, ranging from 3 to 9% across the four treatments containing strobilurins. The third application was completed in June at both the CRS and UCRPS and in July at the OTRS (Table 2). Rainfall was generally less frequent when the last application was completed at the CRS (Table 6). It is plausible that the slight increase in precipitation (Table 6) resulted in less injury at the OTRS and UCPRS after the third application. With an increase in precipitation, injury ranged from 0 to 5% at the OTRS and UCPRS following application three (Table 9). Leaf phytotoxicity was similar among the three treatment programs containing azoxystrobin (8 to 9%), whereas only slight injury was observed with picoxystrobin and cyproconazole (3%) following application three at the CRS (Table 9). Similar to observations reported in 2022, cured leaf yield in 2023 was not impacted by visual injury from FRAC group 11 fungicide treatments (Table 14).

Disease Control (Severity/Incidence)

Severity. The disease severity rating prior to first harvest was conducted on August 1 at the OTRS in 2022 (Table 2). As highlighted in Table 6, rainfall in 2022 was less frequent soon after transplanting, specifically in the month of June (3.32 cm) when compared to each of the 2023 growing environments. The cumulative precipitation (53.51 cm) was also lower in 2022, being 23 to 38 cm less than what was measured in 2023 (Table 6). As a result, disease pressure, and thus treatment differences, were minimal and only identified in the lowest stalk position group (Table 10). Based off leaf samples submitted to the NC State University PDIC (Plant disease and insect clinic), the 2022 severity rating was an evaluation of brown spot control. Foliar leaf spot diseases were not apparent later in the season before third and fourth harvest removal, as such, only one disease rating was conducted. Brown spot severity in the lower stalk position was similar among mancozeb (2.60%), inpyrfluxam (2.44%), difenoconazole and pydiflumetofen (3.28%) and the non-treated control (3.26%) (Table 10). Severity was nearly twice as much or more when comparing *Bacillius subtillus* strain AFS032321 (7.54%), picoxystrobin and cyproconazole (6.23%), and azoxystrobin and flutriafol (5.88%) to the non-treated control (3.26%) (Table 10). Treatment response was similar with *Psueudomonas chlororaphis* strain AFS009 (4.99%), azoxystrobin (5.69%), picoxystrobin and cyproconazole (6.23%), azoxystrobin and flutriafol (5.88%), and azoxystrobin and flutriafol and fluindapyr (5.57%) (Table 10). *Bacillus subtillus* strain AFS032321, *Pseudomonas chlororaphis* strain AFS009, difenoconazole and pydiflumetofen, picoxystrobin and cyproconazole, and azoxystrobin and flutriafol are each registered for use in other cropping systems for diseases caused by pathogens within the *Alternaria* genus. According to information from the inpyrfluxam manufacturer, the main target species of the fungicide are diseases caused by *Rhizoctonia.* Alternatively, as suggested by the Environmental Protection Agency (2021), inpyrfluxam is described to provide control of alternaria blotch, a disease in apples (*Malus pumila*) that is caused by an organism within the same genus as tobacco brown spot. Inpyrfluxam (2.44%) had the lowest brown spot severity in the lower and middle stalk position groups in 2022 (Table 10). Treatment response differences were not identified in the middle stalk position, most likely due to limited disease pressure (Table 10). No foliar diseases were observed in the upper stalk position in 2022 (Table 10).

Disease severity ratings prior to first harvest were affected by treatment programs within each environment in 2023 (Table 10). With an increase in both rainfall and humidity (Tables 6 and 8), target spot pressure was observed at each of the three field sites (Table 10). Each treatment program, excluding the two biological products (*Bacillus subtillus* and *Pseudomonas chlororaphis*) and mancozeb, reduced target spot severity when compared to the non-treated control. In the lower stalk position, inpyrfluxam (4.39%, 4.07%) reduced target spot severity by nearly 41% at the UCPRS and 9.07% at the OTRS relative to the non-treated control (45.27 and 13.14%, respectively) (Table 10). Picoxystrobin and cyproconazole (4.55 to 8.99%), azoxystrobin and flutriafol (5.08 to 14.11%), and azoxystrobin and flutriafol and fluindapyr (6.12

to 6.87%) performed similar to inpyrfluxam across the three locations (Table 10). A 31 to 38% reduction in target spot severity was observed among those treatments in the lower stalk position when compared to the non-treated control (45.27%) at the UCPRS (Table 10). Within the same stalk position group, the treatment response to azoxystrobin alone was similar to inpyrfluxam at two of the three locations and similar to picoxystrobin and cyproconazole, azoxystrobin and flutriafol, and azoxystrobin and flutriafol and fluindapyr at all three locations. Specifically, azoxystrobin accounted for a 31% reduction in target spot at the UCPRS and a 3.2% reduction at the CRS when compared to the non-treated control (45.27 and 8.80%, respectively) (Table 10). Treatment response to difenoconazole and pydiflumetofen was similar to azoxystrobin within each environment. At the UCPRS, applications of difenoconazole and pydiflumetofen (12.76%) accounted for a 33% reduction in target spot severity relative to the non-treated control (45.27%) (Table 10). In addition, a 3.2% reduction was also observed at the OTRS (Table 10). A positive treatment response to mancozeb, in the lower and middle stalk positions was only observed in the highest disease pressure environment (UCPRS) (Table 10). Within the lower stalk position, mancozeb (15.43%) accounted for a 30% reduction in severity when compared to the non-treated control (45.27%) at that location (Table 10). The two biological products (*Bacillus subtillus* strain AFS032321 and *Psueudomonas chlororaphis* strain AFS009) did not impact target spot severity in the lower stalk position at the CRS (Table 10). In contrast, *Bacillus subtillus* strain AFS032321 did reduce target spot severity by 16% at the UCPRS (Table 10).

A treatment response was only identified at one of the three environments (UCPRS) within the middle stalk position group rating. Inpyrfluxam (1.98%) reduced target spot severity relative to the non-treated control and each of the other fungicide programs. When compared to the non-treated control (23.78%), inpyrfluxam reduced severity by 22% (Table 10). A positive treatment response was also identified with the azoxystrobin (11.02%), picoxystrobin and cyproconazole (7.76%), azoxystrobin and flutriafol (10.08%) and azoxystrobin and flutriafol and fluindapyr (8.36%) treatments. In general, severity was reduced by 13 to 16% among these treatments when they were compared to the non-treated control (23.78%) (Table 10). Difenoconazole and pydiflumetofen (18.81%) and mancozeb (16.31%) also reduced target spot severity by 5 to 7%, respectively, relative to the non-treated control (Table 10), but these treatments were less effective than those previously mentioned. A treatment response was not documented with *Bacillus subtillus* strain AFS032321 (19.58%), and *Psueudomonas chlororaphis* strain AFS009

(20.55%) relative to the non-treated control (23.7%) (Table 10). Leaf spot disease was only observed at two of the three locations in the upper stalk position. A treatment response was not reported within those environments, most likely due to limited disease pressure within the stalk position group (Table 10).

A second rating was conducted immediately prior to the third harvest (out of four), following the removal of all lower stalk leaves (Table 2). With target spot being the most problematic during the early stages of plant growth, disease pressure was not as prominent during the second rating. As such, treatment differences were not as large. A second rating was not conducted in 2022, as leaf spot diseases were not observed even though brown spot tends to be more concerning later in the growing season (Marathi and Gorny, 2023). The middle stalk position rating is only reported at the OTRS in 2023. Leaves at each of the other field environments had been removed prior to data collection. Each fungicide program, excluding *Bacillus subtillus* (4.01%), *Psueudomonas chlororaphis* (3.27%) and mancozeb (3.71%) provided a positive response within the middle stalk position at the OTRS in comparison to the non-treated control (4.49%) at the second rating (Table 12). Treatment response was similar among azoxystrobin (2.59%), inpyrfluxam (2.05%), difenoconazole and pydiflumetofen (2.89%), picoxystrobin and cyproconazole (2.00%), azoxystrobin and flutriafol (3.05%), and azoxystrobin and flutriafol and fluindapyr (2.80%). Collectively, target spot severity in the middle stalk position, was reduced by 1.4 to 2.49% with these treatments (Table 12).

Similar trends were also identified in the upper stalk position rating. Despite greater disease pressure at the UCPRS, a treatment response was only identified at the OTRS (Table 12). *Bacillus subtillus* strain AFS032321 (2.13%), *Psueudomonas chlororaphis* strain AFS009 (1.88%), difenoconazole and pydiflumetofen (2.08%) and azoxystrobin and flutriafol (1.98%) were the only treatments that did not reduce severity when compared to the non-treated control (2.66%). Severity was reduced by .94 to 1.5% among the fungicide programs that contributed to a positive treatment response within the upper stalk position (Table 12).

Incidence. Similar trends were identified in the disease incidence ratings as what was previously discussed in the severity section. Collectively, disease pressure was minimal in 2022. As was previously emphasized, and also identified in Tables 6-8, environmental conditions were less conducive for disease infestation, thus, treatment response was minimal when compared to 2023. The 2022 incidence rating was an evaluation of fungicidal efficacy against brown spot. A treatment response was only identified in the lower and middle stalk positions (Table 11). Leaf spot diseases were not observed late in the season before third harvest; thus, a second rating was not conducted. Within the lower stalk position, *Psueudomonas chlororaphis* strain AFS009 (31%), difenoconazole and pydiflumetofen (27%), and mancozeb (33%) were the only fungicides to reduce brown spot incidence in comparison to the non-treated control (51%) (Table 11). The three fungicides accounted for an 18 to 24% incidence reduction (Table 11). Brown spot incidence was 14% higher in plots treated with azoxystrobin (65%) when compared to the non-treated control (51%) (Table 11). Treatment response to *Bacillus subtillus* strain AFS032321 (39%), inpyrfluxam (44%), picoxystrobin and cyproconazole (49%), azoxystrobin and flutriafol (47%), and azoxystrobin and flutriafol and fluindapyr (50%) was similar to the non-treated control (51%) in the lower stalk position (Table 11). In contrast, treatment response was similar with each fungicide in the middle stalk position. Collectively, the treatment programs reduced brown spot incidence by 18 to 27% when compared to the non-treated control (39%) (Table 11). A treatment response was not recorded within the upper stalk position in 2022, most likely due to limited disease pressure. Results from the 2022 incidence rating contradict observations in 2023, likely a result of unknown brown spot suppression potential and limited disease pressure.

Disease incidence ratings prior to first harvest were affected by treatment programs in multiple stalk position groups across each environment in 2023. However, fungicidal response was most apparent at the OTRS and the UCPRS (Table 11). Results were similar to what was discussed previously regarding disease severity. Treatment response with inpyrfluxam (33 to 64%), picoxystrobin and cyproconazole (39 to 71%), and azoxystrobin and flutriafol and fluindapyr (33 to 70%) was similar at the OTRS and UCPRS in the lower stalk position (Table 11). At the OTRS, inpyrfluxam (33%), picoxystrobin and cyproconazole (39%), and azoxystrobin and flutriafol and fluindapyr (33%) reduced target spot incidence by 12 to 18% in comparison to the non-treated control (51%) (Table 11). In addition, incidence was reduced by 26 to 33% at the UCPRS (Table 11). A positive treatment response was also identified with difenoconazole and pydiflumetofen (82%) and azoxystrobin and flutriafol (83%) at the UCPRS. When compared to the non-treated control (97%), difenoconazole and pydiflumetofen (82%) and azoxystrobin and flutriafol (83%) also reduced target spot incidence by roughly 15% (Table 11). The two biological products (*Bacillus subtillus* strain AFS032321 and *Psueudomonas* *chlororaphis* strain AFS009), azoxystrobin, and mancozeb showed results similar to the non-treated control in each environment (Table 11).

A positive treatment response was also identified at each environment in the middle stalk position. Relative to the non-treated control (31 and 87%), inpyrfluxam (0 and 37%) reduced target spot incidence by 31% at the OTRS and 50% at the UCPRS, respectively (Table 11). Treatment response to picoxystrobin and cyproconazole (8%) was similar to inpyrfluxam (0%) at the OTRS, accounting for a 23% reduction when compared to the non-treated control (31%) (Table 11). A positive response was also identified with azoxystrobin and flutriafol and fluindapyr. At the OTRS, azoxystrobin and flutriafol and fluindapyr (15%) reduced target spot incidence by 16% when compared to the non-treated control (31%) (Table 11). In addition, incidence was reduced by 13% at the CRS and 14% at the UCPRS (Table 11). Treatment response to azoxystrobin and flutriafol (16%) was similar to azoxystrobin and flutriafol and fluindapyr (15%) at the OTRS (Table 11). Disease incidence in plots treated with azoxystrobin (19%, 34%, and 89%) and mancozeb (24%, 29%, and 81%) was similar to the non-treated control at the OTRS, CRS, and UCPRS, respectively (Table 11). *Bacillus subtillus* strain AFS032321 (20%) and *Psueudomonas chlororaphis* strain AFS009 (22%) reduced target spot incidence by 13 to 15% at the CRS when compared to the non-treated control (35%) (Table 11). In contrast, both performed similar to the non-treated control at the OTRS and UCPRS (Table 11).

A treatment response was identified at two (OTRS and UCPRS) of the three environments in the upper stalk position group. Inpyrfluxam, picoxystrobin and cyproconazole, and azoxystrobin and flutriafol and fluindapyr reduced target spot incidence relative to the non-treated control (Table 11). At the OTRS, inpyrfluxam (0%), picoxystrobin and cyproconazole (7%), and azoxystrobin and flutriafol and fluindapyr (4%) reduced target spot incidence by 12 to 19% when compared to the non-treated control (19%) (Table 11). Similar observations were identified at the UCPRS where inpyrfluxam (7%) and picoxystrobin and cyproconazole (0%) reduced target spot incidence by 15 to 22% relative to the non-treated control (Table 11). Treatment response to azoxystrobin and flutriafol (4%) was similar to inpyrfluxam and picoxystrobin and cyproconazole at the OTRS (Table 11). A positive response was also identified with azoxystrobin (9%) at the UCPRS (Table 11), which accounted for nearly a 13% incidence reduction when compared to the non-treated control (22%) (Table 11).

A second rating was conducted immediately prior to third harvest (out of four), following the removal of all lower stalk leaves (Table 2). Leaf spot disease was not identified at the OTRS at the time of the second rating, thus a second incidence rating is not reported from the 2022 season. The middle stalk position rating is only reported at the OTRS in 2023. Stalk positions at each of the other field environments had been removed prior to data collection. Within the second disease rating, a treatment response was only identified at the OTRS (Table 13). Each fungicide reduced target spot incidence in the middle stalk position when compared to the non-treated control (83%) (Table 13). Similar to the initial incidence rating, inpyrfluxam (28%) and picoxystrobin and cyproconazole (23%) reduced target spot incidence by 55 and 60%, respectively, relative to the non-treated control (Table 13). Suppression was further highlighted in the upper stalk, where inpyrfluxam (5%) accounted for a 19% reduction, whereas picoxystrobin and cyproconazole (11%) reduced target spot incidence by 13% (Table 13). Treatment response with azoxystrobin and flutriafol and fluindapyr was similar to inpyrfluxam in both the middle (39%) and upper (7%) stalk positions (Table 13). Incidence was reduced by 44% in the middle stalk position and 17% in the upper stalk when compared to the non-treated control (83 and 24%, respectively) (Table 13). A positive response was also identified with azoxystrobin (56%), difenoconazole and pydiflumetofen (47%), mancozeb (47%) and azoxystrobin and flutriafol (57%) in the middle stalk position at the OTRS (Table 13). Collectively, incidence was reduced by 26 to 36% relative to the non-treated control (83%) (Table 13). The same four fungicides performed similar to inpyrfluxam (5%) and picoxystrobin (11%) in the upper stalk position, reducing incidence by 7 to 16 % when compared to the non-treated control (24%) (Table 13). Relative to the non-treated control (83%), incidence was only reduced with the two biological fungicides *(Bacillus subtillus* strain AFS032321 and *Psueudomonas chlororaphis* strain AFS009) at the OTRS. Collectively, these two fungicides reduced incidence by 13 to 16% (Table 13) in the middle stalk position. As highlighted in Table 6, the OTRS received the most precipitation from transplanting to harvest, specifically in the month of July (20.04 cm). In 2023, the cumulative precipitation at the OTRS was 14-19 cm greater than the other field environments (Table 6). While disease pressure may have been more prevalent at other locations, it is plausible to assume that increased rainfall could have led to the treatment differences previously discussed. Insignificant results in the upper stalk position at the CRS and UCPRS during the second disease rating is most likely a result of limited disease pressure and higher temperatures in July (32.1 ^{o}C and 32.5^{o}C, respectively) and August (32.1 ^{o}C and 32.5^{o}C, respectively) (Table 7).

Yield, Quality, and Value

Yield. Collectively, yields were lower than normal in each growing season. Thus, treatment differences did not always align with observations relative to the severity and incidence ratings. In the single 2022 growing environment, cured leaf yield was lowest in the non-treated control (3,099 kg ha^{-1}). Relative to the non-treated control, treatments of *Bacillus subtillus* strain AFS032321 and *Psueudomonas chlororaphis strain* AFS009 were similar (Table 14). This observation aligned with disease severity ratings that occurred before first harvest (Table 10). A positive treatment response was identified with azoxystrobin and flutriafol and fluindapyr (3,375 kg ha^{-1}), azoxystrobin and flutriafol (3,546 kg ha^{-1}) and difenoconazole and pydiflumetofen (3,482 kg ha^{-1}). Collectively, these three fungicide programs improved cured leaf yield by 275 to 450 kg ha^{-1} relative to the non-treated control. The documented yield improvement aligned with the initial disease incidence rating, where difenoconazole and pydiflumetofen (27%) had 24% fewer infected leaves in the lower stalk position (Table 11).

In 2023, a yield response was only recorded at the UCPRS (Table 14). As previously emphasized, this environment had the highest target spot disease pressure and the largest variation in fungicide efficacy. Treatments of azoxystrobin and flutriafol and fluindapyr (3,267 kg ha^{-1}) and azoxystrobin and flutriafol (3,047 kg ha^{-1}) had the highest yields (Table 14). Both fungicide programs improved yield by 803 to 1,023 kg ha^{-1} when compared to the non-treated control (2,244 kg ha^{-1}) (Table 14). Treatment response with inpyrfluxam (2,822 kg ha^{-1}), azoxystrobin (2,749 kg ha^{-1}), and difenoconazole and pydiflumetofen (2,673 kg ha^{-1}) were similar to azoxystrobin and flutriafol (3,047 kg ha^{-1}) (Table 14). Collectively, these three fungicide programs improved cured leaf yield by 429 to 578 kg ha^{-1} when compared to the non-treated control (2,244 kg ha^{-1}) (Table 14).

Quality. A treatment response in cured leaf quality was only observed at one location (UCPRS). The two biological fungicides (*Bacillus subtillus* strain AFS032321 and *Psueudomonas chlororaphis* strain AFS009) (54 and 55, respectively) and the non-treated control (56) had the lowest visual quality index. (Table 14). This observation aligned with what was previously discussed in the disease severity and incidence section. Visual quality in plots treated with difenoconazole and pydiflumetofen (55) was also similar to the non-treated control (56) (Table 14). Quality was improved across each of the remaining treatments, with index values ranging from 61 to 64 (Table 14). Cured leaf quality was not impacted in spite of the chemical injury (Table 9) attributed to each of the FRAC group 11 fungicides (Table 7).

Value. A treatment response in crop value was only observed when lower yields were identified. It is plausible to assume that yield and value are correlated. As such, value was only impacted in situations with significant yield variability across treatments (UCPRS) (Table 14). At the UCPRS environment, crop value followed a similar trend to cured leaf yield. For example, crop value was the highest in plots treated with azoxystrobin and flutriafol and fluindapyr (\$3,434 ha^{-1}), azoxystrobin and flutriafol (\$3,351 ha^{-1}), and inpyrfluxam (\$3,062 ha^{-1}) (Table 14). Collectively, the three fungicides improved crop value by \$927 to 1,299 ha^{-1} when compared to the non-treated control (\$2,135 ha^{-1}) (Table 14). A slight positive response was also identified with azoxystrobin (\$2,878 ha^{-1}), picoxystrobin and cyproconazole (\$2,904 ha^{-1}), and mancozeb (\$2,741 ha^{-1}). The three fungicides improved crop value by \$606 to 769 ha^{-1} relative to the non-treated control (\$2,135 ha^{-1}) (Table 14). Treatments of *Bacillus subtillus* strain AFS032321 and *Psueudomonas chlororaphis* strain AFS009 resulted in a crop value that was similar to the non-treated control (Table 14).

CONCLUSIONS

Results from this study identify multiple new fungicides that have potential for brown spot and target spot suppression in flue-cured tobacco production. Most fungicide treatments were generally not effective at reducing brown spot severity; however, those containing *Psueudomonas chlororaphis* strain AFS032321, difenoconazole and pydiflumetofen, and mancozeb did reduce severity relative to the non-treated control. Further research is warranted to confirm these observations. Relative to target spot, inpyrfluxam, azoxystrobin and flutriafol and fluindapyr, azoxystrobin and flutriafol, and picoxystrobin and cyproconazole were most successful in 2023. Of these fungicides, azoxystrobin and flutriafol and fluindapyr, azoxystrobin and flutriafol, and inpyrfluxam also improved cured leaf yield by 500 to 1,000 kg ha^{-1} and crop value by \$900 to \$1,299 ha^{-1}. Finally, treatments containing inpyrfluxam were absent of leaf phytotoxicity.

Overall, our results identify four potential active ingredients that would help address the current resistance concerns. As previously discussed, inpyrfluxam (group 7), cyproconazole (group 3), flutriafol (group 3), and fluindapyr (group 7) effectively reduced target spot infestation levels. Each of these compounds represent different modes of action that have not been used in tobacco. If use of these compounds were approved for application, the risk of fungicide resistance is minimized.

Lastly, environmental conditions, specifically rainfall timing and relative humidity, appear to have the greatest influence on disease infestation levels. Consistent rainfall and above average humidity immediately prior to and soon after transplanting, specifically from April to June, appear to favor target spot infestation. Having a better understanding of which environmental conditions are most conducive for disease development may aid growers with improving management of these leaf spot diseases. Results highlight the importance of preventative spray applications if forecasted field conditions are favorable for pathogen development early in the growing season.

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Table 1. Soil series, soil texture, taxonomic class, transplanting date, variety, and row spacing for each growing environment.

Environment	Soil Series	Soil Texture	Taxonomic Class	Transplanting Date	Variety	Row Spacing (meters)
OTRS 2022	Helena	Sandy Loam	Fine, mixed, semiactive, thermic Aquic Hapludults	May 12, 2022	GL 365	1.21
CRS 2023	Norfolk	Loamy Sand	Fine-loamy, kaolinitic, thermic Typic Kandiudults	Apr. 19, 2023	NC 1226	1.11
OTRS 2023	Helena	Sandy Loam	Fine, mixed, semiactive, thermic Aquic Hapludults	May 24, 2023	GL 365	1.21
UCPRS 2023	Norfolk	Loamy Sand	Fine-loamy, kaolinitic, thermic Typic Kandiudults	Apr. 24, 2023	NC 960	1.21

[a] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC in 2023.

Table 2. Fungicide application, injury evaluations, harvest, and disease rating dates at each growing environment. [a]

	OTRS 2022	CRS 2023	OTRS 2023	UCPRS 2023
Application date(s)[b]	6/8/2022 6/22/2022 7/6/2022	5/16/2023 5/30/2023 6/13/2023	6/27/2023 7/11/2023 7/26/2023	5/22/2023 6/9/2023 6/19/2023
Injury evaluation	6/15/2022 6/29/2022 7/11/2022	5/23/2023 6/6/2023 6/20/2023	7/5/2023 7/18/2023 8/1/2023	5/31/2023 6/15/2023 6/26/2023
1st Harvest	8/2/2022	7/11/2023	8/8/2023	7/26/2023
2nd Harvest	8/30/2022	7/31/2023	9/1/2023	8/9/2023
3rd Harvest	10/20/22	8/16/2023	9/26/2023	9/5/2023
4th Harvest	10/21/22	9/7/2023	9/28/2023	9/6/2023
1st Disease Rating[c]	8/1/2022	7/10/2023	8/3/2023	6/26/2023
2nd Disease Rating[d]	--[e]	8/7/2023	8/23/2023	7/24/2023

[a] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.

[b] Date at which treatments, harvests, or ratings were made.

[c] Treatment evaluation prior to first harvest.

[d] Treatment evaluation prior to third harvest.

[e] Foliar leaf spot diseases not observed.

Table 3. List of treatments, application rates, timings, and solution volumes used.

Treatments	Application Rate	Application Timing	Solution Volume
	kg ai ha^{-1}	**WAT[a]**	**L ha^{-1}**
Non-treated Control	--	--	--
Bacillus subtillus strain AFS032321	3.36	4, 6, 8	234, 327, 467
Psueudomonas chlororaphis strain AFS009	1.40	4, 6, 8	234, 327, 467
Azoxystrobin	0.15, 0.16, 0.16[b]	4, 6, 8	234, 327, 467
Inpyrfluxam	0.05	4, 6, 8	234, 327, 467
Difenoconazole + pydiflumetofen	0.08 + 0.12[c]	4, 6, 8	234, 327, 467
Picoxystrobin + cyproconazole	0.10 + 0.04	4, 6, 8	234, 327, 467
Mancozeb	1.68	4, 6, 8	234, 327, 467
Azoxystrobin + flutriafol	0.17 + 0.13	4, 6, 8	234, 327, 467
Azoxystrobin + flutriafol + fluindapyr	0.12 + 0.08 + 0.12	4, 6, 8	234, 327, 467

[a] WAT; weeks after transplanting.

[b] "," Indicates that the rate was changed following the first application.

[c] "+" Indicates a premix of multiple compounds.

Table 4. Fungicide active ingredient, trade name, formulation concentration, manufacturer, and mode of action.

Active Ingredient	Trade Name	Concentration	Manufacturer	FRAC Code
Bacillius subtillis	Theia®	>1x10^{9} CFU/g	AgBiome	BM02
Pseudomonas chlororaphis	Howler® EVO	>1x10^{6} CFU/g	AgBiome	BM02
Azoxystrobin	Quadris® F	249 g ai L^{-1}	Syngenta	11
Inpyrfluxam	Excalia®	340 g ai L^{-1}	Valent U.S.A	7
Pydiflumetofen + Difenoconazole	Miravis® Top	82 g ai L^{-1}	Syngenta	7 + 3
Picoxystrobin + Cyproconazole	Aproach® Prima	200 g ai L^{-1}	Corteva	11 + 3
Mancozeb	Manzate-Prostick®	750 g ai kg^{-1}	UPL	M03
Azoxystrobin + Flutriafol	TopGuard® EQ	323 g ai L^{-1}	FMC	11 + 3
Azoxystrobin + Fluindapyr + Flutriafol	VJR90	--[a]	FMC	11 + 7 + 3

[a] Formulation concentration not known, VJR90 is an experimental product.

Table 5. Application type, volume, nozzle arrangement and speed at each interval

Timing	Volume	Spray Nozzles	Application Type	Speed
WAT[a]	**L ha^{-1}**			**kph**
4	234	TG6 (full cone nozzle)	OT[b]	4.82
6	327	XR8004- XR8004-XR8004 (flat fan nozzles)	OT[b]	4.82
8	467	XR8003 + XR11003 (flat fan nozzles)	Drop Nozzle	4.82

[a]WAT, weeks after transplanting

[b]OT, over-the-top application

Table 6. Monthly, cumulative, and 30 year average precipitation at each growing environment from April through September of each calendar year.[a]

Month	2022	2023			30 year average		
	OTRS	CRS	OTRS	UCPRS	CRS	OTRS	UCPRS
	cm						
April	4.67	16.02	22.09	14.35	8.96	9.44	8.99
May	10.82	9.80	8.12	8.17	9.95	8.94	9.22
June	3.32	7.89	10.31	10.43	14.04	12.72	12.21
July	17.09	10.94	20.04	10.69	14.70	10.84	12.95
August	6.42	19.43	10.64	11.55	16.15	12.11	13.41
September	11.17	12.75	20.09	16.35	16.86	10.99	15.62
Cumulative[b]	53.51	76.86	91.33	71.57	80.69	65.07	72.41

[a] OTRS; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS; Cunningham Research Station in Kinston, NC, OTRS; UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[b] Total refers to cumulative precipitation from April through September in each environment.

Table 7. Maximum, minimum, cumulative, and 30 year average air temperature at each growing environment.[a]

Month	2022	2023			30 year average		
	OTRS	CRS	OTRS	UCPRS	CRS	OTRS	UCPRS
	°C						
Maximum[b]							
April	21.4	23.9	22.0	23.4	24.1	21.4	21.8
May	25.5	24.2	22.7	23.8	27.7	25.4	25.6
June	30.1	28.9	27.3	28.3	31.1	29.5	29.6
July	31.4	32.4	31.4	32.5	32.5	31.7	31.3
August	29.9	32.5	30.2	32.1	31.5	30.6	29.6
September	27.0	28.1	26.4	27.4	28.7	26.4	27.4
Minimum[c]							
April	9.6	11.9	10.1	11.3	10.7	8.7	8.5
May	15.3	14.2	13.2	13.9	15.2	14.0	13.9
June	18.9	18.1	17.0	17.7	19.7	18.6	18.6
July	21.7	22.3	21.6	22.3	21.8	20.8	20.7
August	20.1	21.8	20.4	21.3	20.8	19.9	19.7
September	16.4	17.8	16.8	17.5	17.8	16.3	16.8
Cumulative Average[d]	21.9	22.7	21.3	22.3	23.5	22.0	21.9

[a] OTRS; Oxford Tobacco Research Station in Oxford, NC, CRS; Cunningham Research Station in Kinston, NC, OTRS; UCPRS; Upper Coastal Plain Research Station near Rocky Mount, NC.

[b] Average maximum air temperature at each location.

[c] Average minimum air temperature at each location.

[d] Cumulative refers to average air temperature from April through September in each environment.

Table 8. Average monthly relative humidity level at each growing environment.[a]

Month	OTRS 2022	CRS 2023	OTRS 2023	UCPRS 2023
	%			
April	58.5	70.2	68.0	69.5
May	76.4	70.4	70.7	70.1
June	68.1	74.4	73.2	74.5
July	80.6	80.7	78.3	79.1
August	79.3	76.5	77.7	75.7
September	74.5	78.6	81.2	82.6

[a] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.

Table 9. The effect of fungicide program on visual chemical injury one week after each treatment application in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	OTRS 2022 (%)			CRS 2023 (%)		
	1 WAA[d]	3 WAA	5 WAA	1 WAA	3 WAA	5 WAA
Non-treated Control	0	0	0 c	0 c	0 c	0 c
Bacillus subtillus	0	0	0 c	0 c	0 c	0 c
Psueudomonas chlororaphis	0	0	0 c	0 c	0 c	0 c
Azoxystrobin	0	0	6 a	3 ab	8 b	8 a
Inpyrfluxam	0	0	0 c	0 c	0 c	0 c
Difenoconazole + pydiflumetofen	0	0	0 c	0 c	0 c	0 c
Picoxystrobin + cyproconazole	0	0	3 b	4 a	7 b	3 b
Mancozeb	0	0	0 c	0 c	0 c	0 c
Azoxystrobin + flutriafol	0	0	6 a	0.5 bc	11 a	9 a
Azoxystrobin + flutriafol + fluindapyr	0	0	5 ab	5 a	9 ab	8 a
P>F	ns	ns	<0.001	0.006	<0.001	<0.001

Table 9. The effect of fungicide program on visual chemical injury one week after each treatment application in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	OTRS 2023 (%)			UCPRS 2023 (%)		
	1 WAA[d]	**3 WAA**	**5 WAA**	**1 WAA**	**3 WAA**	**5 WAA**
Non-treated Control	0 b	0 d	0 c	--[e]	0 c	0 b
Bacillus subtillus	0 b	0 d	0 c	--	0 c	0 b
Psueudomonas chlororaphis	0 b	0 d	0 c	--	0 c	0 b
Azoxystrobin	5 a	3 b	2 b	--	9 a	5 a
Inpyrfluxam	0 b	0 d	0 c	--	0 c	0 b
Difenoconazole + pydiflumetofen	0 b	0 d	0 c	--	0 c	0 b
Picoxystrobin + cyproconazole	5 a	5 a	3 a	--	6 b	0 b
Mancozeb	0 b	0 d	0 c	--	0 c	0 b
Azoxystrobin + flutriafol	5 a	2 c	1 bc	--	6 b	1 b
Azoxystrobin + flutriafol + fluindapyr	5 a	4 ab	0.5 c	--	6 b	2 b
P>F	<0.001	<0.001	<0.001	--	<0.001	0.010

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant. Injury ratings conducted one week following each treatment application. Ratings assigned on a scale of 0-100, with 100 representing plant death.

[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.

[d] WAA; weeks after first application.

[e] Injury ratings not collected due to ozone damage.

Table 10. The effect of fungicide program on disease severity in lower (LS), middle (MS), and upperstalk (US) leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	OTRS 2022 (%)			CRS 2023 (%)		
	LS	**MS**	**US**	**LS**	**MS**	**US**
Non-treated Control	3.26 c-e	1.88	0	8.80 a-c	2.01	1.92
Bacillus subtillus	7.54 a	1.31	0	10.36 ab	1.32	1.20
Psueudomonas chlororaphis	4.99 b-d	1.49	0	11.31 a	1.46	1.62
Azoxystrobin	5.69 a-c	1.66	0	5.59 de	1.59	1.23
Inpyrfluxam	2.44 e	1.04	0	5.07 de	1.53	1.63
Difenoconazole + pydiflumetofen	3.28 c-e	1.42	0	7.55 cd	1.81	2.13
Picoxystrobin + cyproconazole	6.23 ab	1.09	0	4.55 e	1.71	1.13
Mancozeb	2.60 de	1.27	0	8.03 b-d	1.89	1.28
Azoxystrobin + flutriafol	5.88 ab	1.58	0	5.08 de	1.77	1.74
Azoxystrobin + flutriafol + fluindapyr	5.57 a-c	1.42	0	6.28 c-e	1.46	1.37
P>F	0.002	ns	ns	0.001	ns	ns

Table 10. The effect of fungicide program on disease severity in (LS), middle (MS), and upperstalk (US) leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	**OTRS 2023 (%)**			**UCPRS 2023 (%)**		
	LS	**MS**	**US**	**LS**	**MS**	**US**
Non-treated Control	13.14 a	1.55	0	45.27 a	23.78 a	2.38
Bacillus subtillus	8.09 bc	1.10	0	28.81 bc	19.58 ab	2.83
Psueudomonas chlororaphis	10.41 ab	1.66	0	32.26 ab	20.55 ab	2.79
Azoxystrobin	8.82 bc	0.45	0	14.11 cd	11.02 c	1.84
Inpyrfluxam	4.07 d	0.00	0	4.39 d	1.98 d	1.97
Difenoconazole + pydiflumetofen	9.86 b	1.28	0	12.76 cd	18.81 b	2.13
Picoxystrobin + cyproconazole	6.29 cd	1.27	0	8.99 d	7.76 c	1.34
Mancozeb	10.55 ab	0.94	0	15.43 cd	16.31 b	2.58
Azoxystrobin + flutriafol	6.45 cd	0.95	0	14.11 cd	10.08 c	1.50
Azoxystrobin + flutriafol + fluindapyr	6.12 cd	0.76	0	6.87 d	8.36 c	1.49
P>F	<0.001	ns	ns	<0.001	<0.001	ns

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.
[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.
[c] *Bacillus subtillus*; 3.36 kg ai ha^{-1}, *Psueudomonas chlororaphis*; 1.40 kg ai ha^{-1}, Azoxystrobin 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Inpyrfluxam; 0.05 kg ai ha^{-1}, Difenoconazole + pydiflumetofen; 0.08 + 0.04 kg ai ha^{-1}, Picoxystrobin + cyproconazole; 0.10 + 0.04 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}, Azoxystrobin + flutriafol; 0.17 + 0.13 kg ai ha^{-1}, Azoxystrobin + flutriafol + fluindapyr; 0.12 + 0.08 + 0.12 kg ai ha^{-1}.

Table 11. The effect of fungicide program on disease incidence in lower (LS), middle (MS), and upperstalk (US) leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	OTRS 2022 (%)			CRS 2023 (%)		
	LS	**MS**	**US**	**LS**	**MS**	**US**
Non-treated Control	51 b	39 a	16	77	35 ab	17
Bacillus subtillus	39 b-d	21 b	10	68	20 d	11
Psueudomonas chlororaphis	31 d	19 b	6	68	22 cd	9
Azoxystrobin	65 a	19 b	10	65	34 a-c	11
Inpyrfluxam	44 bc	14 b	9	61	34 ab	5
Difenoconazole + pydiflumetofen	27 d	13 b	5	68	38 a	16
Picoxystrobin + cyproconazole	49 b	17 b	6	54	21 d	7
Mancozeb	33 cd	15 b	11	71	29 a-d	14
Azoxystrobin + flutriafol	47 b	12 b	4	59	26 b-d	12
Azoxystrobin + flutriafol + fluindapyr	50 b	12 b	7	60	22 cd	12
P>F	<0.001	0.026	ns	ns	0.013	ns

Table 11. The effect of fungicide program on disease incidence in lower (LS), middle (MS), and upperstalk (US) leaf position groups immediately prior to first harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	OTRS 2023 (%)			UCPRS 2023 (%)		
	LS	**MS**	**US**	**LS**	**MS**	**US**
Non-treated Control	51 a	31 a	19 a	97 a	87 a	22 ab
Bacillus subtillus	43 a-c	32 a	17 a	92 ab	82 ab	26 a
Psueudomonas chlororaphis	52 a	29 ab	13 ab	93 ab	89 a	17 a-d
Azoxystrobin	47 ab	19 a-d	12 a-c	86 ab	89 a	9 c-e
Inpyrfluxam	33 c	0 e	0 d	64 d	37 d	7 de
Difenoconazole + pydiflumetofen	47 ab	21 a-d	11 a-c	82 bc	86 ab	22 ab
Picoxystrobin + cyproconazole	39 bc	8 de	7 b-d	71 cd	67 c	0 e
Mancozeb	49 a	24 a-c	11 a-c	86 ab	81 a-c	24 a
Azoxystrobin + flutriafol	47 ab	16 b-d	4 cd	83 bc	79 a-c	20 a-c
Azoxystrobin + flutriafol + fluindapyr	33 c	15 cd	4 cd	70 cd	73 bc	12 b-d
P>F	0.003	0.001	0.003	<0.001	<0.001	0.001

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.
[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.
[c] *Bacillus subtillus*; 3.36 kg ai ha^{-1}, *Psueudomonas chlororaphis*; 1.40 kg ai ha^{-1}, Azoxystrobin 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Inpyrfluxam; 0.05 kg ai ha^{-1}, Difenoconazole + pydiflumetofen; 0.08 + 0.04 kg ai ha^{-1}, Picoxystrobin + cyproconazole; 0.10 + 0.04 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}, Azoxystrobin + flutriafol; 0.17 + 0.13 kg ai ha^{-1}, Azoxystrobin + flutriafol + fluindapyr; 0.12 + 0.08 + 0.12 kg ai ha^{-1}.

Table 12. The effect of fungicide program on disease severity in middle (MS) and upperstalk (US) leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	**OTRS 2022 (%)**		**CRS 2023 (%)**	
	MS	**US**	**MS**	**US**
Non-treated Control	--[d]	--	--	1.62
Bacillus subtillus	--	--	--	1.47
Psueudomonas chlororaphis	--	--	--	2.13
Azoxystrobin	--	--	--	2.03
Inpyrfluxam	--	--	--	1.84
Difenoconazole + pydiflumetofen	--	--	--	1.70
Picoxystrobin + cyproconazole	--	--	--	2.12
Mancozeb	--	--	--	1.74
Azoxystrobin + flutriafol	--	--	--	1.49
Azoxystrobin + flutriafol + fluindapyr	--	--	--	1.99
P>F	--	--	--	ns

Table 12. The effect of fungicide program on disease severity in middle (MS) and upperstalk (US) leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	OTRS 2023 (%)		UCPRS 2023 (%)	
	MS	US	MS	US
Non-treated Control	4.49 a	2.66 a	--	2.59
Bacillus subtillus	4.01 ab	2.13 ab	--	5.05
Psueudomonas chlororaphis	3.27 a-d	1.88 a-d	--	4.08
Azoxystrobin	2.59 cd	1.70 b-e	--	3.77
Inpyrfluxam	2.05 d	1.22 c-e	--	3.10
Difenoconazole + pydiflumetofen	2.89 b-d	2.08 ab	--	5.19
Picoxystrobin + cyproconazole	2.00 d	1.13 e	--	3.20
Mancozeb	3.71 a-c	1.72 b-e	--	3.87
Azoxystrobin + flutriafol	3.05 b-d	1.98 a-c	--	3.77
Azoxystrobin + flutriafol + fluindapyr	2.80 cd	1.21 de	--	2.37
P>F	0.004	0.012	--	ns

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.
[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.
[c] *Bacillus subtillus*; 3.36 kg ai ha^{-1}, *Psueudomonas chlororaphis*; 1.40 kg ai ha^{-1}, Azoxystrobin 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Inpyrfluxam; 0.05 kg ai ha^{-1}, Difenoconazole + pydiflumetofen; 0.08 + 0.04 kg ai ha^{-1}, Picoxystrobin + cyproconazole; 0.10 + 0.04 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}, Azoxystrobin + flutriafol; 0.17 + 0.13 kg ai ha^{-1}, Azoxystrobin + flutriafol + fluindapyr; 0.12 + 0.08 + 0.12 kg ai ha^{-1}.

Table 13. The effect of fungicide program on disease incidence in middle (MS) and upperstalk (US) leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	OTRS 2022 (%)		CRS 2023 (%)	
	MS	**US**	**MS**	**US**
Non-treated Control	--[d]	--	--	43
Bacillus subtillus	--	--	--	33
Psueudomonas chlororaphis	--	--	--	45
Azoxystrobin	--	--	--	51
Inpyrfluxam	--	--	--	48
Difenoconazole + pydiflumetofen	--	--	--	44
Picoxystrobin + cyproconazole	--	--	--	56
Mancozeb	--	--	--	44
Azoxystrobin + flutriafol	--	--	--	40
Azoxystrobin + flutriafol + fluindapyr	--	--	--	51
P>F	--	--	--	ns

Table 13. The effect of fungicide program on disease incidence in middle (MS) and upperstalk (US) leaf position groups immediately prior to third harvest in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	OTRS 2023 (%)		UCPRS 2023 (%)	
	MS	**US**	**MS**	**US**
Non-treated Control	83 a	24 ab	--	67
Bacillus subtillus	67 bc	18 bc	--	60
Psueudomonas chlororaphis	70 b	32 a	--	59
Azoxystrobin	56 cd	9 cd	--	62
Inpyrfluxam	28 fg	5 d	--	52
Difenoconazole + pydiflumetofen	47 de	14 b-d	--	65
Picoxystrobin + cyproconazole	23 g	11 cd	--	57
Mancozeb	47 de	17 b-d	--	63
Azoxystrobin + flutriafol	57 b-d	8 cd	--	57
Azoxystrobin + flutriafol + fluindapyr	39 ef	7 cd	--	52
P>F	<0.001	0.002	--	ns

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.
[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.
[c] *Bacillus subtillus*; 3.36 kg ai ha^{-1}, *Psueudomonas chlororaphis*; 1.40 kg ai ha^{-1}, Azoxystrobin 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Inpyrfluxam; 0.05 kg ai ha^{-1}, Difenoconazole + pydiflumetofen; 0.08 + 0.04 kg ai ha^{-1}, Picoxystrobin + cyproconazole; 0.10 + 0.04 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}, Azoxystrobin + flutriafol; 0.17 + 0.13 kg ai ha^{-1}, Azoxystrobin + flutriafol + fluindapyr; 0.12 + 0.08 + 0.12 kg ai ha^{-1}.

Table 14. The effect of fungicide program on cured leaf yield, visual quality, and economic value in 2022 and 2023. Data are presented for each growing environment. [a,b,c]

Fungicide Program	**OTRS 2022**			**CRS 2023**		
	Yield	**Quality**	**Value**	**Yield**	**Quality**	**Value**
	kg ha^{-1}		\$ ha^{-1}	kg ha^{-1}		\$ ha^{-1}
Non-treated Control	3,099 d	83	4,639	2,594	53	2,318
Bacillus subtillus	3,109 d	83	4,671	2,719	59	2,769
Psueudomonas chlororaphis	3,084 d	84	4,648	2,567	58	2,599
Azoxystrobin	3,243 b-d	83	4,595	2,494	55	2,391
Inpyrfluxam	3,227 b-d	80	4,630	2,637	54	2,384
Difenoconazole + pydiflumetofen	3,482 ab	80	5,030	2,655	60	2,812
Picoxystrobin + cyproconazole	3,183 cd	81	4,587	2,695	59	2,693
Mancozeb	3,255 b-d	81	4,721	2,642	54	2,438
Azoxystrobin + flutriafol	3,546 a	83	5,331	2,743	61	2,949
Azoxystrobin + flutriafol + fluindapyr	3,375 a-c	83	5,096	2,837	55	2,690
P>F	0.010	ns	ns	ns	ns	ns

Table 14. The effect of fungicide program on cured leaf yield, visual quality, and economic value in 2022 and 2023. Data are presented for each growing environment (continued). [a,b,c]

Fungicide Program	OTRS 2023			UCPRS 2023		
	Yield	**Quality**	**Value**	**Yield**	**Quality**	**Value**
	kg ha^{-1}		\$ ha^{-1}	kg ha^{-1}		\$ ha^{-1}
Non-treated Control	3,016	55	2,808	2,244 de	56 b-d	2,135 f
Bacillus subtillus	2,917	56	2,743	2,234 e	54 d	2,066 f
Psueudomonas chlororaphis	2,942	54	2,713	2,446 c-e	55 cd	2,305 ef
Azoxystrobin	3,036	54	2,864	2,749 bc	61 a-c	2,878 cd
Inpyrfluxam	3,328	55	3,099	2,822 bc	63 a	3,062 a-c
Difenoconazole + pydiflumetofen	3,178	54	2,886	2,673 bc	55 cd	2,502 d-f
Picoxystrobin + cyproconazole	2,972	56	2,849	2,663 b-d	63 a	2,904 b-d
Mancozeb	3,052	56	2,750	2,544 c-e	62 ab	2,741 c-e
Azoxystrobin + flutriafol	3,041	56	2,906	3,047 ab	64 a	3,351 ab
Azoxystrobin + flutriafol + fluindapyr	3,158	55	2,931	3,267 a	61 a-d	3,434 a
P>F	ns	ns	ns	0.005	0.016	<0.001

[a] Treatment means followed by the same letter within the same column and growing environment are not significantly different at the α=0.05 level. ns; not significant.
[b] OTRS 2022; Oxford Tobacco Research Station in Oxford, NC in 2022, CRS 2023; Cunningham Research Station in Kinston, NC, in 2023, OTRS 2023; Oxford Tobacco Research Station in Oxford, NC, in 2023, UCPRS 2023; Upper Coastal Plain Research Station near Rocky Mount, NC, in 2023.
[c] *Bacillus subtillus*; 3.36 kg ai ha^{-1}, *Psueudomonas chlororaphis*; 1.40 kg ai ha^{-1}, Azoxystrobin 0.15 + 0.16 + 0.16 kg ai ha^{-1}, Inpyrfluxam; 0.05 kg ai ha^{-1}, Difenoconazole + pydiflumetofen; 0.08 + 0.04 kg ai ha^{-1}, Picoxystrobin + cyproconazole; 0.10 + 0.04 kg ai ha^{-1}, Mancozeb; 1.68 kg ai ha^{-1}, Azoxystrobin + flutriafol; 0.17 + 0.13 kg ai ha^{-1}, Azoxystrobin + flutriafol + fluindapyr; 0.12 + 0.08 + 0.12 kg ai ha^{-1}.

Figure 1. Spray pattern of the single solid-cone TG6 nozzle during application one, four weeks after transplanting.

Figure 2. Spray coverage with the single solid-cone TG6 nozzle during application one, four weeks after transplanting.

Figure 3. Spray pattern of the three flat fan nozzle arrangement (XR8004VS) during application two, six weeks after transplanting.

Figure 4. Spray pattern of the seven flat fan nozzles (five XR11003EVS and two XR8003VS) affixed to a dropline arrangement during application three, eight weeks after transplanting.

Figure 5. Spray coverage with the seven flat fan nozzles (five XR11003EVS and two XR8003VS) affixed to a dropline arrangement during application three, eight weeks after transplanting.

Figure 6. Research plots absent of fungicide treatment (non-treated control) at the UCPRS in 2023. Picture was taken before first harvest removal.

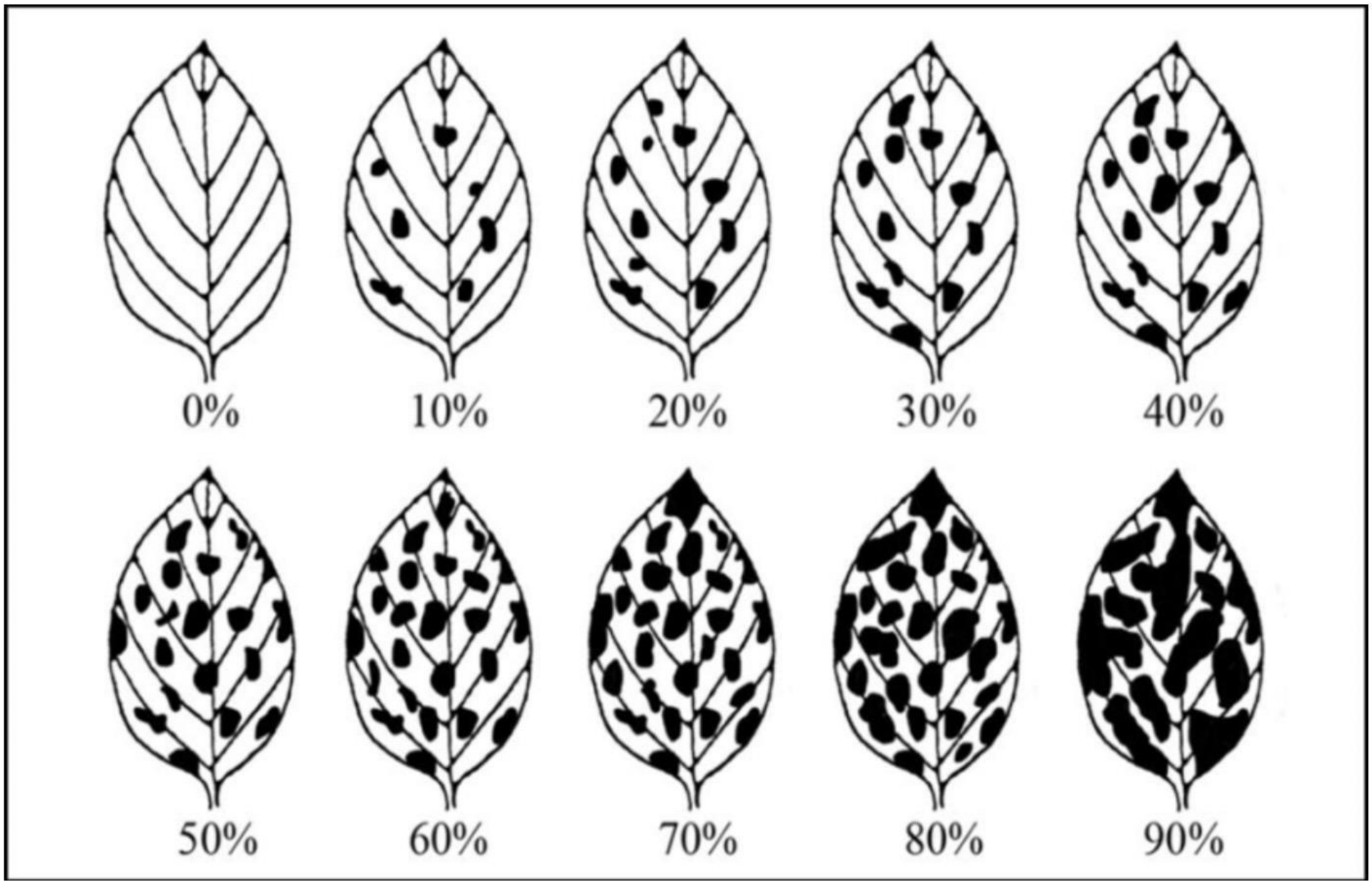

Figure 7. Severity percentage chart used in treatment evaluations (Jadhav and Patil 2016).

Figure 8. Tobacco leaf displaying a 5% disease severity.

Figure 9. Tobacco leaf displaying a 30% disease severity.

Figure 10. Tobacco leaf displaying a 40% disease severity.

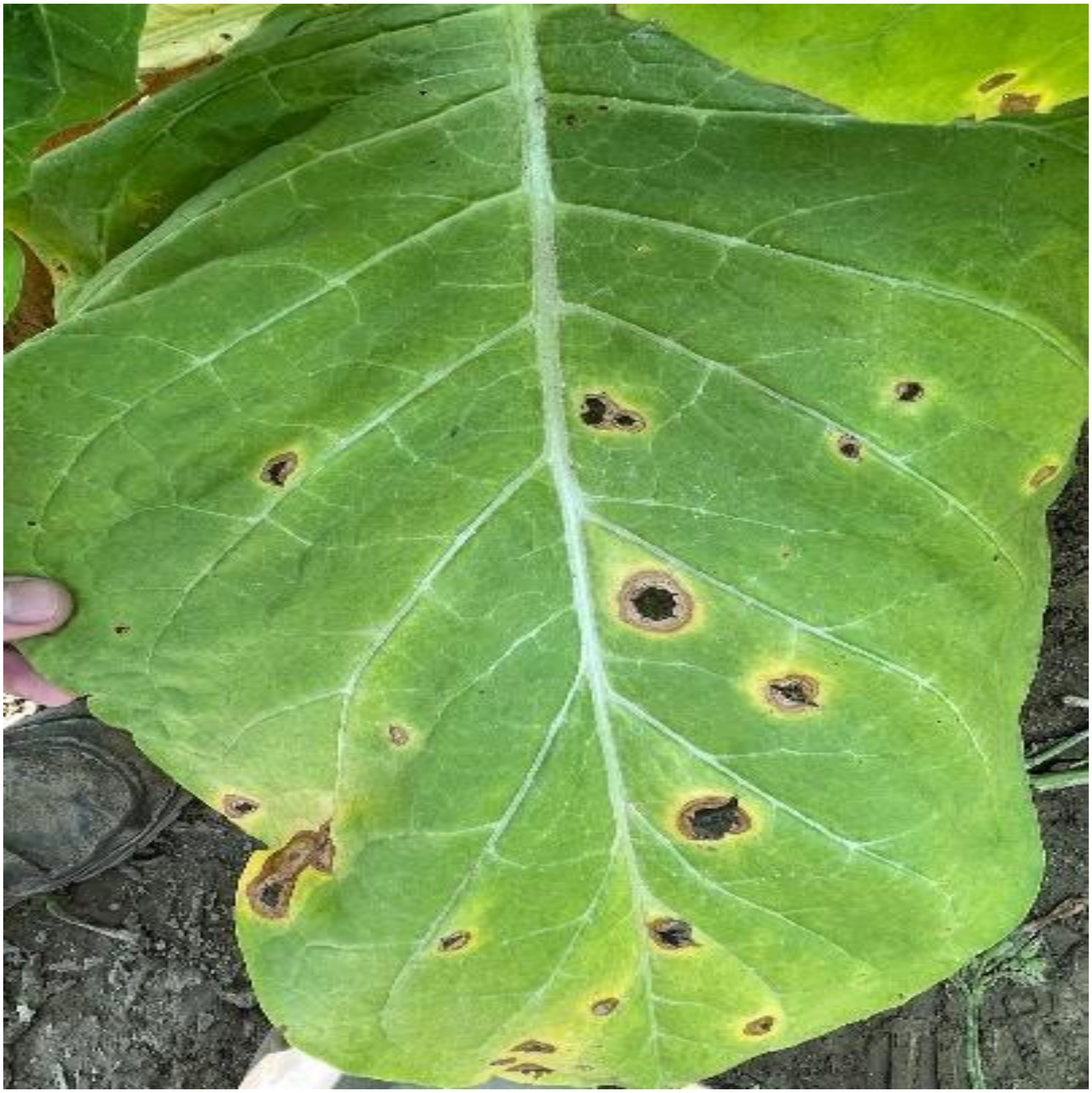

Figure 11. Tobacco leaf displaying a 15% disease severity.

Figure 12. Tobacco leaf displaying a 60% disease severity.