

Review Article

Integrated Management of Peanut Foliar Diseases by *Cercospora*, *Alternaria* and Web Blotch

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Abstract

The concept of integrated management was first conceptualized in the 1950s by entomologists to describe the selection of specific pesticides with adjusted dosages and application timings to control harmful insects and mites while preserving beneficial insects. However, the management of disease conveys the idea of a continuous process that is more preventative in which the loss from disease is kept below some economic level. Over the decades, the concept was eventually expanded to include all relevant biological, cultural, and chemical tactics to manage insect, pathogen and weed pests of crops; a multi-tactic approach to pest management. Notably, the concept of plant health management was taken up by the American Phytopathological Society in the early '90s, which resulted in series of Plant Health Management publications such as "Wheat health Management" and "Potato Health Management". In this review article, we will discuss the closely associated pathogens included *Cercospora*, *Alternaria*, and Web blotch, which cause peanut foliar disease, and its management strategies.

Keywords: Insects; *Cercospora*; Disease; Management; Program

Introduction

A successful disease management program depends on a number of factors; for example; pest management, selection of appropriate varieties, irrigation system that minimizes leaf wetness, fertilizer program that results in optimal plant growth, plant density, and canopy management that facilitate optimum air circulation and pesticide coverage when needed, disease-free seed production program, effective pest monitoring by scouting regularly during the season, minimize transplant shock, a safe and sound harvesting & shipping procedure that maximizes shelf life and produce quality [1]. IPM/IDM refers to all the tactics available to growers for example; cultural, host-plant resistance, biological, field scouting, a chemical which provide acceptable yield and quality at the least cost and compatible with the environment. In a holistic approach to plant health management, accentuating to maximizing total yield and control of pests to maximize total economic return, food safety, and environmental protection. Notably, the production economics becomes the bottom line.

The major elements of an IDM approach are described such as:

Prevention: The entry of the pathogen can be inhibited through planting materials, irrigation water, farming tools or equipment and workers.

Monitoring: Regular field scouting is imperative to recognize disease symptoms and plant disease vectors.

Accurate Disease Diagnosis: Diagnostic clinics and identifying the causal organism for a disease as relevant to determine the appropriate biological and chemical management options.

Development of acceptable disease threshold: Asses the effect of disease and tentative yield loss. For example, 15% disease incidence

result of a specific pathogen may not cause substantial production gap in oilseed crop where their chemical control may be an unnecessary expense.

Optimal Selection of Management Tools: To determine an effectively integrated management plant mostly depends on the disease, crop, and field history. The previous cropping pattern is imperative in assessing the risk involved in the production. Agronomic, cultural, host-plant resistance, biological and chemical control options need to be considered for the specific location [1].

Among the four "fundamental principles of plant disease control" as described by Exclusion is the first principle among the four principles of disease control and is directed to the causal agent/pathogen. It refers to prevent the entrance and establishment of a pathogen in an uninfected area, i.e., by quarantine. Preventing the host plant from coming into contact with a plant pathogen. The avoidance of disease by excluding pathogens from the garden, field, region, state or country.

The use of pathogen-free propagating material is related to quarantines in the implementation of exclusion as a disease management strategy. The goal of both is to prevent the introduction of large numbers of pathogens to an area where a plant or a crop is to be planted. Both approaches are also directed at the initial pathogen population. Both approaches are also largely regulatory in nature. Pathogen-free may be a misnomer for propagating a number of plant materials because a low population of the pathogen is the goal instead of absolute elimination of the pathogen.

Eradication and sanitation have a certain level of difference. Eradication refers to the complete removal of inoculum (pathogen or infected plant materials) from a geographical area. However, it is biologically not feasible. Sanitation replaced the concept of

eradication. It illustrates the removal of pathogen inoculum from the host or from the soil rather than the total eradication of the pathogen. Sanitation is more pragmatic technically and biologically as it reduces the amount of inoculum and reduces the chances of infections in a given ecosystem. One method of managing seed-borne disease using the principle of exclusion is to locate seed production in an isolated area, usually where moisture is not a factor contributing to foliar fungi or bacterial infection. These seed production area is usually isolated away from the commercial production area of that crop.

Before undertaking the disease management approaches, it is necessary to understand the following parameters of disease as follows; Assessment of the amount or efficacy of primary inoculum: This is the most important phenomenon of forecasting plant disease either caused by a monocyclic or polycyclic pathogen. However, this approach is highly emphasized on monocyclic pathogen as it considered mainly the amount of initial inoculum. A large amount of initial inoculum or if the number of generations (secondary cycles) is small. For some plant pathogens, the initial pathogen population is always very large but disease severity still varies from one season to another. The seasonal variability in disease intensity is due to changes in the environment that affect pathogenesis (inoculum efficacy). As a result, there is no direct relation between the size of the initial population (always large) and subsequent disease. Assessment of the speed of the secondary cycles: This is very important for diseases that have very small amounts of initial inoculum and the potential for a large number of rapidly produced secondary cycles are high. Forecast based on the number of secondary cycles or the amount of secondary inoculum that can be produced is useful for plant diseases. For these type of diseases, growers need a forecasting system that will indicate when management tactics should be initiated and what intensity it should be [1].

Cultural methods can be frequently utilized to modify the environment in order to suppress disease development. Cultural practices and its modification can influence crop-climate in several ways. Besides, cultural practices brought very little success in some areas, for example; in a climate with few seasonal changes and all year around rain, in regions with uniform soil conditions and essentially flat land, and or crops grown under conditions approaching their natural habitat or the conditions for which they have been bred. However, the extent to which cultural practices can change the crop-climate depends primarily on the microclimate and the adaptation of crops to it. With the increasing diversity of cropping conditions, the scope for cultural practices to be value for disease control. For example; Use of slopes, adding water via irrigation, plating dates, and planting in different seasons. Cultural practices can influence crop-climate by improving growth and overall health under two primary conditions. For instance; Annual crops are grown out of season in climates with contrasting seasons, for example, hot and dry while rain and cold etc. Mitigating stress or disease by adjusting the planting dates and managing by mulching, tillage, and moisture. Perennial crops grown in areas that differ from natural habitat, crops moved from tropical conditions to warm areas without tree cover, for example, cocoa. Temperate zone crops are grown in warmer climates during winter months, for example, deciduous fruits. Topography, use of slopes, moisture management, and shading to trigger flowering and fruiting. Cultural practices for example; sanitation, crop rotation, tillage have

a positive effect on primary inoculum, and rate of inoculum build up [2]. Crop nutrition has positive effects on length of susceptible phase, length of the latent period, the apparent rate of infection, a period of infectiousness and rate of inoculum build up. While irrigation has a positive effect on primary inoculum, length of susceptible phase, length of the latent period, a period of infectiousness, the apparent rate of infection, and rate of inoculum build up. Time of planting and planting practices have a positive effect on all these parameters. Harvesting practices have a positive effect on primary inoculum. The proximity of inoculum sources has a positive effect on primary inoculum, length of susceptible phase and rate of inoculum build up. Several principles can be demonstrated by the previous parameters. For instance; all cultural practices except crop rotation and planting practices affect inoculum buildup and the subsequent rate of buildup, Secondly, crop rotation and planting practices affect all other factors involved in pathogen infection, Last but not least, the length of time a host remains susceptible is affected by all cultural practices that affect the size of inoculum and rate of inoculum buildup [1]. Seed treatment is assumed to be the simplest and least costly chemical control measure. It is an essential farm practice and treating true seed treatments are very effective in protecting seed and in managing many seed-borne pathogens. This type of treatments is excellent as a control measure when integrated into other strategies and tactics for disease management.

At a Glance Peanut

Peanut (*Arachis hypogaea* L.), also known as groundnut or monkey nut (UK) that belongs to Fabaceae family. It is an important leguminous oilseed crop which is cultivated approximately 23.1 million hectares, with a production of 44 million metric tons in 2016. While China itself produced just 38% of the total production [3]. In the US, peanuts are mainly grown in the southern states where the environmental conditions are conducive for the leafspots epidemics [4]. Peanut providing all source of nutrients for example; protein, fat, oil, and carbohydrates. Peanut has an important natural phenomenon to synthesize stilbene phytoalexins (antibiotic compounds) that resist the fungal invasion. It is believed that the manipulation of host plant resistance mechanisms is much more appreciable to achieve an economical and eco-friendly approach to manage the diseases. Peanut flowers have been reported to trigger a high level of flavonoids and spermidines which is unique, it helps to protect against pests [5]. Several studies have been reported that the peanut pod yields positive correlated with disease severity aggravated by the early and late leaf spot [6,7].

Foliar Diseases of Peanuts

Early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercospora personatum*)

There are a number of foliar diseases that seriously affects the yield return of peanut, for example; Early Leaf Spot (ELS) caused by *Cercospora arachidicola* Hori. Late Leaf Spot (LLS) by *Cercoporiidium personatum* (Berk. & M.A. Curtis) Deighton these diseases have profound importance on a global scale. Both diseases are caused by very closely related pathogens; early leaf spot is the most common foliar disease of peanuts while late leaf spot appears infrequently. Moreover, the disease cycle of these pathogens and the nature of damage by the two diseases are similar. Late leaf spot causing

pathogen is more virulent than the early leaf spot causing pathogen and more difficult to mitigate the disease. These diseases are severity is high if peanuts are grown in the same field year after year, without following any crop rotation. Due to shedding or defoliation of leaflets, drastically reduce the tonnage. In some cases, more than 50% yield loss can happen as a consequence of heavy defoliation. The dropping of leaves affects healthy leaf area that might result in vulnerable stems and pegs undermine the pods to fall off during digging and harvesting. Hence, appropriate control measures are imperative for producing high yielding peanut crop [8].

Symptoms: Both pathogens cause infection any above ground portion of the plant, though the leaf spots are the clearest symptom. Under favorable conditions and the same cropping history leaf symptoms generally, appear between 30-50 days after planting. Early and late spot first appears as brown or black and pinpoint size dots on the upper leaf surface. Symptoms of ELS on the upper leaf surface are irregular to circular, dark brown spots typically surrounded by a yellow halo (Figure 1). On the other hand, symptoms of LLS on the upper leaf surface are irregular to circular, but dark brown to black spots surrounded by a faint yellow halo or without a halo (Figure 2). ELS on the lower leaf surface is smooth in texture, brown spots typically surrounded by a yellow halo, no sporulation (Figure 3). LLS on the lower leaf surface are typically black spots, rough texture surrounded by a faint yellow halo or without a halo, abundant moldy tufts of sporulation (Figure 4).

Disease Cycle of ELS and LLS

Pathogen inoculum of ELS & LLS, for example, microscopic spores infect plants. The production of infective propagules is favored by high humidity. Both pathogens usually produce a profuse number of spores on the infected plant parts. Primary inoculum is the initial source of infection in the growing season as they are produced on plant debris in the soil. Symptoms appear on the leaves about 10-14 days after infection, and new spores are produced in mature spots in short period. The new spores again (secondary inoculum) infect new leaves. These inoculums dispersed by splashing rain, wind, and insects. The number of spots increases under conducive conditions and several secondary cycles occur per growing season. Crop residue of the peanut in the field where the same crop is cultivated continually often results in early and fast development of leaf spot. Frequent irrigation, high humidity, warm temperature, and leaf wetness



Figure 1: Symptoms of ELS on the upper leaf surface are irregular to circular, dark brown spots typically surrounded by a yellow halo [9].



Figure 2: Symptoms of LLS on the upper leaf surface are irregular to circular, dark brown to black spots surrounded by a faint yellow halo or without a halo [9].



Figure 3: ELS on the lower leaf surface is smooth in texture, brown spots typically surrounded by a yellow halo, no sporulation [9].



Figure 4: LLS on the lower leaf surface are typically black spots, rough texture surrounded by a faint yellow halo or without a halo, abundant moldy tufts of sporulation [9].

promote disease development [8].

Management for the ELS and LLS

Control of both diseases is imperative to prevent the resulting heavy defoliation and yield loss. An integrated disease management program comprises with genetic resistance, cultural practices, and a fungicide program. Host genetic resistance; genetic resistance is least expensive and least costly methods of plant disease management. Developing resistance from the host side, the inheritable ability of host

plant to resist a pathogen and it is based on the genetic composition of host and pathogen. Most economic efficient and environmentally friendly way to control the disease. Agriculture producer needs to screen the right variety that is resistant to a particular disease. Genotypes of a host species that differ in response to races of a pathogen. For example; A new peanut variety such as “Danwon” found to be tolerant against early and late spot causing pathogen, this variety has a short stem and lodging resistance compared to reference variety, it has also been reported to yield approximately 4.52 MT/ha which was 6% more than the reference variety [9]. In general, cultural practices will give partial control of leaf spot. For example, crop rotation with other non-host crops and management of crop residue by tillage, it paves the way of delay the onset of disease and minimizes the leaf spot development. As a result, it reduces the level of primary inoculum in the field. Nevertheless, peanut cultivars or varieties differ in reaction to leaf spot, but the levels of resistance in peanut varieties alone are not adequate to provide adequate disease control. For example, Runner varieties are partially resistant while Spanish varieties are most susceptible and Virginia types are intermediate. Free moisture favors the development of many foliar diseases. The rainfall and dew are beyond the control while irrigation can be regulated. For example; Irrigation methods, schedules, and rates can all be managed to control plant disease. Timely application of irrigation will also help reduce leaf spot. If the humidity is high, the application and the adequate amount of water to be considered will help to maintain a drier canopy and soil surface between irrigations.

Besides, cultural controls followed by a fungicide program are usually inevitable to prevent yield loss from leaf spot. Fungicides application to the target is always a challenge. There is a number of fungicides are available which provide an excellent leaf spot control while applied on a recommended schedule. Preventative organic fungicides can be used to control plant pathogens, for example, EBDCs or ethylenebisdithiocarbamates, chloronitriles, QoIs, and sterol biosynthesis inhibitors. Among them, EBDCs or Ethylenebisdithiocarbamates, and chloronitriles are belonged to preventative organics/Organo Metallic class fungicides widely used. They are also known as mancozeb (Manzate, Dithanr M-45, Penncozeb, Fore), maneb (Maneb, Dithane M-22), zineb (Zineb). They have a broad spectrum and multi-site of action; hence, this group has low resistance risk. There are a number of fungicides, which manage foliar diseases also control soil-borne diseases, for instance, limb rot, Sclerotinia blight, and southern blight. Fungicides act to protect healthy leaves and plants from infection, they must be applied preventatively and the purpose of the spray should be to minimize the defoliation at harvest. The recommended application schedules include a 14-day schedule and programs the base applications on weather conditions. There are a number of factors that affect the management practices, for example; cultural practices and application schedule used the need of for soil-borne disease control and the level of control desired, and type of applications and cost of fungicide programs vary depending upon the variety. Recently, many fungicides have been reported to have resistant problems while they used exclusively over a period of time. Notable, fungicides with multiple modes of action can be applied in alternation, in a tank mix, or in a pre-mix to deter the development and build-up of resistance [8].

To manage the peanut disease a meta-analytical approach was applied to quantify relationships of end season defoliation and yield loss in runner and Virginia varieties. In runner types, yield loss increased to 1.3% to 2.8% with the 10% increase in defoliation. On the other hand, Virginia types yield reduced to 1.6% to 3.2% with the 10% increase in defoliation [10].

In another study, disease progress of ELS and the components of tolerance to *C. arachidicola* (Ca) and *C. personatum* (Cp) in Runner-type peanut cultivars were assessed. For example, Georgia Green, Georganic, and DP-1 were subjected to the field study to monitor the progress of leaf spot incidence and severity. Time of disease onset was 9 days delayed in DP-1 compared to Georgia Green and Georganic, which was estimated on the incidence model. To determine the components of resistance in these genotypes a detached leaf assay was used to understand the pathogenesis by Ca and Cp, Georgia Green has a higher infection frequency for both pathogen, lesion diameter, and percent necrotic diameter at 30 days after inoculation. DP-1 showed increased field resistance to early and late leaf spots. On the other hand, Georganic cultivar showed lower infection frequencies [11].

Seven years of field study in Penn State has demonstrated that the impact of intercropping on the epidemics of peanut leaf spots. The disease is often reduced by intercropping as it enhances the plant diversity although the variability can be high. Intercropping, for example, strip patterns with cotton, and reduced fungicide application demonstrate low levels of ELS (25-41%, AUPDC), but this practice was not effective with the intercropping with maize and against the late leaf spot [12].

In recent years, ELS and LLS have been reported in all peanut producing states and multiple application of fungicides are advised to control the diseases. DMI fungicide tebuconazole resistance in the isolates of *Cercospora arachidicola* has been reported in Georgia and neighboring states. EC50 values were used to detect the tebuconazole resistance among *Cercospora* isolates [13].

Introduction of *Alternaria*

The genus *Alternaria* are ubiquitous, sustain under a wide range temperature, and includes nearly 300 species. These fungi either are saprophytic microorganisms or plant pathogenic alternaria leaf blight reported firstly in Germany in 1855; it described as a sporadic disease in carrot in Northern Europe. While in the USA, it was reported first in Louisiana in 1890, the causal agent identified as *Macrosporium carotae*. Subsequently, it was reported in Massachusetts and Florida, and since then the damage was found to be pronounced in carrot growing areas in the other parts of the world [14]. However, few studies on peanut crop have been reported for *Alternaria* in the U.S. The outbreak of *Alternaria alternata* cause leaf blight in peanut has been reported in Gujarat, India in 2012 [15]. *A. alternata* is known as a plant pathogenic saprophyte and regarded as a weak filamentous fungus. Even though, it involves crops damage in the field and escalates post-harvest decay. The pathogenic species produce harmful mycotoxin, which can contaminate plant products and are subject to cancer. *A. alternata* found mainly in an anamorphic stage in nature. The taxonomy is based primarily on the morphology and the development of conidia and conidiophores. Secondly, the host plant association and colony morphology. The typical structure of the

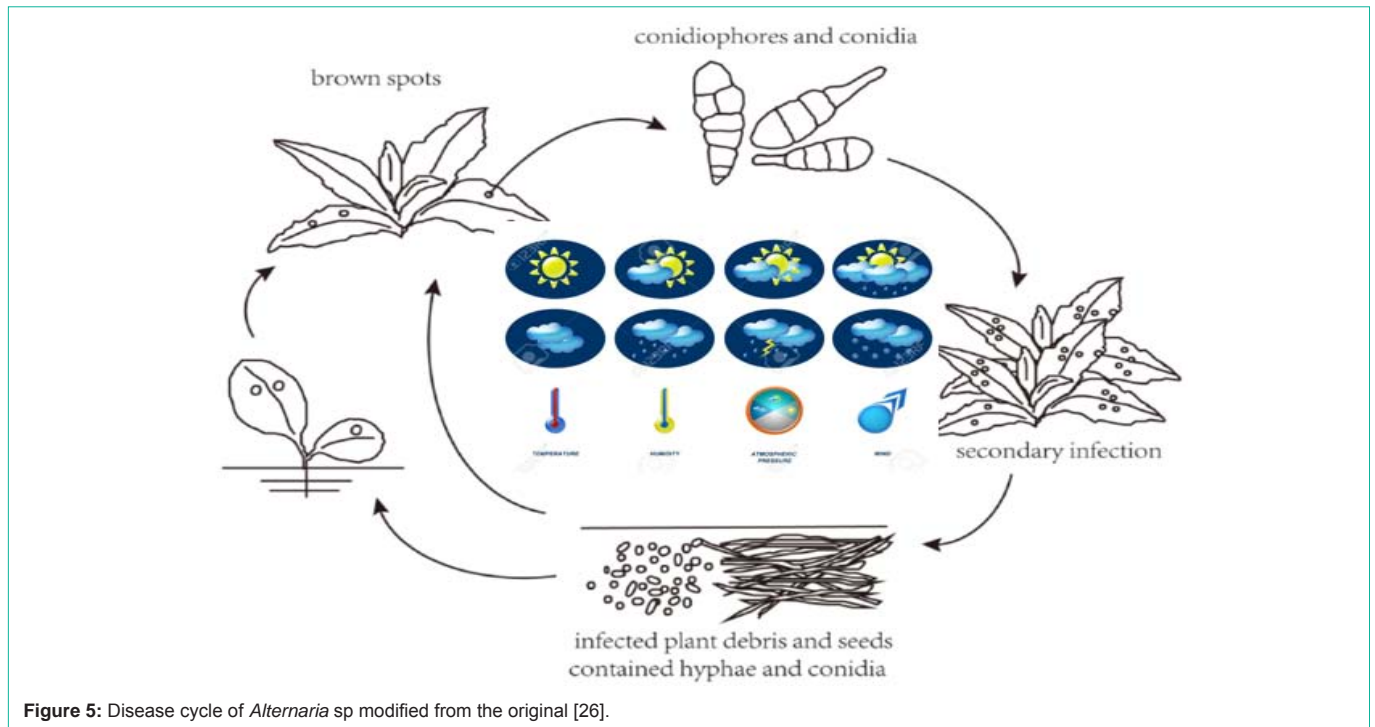


Figure 5: Disease cycle of *Alternaria* sp modified from the original [26].

conidia are long chained (10 or more), polymorphous, transverse (1-9) and several longitudinal or oblique septate [16]. There is variability in conidial shape and size. Therefore, it is challenging to differentiate the *Alternaria alternata* from other *Alternaria* species. *Alternaria* leaf blight of sunflower is considered as a significant paramount disease as it hampers up to 80% and 30% seed and oil production, respectively. Lately, *A. alternata* is synonymized with *A. tenuissima* [17]. Recently, *A. alternata* reported to causing leaf blight in South Africa in 2018; these findings indicated that *Alternaria* genus belongs to a complex phylogenetic grouping. Synonymize certain species is often controversial. Notably, *Alternaria* genus has a large number of recognized species. Hence, the identification is somehow challenging. Apart from that, there is various spore dimension range, and it overlaps among the species. Conidial morphology and catenation are affected by environmental factors; for instance; light and humidity. Therefore, morphological characterization may be unreliable. Furthermore, the genus *Alternaria* was formerly also often characterized based on host association, till now it has been reported to infect more than 4000 host plants all over the world [19].

To achieving sustainable disease management, it is prerequisite to understand the genetic structure and evolutionary trajectory of the pathogen populations in the agroecosystems. Over the last two decades, simple sequence repeats or microsatellite markers have been used for population genetics studies that enriched our understanding in fungal biology and epidemiology of plant pathogens. Nevertheless, it has complicity in mutation-migration-drift equilibrium, null alleles, homoplasy, and genome-wide patterns of diversity [20,21].

On the flip side, Single Nucleotide Polymorphism (SNPs) offers genome-wide coverage, but it requires a higher number of loci to be analyzed. These criteria hindered the use of SNPs in population genetics studies of non-model organisms [21,22].

However, Genotyping by Sequencing (GBS) method involves discovering Single Nucleotide Polymorphisms (SNP) to facilitate Genome-Wide Association Studies (GWAS) and reduce genome complexity. After digestion with restriction enzymes, PCR is followed up to increase fragments pool, and GBS libraries are sequenced using next-generation sequencing technologies. It better reflects the multitude of polymorphic markers that help to understand the genome-wide genetic diversity of populations and improve resolution to identify fine-scale genetic variation or detect rare recombination events [23]. Recently, genotyping by sequencing approach confirmed the global genotype flow in *Cercospora beticola* populations [21].

The disease cycle of *Alternaria*

Alternaria leaf spot disease in peanut is caused by three *Alternaria* species, for example, *A. arachidis*, *A. alternata*, and *A. tenuissima* [24]. The disease cycle of *Alternaria* begins with the asexual spores or long chain conidia produced on brown leaf spots, and often colonizes in plant stems and roots; it results in black rot or wounds. It can disperse through air currents and rainfall. On the flip side, conidia dispersal halted with prolonged high winds, long periods of leaf wetness and cool temperature. High humidity favors disease infection and sporulation, while lower humidity conducive for conidial dissemination. If the environmental condition is favorable, it paves the way of secondary infection, and the conidia spread very rapidly in the field. Temperature study at 24°C has observed increasing damage with increasing hours of leaf wetness from 8 to 56 h. Conidia and conidiophores are produced at a temperature range between 10 to 28 °C and relative humidity from 96 to 100 %. Conidia became airborne and released as the relative humidity drops. Spores deposited on leaf surfaces would germinate, and infection occurs under appropriate temperature and leaf wetness conditions [25].

It causes great loss of agriculture production, and the produced

mycotoxins threaten the health and animals [26]. The pathogen usually overwinters as a mycelial form in seed, crop debris, and diseased volunteer. *Alternaria* leaf blight reduces the photosynthetic surface area of the plants which ultimately affect the desired yields. However, a single control measure is not sufficient to control high disease pressure. Several integrated approaches for instance; clean seed, crop rotation, field sanitation, resistant cultivar selection, and fungicide applications [14].

Alternaria leaf spot of peanut

The leaf spot disease is triggered by three species of the *Alternaria* (soil-borne fungi). The primary source of inoculum is believed to be infected seeds. Severe yield losses can occur while the environmental conditions are conducive for the pathogen. The propagules (conidia) are dispersed by wind movement, rain and by insects, it facilitates the secondary spread between plants. Disease severity increases while the temperature is above 20 °C and high humidity perpetuate for a long time with prolonged leaf wetness. Irrigation after the rainy season provokes the disease incidence on peanut crops. Notable, the severity and occurrence of the disease affect the pod and fodder yields can be reduced by approximately 22 % and 63 %, respectively [27].

Symptoms: *A. arachidis* cause leaf spot, which is small brown, irregular shaped spots surrounded by a yellowish halo on leaves. On the flip side, *A. tenuissima* produces a “V” shaped blighting on the apical portions of leaflets. Subsequently, dark-brown lesion proceeds to midrib and the entire leaves appears to be blighted, curls inward and becomes brittle (leaf blight). The lesions developed by *A. alternata* are small, round to irregular in shape and spread over the whole leaf. Symptoms appear as chlorotic and water-soaked, but in a few days, they enlarge, they turn necrotic and also affect the adjacent veins known as leaf spot and venial necrosis. In some cases, middle portions rapidly withered up and disintegrate, leaving the leaf a ragged appearance that leading to defoliation of the plant [27].

Biology and Genome Size of Alternaria

This species grew well in artificial media, for example in Potato Dextrose Agar (50% PDA) and Water Agar. The hyphae are sub-hyaline to olive-brown and septate. The conidiophores are olive-brown and flexuous with a single terminal or one or two geniculate conidiogenous sites. Conidium is usually borne singly and appeared as long conidial chain. Conidium is medium to dark brown, long ellipsoid to obclavate, 60-100 x 15-25 µm (spore body), with 7 to 11 transepta and 1 to 3 longisepta in fewer than half of the transverse segments. Mature conidium is rostrate with a terminal filamentous beak 80-250 x 5 µm tapering distally. Teleomorph or sexual reproduction stage is mostly unknown [28]. Lately, a draft genome sequence of *A. alternata* (ATCC 34957) was completed by PacBio technology, and it assembled into 27 scaffolds with a total genome size of 33.5 Mb. The largest scaffolds reported up to 3.97 Mb [29].

Molecular Detection Methods and Its Importance

Pathogen detection is an important component of certification/seed regulatory programs. These test usually involve either direct examination of seeds, standard isolation techniques on media (semi-selective if available), grow out tests conducted in glasshouse/growth chamber or in a southern state (FL, CA, or HI) or test of seed or

seed extracts, involving serology or PCR detection technology. The Internal Transcribed Spacer (ITS) region and its metabarcoding pave the way to characterize microbial communities and it is widely used in taxonomy and molecular phylogeny of fungi. The Internal Transcribed Spacer (ITS) regions are the spacer DNA situated between the small-subunit Ribosomal RNA (rRNA) and large subunit rRNA genes in the chromosomes. The rRNA maturation process followed by the excisions of the External Transcribed Sequence (5'ETS) and ITS. So far it has been used in molecular systematics; within the species and also to genus level, for instance; the universal ITS1 + ITS2 primers that allow selective amplification of fungal sequences [30]. A recent study has conducted in the Andalusia region, Southern Spain in 2017, *P. terebinthus* plants observed leaf blight symptoms in a commercial nursery. Later, *A. alternata*, isolate ColPat-420 has been detected via sequencing the Internal Transcribed Spacer region (ITS) and RNA Polymerase Second Largest Subunit (RPB2) with primers ITS4/ITS5 [31,32].

In another study, *Alternaria alternata* infections that cause brown leaf blight of shenguyon in China in 2017. The molecular detection was confirmed via the Internal Transcribed Spacer region (ITS) region of rDNA, and a histone gene was amplified using the primers ITS1 /ITS5 and H3-1a/H3-1b, respectively [33]. Nevertheless, the pathogen can be detected through the ITS, but the multi-gene pathogen detection is strong enough to withstand most challenges.

Against this backdrop, Genotyping-By-Sequencing (GBS) approach now been profoundly promising for highly diversified and large genomic species. There are some merits to this approach, for example; it can be generalized to any species at low-cost, it can be done quickly, extremely specific, simple and highly reproducible. It can reach the important regions of the genome that are inaccessible to sequence capture approaches. Furthermore, genomic libraries developed that contains reduced genomic complexity (Elshire et al. 2011)[23].

This approach considers methylation-sensitive restriction enzymes (REs). Thus repetitive regions of genomes are minimized, and it facilitates to capture low copy regions with two to three times the higher efficiency. Besides, it simplifies computational alignment problems in species with a high level of genetic diversity.

Management of the Alternaria Disease

In a disease triangle host, pathogen and environment are influenced by each other, however many plant diseases are managed by establishing environmental barriers between the host and the pathogen. These barriers are created by modifying or manipulating environmental factors that influence disease development. A current vogue in plant disease management is achieving a level of control by maintaining plant vigor since it is generally believed that healthy vigorous plants are more able to repel pathogens. This phenomenon is referred to as a holistic approach to plant disease management. Free moisture favors the development of many foliar diseases. The rainfall and dew are beyond the control while irrigation can be regulated. For example; Irrigation methods, schedules, and rates can all be managed to control plant disease. In recent years, irrigation practices switched from furrow to sprinkler, by either solid set or center pivot. The shortcomings of sprinkler methods are to enhance the dispersal of inoculum.



Figure 6: Web blotch symptoms on the upper leaf of peanut, typically irregular shaped brown blotches [9].

So far, there is no effective biological control available to manage the disease. However, an integrated approach with preventative measures has some success. For example; use of resistant or tolerant cultivars. In the US, private companies are mainly developing the resistant varieties but, in many cases, they do not want to disclose the genetic background of the resistant gene. Healthy disease-free seeds or certified pathogen-free seeds grew to manage the disease. Cultural practices for example; unwanted crop debris, weeds, alternate host, and volunteer plants are recommended to remove from cultivation area. The field visit is imperative for controlling the foliar disease, handpicking and destroying of the leaf and plant is necessary for the seedbeds. Agronomic practices such as; cropping sequences dramatically affect the intensity of plant diseases. For example; Mono cropping, growing a single crop year after year will obviously lead to deleterious effects, crop rotation with non-host crops is usually practiced for three years to control the disease. Tillage also affects the development of plant disease. Good seedbed preparation loosens soil and improve drainage, promotes vigorous, uniform and rapid emergence of seedlings. Conventional tillage operations, such as moldboard plowing, disking, harrowing, deep shanking, chisel plowing, will all invert or mix soil to depths of 8-14 inches. These operations either bury plant residues or dilute them (mix) plant residues, thereby reducing the population of initial inoculum for a number of diseases. Depending on the method of conventional tillage, plant diseases may also be suppressed because of improved soil drainage and seedbed preparation will enhance emergence. However, in the case of moldboard plowing, a hardpan/plow-pan compacted zone can form resulting in more moisture due to decreased percolation, less oxygen in the root zone which will impede root formation and promote infection by certain fungi (water molds). Deep plowing is advised to reduce the propagules of the pathogen. Chemical Control, for example, foliar application of copper oxychloride (3 g/l) after the emergence of symptoms which is effective against the disease. Mancozeb (3 g/L of water) application found to be effective to control the disease [24,34].

Web Blotch (*Phoma arachidicola*)

Peanut web blotch is an emerging threat for the peanut cultivation worldwide and it has been considered as one of the most important fungal diseases in China. To undertake effective integrated disease management, it is necessary to unveil the pathogenicity-related genes,

but there is very little genomic information is available. Recently, the draft genome of WB2 has been developed which is about 34.11 Mb and contains 37330 Open Reading Frame (ORFs), with G+C content 49.23 %. It reveals number secreted enzymes for example; oxidases, peroxidases, carbohydrate-active enzymes for degrading cell wall. Genome-based plant-pathogen interaction in-depth analysis would provide clues for disease management which is profoundly important peanut production [35].

In the U.S, this disease occurs in less frequency than the leaf spot but it can cause severe defoliation and yield loss when and where it occurs. For instance, the disease is profoundly observed on Spanish and Virginia varieties compare to runner types in Oklahoma. As they are moderately resistant that helps to reduce the damage [8].

Symptoms

The symptoms mostly found on the upper leaf surface, the typical characters are; roughly circular tan to dark brown blotches or net-like spots with irregular and light brown margins. The growth of the fungal strands appears on the lower surface, often net-like webbing. However, the blotches size varies from 1 inch or more in diameter (Figure 6) which is larger the leaf spot disease. Infected leaflets turn dry and become brittle and drop from the plant. Defoliation may lead up to 50% yield loss on Spanish varieties if the disease severity is not controlled in the right way [8].

Disease cycle

The disease cycle of the pathogen is not very clearly defined. The pathogen can perpetrate on crop debris or in residue in the soil. The disease is favored by cool (60 F to 70 F) wet weather and generally become a problem in the fall if the harvesting is delayed due to rainy weather. High humidity and prolonged wetness with lower temperature cause early leaf spot [8].

Disease management

Agronomic and cultural practices, for example; use of crop rotation and residue management practices recommended for controlling the leaf spot diseases that would help to minimize the web blotch. Genetic resistance is the least expensive and least costly methods of plant disease management. Developing resistance from the host side, the inheritable ability of host plant to resist a pathogen and it is based on the genetic composition of host and pathogen. Most economic efficient and environmentally friendly way to control the disease. Agriculture producer needs to screen the right variety that is resistant to a particular disease. For example; Korean research group recently developed a Virginia typed short stem and Large grain peanut cultivar, named "Ahwon" found to be resistant to web blotch and early leaf spot [36]. A new peanut variety such as "Danwon" found to be tolerant against web blotch [9]. Another cultivar/varieties such as runner varieties are moderately resistant to web blotch, levels of web blotch rarely reach damaging levels and fungicide programs for leaf spot generally provide adequate control of web blotch. On the other hand, Virginia and Spanish cultivars along with good fungicide programs may be necessary to achieve management of the disease, particularly when web blotch pressure is severe. A strict 14-day schedule with a fungicide is required until harvest, while web blotch found on Spanish and Virginia varieties [8]. Fungicides cause the death of fungi by damaging the cell membrane or inactivating critical

enzymes or proteins or by disrupting the key process; for instance, energy production or respiration. QoIs hinders the mitochondrial (power house of the cell) respiration in fungi by binding the Quinol Oxidation (Qo) site of the cytochrome bc1 complex, blocking electron transfer and inhibit the ATP synthesis. QoI- Analogue based on the structure of the naturally occurring fungicides are known generally as strobilurins but belong to the larger fungicide group known as Quinone outside Inhibitors (QoI). For example; Azoxystrobin, pyraclostrobin, kresoxim-methyl, and trifloxystrobin, all these fungicides are found to be preventative and curative.

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