R. Pandey (2023) Int. J. Appl. Sci. Biotechnol. Vol 11(1): 1-7.
DOI: 10.3126/ijasbt.v11i1.52440

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Mini Review

Controlling Tobacco Diseases: An Overview of Black Shank and Fusarium Wilt

Roman Pandey*

Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal

Article Information

Received: 12 February 2023
Revised version received: 23 march 2023
Accepted: 25 March 2023
Published: 31 March 2023

Cite this article as:
R. Pandey (2023) Int. J. Appl. Sci. Biotechnol. Vol 11(1): 1-7. DOI: 10.3126/ijasbt.v11i1.52440

***Corresponding author**
Roman Pandey,
Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal.
Email: romanpandey2018@gmail.com

Peer reviewed under authority of IJASBT

Abstract

Black shank, caused by Phytophthora nicotianae, and Fusarium wilt, caused by the Fusarium oxysporium f. sp. nicotianae, are major diseases affecting tobacco crops globally. Phytophthora nicotianae is primarily found in tropical and subtropical regions and infects tobacco plants by producing zoospores that swim to root tissue and form cysts. Symptoms of black shank include root and crown rot, wilting, leaf chlorosis, stem lesions, and pith necrosis. Management strategies for black shank include cultural practices, chemical treatments, and host resistance. Chemical fungicides, such as metalaxyl or mefenoxam, oxathiapiprolin, and fluopicolide, can be used but there is a risk of fungicide resistance. Fusarium wilt is characterized by yellowing, drying, and death of leaves, leading to the death of the entire plant. Control measures for Fusarium wilt include plant resistance, cleanliness, crop rotation, nutrition, nematode management, and fumigation or biofumigation. The most successful control of Fusarium wilt has been through the use of resistant tobacco cultivars. The impact of rotation crops and resistant plants on the pathogen populations in the soil must be evaluated and soil pH and calcium levels may also impact the disease. The presence of the fungus in a field can last for several years without a vulnerable host.

Keywords: Tobacco diseases; Black shank; Phytophthora nicotianae; Fusarium wilt

Introduction

Tobacco, a herbaceous plant native to South and Central America, is grown globally as a major non-food, cash crop. It is known scientifically as *Nicotiana tabacum* (McCants & Woltz, 1967). Tobacco belongs to the Solanaceae family and is grown in approximately 125 countries worldwide. It is a widely cultivated and produced crop (Eriksen *et al.*, 2015). There are two main types of tobacco grown as cash crops are flue-cured and burley tobacco (Tso, 1972). Tobacco plant grows to a height of around 2 metres and has elliptical, simple leaves that are alternate and can be up to 45 centimetres long (Garner, 1951). Tobacco plants produce clusters of white or pink flowers at the apex of the plant, which have pedicels that are 5-7 mm long. Each plant can produce a large number of small seeds, with a single plant potentially producing up to 150,000 seeds. Tobacco is a hardy plant that can thrive in many different environments and habitats, including forests, plains, mountainous regions, wetlands, and savannahs (Tso, 1972). Moreover, *Nicotiana tabacum* can be grown in various types of soil, including sandy, loamy, and clay, and can tolerate a range of pH values. However, the optimal conditions for tobacco production include sandy, well-draining soil that is high in

nutrients, full sun exposure, temperatures between 20-30 degrees Celsius, and a soil pH between 5.0-6.0 (Garner, 1951). In order to be successfully harvested, tobacco plants need to be protected from frost for a period of 90-120 days after transplanting and have a total growing season of 70-110 days (Tso, 1972). The cultivation of tobacco plants is widespread worldwide and it is one of the most important crops in terms of economic significance. However, like any other crops, tobacco plants are susceptible to various diseases that can severely affect their growth and production. The diseases caused by pathogens, such as fungi, bacteria, viruses, and nematodes, can lead to significant losses in yield and quality of the harvested tobacco leaves. In this article, we will delve into black shank and Fusarium wilt of tobacco plants, their causes, symptoms, and control measures.

Black Shank of Tobacco

Black Shank is one of the most important threatening diseases (Cartwright & Spurr Jr, 1998; Jin & Shew, 2022) for tobacco (*Nicotiana tabacum* L.) plants. It is caused by the fungus *Phytophthora parasitica* var. *Nicotianae* which affects most tobacco-growing regions (Lucas, 1975). *Phytophthora nicotianae* is classified in the Kingdom Chromista, phylum Oomycota, class Oomycetes, and order Peronosporales. Recent research using genetic markers such as 60S Ribosomal protein L10, beta-tubulin, elongation factor 1 alpha, enolase, heat shock protein 90, 28S ribosomal DNA, and tigA identified 10 distinct clades within the genus *Phytophthora* (Yang *et al*., 2017). *Phytophthora nicotianae* whose hyphae (filaments) are branched, white, and have a diameter ranging from 3-11 micrometres (Lucas, 1975; Gallegly & Hong, 2008). As the hyphae mature, they may turn pale yellow and have a fluffier colony appearance. The hyphae do not have septa (partitions) but older cultures may develop pseudo septa (apparent partitions). The hyphae become granular and develop oil globules as they age. The sporangia (spore-producing structures) of this fungus are sympodial (occurring in pairs), ovoid, lemon-shaped, or pear-shaped and range in size from 18-61 by 14-39 micrometres (Lucas, 1975; Gallegly & Hong, 2008). Sporangia are transparent to light yellow and are formed on short pedicels from the hyphae. These sporangia have an apical papilla and can produce 5 to 30 zoospores, which are small, mobile spores that are 7-11µm in size (Shoemaker & Shew, 1999).

Phytophthora nicotianae, has a number of different spore types. Zoospores are biflagellate and have flagella attached to the concave side of the spore. Chlamydospores, which can range in diameter from 14-43 µm and are typically 30 µm in size, are non-papillate and spherical or ovoid in shape (Lucas, 1975). They are initially thin-walled and hyaline, but turn yellow to brown and thicken as they mature. Chlamydospores form on short lateral hyphae that are perpendicular to vegetative hyphae. Oospores, which have been recorded in lab settings but are not well documented in the environment, are spherical to pyriform and hyaline to pale yellow in color (Lucas, 1975). They are produced inside oogonia, which are hyaline to pale yellow structures that enclose the oosphere. After fertilization, the oosphere develops into an oospore, which is typically 23-30 µm in diameter (Gallegly & Hong, 2008).

P. nicotianae is a species of plant pathogen that is commonly found in tropical and subtropical regions where the humidity is high and the temperatures are warm (Lucas, 1975). They typically grow best at temperatures between 28-32°C and pH levels between 5.7-7.0. They also require a temperature of at least 20°C for optimal infection to occur (Dukes & Apple, 1965; Lucas, 1975). Sporangia growth and formation occurs when there is sufficient oxygen and water present in the environment, with optimal temperature being between 24-28°C. Sporangia can appear within 48 hours after the mycelium has developed (Lucas, 1975; Shoemaker & Shew, 1999). At the same temperature, sporangia will produce secondary sporangia when they germinate. Inside the sporangia, kidney-shaped zoospores with two flagella will form and exit through a small protrusion called a papilla. These zoospores will swim in circles and, when they land on a host's tissue, will germinate and produce new sporangia that will produce another generation of zoospores within 72 hours (Lucas, 1975).

Phytophthora nicotianae is a type of plant pathogen that causes diseases in a polycyclical manner, primarily attacking the roots of its host plant but also capable of infecting leaves and flowers. It is classified as a hemibiotrophic pathogen, meaning it causes both biotrophic (living off a host) and necrotrophic (causing death in the host) stages during its disease cycle (Gallup *et al*., 2006). *P. nicotianae* is a pathogen that initially forms a mutually beneficial relationship with its host. However, it eventually causes the host cells to die and enters a phase in which it feeds on the host tissue that has died. This is known as the necrotrophic phase (Panabieres *et al*., 2016). Infection happens when zoospores, which are mobile and don't have a cell wall, are released from asexually produced, multi-nucleated sporangia. The amount of zoospores in soil is related to the severity of the infection (Gooding & Lucas, 1959). The zoospores are attracted to specific chemicals in the soil and move towards root tissue, where they form a cyst on the plant. The growth of a germ tube, which eventually develops into an appressorium, is essential for the disease cycle of *P. nicotianae*. These structures allow the pathogen to enter and infect host cells, leading to the death of the host cells. *P. nicotianae* can also survive in the soil as chlamydospores for extended periods of time, serving as a source of infection for future growing seasons. (Lucas, 1975) Management strategies often target the formation of germ tubes in an effort to control the spread of this pathogen (Shan *et al*., 2004).

P. nicotianae primarily reproduces asexually, although sexual reproduction through the fusion of male and female gametangia does occur occasionally (Gallup *et al*., 2018; Meng *et al*., 2014; Gallup *et al*., 2006). The production of thick-walled oospores during sexual reproduction requires the presence of both A1 and A2 mating types (Lucas, 1975; Gallup *et al*., 2018). However, the unequal frequency of these mating types in the environment suggests that the production of oospores may not be significant in the pathogen's life cycle and that concerns about changes in virulence and pathogenicity due to sexual reproduction may be overestimated (Meng *et al*., 2014).

The pathogen can infect all parts of the tobacco plant, including the roots, stems, and leaves, at any stage of growth. This can significantly reduce the yield and quality of the tobacco crop (Csinos & Minton, 1983). If tobacco plants become infected with the Black Shank disease early in the growing season, they may become stunted and topple down before the leaves are mature enough to be harvested (Csinos & Bertrand, 1994). *P. nicotianae* primarily infects tobacco plants that are 6 to 8 weeks old, but it can also infect plants at any growth stage (Lucas, 1975; Jacobi *et al*.,1983; Shoemaker & Shew, 1999). The symptoms of black shank can be observed within 48 hours of pathogen introduction and the plant may die within a week of infection (Lucas, 1975). It primarily causes root and crown rot, with uniform wilting and leaf chlorosis leading to water-soaked lesions on stem tissue 15-20 centimeters above the soil line (Shoemaker & Shew, 1999). The disease progresses, root necrosis may also be observed (Todd, 1981). The hallmark symptom of black shank is pith necrosis and brown to black disking of the vascular (Shoemaker & Shew, 1999). A large, circular, concentric dark-brown lesions that are 7 to 8 cm in diameter may also appear on older foliage due to rain splash dispersal of inoculum (Lucas, 1975; Shoemaker & Shew, 1999; Gallup *et al*., 2006). In order to accurately diagnose *P. nicotianae* infection, the macroscopic symptoms described above should be used in conjunction with microscopic visualization and molecular characterization, as other tobacco diseases such as Fusarium Wilt and Granville Wilt can present similar symptoms.

P. nicotianae can be found in plant tissue that shows symptoms of infection, in water, or in soil. There are several baiting methods for recovering Phytophthora nicotianae from infected plants and soil (Klotz & DeWolfe, 1958), but it is important to note that different strains of this pathogen may have varying levels of pathogenicity on different host plants (Erwin & Ribeiro, 1996). In the past, it was challenging to culture Phytophthora species, but the use of hymexazol and low levels of pimaricin in media has made it easier to selectively isolate these pathogens (Tsao & Ocana, 1969). There are certain media that contain antifungal and antibacterial compounds that can help to isolate *P. nicotianae* by inhibiting the growth of other contaminants like Pythium spp (Erwin *et al*., 1983). One example of this type of semi-selective medium is PARPH-V8, which includes pimaricin, ampicillin, rifampicin, PCNB, and hymexazol. This medium is effective at helping to separate *P. nicotianae* from other organisms (Jeffers & Martin, 1986).

Management

Effective management of black shank requires the integration of various techniques, including cultural practices, chemical treatments, and host resistance (Shoemaker & Shew, 1999). Cultural methods such as raised beds, crop rotation, and disposal of plant debris are essential for long-term management of the disease (Gallup *et al*., 2006; Gallup, 2009; Thiessen *et al*., 2022). Using raised beds helps to prevent soil saturation and restrict the spread of zoospores to roots, while rotating crops that are not susceptible to black shank reduces the amount of pathogen present in the soil and reduces the pathogens' survival needs (Gallup, 2009). Commonly used crop rotation strategies include alternating between forage crops, cotton, corn, or peanuts for 2 to 4 years (Thiessen *et al*., 2022). Clearing debris from previous crops also helps to prevent the buildup of inoculum (Gallup, 2009; Thiessen *et al*., 2022). Disease-free transplants are also important for maintaining sanitary conditions. Alongside cultural practices, chemical controls are useful for reducing disease losses.

Managing black shank in tobacco crops can be achieved through the use of chemical treatments as part of an integrated management system. However, it is important to note that this approach may not be effective when used on susceptible varieties (Gallup *et al*., 2006). There are three active ingredients and modes of action commonly used for black shank management: metalaxyl or mefenoxam, oxathiapiprolin, and fluopicolide (Rivera & Thiessen, 2017; Thiessen *et al*., 2022). Metalaxyl and mefenoxam, which belong to the FRAC group 4 phenylamide fungicides, inhibit the growth and development of hyphae, haustoria and sporangia in oomycetes (Wollgiehn *et al*., 1984). These fungicides are highly systemic and are most effective when applied at transplant or in the early growth stages of the crop (Antonopoulos *et al*., 2010; Rivera & Thiessen, 2017). However, their site specificity can lead to the development of fungicide resistance in pathogen populations (Hu *et al*., 2008), so it is important to monitor for this. Oxathiapiprolin and fluopicolide are newer fungicides that are available for managing *P. nicotianae* (Bittner & Mila, 2017). Oxathiapiprolin, a FRAC group 49 fungicide, is most effective when applied in transplant water and can reduce disease severity when applied at first and last cultivation (Bittner & Mila, 2016). Meanwhile, fluopicolide, a FRAC group 43 benzamide fungicide, is most effective when applied to the soil during first cultivation or layby. Recent studies have suggested that combining fluopicolide with

propamocarb hydrochloride can be more effective than using fluopicolide alone (Ren *et al*., 2018).

The use of chemical fungicides to control the *Phytophthora nicotianae* fungus, may be challenged by the potential development of fungicide resistance. Other Phytophthora species have already shown resistance to metalaxyl, mefenoxam, and oxathiapiprolin (Shattock, 1988). A study by Bittner *et al*. found that while oxathiapiprolin was still effective in controlling *P. nicotianae*, repeated applications of the fungicide led to mutations that reduced its sensitivity and resulted in a cost to the fungus' overall fitness (Bittner & Mila, 2016; Bittner & Mila, 2017). Fluopicolide resistance has not yet been identified in *P. nicotianae*, and its potential for resistance development remains unknown (Qu *et al*., 2016). To effectively manage black shank, a combination of strategies should be employed, including utilizing host resistance, in addition to fungicide treatments.

The most efficient way to combat black shank disease is by using resistant varieties (Todd, 1981). These cultivars possess genes or characteristics that decrease the likelihood of infection, either by reducing the density of *P. nicotianae* or through other physiological mechanisms (Jones & Shew, 1995). For instance, smaller and more condensed root systems decrease the incidence of disease as they decrease the chance of direct contact with *P. nicotianae* (Jones & Shew, 1995). However, these types of root systems are not optimal for tobacco crop production as they can negatively impact yield by compromising the structural integrity of the host plant (Jones & Shew, 1995).

Fusarium Wilt of Tobacco

Fusarium wilt, a disease caused by the *Fusarium oxysporum* species complex, causes severe damage in many countries worldwide (Lucas, 1975). The first case of the disease in the US was recorded in 1916 in Maryland (Johnson, 1921), and in 1943 it was documented as a peculiar occurrence in Connecticut on a sweet potato research plot (Anderson, 1944). By the 1980s and early 1990's, it had evolved into the most devastating and significant ailment of broadleaf cigar wrapper tobacco in the states of Connecticut and Massachusetts. The disease affected around 20% of the tobacco production areas, resulting in severe harm and the elimination of fields heavily plagued with the disease from tobacco production.

The three species of the wilt-causing Fusarium fungus, which affects tobacco plants, have been identified as *Fusarium oxysporium f. sp. nicotianae, Fusarium oxysporium f. sp. batatas, and Fusarium oxysporium f. sp. vasinfectum* (Armstrong & Armstrong, 1968; Smith & Shaw, 1943). These species were differentiated based on their impact on different hosts such as sweet potato and cotton in addition to tobacco. Four races of the *Fusarium oxysporum* fungus were found to be pathogenic to tobacco. Recent research through cluster analysis has revealed that the pathogen is made up of at least three different groups of isolates, which may be separate lineages from sweet potato and tobacco (Clark *et al*., 1998). One of these clusters, *Fusarium oxysporium. f. sp. nicotianae*, includes all isolates originally found in tobacco. The second and third clusters, *Fusarium oxysporium. f. sp. batatas*, consist of sweet potato isolates from Louisiana and North Carolina (Race 0) or from California (Race 1). Race 0 of *Fusarium oxysporium. f. sp. batatas* did not cause disease in flue-cured tobacco or resistant sweet potato, while Race 1 did not affect tobacco but caused wilt in resistant sweet potato. This highlights the variability of the tobacco wilt pathogen.

Fusarium wilt in tobacco plants is characterized by a range of symptoms, such as yellowing, drying, and death of leaves that may appear in a vertically aligned pattern, typically on one side of the plant or leaf midvein (Lucas, 1975;). The disease causes a distinctive chocolate-brown discoloration of the plant's vascular tissue, which can spread up to the top of the plant. Over time, this discoloration becomes visible on the green stalk's exterior. The leaves will not rot but instead cure on the stalk, causing the stalk to bend over at the bud, resulting in a distinctive "crookneck" appearance (Anderson, 1944). Ultimately, the entire plant will die, becoming dry and necrotic. The severity of the disease is heightened in warm conditions and sandy loam soils (Lucas, 1975).

There are several methods for controlling Fusarium wilt in tobacco, including plant resistance, cleanliness, crop rotation, nutrition, nematode management, and fumigation or biofumigation. However, not all methods are equally effective. Currently, there are no chemical controls for *Fusarium oxysporium. f. sp. nicotianae* in soil that are completely effective. Fumigation of soil may lead to some reduction in disease severity, but the most reliable fumigants, methyl bromide and chloropicrin (Bennett *et al*., 2011), are being phased out and have negative effects on the quality and appearance of wrapper leaves (LaMondia & Taylor, 1987). Additionally, fumigation with metam sodium is not effective at reducing cotton wilt caused by *Fusarium oxysporum f. sp. vasinfectum* or soilborne Fusarium (Bennett *et al*., 2011). Chlamydospores may also be resistant to fumigation, particularly in crop residue. Fumigation may control disease expression indirectly through its effect on plant parasitic nematodes, but the results have been inconsistent in Connecticut, particularly when tobacco is continuously grown in the same field.

The most successful method of controlling Fusarium wilt globally has been through the creation and widespread adoption of tobacco cultivars that are resistant to the disease (Lucas, 1975). In Connecticut, broadleaf cigar wrapper varieties such as 'C9' and 'B2' were introduced with a resistance to the Fusarium wilt pathogen (LaMondia, 2013; LaMondia & Taylor, 1991; Lucas, 1975). This resistance is due to multiple genes and the accumulation of these genes

for increased effectiveness (Jones *et al*., 1972). However, these resistant cultivars are not completely immune to Fusarium infection and may still develop wilt symptoms under extreme conditions (LaMondia & Taylor, 1987). Studies have repeatedly found *Fusarium oxysporum* in wilt-resistant broadleaf tobacco and have observed that the resistance to Fusarium wilt is related to the pathogen's slower movement in the plant's vascular tissues. Additionally, quick responses such as vesicle formation to block xylem, callose deposition, and lipoidal material secretion have been observed in resistant plants within 24 hours of inoculation, but not in susceptible plants (LaMondia, 2013).

The rapid dissemination of the pathogen may be attributed to various reasons, such as the custom of using tobacco stalks as fertilizer and waste disposal, as well as the transfer of chlamydospores through soil on farming equipment that moves between fields. Research has indicated that *Fusarium oxysporum* can endure composting, which is not the case for many other plant pathogens. Currently, tobacco stalks are either spread on non-tobacco fields or buried, instead of being left on the tobacco fields. Implementing sanitation measures, like removing soil clumps from field equipment before moving to another field, can help prevent not only the spread of the wilt pathogen but also other soil-borne pathogens like tobacco cyst nematodes. Despite these practices, the spread of *Fusarium oxysporum* between farms is still more prevalent and occurs at a quicker rate than expected (Wichuk *et al*., 2011).

Wounding roots from close cultivation, hoeing or laying drip tape irrigation creates openings for infection, leading to heightened Fusarium wilt (Lucas, 1975). Decreased wounding leads to a decrease in wilt incidence and severity. Root knot nematodes (Meloidogyne spp.) and tobacco cyst nematode (*Globodera tabacum*) also worsen Fusarium wilt, beyond just creating new infection sites from wounds (LaMondia & Taylor, 1991). Plants infested with nematodes prior to fungal exposure showed higher wilt rates and severity compared to simultaneous exposure. G. tabacum infestation was found to cause greater wilt compared to equivalent numbers of *Meloidogyne hapla*. Early season control of nematodes in field trials resulted in lower incidence and severity of Fusarium wilt in tobacco (LaMondia & Taylor, 1987).

The management of various Fusarium wilt diseases has been achieved by adjusting soil pH and nutrient levels (Engelhard & Woltz, 1973). Factors such as nitrogen sources, lime, calcium, and soil pH have been linked to the occurrence and severity of the disease in tomatoes and chrysanthemums (Engelhard & Woltz, 1973; Woltz & Jones, 1973). LaMondia and Rathier (1995) found that calcium was the most effective of the tested nutritional factors for managing Fusarium wilt in tobacco. Lack of calcium has been linked to the development of the disease, as it regulates the production of callose, a defence response in plants against vascular wilt pathogens. Calcium is also believed to be the easiest nutrient to manipulate in the production of Connecticut broadleaf tobacco (Woltz & Jones, 1973). Fusarium wilt can occur in different soil pH levels, but increasing the pH to over 6.4 may have an impact on the disease. However, black root rot can become severe in soils with a pH between 5.6 and 6.0 (Lucas, 1975). While adding large amounts of gypsum to the soil can suppress Fusarium wilt in susceptible broadleaf tobacco, it had little effect at low levels of disease incidence. The amount of gypsum required to reduce wilt (13,400 kg/ha) is much higher than what is commonly used by tobacco growers (340-560 kg/ha) and may have negative agronomic effects, such as reduced crop growth (LaMondia & Taylor, 1991).

The presence of the fungus in a field lasts for several years without a vulnerable host. The success of using crop rotation to decrease the density of Fusarium wilt pathogens in the soil varies greatly, based on the survival of the chlamydospores (Nelson, 1981) and the pathogen's ability to infect the roots of resistant crops and other non-affected plant species (Gordon *et al*., 1989). The impact of rotation crops and resistant plants on the pathogen populations in the soil over time must be evaluated for each specific pathosystem and crop. For fields affected by *F. oxysporum*. f. sp. nicotianae, it is not recommended to grow tobacco in rotation with sweet potatoes as both are susceptible to the same wilt pathogen. On the other hand, flue-cured tobacco can be grown with cotton, but burley and dark tobacco should not as they are also susceptible to the same strains of the pathogen as cotton (Shoemaker & Shew, 1999).

Conclusion

Black Shank and Fusarium Wilt are serious diseases affecting tobacco crops caused by *Phytophthora nicotianae* and *Fusarium oxysporium f. sp. nicotianae*, respectively. Both diseases cause significant damage to tobacco plants and have a significant impact on production. To manage these diseases, an integrated approach that includes cultural practices, chemical treatments, and host resistance is needed. The most efficient approach is the use of resistant varieties, but this can negatively impact yield. Chemical fungicides can also be used, but there is a risk of resistance development. The successful control of Fusarium wilt has been through the use of resistant tobacco cultivars. Soil management practices, such as crop rotation and adjusting soil pH and nutrition, may also play a role in reducing the impact of these diseases. The presence of the pathogens can last for several years and the impact of rotation crops and resistant plants on their populations over time must be evaluated.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

References

Anderson PJ (1944) Diseases of tobacco in 1943. *CAES Bulletin* ***478***: 756-757.

Antonopoulos DF, Melton, T & Mila AL (2010) Effects of chemical control, cultivar resistance, and structure of cultivar root system on black shank incidence of tobacco. *Plant disease* ***94***(5): 613-620. DOI: 10.1094/PDIS-94-5-0613

Armstrong GM & Armstrong JK (1968) Formae speciales and races of Fusarium oxysporum causing a tracheomycosis in the syndrome of disease. *Phytopathology* ***58***: 1242-1246.

Bennett RS, Spurgeon DW, Detar WR, Gerik JS, Hutmacher RB & Hanson BD (2011) Efficacy of four soil treatments against Fusarium oxysporum f. sp. vasinfectum race 4 on cotton. *Plant disease* ***95***(8): 967-976. DOI: 10.1094/PDIS-09-10-0696

Bittner RJ & Mila AL (2016) Effects of oxathiapiprolin on Phytophthora nicotianae, the causal agent of black shank of tobacco. *Crop Protection* ***81***: 57-64. DOI: 10.1016/j.cropro.2015.12.004

Bittner RJ & Mila AL (2017) Efficacy and timing of application of oxathiapiprolin against black shank of flue-cured tobacco. *Crop Protection* ***93***: 9-18. DOI: 10.1016/j.cropro.2016.10.019

Cartwright DK & Spurr Jr HW (1998) Biological control of Phytophthora parasitica var. Nicotianae on tobacco seedlings with non-pathogenic binucleate Rhizoctonia fungi. *Soil Biology and Biochemistry* ***30***(14): 1879-1884. DOI: 10.1016/S0038-0717(98)00019-4

Clark CA, Hyun JW & Hoy MW (1998) Relationships among wilt-inducing isolates of Fusarium oxysporum from sweetpotato and tobacco. *Plant disease* ***82***(5): 530-536. DOI: 10.1094/PDIS.1998.82.5.530

Csinos AS & Bertrand PF (1994) Distribution of Phytophthora parasitica var. nicotianae races and their sensitivity to metalaxyl in Georgia. *Plant Dis.* ***78***: 471-474. DOI: 10.1094/PD-78-0471

Csinos AS & Minton NA (1983) Control of tobacco black shank with combinations of systemic fungicides and nematicides or fumigants. *Plant disease* ***67***(2), 204-207. DOI: 10.1094/PD-67-204

Dukes PD & Apple JL (1965) Effect of oxygen and carbon dioxide tensions on growth and inoculum potential of Phytophthora parasitica var nicotianae. *Phytopathology* ***55***(6): 666.

Engelhard AW & Woltz SS (1973) Fusarium wilt of chrysanthemum: Complete control of symptoms with an integrated fungicide-limenitrate regime. *Phytopathology* **63**: 1256-1259. DOI: 10.1094/Phyto-63-1256

Eriksen MP, Mackay J, Schluger NW, Islamic F, & Drope J (2015) The tobacco atlas. *Food and Agriculture Organization of the United Nations*. http://www.fao.org/land-water/databases-and-software/crop-information/tobacco/en/

Erwin DC & Ribeiro OK (1996) Phytophthora diseases worldwide. APS Press.

Erwin DC, Bartnicki-Garcia S, & Tsao PHT (1983) Phytophthora: its biology, taxonomy, ecology, and pathology. *American Phytopathological Society.*

Gallegly ME & Hong C (2008) Phytophthora: Identifying Species by Morphology and DNA Fingerprints. American Phytopathological Society.

Gallup CA (2009) The impacts of black shank resistance management on the population biology of Phytophthora nicotianae in Tobacco. North Carolina State University.

Gallup CA, McCorkle KL, Ivors KL & Shew D (2018) Characterization of the black shank pathogen, Phytophthora nicotianae, across North Carolina tobacco production areas. *Plant disease* ***102***(6): 1108-1114. DOI: 10.1094/PDIS-02-17-0295-RE

Gallup CA, Sullivan MJ & Shew HD (2006) Black shank of tobacco. *Black shank of tobacco*. DOI: 10.1094/PHI-I-2006-...

Garner WW (1951) *The production of tobacco.* (Rev. 1st ed. ed.). Philadelphia. http://hdl.handle.net/2027/uc1.b4597241

Gooding GV & Lucas GB (1959) Factors influencing sporangial formation and zoospore activity in Phytophthora parasitica var, nicotianae. *Phytopathology* ***49***(5): 277-281.

Gordon TR, Okamoto D & Jacobson DJ (1989) Colonization of muskmelon and nonsusceptible crops by Fusarium oxysporum f. sp. melonis and other species of Fusarium. *Phytopathology* ***79***(10), 1095. DOI: 10.1094/Phyto-79-1095

Hu JH, Hong CX, Stromberg EL & Moorman GW (2008) Mefenoxam sensitivity and fitness analysis of Phytophthora nicotianae isolates from nurseries in Virginia, USA. *Plant Pathology* ***57***(4), 728-736. DOI: 10.1111/j.1365-3059.2008.01831.x

Jacobi WR, Main CE & Powell NT (1983) Influence of temperature and rainfall on the development of tobacco black shank. *Phytopathology* ***73***(2): 139-143. DOI: 10.1094/Phyto-73-139

Jeffers SN & Martin SB (1986) Comparison of two media selective for Phytophthora and Pythium species. *Plant disease* ***70***(11): 1038-1043. DOI: 10.1094/PD-70-1038

Jin J & Shew HD (2022) Impacts of Continued Exposure to a Susceptible Host Genotype on Aggressiveness of Phytophthora nicotianae Isolates Adapted to Multiple Sources of Partial Resistance. *Plant Disease* ***106***(2), 373-381. DOI: 10.1094/PDIS-09-20-1972-RE

Johnson J (1921) Fusarium-wilt of tobacco. *Journal of Agricultural Research* ***20***(1): 515-535.

Jones JL, Gupton CL & Terrill TR (1972) Inheritance of Resistance to Isolates of Fusarium oxysporum var. batatas in Burley Tobacco 1, 3. *Crop science* ***12***(4): 522-525. DOI: 10.2135/cropsci1972.0011183X001200040042x

Jones KJ & Shew HD (1995) Early season root production and zoospore infection of cultivars of flue-cured tobacco that

differ in level of partial resistance to Phytophthora parasitica var. nicotianae. *Plant and soil* ***172***(1): 55-61. DOI: 10.1007/BF00020859

Klotz LJ & DeWolfe TA (1958) Techniques for isolating Phytophthora spp. which attack citrus. *Plant Disease Reporter* ***42***(675), 41-46.

LaMondia JA & Taylor GS (1987) Influence of the tobacco cyst nematode (Globodera tabacum) on Fusarium wilt of Connecticut broadleaf tobacco. *Plant Disease*. DOI: 10.1094/PD-71-1129

LaMondia JA & Taylor GS (1991) New Fusarium wilt-resistant Connecticut broadleaf tobacco varieties. Bulletin-Connecticut Agricultural Experiment Station. DOI: 10.5962/bhl.title.51058

LaMondia JA (2013) Registration of 'B2'Connecticut Broadleaf Cigar-Wrapper Tobacco Resistant to Fusarium Wilt, Tobacco Mosaic Virus, Cyst Nematodes, and Blue Mold. *Journal of Plant Registrations* ***7***(1): 58-62. DOI: 10.3198/jpr2011.12.0656crc

Lucas GB (1975) *Diseases of tobacco* (3rd ed.). Biological Consulting Associates. Raleigh, NC.

McCants CB, & Woltz WG. (1967) Growth and Mineral Nutrition of Tobacco. *In Advances in Agronomy, Elsevier* ***19***: 211-265. DOI: 10.1016/S0065-2113(08)60736-1

Meng Y, Zhang Y, Ding Q & Shan W (2014) Phytophthora parasitica: a model oomycete plant pathogen. *Mycology* ***5***(2), 43-51. 10.1080/21501203.2014.917734

Nelson PE (1981) Life Cycle and Epidemiology of Fusarium oxysporum. *Fungal Wilt Diseases of Plants* 51-80. DOI: 10.1016/B978-0-12-464450-2.50008-5

Panabieres F, Ali GS, Allagui MB, Dalio RJ, Gudmestad N, Kuhn ML, Guah Roy S, Schena L & Zampounis A (2016) Phytophthora nicotianae diseases worldwide: new knowledge of a long-recognised pathogen. *Phytopathologia Mediterranea* ***55***(1), 20-40. http://www.jstor.org/stable/43871852

Qu T, Shao Y, Csinos AS & PJ (2016) Sensitivity of Phytophthora nicotianae from tobacco to fluopicolide, mandipropamid, and oxathiapiprolin. *Plant Disease* ***100***(10): 2119-2125. DOI: 10.1094/PDIS-04-16-0429-RE

Ren X, Ji X, Qi W, Meng Z, & Qiao K. (2018) Evaluation of the combination of propamocarb hydrochloride and fluopicolide for management of black shank on tobacco. *Crop Protection* ***114***: 12-17. DOI: 10.1016/j.cropro.2018.08.013

Rivera YR & Thiessen L (2017) Black Shank of Tobacco. NC State Extension Publications.

Shan W, Marshall JS & Hardham AR (2004) Gene expression in germinated cysts of Phytophthora nicotianae. *Molecular Plant Pathology* ***5***(4): 317-330. DOI: 10.1111/j.1364-3703.2004.00231.x

Shattock RC (1988) Studies on the inheritance of resistance to metalaxyl in Phytophthora infestans. *Plant pathology* ***37***(1): 4-11. DOI: 10.1111/j.1365-3059.1988.tb02188.x

Shoemaker PB & Shew HD (1999) Major Tobacco diseases: Fungal and bacterial diseases. *Tobacco: Production, Chemistry and Technology*. 189-191.

Smith TE & Shaw KJ (1943) Pathogenicity studies with Fusaria isolated from tobacco, sweet potato and cotton. *Phytopathology* ***33***: 469-483.

Thiessen L, Gorny A & Moparthi S (2022) Managing diseases. NC State Extension Publications.

Todd FA (1981) Flue-cured Tobacco-Producing A Healthy Crop.

Tsao PH & Ocana G (1969) Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. *Nature* ***223***(5206): 636-638. DOI: 10.1038/223636a0

Tso TC (1972) Physiology and biochemistry of tobacco plants. Dowden, Hutchinson & Ross.

Wichuk KM, Tewari JP & McCartney D (2011) Plant pathogen eradication during composting: a literature review. *Compost Science & Utilization* ***19***(4): 244-266. DOI: 10.1080/1065657X.2011.10737008

Wollgiehn R, Bräutigam E, Schumann B & Erge D (1984) Effect of metalaxyl on the synthesis of RNA, DNA and protein in Phytophthora nicotianae. *Zeitschrift fûr allgemeine Mikrobiologie* ***24***(4): 269-279. DOI: 10.1002/jobm.3630240417

Woltz SS & Jones JP (1973) Interactions in Source of Nitrogen Fertilizer and Liming Procedure in the Control of Fusarium Wilt of Tomato1. *HortScience* ***8***(2): 137-138. DOI: 10.21273/HORTSCI.8.2.137

Yang X, Tyler BM & Hong C (2017) An expanded phylogeny for the genus Phytophthora. *IMA fungus* ***8***(2): 355-384. DOI: 10.5598/imafungus.2017.08.02.09