UNIVERSITY FOR DEVELOPMENT STUDIES

LEAFSPOT DISEASE EPIDEMICS AND COMPONENTS OF RESISTANCE OF SELECTED GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES IN GHANA

ISAAC BOATEY AKPATSU

2022

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BY

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(UDS/MCS/0002/20)

THESIS SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE

OCTOBER, 2022

DECLARATION

I, hereby declare that this thesis is the result of my original work and that no part of it has been presented for another degree in this university or elsewhere:

Candidate:

Signature:……...…… Date:…….……

Name: **Isaac Boatey Akpatsu**

Supervisors'

We, hereby declare that the preparation and presentation of the thesis was supervised in following the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Leafspot disease is a major yield limiting factor in groundnut growing areas in Northern Ghana. This study was to monitor the progression of the disease in ten selected groundnut genotypes. Six were derived from a cross between BC_3F_6 interspecific introgression lines (43-09-03-02 or 60-02-03-02) and Spanish groundnut genotypes (Schubert and TS32-1) while the remaining four are released groundnut varieties in Ghana. Nkatiesari and Chinese served as the resistant and susceptible checks, respectively. The experiment was conducted on an experimental field of CSIR- Savanna Agricultural Research Institute in Ghana during the 2020 and 2021 cropping seasons under natural field infections. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Disease incidence and progress, as well as severity were monitored for both 2020 and 2021 cropping seasons. Leafspot epidemics measured as AUDPC for disease incidence and severity were more severe for L0104B, Chinese and Sarinut-2. AUDPC values for resistant check (Nkatiesari) were comparable to Sarinut-1, L027, L076J and L010A1. Initial inocula and infection rates, lesion number, lesion diameter, and percentage necrotic areas, were higher for the susceptible genotypes. Based on disease rating and yield data, Chinese, Sarinut-2, L046 were classified as leafspot tolerant genotypes while Nkatiesari, Sarinut-1, L027B and L076J considered resistant genotypes. A molecular study to determine the genes responsible for resistance in the resistant genotypes should be considered in future studies.

ACKNOWLEDGEMENT

My profound gratitude goes to my supervisor, Prof. Frederick Kankam for encouraging and guiding me throughout this work. May the God I serve richly bless him. I am extremely grateful for the support of my co-supervisor, Dr Theophilus Kwabla Tengey of CSIR-Savanna Agriculture Research Institute who played a very significant role in executing this study. I acknowledge the University for Development Studies and the CSIR-Savanna Agriculture Research Institute for the opportunity to have my MPhil studies. I would also like to acknowledge the international foundation for science award to Dr. Theophilus Kwabla Tengey, which partly supported this work. I appreciate the support I receive from Dr. Joseph Kwowura Kwodaga, Mr. Francis Abbas Senyabor, Mr. Emmanuel Israel Affram, Mr. Matthew A. Achaglinkame, Mr. Emmanuel Dear Akpatsu, and Mr Dominic Ndela. I am grateful to the staff and my mates in the Department of Crop Science, UDS for their cooperation. I also appreciate the advice and encouragement from Dr. Edward Martey of CSIR-Savanna Agriculture Research Institute. I extend my utmost gratitude to my family for their support, advice, guidance and love. I ask God's blessing for Ms. Millicent Ekpe Amenyega for the care and support for me over the period. The Church of Pentecost, Nyankpala Central, has inspired me to push harder and may God bless you for the love showed to me.

DEDICATION

This thesis is dedicated to Ms. Millicent Ekpe Amenyega. May God bless her for everything.

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LIST OF ACRONYMS

AUDPC:	Area under disease progress curve
CLS:	Cercospora leafspot
DI:	Disease incidence
DS:	Disease incidence
ELS:	Early leafspot
HI:	Harvest index
LLS:	Late leafspot
MoFA:	Ministry of Food and Agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background

The leafspot disease, caused by the *Cercospora arachidicola* and *Cercosporidium personatum* (McDonald & Subrahmanyam, 1985) is a major fungal disease that has gained a noticeable economic importance across groundnut producing areas in Ghana (Nutsugah *et al*., 2007; Tshilenge-Lukanda *et al*., 2012) and the world at large. Depending on the susceptibility of a variety, infected plants suffer a severe damage of leaf tissues leading to defoliation, or greatly interfering with both the photosynthetic and photosynthate process of the plant. The disease in many cases has been reported to be responsible for a drastic reduction in both the quantity and quality of groundnut yield and its components (Nutsugah *et al.*, 2007; Kankam *et al*., 2020). According to Nutsugah *et al*. (2007), the combination of the early and the late leaf spots was responsible for up to 70 % yield loss among susceptible groundnut varieties in northern Ghana.

Farmers usually do not implement any control measure to curb the disease because they consider it as a sign of pod maturity (Tsigbey *et al.*, 2004; Nutsugah *et al.*, 2007). The use of chemicals to control this disease poses serious environmental and health issues. Breeding for cultivars with higher resistant levels to the leafspot disease will be of an immense relevance, with perhaps, partial or quantitative resistance possibilities, which will be a sure-enough approach in putting under control the menace caused by the disease.

Some groundnut genotypes with resistance/tolerance to leafspot disease have been developed and released. However, farmers still use Chinese variety despite its susceptibility to leafspot disease. Efforts have been made to develop Spanish type peanuts with resistance to leafspot disease (Tengey, 2018).

In leafspot resistant cultivars, there is a slower rate of infection and progression of disease compared to highly susceptible ones. That is to say the development of leafspot disease among groundnut genotypes can not only be attributed to environmental factors such as higher temperatures and humidity but also on an array of changes that occurs in the process of infection due to genotypic effects. These changes may be those that begin shortly after contact of the pathogen and the host plant, such as incubation period, infection rate, lesion expansion rate, production of spores, lesion number, lesion size, and percentage necrotic area. Both the individual and combined effects of these components of resistance cause a very large variation in the final resistance or susceptibility to the disease on the field. Components of resistance are very useful tools in the selection of suitable materials in breeding programs by allowing resistant genotypes to be easily identified and selected for future evaluations (Tshilenge-Lukanda *et al*., 2012). These components are usually estimated either by natural infections on the fields or by monocyclic infections carried out in growth chambers (Deadman, 2006). This approach could also be a sure way to speed up the process of selecting groundnut cultivars with higher resistance levels in groundnut breeding programs. This criterion must, however, be associated with an on-field development of the disease, as mostly assessed by the computation of the area under the disease progress curve (AUDPC).

In Ghana, not much attention has been paid to the utilization of components of resistance and disease epidemics in the selection of groundnut cultivars resistant to leafspot disease (*C. arachidicola* and *C. personatum*).

There is a need therefore to study disease progression and components of resistance in these selected candidate lines and released varieties to provide relevant information for groundnut breeding programmes.

1.2 Objectives of study

The aim of this study was to assess the progression of the leafspot disease intensity on the field and estimate some components of resistance of the evaluated genotypes.

The specific objectives were to;

i. Determine the progression of the early and late leafspot diseases on selected groundnut genotypes

ii. Estimate leafspot symptom related components of resistance among genotypes and

iii. Rank the selected lines based on their resistance or susceptibility to early and late leafspot disease

CHAPTER TWO

LITERATURE REVIEW

2.1 Leafspot diseases

Early and late leafspots (ELS and LLS) diseases are caused by *Cercospora arachidicola* S. Hori (teleomorph: *Mycosphaerella arachidis* Deighton) and *Cercosporidium personatum* (Berk. & Curt) Deighton (teleomorph: *Mycosphaerella berkeleyi* Jenk.) respectively. Due to the susceptibility of groundnut crop to these pathogens, they are found at almost all groundnut growing regions across the globe. Higher incidence of both early and late leafspot diseases categorises them among important constraint in groundnut production (McDonald & Subrahmanyam, 1985; Zhang *et al.*, 2001; Chaube & Pundhir, 2009). Symptoms of both leafspots include intense lesions on above ground parts of the crop, with the exception of the flower. These includes the stipules, leaves, petioles, stems and pegs (McDonald *et al.*, 1985). Symptoms starts as small necrotic spots (usually as a pinhead size) which under favourable conditions enlarges in a faded to dark brown or black circular spots. These lesions, depending on the cultivar and climatic conditions may range from 1.01 – 10 mm in diameter (Foster *et al.*, 1980; McDonald *et al.*, 1985). In the case of the early leafspot (*C. arachidicola*), the lesions are mostly surrounded by a yellow halo, usually on the upper surface of the leaves. At advanced stages, the lesions spread and coalesce with each other and cause defoliation of leaves and total death of plant under severe conditions. Symptoms of late leafspot is characterised by dark-brown to black spots with no yellow halo. These spots usually emanate with a rough and tufted appearance at the lower surface of the leaf

whilst the upper surface remains smooth. Generally, the early leafspot is formed on the upper part of the leaf whilst the late leafspot is formed on the lower surface of the leaf (Foster *et al.*, 1980; Subrahmanyam *et al.*, 1982). According to Ngegba e*t al.* (2017), early and late leafspots lesions reduced the uptake of carbon dioxide by 85 %. The same source revealed that canopy carbon exchanged rate was reduced by 93% in a study into the components of resistance of the early and late leafspots on some groundnut genotypes in Sierra Leone. Reductions in uptake of carbon dioxide and canopy carbon according to Agrios (2005) has a higher potential of reducing the photosynthetic ability of the crop, hence reducing plant growth, development and yield in terms of both biomass and pods or grains. According to Tshilenge-Lukanda *et al.* (2012), yield loss of about 50% in groundnut were associate with uncontrolled leafspot disease.

2.2 Morphological characteristics of *C. arachidicola* and *C. personatum*

The *C. arachidicola* can be identified in two main forms. Thus, the anarmoph and the perfect stages. In most cases, the anarmoph stage is called the *C. arachidicola* whilst it is called *Mycosphaerella arachidis* in the perfect or teleomorph stage. Conidiophores are usually present in a short, one or few septate, unbranched and geniculate, yellowish brown to brown and in clusters. Its conidia are usually pale yellow or sub-hyaline, obclavate, usually curved with 3-12 septate, with a size of 35 - 110 x 2.5 - 5.4 µm. It also come in a rounded or truncated base and a sub-acute tip (McDonald & Subrahmanyam, 1985; Chaube & Pundhir, 2009). According to Shokes *et al*. (1982), the *C. personatum* is usually seen in the imperfect state. Its distinctive features are fasciculated conidiophores, 1 – 3 geniculate coupled with

conspicuous conidial scars, they are darker in colour and are usually arranged in concentric rings and are arranged on the lower surface of the leaves. The conidia are cylindrical with one or two septate, obclavate and olivaceous (Ijaz, 2011).

2.3 Survival of early and late leafspot pathogens

Just as any other pathogen, the ELS and LLS pathogens overwinter from seasons to seasons. The ELS and LLS pathogens overwinters mainly on infected plant debris that are left undestroyed and volunteer groundnut plants. These pathogens, depending of other environmental conditions, either become dormant or keep building up their inoculum bank in waiting for conducive conditions such as susceptible host, temperature, humidity etc. (Subrahmanyam *et al.*, 1992).

2.4 Leafspot disease epidemiology

Early and late leafspot diseases usually emanate from an active or overwintered inoculum. Primary inoculum may comprise of conidia, ascospores, or mycelia on crop debris such as pods, stems, branched, leaves, and petioles which have overwintered from previous season. These pathogenic components may be disseminated by splashing raindrops, insects, wind, and other factors from the source of overwintering to the new host. Shortly after setting of inoculum (mostly a multi-celled conidia) on groundnut tissues, they germinate with the help of their germ tubes (Shokes *et al*. 1982). Shortly after germination, they enter plants through openings such as the stomata, wounds, and lateral surfaces of epidermal cells. Infections may occur on both the adaxial and abaxial (lower and upper) leaf surfaces and penetration pegs (Jenkins, 1938). *C. arachidicola* is nectrotroph, and intracellular hyphae are only found in cells that have been lethal to pathogens.

However, the *C. personatum* always remains intercellular and usually known to develop haustoria in living cells (Jenkins, 1938; Abdou *et al.*, 1974; Mims *et al*., 1989) Under favourable conditions, plants infected with early leafspot develops visible symptoms between 7 and 9 days after the inoculation whilst symptoms of the late leafspot are seen only after 10 days after inoculation (Shokes *et al*., 1982).

2.5 Disease progression models

Quantitative descriptions and analysis of temporal disease progression among plant species gained some recognisable priority prior to the 1950s. Ware *et al*. (1932) and Ware & Young (1934) in their respective reports presented curves that were purported to illustrate the impacts of genotypic resistance under fertilizer treatment on the epidemiology of cotton wilt. The use of disease progress curves and rate of disease progress data to demonstrate the influence on of fungicides on the development of late potato blight was also reported by Large (1945, 1952). The study revealed the adoption of transformed data to linearise the observed disease progress curve. This transformation was based on the normal distribution of the observed data. His report further revealed that the transformation was very useful as it was able to indicate the half-decay point when different models were compared. In contrast to this, other literature has revealed that quantitative data analysis in epidemiological studies were fully adopted in plant disease measurement after the input of Van der Plank (1963) and the inception of higher end computers and statistical packages. His input did not only bring about a new way of conceptualising the increase of disease among plant populations but also brought about the inception of very relevant models into plant disease epidemiological studies. Other aspects he

introduced to the studies of disease epidemiology were latent and infection periods, apparent infection rates, and the use of model parameter estimates in comparing the influence of treatments on disease development. In addition, he also introduced the use of different equations to form the fundamentals of quantifying disease progress curves. The disease progress curve has been extensively used by plant disease epidemiologists. This is because it makes it easy to understand plant disease epidemic processes and also compare two or more epidemics among populations. Plant disease progress curves are usually quantified with the help of statistical or mathematical models to interpret temporal disease progress over time. These curves are useful due to the fact that most plant disease incidence and severity starts from lower levels and increases over time to attain higher levels. Literature has revealed that there are always patterns of disease development over time which is always evidenced in the disease progress curves (Lalancette & Hickey, 1986: Contreras-Medina *et al*., 2009).

According to Xu (2006), the four main models used in plant disease epidemiological studies are the Gompertz, Logistic, Exponential, and the Monomolecular models. The logistic model, as postulated by Van der Plank (1963) is widely used to describe epidemics in most polycyclic disease. The Gompertz model has an almost absolute rate curve to reach maximum more rapidly. Adding to it, it also declines in a slower rate as compared to the logistic model. Nevertheless, this model serves as a perfect alternative for the Logistic model (Forrest, 2007). The exponential model, also known widely as the logarithmic model is mostly appropriate when applied in describing early stages of polycyclic epidemics (Forrest, 2007). The monomolecular

on the other hand is very useful in modelling epidemics that has a unicyclic epidemic, this model could also be termed as the negative exponential model (Campbell & Madden, 1990; Forrest, 2007).

2.6 Area under the disease progress curve (AUDPC)

It has long been the interest of plant disease epidemiologists when it comes to the increase in disease as characterised by a standard disease progress curve. According to literature, simple descriptive plant growth models has always served as a tool for the characterisation of the overall patterns of disease increase over time (Pennypacker *et al.*, 1980; Berger, 1981a; Luke & Berger, 1982; Subba Rao *et al.*, 1990) or in space and time (Damicone *et al*., 1976; Berger & Luke, 1979; Jeger, 1984; Headrick & Pataky, 1988). In some instances, these models have been used by epidemiologists in the estimation of rate parameter, and or other relevant disease progress parameters that could be useful in identifying and selection of genotypes that exhibits divers patterns of disease progress (MacKenzie, 1976). It has also been useful in the estimation of the relationship between these patterns and the various components of partial or quantitative resistance (Das *et al.*, 1993; Aquino *et al.*, 1995). In the accounts of Leonard & Mundt (1984), disease progress was attributed to components of partial resistance among genotypes. However, this was true only at the exponential development phase of an epidemic.

Over the years, the computation of the area under the disease progress curve (AUDPC) has been an advance and more developed form of disease progress data (Ferrandino & Elmer, 1992) in most field assessment of partial resistance. It has also been a useful tool in averaging the inevitable variations and idiosyncrasies (Royle,

1994) that are mostly observed in disease progress curves. Furthermore, it has also been useful in the integration of almost all other aspects of disease progress that relates with plant growth and development. Applications of this has therefore been made in most field studies requiring higher levels of resistance. In attempt to come out with the best method in calculation of the AUDPC, several techniques have been developed based on simple rules and formulae of which an example is the trapezoidal rule for computing areas (Wilcoxson *et al.*, 1975; Shaner & Finney, 1977; Bjarko & Line, 1988; Das *et al*., 1993; Chen & Line, 1995; Miedaner & Sperling, 1995; Broers *et al*., 1996). In other studies, Hernandez *et al*. (1993) also proposed the area under the linear regression function of genotype yield against an index of environmental productivity as a criterion for the selection of higher resistant cultivars in breeding programs. In recent studies, Nainwal *et al.* (2020) reported AUDPC to be the most effective tool for the quantitative measurement of disease severity over time when they studied into the epidemiology of Rhizoctonia aerial blight disease in soybean.

2.7 Components of resistance

Components of resistance to groundnut leafspot pathogens (*C. arachidicola* and *C. personatum*) have been identified. A number of such components have been reported to have a negative relationship with the rate of disease development, both in controlled environments and in the field (Foster *et al.*, 1980; Johnson *et al.*, 1986). In the account of Foster *et al*. (1980), sporulation percentage and latent periods have been found to be useful components for selection of resistant genotypes in groundnut. Watson (1987) also identified a number of components of resistance to

the late leafspot when he studied into the components of resistance to late leafspot among some groundnut populace. In the same study, it was observed that most resistant cultivars had a relatively reduced sporulation rate, longer incubation rates, smaller lesion number and sizes as compared to susceptible ones. Similarly, Chiteka *et al*. (1988) reported that, latent period, percentage sporulation, and lesion size were the most reliable components of resistance that contributed to leafspot disease severity. Recently, Dwivedi *et al.* (2002) and Cantonwine *et al*. (2008) reported a significant positive correlation among some components of resistance such as sporulation rate, lesion number, lesion size, latent period, and percentage necrotic area. This could mean that a careful study of these component of resistance could be a useful asset in the selection of resistant cultivars, both for commercial and breeding purposes. For example, lesion expansion rate was used to model and validate epidemics of *Exserohilum turcicum* on maize and *Cercospora medicaginis* on alfalfa (Berger, 1981b), and determine the appropriate time for chemical intervention to control *Pyrenophora teres* and *Bipolaris sorokiniana* causing barley leafspots (Menegon *et al.* 2005). Several studies have further proven a distinctive variation in the levels of severity among resistant and susceptible genotypes when assessed with components of resistance (Chiteka e*t al*., 1988; Dwivedi *et al.*, 2002; Cantonwine *et al.*, 2008).

2.8 Management of Cercospora leafspot diseases

Fungicides application over the years have proven to have contributed to the achievement of higher yields among many crops (Chiteka e*t al*., 1988; Dwivedi *et al.*, 2002; Nutsugah, *et al*., 2007). Before the 1970s, systemic fungicides gradually

replaced most indigenous and non-systemic fungicides which were more effective and with higher specificity in disease management (Agrios, 2005; Khoury & Makkouk, 2010). In as much as chemical disease control is very effective, it also has enormous negative impacts on the society (Awurum *et al.*, 2005; Okwu *et al.*, 2007; Amadioha, 2012). This has compelled policy makers to introduce some regulations on the use of most fungicides (Jordan *et al.*, 2012).

Aside the use of chemicals, various cultural practices have also been used to manage plant diseases (Khoury & Makkouk, 2010) Furthermore, the use of antagonistic living organisms, other than resistant host plant, has been successful in controlling populations and activities of specific or a range of phytopathogens (Kerr, 1980; Björkman *et al.*, 1998; Pal & Gardener, 2006).

Host plant resistance, according to Khoury & Makkouk (2010) serves as a very important approach to controlling diseases in many annual and biennial crops. Field results from various trials have reported a 55 – 60 % increase in yields of peanut cultivars that are resistant to Cercospora leafspot disease compared to susceptible cultivars (Khoury & Makkouk, 2010; Desmae & Sones, 2017; Kankam *et al.*, 2020).

2.9 Types of resistance

Disease resistance are generally controlled by the presence of a single, few or many genes in the plant. This is termed as true resistance. In this, both the host and pathogen exhibit qualities that renders them incompatible to each other. This could be explained as lack of chemical recognition between the pathogen and the host, or the host activates pre-existing defence mechanisms against the activities of the

pathogen. There are two main categories of true resistance; thus, horizontal and vertical resistance.

In horizontal resistance, several genes are responsible for resistance in the plant. These genes, singly may have just little or negligible effect against pathogens in the overall horizontal resistance. Contrary to horizontal resistance, vertical resistance, is basically controlled by a single or major gene. In this type of resistance, genes control major steps in the recognition of pathogens. This makes them very important component of the plants ability to express resistance. In most cases, this form of resistance is also explained as host-pathogen incompatibility. Hosts responds to pathogen in the form of hypersensitivity, immune, or sometimes suppression of pathogens activities and reproduction. Activated resistance mechanisms either prevents or inhibits the settling and establishment of pathogens on any part of the host. Subsequently, they also inhibit or supress the development of epidemics. This is achieved by limiting the amount of initial inoculum load and/or reproduction rate proceeding infection (Agrios, 2005).

However, there are instances where known susceptible cultivars appear to be free from infection and/or symptoms of their target pathogens. This is as a result of escape from the most virulent phase of the pathogen. This escape is achieved when the plant grows faster to pass its most susceptible stage before the pathogen establishes itself. However, three most important factors that can influence this are the susceptibility of the host, the virulence of the pathogen and the favourability of the environment (Agrios, 2005).

According to Maloy (1993) tolerance to disease is defined as the plants ability to grow vegetatively or produce appreciably under a deteriorative disease pressure. This is as a result of heritable characteristics of the host that allows pathogens to establish itself and multiply. Tolerant cultivars are mostly susceptible to the pathogen. However, they are show little damage and are not killed by the disease and also produce appreciable yields (Agrios, 2005)

2.10 Mechanism of resistance

The mechanisms of resistance can be categorized into chemical, mechanical and functional. Maloy (1993) proposed that these categories could be grouped into two forms; thus, active and passive. These mechanisms come in a form of barrier that prevents the pathogen from either attaching or establishing itself on the host. According to Waller & Lenné (2001), these barriers could be such as epidemic tissues, thickened cell walls, cork layers, and cuticles. Also, plants defend themselves against plant pathogens by limiting their growth and development, thus providing resistance of host plants to pathogens (Agrios, 2005). According to Pattee & Young (1982), a necrotic defence was observed to be highly operative in resistant genotypes of groundnuts in response to both the early and late leafspot pathogens. Pre-infection resistance to both early and late leafspots, as observed by Abdou *et al.* (1974), was also found to be associated with a non-directional germ tube growth.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted at the Plant Pathology laboratory and experimental field of the Council for Scientific and Industrial Research – Savanna Agricultural Research Institute (CSIR-SARI) at Nyankpala (Latitude 9˚24'26.526'' North, longitude 0˚59'22.338'' West) during the 2020 and 2021 cropping seasons under rainfed conditions. The area t falls under the Guinea savannah ecological zone with an average annual rainfall of 800 – 1200mm. The soil type of the area is a well-drained sandy loam. The area experiences a unimodal rainfall patten. Early and late leafspot disease have previously been reported on the field and the area is a known hotspot for the disease (Owusu & Waylen, 2013).

3.2 Experimental design and treatments

Ten groundnut genotypes (Table 3.1) were used for the experiment. Six were derived from a cross between BC_3F_6 interspecific introgression lines (43-09-03-02 or 60-02-03-02) and Spanish groundnut genotypes (Schubert and TS32-1) selected based on preliminary yield trial in 2019 at Nyankpala. The remaining four are released varieties (Nkatiesari, Sarinut-1, Sarinut-2 and Chinese) among which Chinese is known to be susceptible to the disease whilst the remaining are resistant.

Table 3.1: List of genotypes

Genotypes	Source	Pedigree
L046	TxL164304-46	TS32-1 x 43-09-03-02
L030	TxL164302-30	TS32-1 x 43-09-03-03
L027B	TxL164302-27	TS32-1 x 43-09-03-04
L076J	TxL164305-76	Schubert x 60-02-03-02
L104B	TxL164306-104	Schubert x 60-02-03-03
L010A1	TxL164301-10	TS32-1 x 43-09-03-04
Sarinut-1	SARI	
Sarinut-2	SARI	
Nkatiesari	SARI	
Chinese	SARI	

The ten genotypes were laid in a Randomized Complete Block Design (RCBD) with three replications. An alley of 1 m was created between blocks and between experimental units within blocks. Each experimental unit measured 1.5 x 3 m. Seeds were planted on 25th June in 2020 and 20th June 2021with a planting distance of 0.5 x 0.2 m with one seed per hill. Each experimental unit consisted of four rows.

Triple Super Phosphate (TSP) was applied at a rate of 60 kg/ha as a blanket treatment at 2 WAP to boost plant metabolism and increase growth rate. A pre-emergence herbicide (Pendimethalin) was applied at a rate of 1 kg active ingredient per hectare immediately after planting. Post-emergence weed control was carried out at 4 WAP and followed by ridging to make it easy for the young pegs to pod successfully. Both the weeding and the ridging were done manually using a hoe.

3.3 Data collection and analysis

Data were collected on various agronomic and yield parameters and pathological indices. The agronomic parameters were days to first flower, days to 50 % flowering, pod yield, grain yield, 100 seed weight, biomass yield, shelling percentage, and harvest index. Shelling % = (Grain yield (obtained after shelling))/(Pod yield (unshelled)) x 100, and Harvest index = (Economic yield (grain yield))/(Total plant biomass), where total plant biomass = Biomass yield + pod yield.

Disease parameters such as disease incidence and disease severity were taken. Disease severity was assessed based on a modified 1-10 Florida scale according to Chiteka *et al.* (1988), where 1 = no disease present, 2= Very few lesions (none on upper canopy), 3= Few lesions (very few on upper canopy), 4=Some lesions with more on upper canopy than rank of 3 and slight defoliation noticeable, 5= Lesions noticeable even on upper canopy with noticeable defoliation, 6= Lesions numerous and very evident on upper canopy with significant defoliation (50%+), 7= Lesions numerous on upper canopy with much defoliation (75%+), 8= Upper canopy covered with lesions with high defoliation (95%+), 9= Very few leaves remaining and those covered with lesions (some plants completely defoliated), and 10 = plants completely killed by the disease. Final disease rating was obtained by computing the average of the final two ratings. Resistance otherwise susceptibility levels were ranked such that 1 – 1.9 = highly resistant (HR), 2 – 3.9 = resistant (R), 4 – 5.9 = moderately resistant (MR), 6 – 7.9 = Susceptible (S), and above 8 = highly susceptible (HS). Number of lesions, lesion size, and percentage necrotic area were

also measured as components of resistance. These were determined by scanning sampled leaves with a laser jet scanner, followed by a photographical analysis using imgaeJ software (edition 1.53e). Percentage necrotic area = $\frac{Total\ necrotic\ area}{Total\ leaf\ area} x\ 100$

Total necrotic area = Average size of lesion x number of lesion per leaf.

Area under the disease progress curve (AUDPC) was computed for each plot for percent incidence and severity separately (Shaner & Finney, 1977). AUDPC = $\sum_{i=1}^{n-1} \frac{(y_i+y_{i+1})}{2}) t_{i+1} - t_i)$ Where y_i is the disease severity cores or percentage incidence observed at $_i$th observation, whiles t_i is the time (days) at the different evaluation dates (nine occasions on a weekly bases for ELS and five occasions on a weekly bases for the LLS) ($_i$th observation), *n* is the total number of observations. Disease assessments were converted to proportions [proportion of incidence = percent incidence / 100; proportion of severity assessment = (Florida rating - 1) / 9], and linearized forms of the Gompertz (-In(-In(y), logistic [ln(y/1-y)] and monomolecular [In(1 / (1 – y)], exponential model logy = $logy_0$ + rt , models were fitted using linear regression of the disease intensity proportions on time (DIP). Where *y* = disease severity at a range of 0 < *y* < 1, *r* = infection rate, and *t* = time. The model that significantly ($P < 0.05$) fit all of the curves within each experiment was selected. If more than one model fit all of the curves within an experiment, that model with the highest recalculated R^2 is selected. These were done using the ‘‘epifitter’’ in R as described by Alves & Del Ponte (2021). Data collected were also subjected to ANOVA using GenStat statistical package (12^{th} edition). Means were separated using Duncan multiple range test at 5 %. Correlation analysis was run

using R statistical package (version 4.1.2), and data were presented in graphs and tables.

3.3.1 Isolation of *C. arachidicola* and *C. personatum* and pathogenicity of isolates

A laboratory study was conducted to isolate and characterize the causal organism of the diseases (early and late leafspots). This was preceded by preparation of growth media. An identification manual was used to identify the isolated organism by comparing of morphological characteristics. The laboratory studies were conducted at the Spanish laboratory complex of the University for Development Studies.

3.3.1.1 Media preparation

A Potato Dextrose Agar medium was prepared by adding 39 g of dehydrated PDA in 1 L of sterile distilled water, and stirred thoroughly in a conical flask to facilitate dissolving. To inhibit the growth of any bacteria, 25 mg of amoxicillin was added as an antibiotic. The mixture was autoclaved at 121 ^{o}C for 15 minutes. The prepared PDA was poured into sterilized Petri dishes under a laminar flow-hood cabinet and stored overnight on working bench for later use.

3.3.1.2 Isolation of *C. arachidicola* and *C. personatum*, causing leafspot diseases of groundnut

Sample leaves which contained actively developing lesions of both early and late leafspot diseases were collected from the experimental field and transported to the laboratory immediately in paper envelopes. Small pieces (about 5 mm^2) of diseased leaf tissues were cut at the margins so that they contain both diseased and healthy parts of the leaf tissue. This was to ensure that an active growth and multiplication phase of the pathogen is collected. The cut leaves were dipped in a 5 % sodium

hypochlorite solution for 5 minutes for surface sterilisation. This is to ensure that saprophytes and other pathogen that may be available or present on the lesions are eliminated or killed. After 5 minutes, the pieces leaf samples were rinsed in sterile distilled water immediately and blotted with dry tissue paper. The sterilised leave tissues were plated on the amended PDA media and incubated at room temperature and observed daily. With the exception of the inoculation process, all procedures were carried out under the laminar flow.

3.3.1.3 Sub-culturing of pathogen

At seven days after incubation, the mycelia growth emanating from leaf tissue were transferred onto new media using a sterilized transfer needle. Sub-culturing was conducted several times until pure culture was obtained.

3.3.1.4 Identification of isolates

Identification manual according to Barnett & Hunter (1972) was used for the identification. The isolate's conidia and conidiophores were compared with the pictorial descriptions and drawings of the spores of the fungi in the manual.

3.3.1.5 Pathogenicity test

A detached leaf assay method as described by Winstead & Kelman (1952) was used to test for the pathogenicity of both the early and late leafspot pathogen isolates. Fully developed healthy and clean terminal leaves from a susceptible genotype (Chinese) were plucked and used for the experiment. The leaves were washed thoroughly with distilled water to remove surface dirt. The washed leaves were surface sterilize in 70 % alcohol for 5 minutes and further washed in a sterilized distilled water to remove alcohol from the surface of the leaves. Leaves were then

placed in a sterilized Petri dish containing a wet tissue paper. The wet tissue paper was to retain the moisture level in the Petri dish. One to two colonies of *Ca* and *Cp* were transferred to 3 mL of distilled water, and agitated in a test tube for 5 minutes to obtain the their inocula. Both early and late leafspot isolates (inocula) were inoculated on leaves in separate Petri dishes using syringe and needle. Fully developed healthy and clean terminal leaves from the susceptible check (Chinese) were inoculated with a suspension of *C. arachidicola* and *C. personatum* except the uninoculated control. The control leaf was inoculated with only distilled sterilized water. They were further incubated at room temperature and were observed for the appearance of symptoms of both the early and late leafspots.

CHAPTER FOUR

RESULTS

4.1 Laboratory studies

4.1.1 Isolation and identification of Cercospora leafspot (CLS) disease causing agents

The fungi pathogens isolated from leaves of the groundnut cultivar "Chinese" were identified as *C. arachidcola* and *C. personatum.* Cultures for the early leafspot (*C. arachidcola*) were observed to have a brown shade in appearance whilst the late leafspot (*C. personatum*) was observed to appear as dark brown to black shade (Plate 4.1).

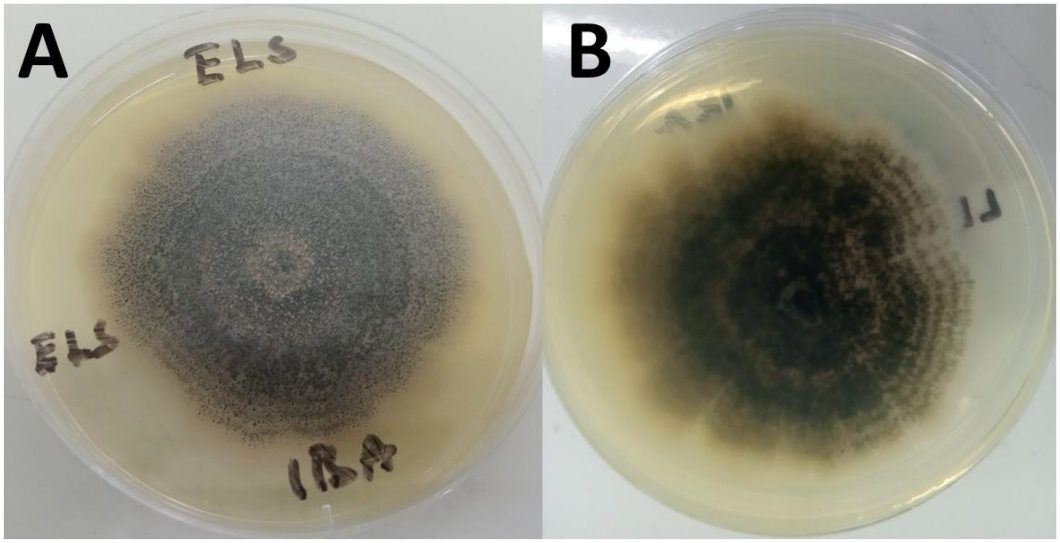

Plate 4.1: Cultures from infected leaves of groundnut in Petri plates. A = *C. Arachidicola,* B = *C. personatum*

Conidia of *C. arachidicola* was observed to be cylindrical or obclavate and septate accompanied with rounded base and sub-acute tip. On the other hand, *C. personatum*

conidium was observed to be cylindrical or obclavate and light coloured and non-septate (Plate 4.2)

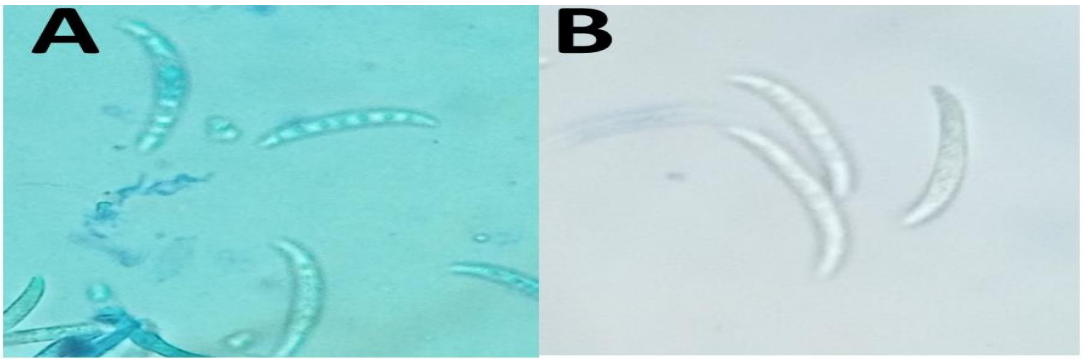

Plate 4.2: Microscopic observation of isolates. A = *C. arachidicola*, B = *C. personatum*

4.2 Evaluation of *C. arachidicola* and *C. personatum* isolates for pathogenicity

Ten days after inoculation, leafspot symptoms were observed on inoculated leaves whilst the uninoculated leave, showed no symptom of leafspot disease (Plate 4.3).

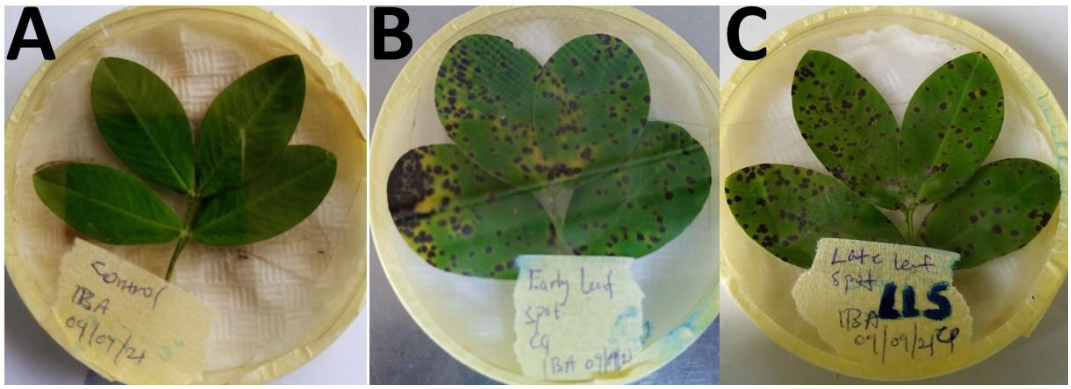

Plate 4.3: Pathogenicity test of *C. arachidicola* and *C. personatum* on a susceptible genotype (Chinese). A = Control, B = *C. arachidicola*, C = *C. pesonatum*

Symptoms for both *C. arachidicola* and *C. personatum* appeared as small to medium chlorotic spots on leaflets. Whilst symptoms for *C. arachidicola* appeared as sub-

circular to irregular in shape, brown to dark-brown, and accompanied with a conspicuous yellow halo, symptoms of the *C. personatum* appeared circular in shape, dark-brown to black in colour with just little to no halo around it (Plate 4.3). Pure cultures of both pathogens that were re-isolated from lesions caused by both pathogens had same characteristics as the initial cultures that were inoculated on the leaves.

4.3 Leafspot disease incidence

There were significant differences among genotypes in terms of leafspot epidemics, measured as AUDPC based on incidence (DI-AUDPC) in both 2020 and 2021 ($P < 0.05$ and $P < 0.001$, respectively). In 2020, DI-AUDPC was higher for Sarinut-2 (4251) than both the susceptible and resistant controls (Chinese = 2835, Nkatiesari = 1127, respectively) (Table 4.1). However, Sarinut-2 was statistically comparable to Chinese but not to the remaining eight genotypes. DI-AUDPC values for L010A, L027B and Sarinut-1 were also comparable to that of the resistant control (Nkatiesari) (Table 4.1).

DI-AUDPC values in 2021 revealed a clear distinction between the susceptible and resistant controls (Chinese and Nkatiesari, respectively) as it was higher for Chinese (3069) as compared to that of Nkatiesari (1233). DI-AUDPC values for L027B, L010A1 and Sarinut-1 were comparable to that of Nkatiesai, just as observed in 2020 (Table 4.1). Also, the relationship between Chinese and Sarinut-2 in 2021 was the same as observed in 2020. Between L076J and 104B was no statistical variation even though L076J had a comparably lower DI-AUDPC value. However, they

(L076J and L104B) were incomparable to the susceptible control (Chinese) (Table 4.1)

4.4 Leafspot disease variables measure as S-AUDPC severity

There were significant differences ($P < 0.001$) among genotypes with reference to leafspot epidemics, measured as AUDPC based on severity (S-AUDPC) with regards to early leafspot (ELS-AUDPC) and late leafspot (LLS-AUDPC) disease in both 2020 and 2021. ELS-AUDPC in 2020 was significantly higher for L104B (317.3) than Chinese (255.5), the susceptible check. The resistant control, Nkatiesari had the lowest ELS-AUDPC value (103.8). However, this is not significantly different from Sarinut-1, L027B, L076J and L046. L104B was significantly higher than the susceptible check (Chinese) (Table 4.1). In 2021, the highest ELS-AUDPC value was observed for Chinese (269.5) whilst the lowest was for Nkatiesari (161.0), following a similar trend as in 2020. ELS-AUDPC values for L010A1, L076J, L027B and Sarinut-1 were comparable to that of Nkatiesari. Similarly, ELS-AUDPC values for L030 and L104B were also comparable to each other, whilst that of L046 was also comparable to Sarinut-2. Despite the higher ELS-AUDPC values for Sarinut-2, L046, and L030, they were significantly lower than the susceptible check (Chinese). Generally, it was also observed that mean ELS-AUDPC values for 2021 was appreciably higher as compared to that of 2020 (190.8 and 174.2, respectively) (Table 4.1).

LLS-AUDPC values in 2020 ranged between 39 (lowest) and 84 (highest). LLS-AUDPC was severe for Chinese (84) than for Sarinut-1 with the lowest AUDPC. However, LLS-AUDPC values for L010A1, L076J, L027B and Sarinut-1 were

comparable to that of the resistant check (Nkatiesari). Similarly, the value for L030 was also comparable to that of L046. Also, their values did not differ from the susceptible check. Generally, the maximum and minimum LLS-AUDPC values for 2021 (154.00 and 44.33, respectively) were higher as compared to 2020 (105.50 and 39.67, respectively). Chinese suffered the highest severity whilst Nkatiesari experienced the lowest, based on LLS-AUDPC values (154 and 44.33 for Chinese and Nkatiesari, respectively) (Table 4.1). In a similar trend as 2020, LLS-AUDPC values for L010A1, L076J and Sarinut-1 were comparable to that of Nkatiesari. L027B was comparable to L076J and L010A1, whiles L030 was also comparable to L104B. Furthermore, Sarinut-2 and L046 were also comparable to L104B but not to L030. Similarly, LLS-AUDPC, values for 2021 are comparatively higher to 2020 (Table 4.1).

Table 4.1: Early and late leafspot disease incidence and severity of groundnut genotypes measured as area under disease progress curve (AUDPC)

	DI-AUDPC		ELS-AUDPC		LLS-AUDPC	
Genotypes	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
L010A1	1460 a	1524 ab	149.3 c	150.5 a	40.83 a	63.00 ab
L027B	1232 a	1288 a	119.0 a-c	155.2 a	51.33 ab	68.83 b
L030	2116 a	2199 cd	142.3 bc	191.3 b	96.83 de	101.50 c
L046	2373 a	2540 d	120.2 a-c	226.3 cd	101.50 e	123.67 d
L076J	1797 a	1948 bc	124.8 a-c	161.0 a	45.50 a	51.33 ab
L104B	2308 a	2468 d	317.3 f	211.2 bc	65.33 bc	112.00 cd
Chinese	2835 ab	3069 e	255.5 e	269.5 e	84.00 d	154.00 e
Nkatiesari	1127 a	1233 a	103.8 a	141.2 a	40.83 a	44.33 a
Sarinut-1	1433 a	1532 ab	116.7 ab	161.0 a	39.67 a	51.33 ab
Sarinut-2	4251 b	2682 de	179.7 d	240.3 d	68.83 c	123.67 d
Mean	2093	2048	162.9	190.8	63.5	89.4
CV	44.6	14	9.5	6.1	13.1	12.9

DI-AUDPC = Incidence, ELS-AUDPC = Early leafspot severity, and LLS-AUDPC = Late leafspot severity, figures showing same alphabets in a column are statistically not different from each other.

4.5 Leafspot disease incidence and progress

Genotypes were assessed for the incidence of leafspot disease at 59, 73, 87 and 101 DAP in both 2020 and 2021 Genotypes exhibited significant variations ($P < 0.05$) in terms of percentage disease incidence across all the assessment dates. In 2020, percentage disease incidence ranged from 18 – 87 % (Figure 4.1). Chinese had the highest across all the sampling occasions with values ranging from 31-87 %. Nkatiesari, L027B, and L010A1 had a percentage disease incidence lower than 50 % at 101 DAP. L046, Sarinut-2, L104B, L046, and Chinese had a percentage disease incidence above 70 % whilst the remaining genotypes had their percentage disease incidences between 50 - 70 %. In 2021, percentage disease incidence ranged between 7- 92 %. Chinese had the highest percentage disease incidence ranging between 38 and 92 %. Nkatiesari, L027B, and L010A1 had a percentage disease incidence lower than 50 % at 101 DAP. L046, L104B, Sarinut-2, L030, and Chinese had a percentage disease incidence above 70 % whilst the remaining genotypes had their percentage disease incidences between 50 and 70 % (Figure 4.1).

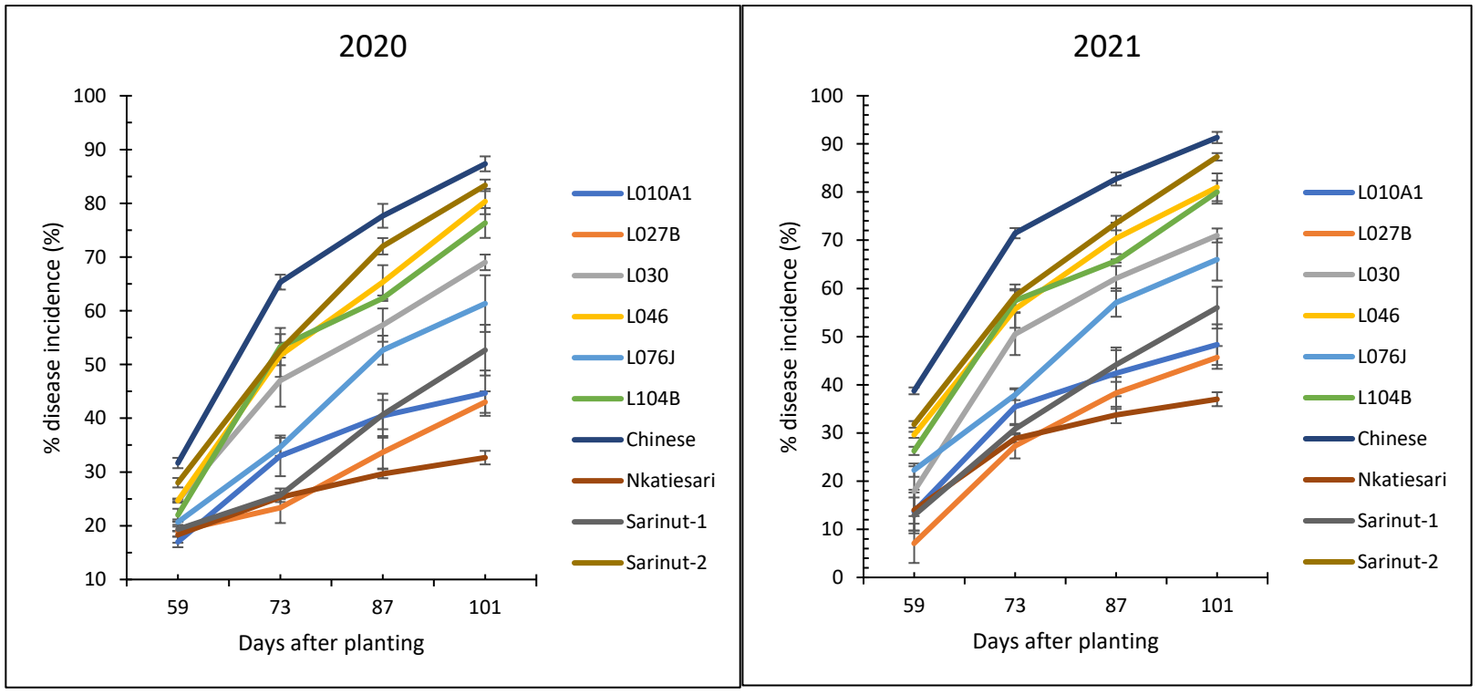

Figure 4.1: Progress of leafspot disease incidence in 2020 and 2021 over time. Data points are means of three replications. Error bars represents SEM.

4.6 Early leafspot disease progress among genotypes

Genotypes reacted variably to the early leafspot disease across the period of assessment in both years. In 2020, the disease was more severe on L104B and Chinese with a severity score of 2 - 9 across the assessment period. Apart from L104B and Chinese, all the other genotypes had a severity score of 5 or lower at 101 DAP. In 2021, the highest disease severity score was observed for Chinese and it ranged from 1.3-9 across the assessment period. Nkatiesari, L010A1, L027B, L076J and Sarinut-1 had a severity score of 5 or below at 101 DAP. L030 had a severity score of 6, while L046, L104B and Sarinut-2 had a severity score between 7-8 at 101 DAP (Figure 4.2).

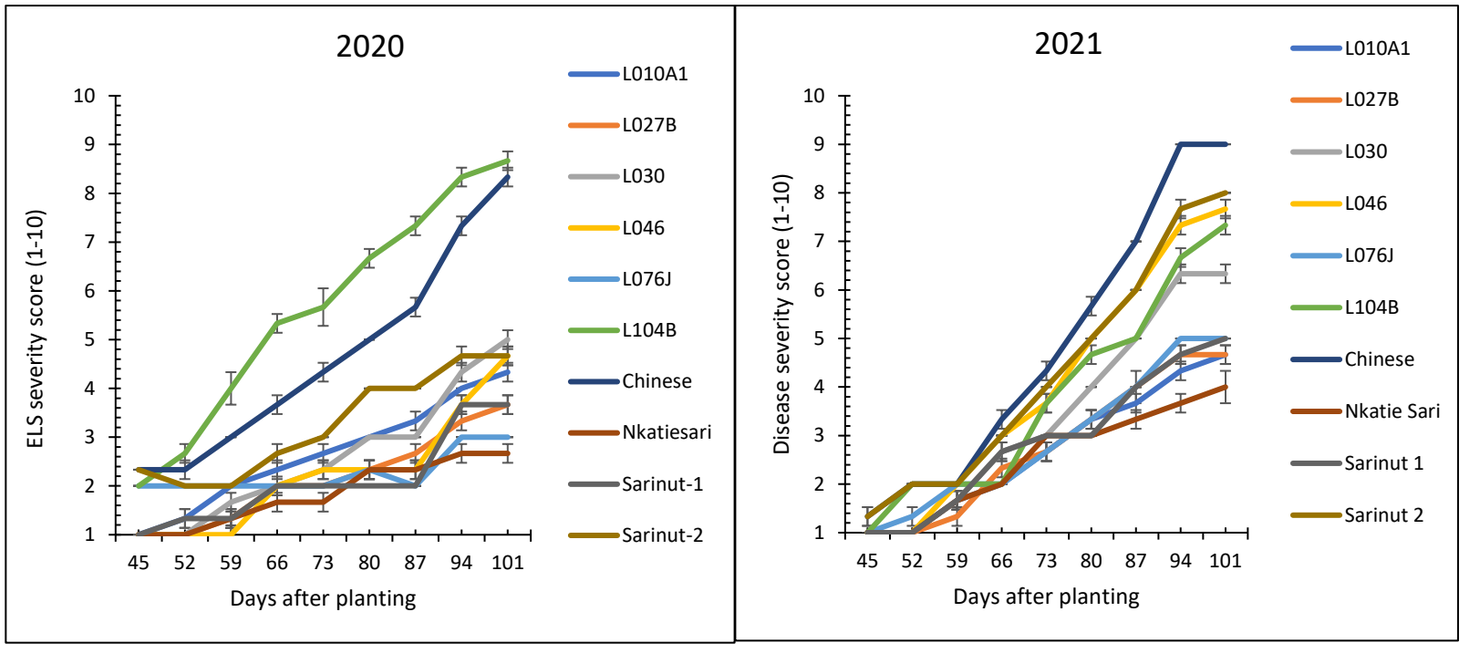

Figure 4.2: Early leafspot disease progression among genotypes in 2020 and 2021. Data points are means of three replications. Error bars represents SEM.

4.7 Late leafspot progress among genotypes

Responds to the late leafspot disease varied significantly ($P < 0.05$) among genotypes across the assessment period. In 2020, LLS disease severity score at 80 DAP ranged between 1 and 3. At 101 DAP, L046 suffered the highest severity level of the disease with a score of 5.33. This was followed by Chinese and L030 with a score of 4.67. L027B, Sarinut-2, and L104B had a score between 3 and 4, whilst L10A1, L027B, Sarinut-1, and L076J had a score below 3. In 2021, the highest severity score was observed for Chinese across all the assessment occasions with scores between 3 and 9. At 101 DAP, L104B, and L030 had a severity score between 6 and 7, Sarinut-2 and L064 had a score of 8 whilst the remaining five genotypes (Nkatiesari, Sarinut-1, L027B, L010A1, and L076J) had a severity score below 5 (Figure 4.3).

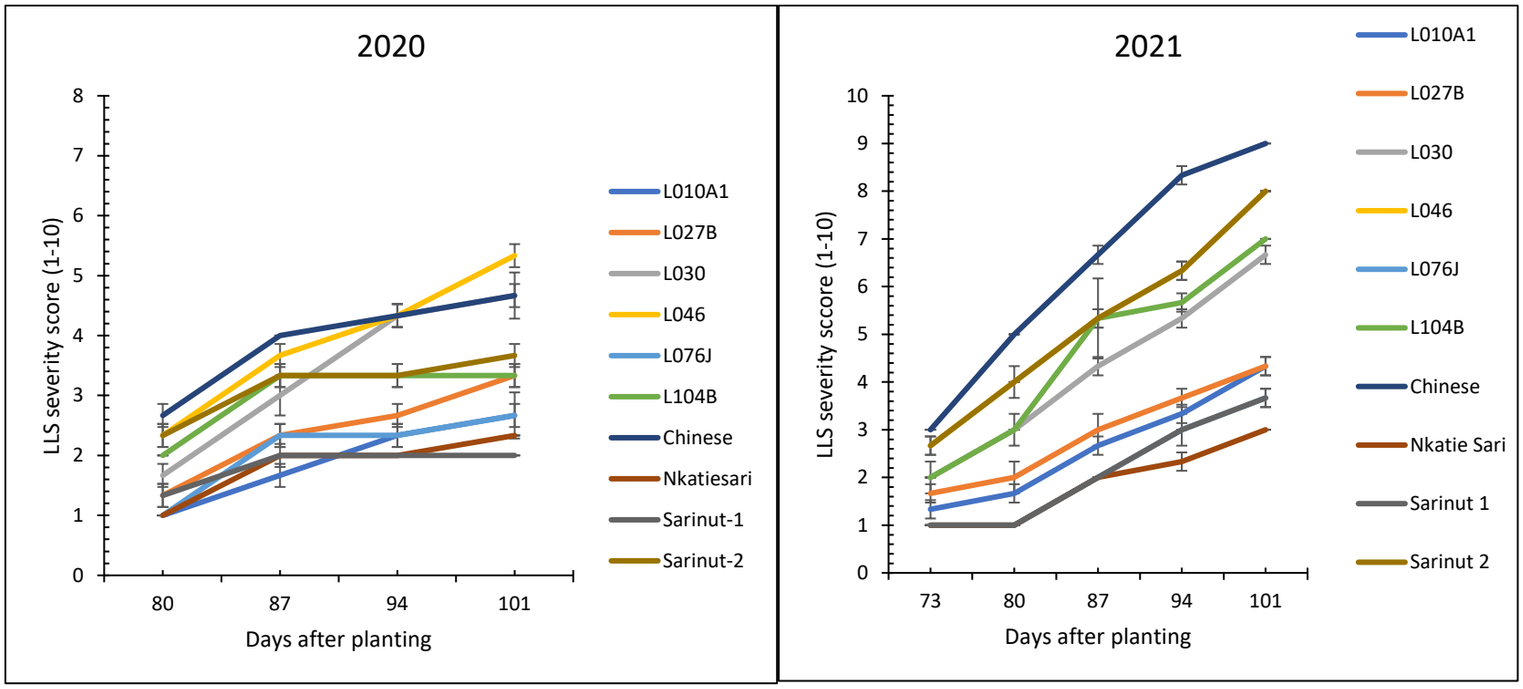

Figure 4.3: Late leafspot disease progression among genotypes in 2020 and 2021. Data points are means of three replications. Error bars represents SEM.

4.8 Fitting models

A summary of R^2 values of disease intensity of groundnut genotypes fitted using the Exponential, Logistic, Gompertz and monomolecular models are displayed on (Table 4.2). The best models (models with the highest R^2 value) for each genotype were used in determining the initial inoculum and infection rate (Table 4.3 - Table 4.5)

Table 4.2: Summary R^2 for the various models for disease incidence, ELS severity, LLS severity

		R^2					
		LS disease incidence		ELS severity		LLS severity	
Genotype	**Model**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
L010A1	Exponential	0.6302	0.5446	0.8524	0.8963	0.7188	0.8174
	Gompertz	0.5573	0.555	0.8904	0.9116	0.7101	0.7736
	Logistic	0.5953	0.5594	0.8749	0.9094	0.7171	0.7987
	Monomolecular	0.4942	0.5077	0.8815	0.8852	0.6851	0.8122
L027B	Exponential	0.7181	0.7197	0.8587	0.8703	0.6492	0.6181
	Gompertz	0.7154	0.7579	0.855	0.8789	0.6721	0.6842
	Logistic	0.7185	0.7439	0.8612	0.8803	0.6617	0.6523
	Monomolecular	0.7042	0.7488	0.818	0.8492	0.668	0.7078
L030	Exponential	0.8006	0.6857	0.9098	0.9258	0.8035	0.9525
	Gompertz	0.8049	0.8	0.9095	0.9425	0.8441	0.9414
	Logistic	0.8098	0.7682	0.9169	0.9485	0.8365	0.9557
	Monomolecular	0.7907	0.8139	0.8611	0.8928	0.8393	0.9059
L046	Exponential	0.8405	0.8209	0.8283	0.9407	0.8398	0.9113
	Gompertz	0.858	0.7968	0.8025	0.9589	0.8381	0.9415
	Logistic	0.8701	0.8201	0.8232	0.9733	0.8442	0.9488
	Monomolecular	0.8272	0.7568	0.7417	0.9001	0.8216	0.9152
L076J	Exponential	0.8425	0.8555	0.5186	0.9144	0.5187	0.8745
	Gompertz	0.7792	0.8183	0.5186	0.9402	0.4623	0.8427
	Logistic	0.813	0.8436	0.5186	0.9341	0.4988	0.8643
	Monomolecular	0.7214	0.7685	0.5186	0.9205	0.3934	0.7886
L0104B	Exponential	0.7966	0.8199	0.8653	0.9319	0.4642	0.6838
	Gompertz	0.8616	0.889	0.908	0.9185	0.4121	0.6664
	Logistic	0.8561	0.886	0.9204	0.9418	0.4385	0.6852
	Monomolecular	0.8462	0.874	0.8725	0.8564	0.3709	0.6148
Chinese	Exponential	0.9068	0.8211	0.9544	0.9375	0.5551	0.9115
	Gompertz	0.9068	0.925	0.8669	0.9073	0.5437	0.9561
	Logistic	0.903	0.9242	0.9148	0.9457	0.5538	0.9643
	Monomolecular	0.8985	0.9167	0.7912	0.8432	0.5161	0.9372
Nkatiesari	Exponential	0.7224	0.6203	0.6841	0.841	0.4132	0.8827

	Gompertz	0.7622	0.6837	0.6781	0.8553	0.3432	0.8838
	Logistic	0.742	0.6514	0.6833	0.8521	0.3876	0.8847
	Monomolecular	0.7809	0.7135	0.6569	0.8279	0.2581	0.8693
Sarinut1	Exponential	0.8345	0.8403	0.7726	0.8861	0.36	0.9203
	Gompertz	0.7355	0.7808	0.7547	0.9324	0.36	0.9195
	Logistic	0.7855	0.8182	0.7698	0.9143	0.36	0.923
	Monomolecular	0.6492	0.7012	0.7032	0.9274	0.36	0.893
Sarinut2	Exponential	0.8974	0.8872	0.8864	0.9443	0.2319	0.8682
	Gompertz	0.9657	0.9596	0.8822	0.9396	0.196	0.9173
	Logistic	0.9625	0.9596	0.8869	0.9629	0.2137	0.9182
	Monomolecular	0.9561	0.948	0.8665	0.8822	0.1702	0.8987

4.8.1 Disease incidence

In both 2020 and 2021, the Gompertz model fitted best for the description of the presence of the disease on the field ($0.60 < R^2 < 0.97$ for 2020 and $0.50 < R^2 < 0.97$ for 2021, respectively) (Table 4.2 and Table 4.3). However, the logistic model, in this study can also be compared with the Gompertz model. In 2020, initial inoculum was higher for L104B, Sarinut-2, Chinese, and L046 (> 1.00) (Table 4.3). The lowest initial inoculum was respectively, observed for Nkatiesari (-0.0556) (Table 4.3). Infection rate in L046 was higher (0.0593) as compared to the susceptible check (0.0507). Nkatiesari had the least infection rate at 0.00457. The trend for initial inoculum observed for 2021 differed from that of 2021. Chinese, L046, L076J, L104B, Sarinut-2, had the highest initial inoculum (> 1.00). The least was observed for L030 and Nkatiesari (-1.86E+00 and -0.15767757, respectively). In general, both initial inoculum and infection rate for genotypes with higher resistance were lower compared to the susceptible genotypes (Table 4.2 and Table 4.3).

4.8.2 Early leafspot

The best fitted models that describe the severity of the early leafspot disease among the 10 groundnut genotypes in 2020 were the exponential and logistic models ($0.50 < R^2 < 0.96$) (Table 4.4). Initial inoculum in the susceptible genotype, Chinese, was higher (0.0369) when compared to that of the resistant check (0.039974). In relation with other genotypes, L104B had an initial inoculum that is almost equal to the susceptible check (0.0183). Also, the results revealed that L010A1, L027B, L030, L046 and L076J had an initial inoculum that were lower than that of the resistant check (Nkatiesari). Infection rates for L076J and L010A1 were lower compared to the resistant check. However, infection rates in Sarinut-1, Sarinut-2 and L046 were comparable to Chinese (susceptible check). Furthermore, L027B, L030 and L104B had an infection rate higher than the susceptible check. In 2021, the Logistic model was the best fitted model to describe the severity of the early leafspot disease among the 10 groundnut genotypes ($0.85 < R^2 < 0.98$) (Table 4.4). Initial inoculum and infection rates for most resistant genotypes were lower as compared to those of the susceptible genotypes. The highest initial inoculum was observed for L104B (0.00976) whilst the lowest was observed for Sarinut-1. Initial inocula for L104B, Sarinut-2, L030 and L046 were higher (> 0.00334) (Table 4.4) as compared to the susceptible check (> 0.00334). Furthermore, the results revealed that initial inocula for Sarinut-1 and L010A1 were lower than that of the resistant check (< 0.00559). Following a similar trend, the initial inoculum and infection rates were higher for most susceptible genotypes when compared with the resistant genotypes. The highest infection rate was observed for Chinese (0.0778) whilst the lowest was

observed for Nkatiesari (0.0178). Comparatively, the rates of infection for L030, L046, L104B, Sarinut-2 were not different from the susceptible control. However, L010A1, Sarinut-1, and L076J had an infection rate comparable to the resistant check (Nkatiesari) (Table 4.2 and Table 4.4)

Table 4.4: Best fitted models of early leafspot disease progress in 2020 and 2021 as revealed by their Linear coefficients

	2020					2021				
Genotypes	**Initial inoculum**	**Infection rate**	**Standard error**	**R^2**	**Model**	**Initial inoculum**	**Infection rate**	**Standard error**	**R^2**	**Model**
L010A1	0.00744	0.01767	0.00124	0.8904	Gompertz	0.00155	0.02152	0.00134	0.9116	Gompertz
L027B	0.02494	0.03107	0.0025	0.8612	Logistic	0.0144	0.04276	0.00315	0.8803	Logistic
L030	0.01786	0.03854	0.00232	0.9169	Logistic	0.00795	0.0552	0.00257	0.9485	Logistic
L046	0.02607	0.02755	0.00251	0.8283	Exponential	0.00486	0.066	0.00219	0.9733	Logistic
L076J	0.1264	0.00941	0.00181	0.5186	Logistic	0.00134	0.02275	0.00115	0.9402	Gompertz
L104B	0.0183	0.05895	0.00347	0.9204	Logistic	0.00973	0.05519	0.00274	0.9418	Logistic
Chinese	0.0639	0.02565	0.00112	0.9544	Exponential	0.00334	0.07782	0.00373	0.9457	Logistic
Nkatiesari	0.03997	0.01977	0.00269	0.6841	Exponential	0.00559	0.0178	0.00146	0.8553	Gompertz
Sarinut-1	0.03814	0.02206	0.00239	0.7726	Exponential	0.00117	0.02283	0.00123	0.9324	Gompertz
Sarinut-2	0.05943	0.027	0.00193	0.8869	Logistic	0.00855	0.06063	0.00238	0.9629	Logistic

4.8.3 Late leafspot

In 2020, the exponential model was the most appropriate model to describe the severity of the late leafspot disease among the 10 groundnut genotypes ($0.22 < R^2 < 0.85$) (Table 4.5) with infection rates between 0.014325 and 0.059482. The most appropriate that best describes the severity of the late leafspot disease of most of the 10 groundnut genotypes in 2021 was the Logistic model ($0.65 < R^2 < 0.97$) (Table 4.4 and Table 4.5). Infection rates in 2021 were between 0.014378 and 0.082639. Infection rate observed for the susceptible check, Chinese, was 0.110652, whilst that for the resistant check was 0.052721. Infection rate among genotypes followed the same trend as most susceptible genotypes such as Sarinut-2 and L104B also had an infection rate greater than 0.08. With reference to the resistant check, L010A1, L027B and L076J had an infection rate equal to or less than 0.05, observed for the resistant check.

Table 4.5: Best fitted models of late leafspot disease progress in 2020 and 2021 as revealed by their Linear coefficients

	2020					2021				
Genotypes	**Initial Inoculum**	**Infection rate**	**Standard error**	**R2**	**Model**	**Initial Inoculum**	**Infection rate**	**Standard error**	**R2**	**Model**
L010A1	$2.61x10^{-3}$	0.046523	0.009202	0.7188	Exponential	$7.59x10^{-11}$	0.032727	0.00429	0.8174	Gompertz
L027B	$1.34x10^{-8}$	0.027955	0.006175	0.6721	Gompertz	-1.43	0.014378	0.002562	0.7078	Monomolecular
L030	$1.11x10^{-35}$	0.051433	0.00699	0.8441	Gompertz	$1.18x10^{-3}$	0.073644	0.004396	0.9557	Logistic
L046	$2.62x10^{-4}$	0.059482	0.00808	0.8442	Logistic	$8.57x10^{-4}$	0.082639	0.005323	0.9488	Logistic
L076J	$5.29x10^{-3}$	0.039608	0.012065	0.5187	Exponential	$1.99x10^{-3}$	0.052002	0.005463	0.8745	Exponential
L104B	0.04167	0.021487	0.007301	0.4642	Exponential	$6.00x10^{-4}$	0.082835	0.015572	0.6852	Logistic
Chinese	$3.96x10^{-2}$	0.024977	0.007071	0.5551	Exponential	$1.38x10^{-4}$	0.110652	0.005909	0.9643	Logistic
Nkatiesari	0.014085	0.027776	0.010467	0.4132	Exponential	$2.12x10^{-3}$	0.052721	0.005278	0.8847	Logistic
Sarinut-1	0.026128	0.023169	0.009769	0.36	Logistic	$7.45x10^{-4}$	0.06627	0.005309	0.923	Logistic
Sarinut-2	0.084414	0.014325	0.008243	0.2319	Exponential	$8.42x10^{-4}$	0.082812	0.006854	0.9182	Logistic

4.9 Components of resistance

4.9.1 Number of lesions

Number of lesions varied significantly among genotypes ($P < 0.05$ and $P < 0.001$, respectively) for both 2020 and 2021. In 2020, 76J had the least number of lesions (63.8) and was significantly different from Sarinut-2 which had the highest (430.9). It was however not significantly different from Nkatiesari, Sarinut-1, L027B, L010A1, L030 and L046. Lesion numbers of L104B and Sarinut-2 did not differ from the susceptible check (Chinese). A similar trend was also observed for 2021. Although, Chinese had the highest number of lesions and more lesions than Sarinut-2 in 2021 there was no significant difference ($P > 0.05$) between them. Sarinut-1, L010A1, L027B and L076J were all comparable to the resistant check. L0104B had significantly lower number of lesions in 2021 as compared to Chinese and was comparable to L030 and L040. Conclusively, number of lesions were observed to be higher for most genotypes in 2021 as compared to 2020 (Table 4.6).

4.9.2 Lesion diameter

There were significant differences ($P < 0.05$ in 2020 and $P < 0.001$ in 2021) among genotypes in terms of lesion diameter (Table 4.6). The mean lesion diameter among genotypes ranged from 0.1827 - 1.3960 mm in 2020 and 1.090 - 1.664 mm in 2021. In 2020, the largest lesion diameter was observed for Sarinut-2 (1.3960 mm) whilst the lowest was observed for L010A1 (0.1827 mm). Lesion diameter observed for the susceptible check (Chinese) was comparable to Sarinut-2. L010A1 and Sarinut-1 had a smaller lesion diameter as compared to Nkatiesari (resistant check). L027B and L076J were also comparable to the resistant check. This trend varied a bit in 2021. The largest lesion diameter (1.664 mm) was observed for the susceptible

check (Chinese) and this was statistically similar to Sarinut-2 (1.527 mm) (Table 4.6). Lesion diameter for the rest of the genotypes is statistically similar ($P > 0.05$).

4.9.3 Percentage necrotic area

There were significant ($P < 0.001$) differences in percentage necrotic area among groundnut genotypes for both 2020 and 2021 as induced by the disease. In 2020, percentage necrotic area was lower for L076J (3.44 %) than for both the resistant and susceptible checks (5.91 and 74.85 %, respectively). L010A1, L027B, Sarinut-1, L046 and L030 were all comparable to the resistant control (Nkatiesari). On the other hand, L0104B were comparable to the susceptible control whilst Sarinut-2 was also comparable to L104B. In 2021, percentage necrotic area for L027B, L010A1, L076J, Sarinut-1 and Nkatiesari were not significantly different. There was no significant difference between Sarinut-2 and Chinese. (Table 4.6)

Table 4.6: Lesion number, lesion diameter, and percentage necrotic area of groundnut genotypes under field infections in 2020 and 2021

	No. of Lesions/leaf				Lesion diameter (mm)		% Necrotic area (%)	
Genotypes	**2020 (BT)**	**2021 (SQRT)**	**2021 (BT)**	**2021 (SQRT)**	**2020**	**2021**	**2020**	**2021**
L010A1	116.5 ab	10.79 ab	83.3 a	9.1 a	0.18 a	1.11 a	4.23 a	8.02 a
L027B	77.2 ab	8.78 ab	83.5 a	9.1 a	0.37 ab	1.15 a	4.10 a	7.47 a
L030	202.6 a-c	14.2 a-c	199.0 b	44.6 b	1.06 a-c	1.19 a	20.30 a	17.08 bc
L046	197.6 a-c	14.05 a-c	214.4 b	14.6 b	0.98 a-c	1.26 a	19.05 a	19.93 c
L076J	63.8 a	7.98 a	66.5 a	8.1 a	0.23 ab	1.09 a	3.44 a	7.71 a
L104B	283.7 b-d	16.84 b-d	214.7 b	14.6 b	0.98 a-c	1.25 a	63.08 bc	37.51 d
Chinese	349.8 cd	18.70 cd	659.9 c	25.6 c	1.19 bc	1.66 b	74.85 c	67.09 e
Nkatiesari	70.0 ab	8.36 ab	74.0 a	8.6 a	0.32 ab	1.10 a	5.91 a	9.52 ab
Sarinut-1	81.8 ab	9.04 ab	60.8 a	7.7 a	0.19 a	1.12a	4.40 a	7.50 a
Sarinut-2	430.9 d	20.75 d	628.7 c	25.0 c	1.39 c	1.52 b	50.77 b	60.84 e
Mean	187	12.95	228.5	16.7	0.69	1.25	25	24.27
CV	59.4	27.2	20.9	11.4	72.1	11	40.9	20.1

BT = Back transformed, SQRT = Square root transformed. Figures sharing the same alphabet in a column are statistically the same

4.10 Disease severity rating

4.10.1 Early leafspot

Early leafspot disease symptoms were observed on all ten groundnut genotypes screened. However, they responded differently ($P < 0.001$) to the pressure of the disease in their level of resistance or otherwise. In 2020, L027B, L076J, Sarinut-1, and Nkatiesari (resistant check) were classified resistant (2-3.9), L104B was highly susceptible (8-10) whilst the susceptible check remained susceptible (6-7.9). The remaining four genotypes; Sarinut-1, Sarinut-2, L030, and L046 were moderately resistant (4-5.9) (Table 4.7). In 2021, Nkatiesari was classified as resistant variety (2-3.9), Four genotypes; L027B, Sarinut-1, L010A1, and L076J were moderately resistant (4-5.9). The susceptible check (Chinese) was highly susceptible (8-9), whilst the remaining four genotypes; Sarinut-2, L104B, L030, and L046 were susceptible (6-7.9) (Table 4.7). Across the years, Nkatiesari was classified as resistant, five genotypes namely, L010A1, L024B, L030, L046, L076J, and Sarinut-1 were moderately resistant. Sarinut-2 and L104B were classified susceptible genotypes whilst Chinese was classified highly susceptible (Table 4.7).

4.10.2 Late leafspot

Groundnut genotypes varied ($P < 0.001$) in their response to the late leafspot disease in both 2020 and 2021. In 2020, three genotypes; L030, L046, and Chinese were moderately resistant (4-5.9) to the disease. The seven remaining genotypes were resistant (2-3.9) to the disease (Table 4.7). In 2021, three genotypes (L010A1, Sarinut-1, and Nkatiesari) were resistant (2-3.9) to the late leafspot disease. L076J and L027B were moderately resistant (4 -5.9) to the disease. Chinese was highly susceptible (8-9) to the disease whilst the remaining four genotypes; Sarinut-2,

L030, L046, and L104B were susceptible (6 -7.9) to the disease. Across the years, L010A1, L027B, L076J, Nkatiesari and Sarinut-1 were classified resistant genotypes. L030, L104B, and Sarinut-2 were moderately resistant, whilst L046 and Chinese were susceptible to the disease. However, based on disease rating and yield and its components, Chinese, Sarinut-2, L046 can be classified as leafspot tolerant genotypes as they gave an appreciably good yield even though they were susceptible to the early and late leafspot diseases (Table 4.7 and Table 4.9).

Table 4.7: Classification of resistance status of groundnut genotypes based on early and late leafspot disease severity scores

Genotypes	Early leafspot									Late leafspot								
	2020 (BT)	2020 (LT)	Rating	2021 (BT)	2021 (LT)	Rating	Across years (BT)	Across year (LT)	Rating	2020 (BT)	2020 (LT)	Rating	2021 (BT)	2021 (LT)	Rating	Across years (BT)	Across years (LT)	Rating
L010A1	4.17 cd	0.62 cd	MR	4.50 ab	0.65 ab	MR	4.31 b	0.63 b	MR	2.50 ab	0.39 ab	R	3.83 b	0.58 b	R	3.167 bc	0.49 bc	R
L027B	3.50 bc	0.54 bc	R	4.67 ab	0.66 b	MR	4.01 b	0.60 b	MR	3.00 b-d	0.47 b-d	R	4.00 b	0.60 b	MR	3.50 c	0.53 c	R
L030	4.67 d	0.66 d	MR	6.33 c	0.80 c	S	5.50 c	0.73 c	MR	4.50 e	0.65 e	MR	6.00 c	0.77 c	S	5.25 d	0.71 d	MR
L046	4.17 cd	0.61 cd	MR	7.50 d	0.87 cd	S	5.83 cd	0.76 cd	MR	4.83 e	0.68 e	MR	7.17 d	0.85 d	S	6.00 e	0.76 e	S
L076J	3.00 ab	0.47 ab	R	5.00 b	0.69 b	MR	4.00 b	0.60 b	MR	2.50 a-c	0.39 a-c	R	3.33 ab	0.52 ab	MR	2.91 a-c	0.46 c	R
L104B	8.50 e	0.92 e	HS	7.00 cd	0.84 cd	S	7.75 e	0.88 e	S	3.33 d	0.52 d	R	6.33 c	0.80 c	S	4.83 d	0.66 d	MR
Chinese	7.83 e	0.89 e	S	9.00 e	0.95 e	HS	8.41 f	0.92 e	HS	4.50 e	0.65 e	MR	8.67 e	0.93 e	HS	6.58 e	0.79 e	S
Nkatiesari	2.67 a	0.41 a	R	3.83 a	0.57 a	R	3.25 a	0.51 a	R	2.00 a	0.30 a	R	2.67 a	0.42 a	R	2.33 a	0.36 a	R
Sarinut-1	3.67 bc	0.56 bc	R	4.83 b	0.68 b	MR	4.25 b	0.62 b	MR	2.00 a	0.30 a	R	3.33 ab	0.52 ab	R	2.67 ab	0.41 ab	R
Sarinut-2	4.67 d	0.66 d	MR	7.83 d	0.89 de	S	6.25 d	0.79 d	S	3.33 b-d	0.52 b-d	R	7.17 d	0.85 d	S	5.25 d	0.68 d	MR
Mean	4.68	0.63		6.05	0.76		5.36	0.7		3.25	0.49		5.25	0.68		4.25	0.58	
CV	9.9	7.9		8.1	5.8		6.4	4.3		13.7	9.4		8.4	7.5		8.1	5.6	

BT = Back transformed, LT = Log transformed, HR = Highly resistant, R = Resistant, MR = Moderately resistant, S = Susceptible, HS = Highly susceptible

4.11 Agronomic parameters and yield

4.11.1 Days to first flowering

Days to first flowering was significantly different ($P < 0.001$) among genotypes in both 2020 and 2021. In 2020, L046 took as much as 26 days to reach first flowering while L027B took the least days (20) to reach first flowering. In 2021, Chinese was the earliest to reach first flowering (23.33 days) as compared to that of L010A1 and L027B which took 28 days **(**Table 4.8**)**

4.11.2 Days to 50% flowering

Days to fifty percent flowering varied significantly in both 2020 and 2021 ($P < 0.05$ and $P < 0.001$, respectively). Days to 50% flowering among genotypes in 2020 was between 27 and 33 days. L030 and L027B had the least and highest number of days to reach 50% flowering respectively. In 2021, Chinese was the earliest to attain 50% flowering at 26 days) whilst L010A1 and L027B took the highest number of days (31) to reached 50% (

Table 4.8).

Table 4.8: Effect of groundnut genotype on number of days to flowering

Genotypes	DFF		D50%F	
	2020	**2021**	**2020**	**2021**
L010A1	22.00 b	28.33 d	31.67 b	31.00 f
L027B	20.00 a	28.33 d	33.00 b	31.00 f
L030	24.67 d	25.00 b	27.00 a	26.67 ab
L046	26.00 d	26.33 bc	27.67 a	29.00 de
L076J	25.00 d	26.33 bc	30.33 ab	29.33 e
L104B	22.00 b	27.67 cd	31.67 b	30.67 f
Chinese	22.00 b	23.33 a	30.33 ab	25.67 a
Nkatiesari	24.33 cd	26.00 bc	31.67 b	29.00 de
Sarinut-1	22.67 bc	25.00 ab	31.67 b	28.00 cd
Sarinut-2	22.67 bc	25.00 ab	30.33 ab	27.00 bc
Mean	23.13	26.13	30.53	28.73
CV	4.3	3.6	6.5	2.3

DFF = Days to 1st flowering, D50%F = Days to 50% flowering

4.12 Yield assessment

Yield among genotypes were assessed in terms of number of pods per plant, pod weight (kg/ha), hundred seed weight (g), grain yield (kg/ha), and Biomass yield (kg/ha)

4.12.1 Number of pods per plant

There were significant ($P < 0.05$) among genotypes for number of pods per plant for both 2020 and 2021. In 2020, L046 had a significantly higher average number pods per plant than both the resistant and susceptible checks (Nkatiesari and Chinese, respectively). However, this did not differ from Sarinut-1 and L076J. There was no significant deference between the resistant and susceptible checks, as well as L104B in terms of average number of pods per plant (Table 4.9). In 2021, number of pods for L046 (29.12), just as in 2020 was significantly higher than that of both the resistant and susceptible checks (12.93 and 13.07, respectively). However, all the other seven genotypes didn't show any significant variation for pods per plant when compared to both the resistant and susceptible checks. Notwithstanding this, number of pods were higher in 2021 as compared to 2020 (Table 4.9).

4.12.2 Pod yield

Across the years, genotypes responded differently ($P < 0.001$) to the impact of the disease in terms of pod yield. In 2020, the highest pod yield was observed for L030 (2235 kg/ha), and was significantly higher than L014B which had the lowest pod yield (650 kg/ha). However, pod yields for L046, Sarinut-1, Sarinut-2, Chinese and L076J were comparable to L030. There was no significant difference between the resistant and susceptible controls in terms of pod yield (Table 4.9). As observed in 2020, L030 had the higher pod yield as compared to the resistant control in 2021. Again, pod yields for L046, Sarinut-1, Sarinut-2, Chinese and L076J were comparable to that of L030. As observed in number of pods per plant, pod yields were also higher for 2021 compared to 2020 (Table 4.9).

4.12.3 Grain yield

Grain yield among genotypes were significantly different ($P < 0.001$) for both years. In 2020, grain yield observed for L046 (1914 kg/ha) was significantly higher as compared to the resistant check (Nkatiesari) (1121 kg/ha). However, this was comparable to those of L076J, Sarinut-1, Sarinut-2 and the susceptible control (Chinese). L104B had the lowest grain yield (568 kg/ha). However, this was comparable to that of L010A1, L027B (643 and 1053 kg/ha, respectively), and the resistant control (1121 kg/ha) (Table 4.9). The results in 2021 followed a similar trend as 2020. The highest grain yield was observed for L046 (2603 kg/ha) whilst the lowest was obtained for L104B (772 kg/ha). However, the yields of L076J, Chinese, Sarinut-2 and Sarinut-1 were comparable to that of L046. Just as 2020, grain yield was very low for L104B (772 kg/ha). However, it was comparable to L010A1, L027B and the resistant check (Table 4.9).

4.12.4 Hundred seed weight

Hundred seed weight varied significantly among genotypes in both 2020 and 2021 ($P < 0.001$). Hundred seed weight in 2020 ranged from 24 – 46 g. L076J had a significantly higher hundred seed weight as compared to Nkatiesari (resistant check), however, it is not significantly different from Sarinut-2 and Sarinut-1. Hundred seed weights observed for L010A1, L027B, L030, L046, L104B and Nkatiesari were appreciably lower as compared to the susceptible check which were statistically similar (Table 4.9). In 2021, both the highest and lowest hundred seed weights were higher as compared to that of 2020 (46.00 and 26.33 versus 45.33 and 24.33, for 2021 and 2020 respectively). Just as in 2020, L076J had the highest hundred seed weight whilst L104B had the lowest. Hundred seed weight for Sarinut-

1 is higher than the resistant check but comparable to L076J. Also, hundred seed weights for L010A1, L027B, L030, L046 Sarinut-2 and the susceptible check were comparable to the resistant check (Table 4.9).

4.12.5 Biomass yield

Genotypes exhibited significant variations ($P < 0.05$) in terms of biomass yields in both 2020 and 2021. The highest biomass was observed for Sarinut-1 in both 2020 and 2021 (3841 and 6597 kg/ha respectively). The lowest biomass yield was observed for Chinese in both 2020 and 2021 (1129 and 3062 kg/ha respectively). However, in 2020, biomass yield for Chinese was comparable to that of L010A1, L030, L046, L076J, L104B, Sarinut-2 and Nkatiesari. L027B was also comparable to Sarinut-1 (Table 4.9). In 2021, there were no significant differences among L076J, L010A1, L027B, L076J and Nkatiesari (resistant check) and Sarinut-1. Similarly, there was no significant difference between L104B and Sarinut-2 compared to Chinese (susceptible check) (Table 4.9).

4.12.6 Shelling percentage

Shelling percentage was estimated for both 2020 and 2021. In 2020, Chinese had the highest shelling percentage (90.34%) whilst the lowest was observed for L030 (54.45 %). In 2021, the highest shelling percentage was 92.63 %, observed for L046 whilst the lowest was 59.23 % and was observed for L030. Despite these numerical variations, genotypic variations were not statistically different for both years ($P > 0.05$) (Table 4.9).

4.12.7 Harvest Index

Harvest index was computed for both 2020 and 2021. In 2020, harvest index varied significantly ($P < 0.05$) among genotypes. Chinese had the highest harvest index of0.58 whilst the lowest was observed for L010A1 (0.22). L076J, Sarinut-2 and L046 had comparable harvest indices (0.40, 0.45 and 0.45, respectively) but were lower as compared to that of Chinese. At the lower end, HI of L027B, L030, Sarinut-1, and L104B were comparable to L010A1. Genotypic variation again was significant for harvest index in 2021. Again, the harvest index for Chinese (0.38) was the highest. However, this did not differ from that of Sarinut-2 and L046. Again, L010A1 was the least in terms of harvest index with an index of 0.11 even though this was not different from L027B, L104B, and Nkatiesari. A careful observation revealed that harvest indexes among genotypes in 2020 were higher as compared to that of 2021 (Table 4.9).

Table 4.9: Yield and yield components of groundnut genotypes under leafspot disease pressure

Genotypes	No. of pods/plant		Pod weight (kg/ha)		Grain yield (kg/ha)		100 SW (g)		Shelling % (%)		Biomass yield (kg/ha)		Harvest Index	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
L010A1	4.022 c	10.37 b	796 cd	1125 c-e	643 ef	875 de	24.33 e	28.00 c	80.30 ab	68.56 ab	2371 bc	6432 ab	0.22 d	0.11 f
L027B	7.275 bc	10.02 b	1339 b-d	1800 bc	1053 d-f	1432 c-e	31.33 c-e	32.00 c	78.27 ab	79.78 ab	2735 ab	6165 ab	0.27 d	0.18 ef
L030	5.771 c	10.47 b	2235 a	2850 a	1175 b-e	1599 b-d	26.67 de	27.67 c	54.45 b	59.23 b	1364 bc	3699 cd	0.33 cd	0.25 b-e
L046	15.311 a	29.12 a	2200 a	2683 a	1914 a	2603 a	31.00 de	32.33 c	87.30 a	92.63 a	2045 bc	5548 a-c	0.45 b	0.30 a-c
L076J	10.338 a-c	18.47 b	2085 ab	2558 ab	1708 ab	2450 a	45.33 a	46.00 a	82.30 ab	91.14 a	2250 bc	6103 ab	0.40 bc	0.28 b-d
L104B	4.217 c	12.80 b	650 d	983 ce	568 f	772 e	27.33 de	26.33 c	87.29 a	74.98 ab	1227 bc	3329 d	0.31 cd	0.19 d-f
Chinese	8.987 bc	13.07 b	2032 ab	2467 ab	1794 a	2440 a	34.00 b-d	33.33 bc	90.34 a	85.65 ab	1129 c	3062 d	0.58 a	0.38 a
Nkatiesari	8.615 bc	12.93 b	1440 a-c	1792 b-d	1121 c-f	1524 b-e	30.67 de	30.33 c	78.45 ab	84.71 ab	2356 bc	6391 ab	0.29 cd	0.19 d-f
Sarinut-1	12.827 ab	12.70 b	2218 a	2358 ab	1402 a-d	1907 a-c	38.67 a-c	40.33 ab	64.09 ab	80.86 ab	3841 a	6597 a	0.26 d	0.21 c-e
Sarinut-2	8.925 bc	12.71 b	2088 ab	2567 ab	1634 a-c	2222 ab	40.67 ab	33.67 bc	78.31 ab	85.08 ab	1606 bc	4357 b-d	0.45 b	0.33 ab
Mean	8.63	14.27	1708	2118	1301	1782	33	33	78.1	80.3	2092	5168	0.36	0.24
CV	39	32.2	24.3	20.9	23.4	23.2	12.4	13	18.5	18.4	37.7	21.6	18.2	20.4

4.13 Correlation Analysis

The results of this study revealed that there were significant relationships among most of the variables measured. In 2020, both ELS_AUDPC and LLS_AUDPC significantly and positively correlated with LLS_AUDPC, number of lesions, lesion diameter, and % necrotic area but is negatively correlated with biomass yield. DI_AUDPC was significantly and positively correlated with lesion diameter, % necrotic area and harvest index. Number of lesions was significant and positively correlated with % necrotic area and harvest index but negatively correlated with biomass yield. Lesion diameter correlated positively with % necrotic area. Percentage necrotic area significantly and positively correlated with harvest index but is negatively correlated with biomass yield. Number of pods per plant is significantly and positively correlated with pod yield and grain yield. Pod yield is significantly and positively correlated with 100 seed weight and grain yield. Hundred seed weight is significantly and positively associated with Grain yield. Grain yield is significantly and positively correlated with harvest index. Biomass is significantly and negatively correlated with harvest index, whilst % shelling is significantly and positively correlated with harvest index (Table 4.10). In 2021, DI_AUDPC is significantly and positively correlated with ELS_AUDPC, LLS_AUDPC, number of lesions per leaf, lesion diameter, % necrotic area, pod yield, grain yield, and harvest index but it negatively associated with biomass yield. ELS_AUDPC is significantly and positively correlated with LLS_AUDPC, number of lesions per leaf, lesion diameter, % necrotic area, grain yield, biomass yield, and harvest index. LLS_AUDPC is significantly and positively correlated with number of lesions per leaf, lesion diameter, % necrotic area grain yield and harvest index

but it is negatively correlated with biomass yield. Number of lesions is significantly and positively correlated with lesion diameter, % necrotic area, and harvest index but is negatively correlated with biomass yield. Lesion diameter is significantly and positively correlated with % necrotic area and harvest index but it is negatively correlated with biomass yield. Percentage necrotic area is significantly and positively correlated with harvest index but is negatively associated with biomass yield. Number of pods per plant is significantly and positively correlated with grain yield. Pod yield is significantly and positively associated with grain yield and harvest index. Grain yield is significantly and positively correlated with 100 seed weight, shelling %, and harvest index. Hundred seed weight is significantly and positively correlated with shelling percentage. Biomass yield is significantly and negatively correlated with harvest index, whist shelling percentage is significantly and positively correlated with harvest index (Table 4.11).

Table 4.10: Correlation among some variables in 2020

Variables	ELS_AUDPC	LLS_AUDPC	DI_AUDPC	No of lesion	Lesion diameter	% Necrotic area	No of pods/plant	Pod weight (kg/ha)	100 seed weight	Grain weight (kg/ha)	Biomass (kg/ha)	Shelling %	Harvest index
ELS_AUDPC	1												
LLS_AUDPC	**0.41***	1											
DI_AUDPC	0.32	**0.43***	1										
No of lesion	**0.53****	**0.59*****	0.34	1									
Lesion diameter	**0.37***	**0.56****	**0.85*****	0.29	1								
% Necrotic area	**0.81*****	**0.59*****	**0.58*****	**0.77*****	**0.59*****	1							
No of pods/plant	-0.36	0.19	0.08	-0.09	-0.02	-0.16	1						
Pod weight (kg/ha)	-0.34	0.35	0.22	0.02	0.21	-0.05	**0.69 *** **	1					
100 seed weight	-0.13	-0.13	0.16	-0.05	-0.05	-0.08	0.27	**0.40 ***	1				
Grain weight (kg/ha)	-0.23	**0.43***	0.26	0.12	0.19	0.09	**0.72 *** **	**0.84*****	**0.51 ***	1			
Biomass (kg/ha)	**-0.49****	**-0.55***	-0.33	**-0.41***	-0.36	**-0.48****	0.31	0.18	0.16	0.05	1		
Shelling %	0.28	0.18	0.04	0.19	-0.01	0.29	-0.06	-0.32	0.17	0.19	-0.28	1	
Harvest index	0.27	**0.68 *** **	**0.39***	**0.45***	0.35	**0.51****	0.26	0.36	0.32	**0.66*****	**-0.59*****	**0.53****	1

=significant at 0.05, **= significant at 0.01, * = significant at 0.001*

Table 4.11: Correlation among some variables in 2021

Variables	DI_AUDPC	ELS_AUDPC	LLS_AUDPC	No. Lesion/Leaf	Lesion diameter	% Necrotic area/leaf	No of pods/plant	Pod weight (kg/ha)	Grain weight (kg/ha)	100 Seed weight	Biomass (kg/ha)	Shelling %	Harvest index
DI_AUDPC	**1**												
ELS_AUDPC	**0.89*** **	**1**											
LLS_AUDPC	**0.83*** **	**0.94*** **	**1**										
No. Lesion/Leaf	**0.75*** **	**0.85*** **	**0.79*** **	**1**									
Lesion diameter	**0.62*** **	**0.74*** **	**0.72*** **	**0.79*** **	**1**								
% Necrotic area/leaf	**0.76*** **	**0.86*** **	**0.79*** **	**0.95*** **	**0.83*** **	**1**							
No of pods/plant	0.19	0.21	0.17	-0.01	-0.03	-0.04	**1**						
Pod weight (kg/ha)	**0.41***	0.31	0.19	0.28	0.25	0.14	0.24	**1**					
Grain weight (kg/ha)	**0.36***	**0.37***	0.21	0.32	0.17	0.20	**0.41***	**0.81***	**1**				
100 Seed weight	-0.12	-0.10	-0.23	-0.11	-0.11	-0.12	0.19	0.24	**0.43***	**1**			
Biomass (kg/ha)	**-0.54****	**-0.64 *** **	**-0.67*** **	**-0.59*** **	**-0.58*** **	**-0.63*** **	0.02	-0.04	0.08	0.29	**1**		
Shelling %	0.03	0.21	0.06	0.14	-0.07	0.15	0.32	0.02	**0.56****	**0.41***	0.09	**1**	
Harvest index	**0.59*** **	**0.72*** **	**0.58*** **	**0.700**	**0.57*** **	**0.65*** **	0.290	**0.58*** **	**0.73*** **	0.220	**-0.56****	**0.49****	**1**

=significant at 0.05, **= significant at 0.01, * = significant at 0.001*

CHAPTER FIVE

DISCUSSION

5.1 Disease epidemics assessed as incidence, severity and progress

The study provided a very useful insight on early and late leafspot disease progress and categorisation of field resistance of the ten groundnut genotypes. Nkatiesari was classified as resistant, L010A1, L027B, L030, L046, L076J, and Sarinut-1 were classified as moderately resistant. Sarinut-2 and L104B were classified susceptible genotypes whilst Chinese was classified highly susceptible. Based on yield data Sarinut-2 and Chinese could be considered as tolerant genotypes. These results are similar to what has been reported in previous field trials (Tengey *et al*., 2020, unpublished data).

DI-AUDPC values for Sarinut-2, L104B and the susceptible check (Chinese) were higher as compared to that of most resistant genotypes. Similar trends were observed for ELS-AUDPC and LLS-AUDPC. Lower disease incidence and severity in most resistant genotypes except for the resistant check could be as a result of the transfer of leafspot resistance genes present in these genotypes transferred through the cross with interspecific introgression lines 43-09-03-02 and 60-02-03-02 (Tengey, 2018; Denwar *et al*., 2021). Apart from the presence of resistance genes, defensive mechanism such as cuticular wax on groundnut leaves as reported by (Waller & Lenné, 2001) could also be a reason for a slower rate of disease progression for some genotypes in this study. The increased levels of incidence and severity in 2021 is as a result of increased level of initial or primary inoculum as the experiment was repeated on the same field. Agrios (2005) reported that initial inoculum loads

determine the amount of disease caused. The steady progression of disease incidence and severity in Sarinut-2, L104B and the susceptible check (Chinese), confirms the trend of disease severity ranking such that most susceptible genotypes had higher severity scores whilst resistant genotypes had lower or moderate severity scores. Impacts of the disease were observed as distorted leaves, accompanied with multiple yellow to dark brown spots with or without halos and defoliation. (Ngegba *et al.*(2017) reported a similar trend when they evaluated some groundnut genotypes for severity to both the early and late leafspot diseases. Wide variation in the ecosystems, such as temperature, vegetation, rainfall, inoculum load, among others, attributes to a very dynamic progression or retardation of plant diseases. In 2020, the best fitting model for severity was the logistic model ($0.50 < R^2 < 0.96$) although Gompertz model best fitted for genotypes that had lower infection rates. According to results of the current study, 2021 infection rates for the logistic model was higher than that of the Gompertz model. This is also to say that regression lines in the Logistic fittings were steeper as compared to that of the Gompertz model. This finding corroborates to that of Mohapatra *et al*. (2008) who reported that Gompertz model fitted best for lower infection rates in rice blast disease progress. Initial inocula for disease incidence in this study did not follow any defined pattern. This is because the disease was present on all the ten genotypes. Also, disease incidence is the presence or absence of the disease on the plant, hence is not really influenced by the amount of initial inocula. Resistant genotypes identified in the present study had slower infection rate compared to susceptible ones. Perhaps, the slower rate of infection rates and progress of the disease in Nkatiesari, L010A, L027B, L076J and Sarinut-1(resistant genotypes) could be as a result of programmed cell death (PCD)

or hypersensitive response of leaf cells. This response compels cells at a site of infection to automatically shut down or cascade to prevent further spread to other healthier cells (Whalen *et al*., 1993). Sarinut-2, L046, L030 and the susceptible check (Chinese), gave an appreciably higher yield despite suffering severe effects of the disease. These genotypes are therefore categorised as tolerant genotypes. A similar trend was observed by Cantonwine *et al.* (2008) and Thakur *et al.* (2012)

5.2 Components of resistance

Components of resistance such as number of lesions per leaf, lesion size, and percentage necrotic area are some of the very relevant parameters in assessing resistance or susceptibility to foliar diseases (Chiteka *et al*., 1988).

The results of this study revealed a significant variation among genotypes when assessed in 2020 and 2021. Resistant genotypes had fewer number of lesions as compared to the susceptible genotypes in both years. Similarly, lesion diameter in susceptible genotypes were also larger compared to resistant genotypes. Also, a trend of reduction and/or similarities in the number of lesions per leaf in most resistant genotypes whilst susceptible genotypes saw some surge in their number of lesions per leaf in the second year of evaluation. The increase in number of lesions and lesion diameter in susceptible genotypes can be associated with a faster rate of infection by disease-causing pathogens in the susceptible genotypes. This result is in line with Tshilenge-Lukanda *et al.* (2012) who observed a higher lesion number in susceptible groundnut genotypes. Furthermore, programmed cell death (hypersensitive response) as a defensive mechanism of plants against phytopathogens (Agrios, 2005) could also be a major reason for lesser lesion

diameter observed in resistant genotypes. However, this observation is in contrast to that of Johnson *et al.* (1986) and Cantonwine *et al.* (2008) who reported that lesion diameter did not increase with an increase in lesion number.

Percentage necrotic area in this experiment followed a similar trend as number of lesions per leaf and lesion diameter. Larger necrotic areas as observed for Chinese and Sarinut-2 could mean that a larger proportion of leaf tissues were lost to the disease. This can translate into a reduced rate in photosynthesis as a result of high defoliation. L010A1, L027B, Sarinut-1, L076J and Nkatiesari (resistant check) had percentage necrotic areas below 10 %. This could be attributed to a slower rate of infection due to their resistance accounting for higher biomass yields in these genotypes

5.3 Impacts of leafspot disease on yield and yield components

Early and late leafspots have been one of the major limiting factors in groundnut production across all groundnut producing regions in Ghana. Under severe conditions as usually observed in susceptible varieties, the disease has been reported to influence the number of days of flowering, average number of pods per plant, pod weight, hundred seed weight, grain yield, shelling percentage, harvest index, and biomass weight and quality (Sinsiri *et al*., 2006; Plantwise, 2011). Days to flowering was not influenced by disease pressure as most of the genotypes reached 50 % flowering before the onset of the disease at 45 DAP thus the crops grew pass a stage where the disease could have influenced flowering (Awurum & Emechebe, 2001). This could also mean that the cultivation of early maturing cultivars could be a great deal in escaping the higher impact of the disease. Most genotypes however, spent

more days to flower in 2021 as compared to 2020. This could be as a result of residual nitrogen from the previous season trial which resulted in an improved soil fertility in 2021. According to Frankow-Lindberg & Dahlin (2013), legumes fix up to 180 kg/ha nitrogen into the soil through their symbiotic association with nitrogen fixing bacteria in the soil. Where conditions are very favourable crop plants take time to flower (Wada & Takeno, 2010).

5.3.1 Pod and grain yield

Pod and grain yield was higher for L046 in both 2020 and 2021 compared to both the resistant and susceptible checks. Most susceptible genotypes in the current study had yields that are comparable, or even higher than that of the resistant genotypes. This finding is in contrast Alidu *et al.* (2019) who observed a significantly higher yield in resistant genotypes as compared to susceptible genotypes. The differences could be attributed to differences in genetic makeup of various genotypes used in both experiments. L104B (susceptible genotype) had a significantly low yield. This is in agreement with Waliyar *et al.* (2000) that higher severity levels of foliar diseases, such as early and late leafspot distorts leaf cells and thus, interrupts with the photosynthetic processes of the plant, hence reducing its reproductive potential. The yields of L104B and L010A1 although were low compared to what is reported on farmers field (800 kg/ha) (Kombiok *et al*., 2012; MOFA, 2016), grain yield in this study was between 568 – 1914 kg/ha in 2020 and 772 – 2603 kg/ha in 2021. Generally, yield was not substantially affected by the effects of the early and late leafspot disease because of the resistance/tolerance of the genotypes to the disease. L046, Sarinut-2, and Chinese (susceptible check) had grain yields that were comparable to L076J and Sarinut-1 which exhibited some competent resistant levels

to the diseases. The ability of L046, Sarinut-2, and Chinese to give higher yields amidst higher disease pressures could also be attributed to tolerance as described by Maloy (1993). In most susceptible genotypes, low yields were recorded as a result of higher foliar disease pressures. This is because foliar diseases inhibit or interrupts the photosynthetic processes as a result of an intense defoliation or reduction in leaf area. Also, there is usually a reduction in plant chlorophyll contents as well as carbon exchange when they are under intense disease pressures (Hwang *et al*., 2006). Perhaps L046, Sarinut-2, and Chinese could also be said of as early maturing accessions which might have already podded before disease pressures rose to detrimental levels. The poor yield observed for L104B is evidence to the effect of higher disease pressures. Tolerance, therefore, could be a major reason for observed trend.

The large seed size of L076J over its contemporaries, Sarinut1 and Sarinut-2 could be attributed to its genetic make-up and also resistance to early and late leafspot disease, this is because in 2021 specifically where disease incidence and severity were higher, seed weight of L076J (46 g) was significantly higher than Sarinut-2 (33.67 g). Seed weight of the remaining genotypes were comparable to Nkatiesari, and therefore fall within the expected market class of seed size.

5.3.2 Shelling percentage

Shelling percentage reveals the actual proportion of pod yield that transcends into actual grain yield. In this study, even though genotypes did not vary greatly in terms of shelling percentage, it was observed that Chinese and L046 had the highest shelling percentages in both 2020 and in 2021, L046 and L076J were respectively

the highest. These genotypes were also found to have higher grain yield confirming. A similar trend was observed by other authors (Igze *et al.*, 2007; Chintu, 2013). Higher shelling percentage also means these genotypes either have a relatively thinner pod shell or seeds have higher fibre. Memon *et al.* (2005) reported that seeds with less fibre shrink in size and weight quickly.

5.3.3 Biomass yield and harvest index

Groundnut biomass serves as feedstock for farm animals among most poor and middle resourced farmers in most developing countries. The quantity and quality obtained, however, could be as a result of several factors. These factors could be crop species, rainfall pattern during cropping season, soil nutrient, pest and disease that causes defoliation, among others. Genotypes showed some considerable variations in terms of biomass yield in this study. The results revealed that the use of disease resistant cultivar as planting materials would be one of the sure ways in achieving higher biomass yields as the highest biomass yields from this study were observed for genotypes resistant to leafspot disease (Sarinut-1, Nkatiesari, L010A1, L027B, and L076J). The least biomass yields were observed for Chinese (susceptible check) and L104B (susceptible genotype). This is clear evidence that higher leafspot disease severity levels reduce or retards the rate of vegetative growth and development in most green plants due to reduced rate of photosynthesis (Denwar *et al*., 2021).

Chinese gave the highest harvest index in both years (0.58 and 0.38, respectively), even though did not differ from L046 and Sarinut-2. This could mean that about 58 and 38 % of the total output of Chinese was translated into grain yield in 2020 and

2021, respectively. Same explanation could also be applicable to L046 and Sarinut-2. The differences between these genotypes and L010A1 with the lowest harvest index for both of the years is related to individual shoot biomass yields. This result corroborates with that of Swetha & Bhunia (2019) who reported that grain yields transcended into a higher harvest index when proportionated to its total plant biomass. A higher shoot biomass could possibly lessen the index value when grain yield is proportionated to total output. Perhaps, higher disease severity levels of Chinese, L046 and Sarinut-2 reduced their biomass yield, hence their total plant biomass and thereby having high harvest index. This is evidenced in a study by Sripunitha *et al.* (2011) that a higher grain yield, with a relatively moderate shoot biomass gives a relatively higher harvest index. Furthermore, L010A1 in this study gave significantly higher shoot biomass as compared to Chinese, L046, and Sarinut-2 explaining its low harvest index.

5.4 Implications of association of traits

As number of lesions, lesion diameter, and percentage necrotic area increases AUDPC increases. These components of resistance and AUDPC were found to rather have a more negative impact on biomass rather than pod yield. Reduced biomass although means reduced rate of photosynthesis and therefore low yield. Studies by Denwar *et al.* (2021) did not find any significant negative association between AUDPC and pod yield. A reason for the positive correlation with yield in this study is because of the tolerance of some genotypes, and how resistance is sacrificed for low yield in some crop varieties. High defoliation leading to a reduced rate of photosynthesis among susceptible genotypes. High biomass yield reduces the

harvest index as was observed for L010A1, L027B, and Nkatiesari. A similar pattern was observed by Tengey (2018) among earlier generations of this cross.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Disease progress and components of resistance can help plant breeders select groundnut varieties that are resistant to the early and late leafspots diseases of groundnut for farmers use.

In this study, groundnut genotypes that had the best ratings through the components of resistance also had lower disease epidemic development rates and severity levels, measured as AUDPC. DI_AUDPC, ELS_AUDPC, LLS_AUDPC, and components of resistance such as number of lesions, lesion diameter, and percentage necrotic area all correlated significantly and negatively with biomass weight. The Gompertz and Exponential models suggested in the current study fitted best to disease incidence whilst the Logistic model fitted best to disease severity estimated over time. The models allowed the estimation of initial inocula and infection rates which could further be used in the classification of groundnut genotypes as susceptible or resistant. AUDPC values for resistant check (Nkatiesari) were comparable to Sarinut-1, L027B, L076J and L010A1. Based on disease rating and yield data, Chinese, Sarinut-2 and L046 can be classified as leafspot tolerant lines while Nkatiesari, Sarinut-1, L027B and L076J considered resistant lines. Among the candidate lines, L076J combines leafspot resistance with large seed size, and high biomass and grain yield. Despite the susceptibility of L046 and L030 to the disease, they also have high yielding potentials.

6.2 Recommendation

Susceptible genotypes such as Sarinut-2 and Chinese produced yields which were comparable to the resistant genotypes. This could be due to escape or tolerance. Further studies on the influence of planting date on the incidence and severity of the early and late leafspot disease should therefore be conducted.

Further studies on the influence of planting date on the incidence and severity of the early and late leafspot disease should be conducted with the evaluated genotypes.

Molecular studies should be conducted to identify the genes responsible for the resistance in the resistant genotypes.

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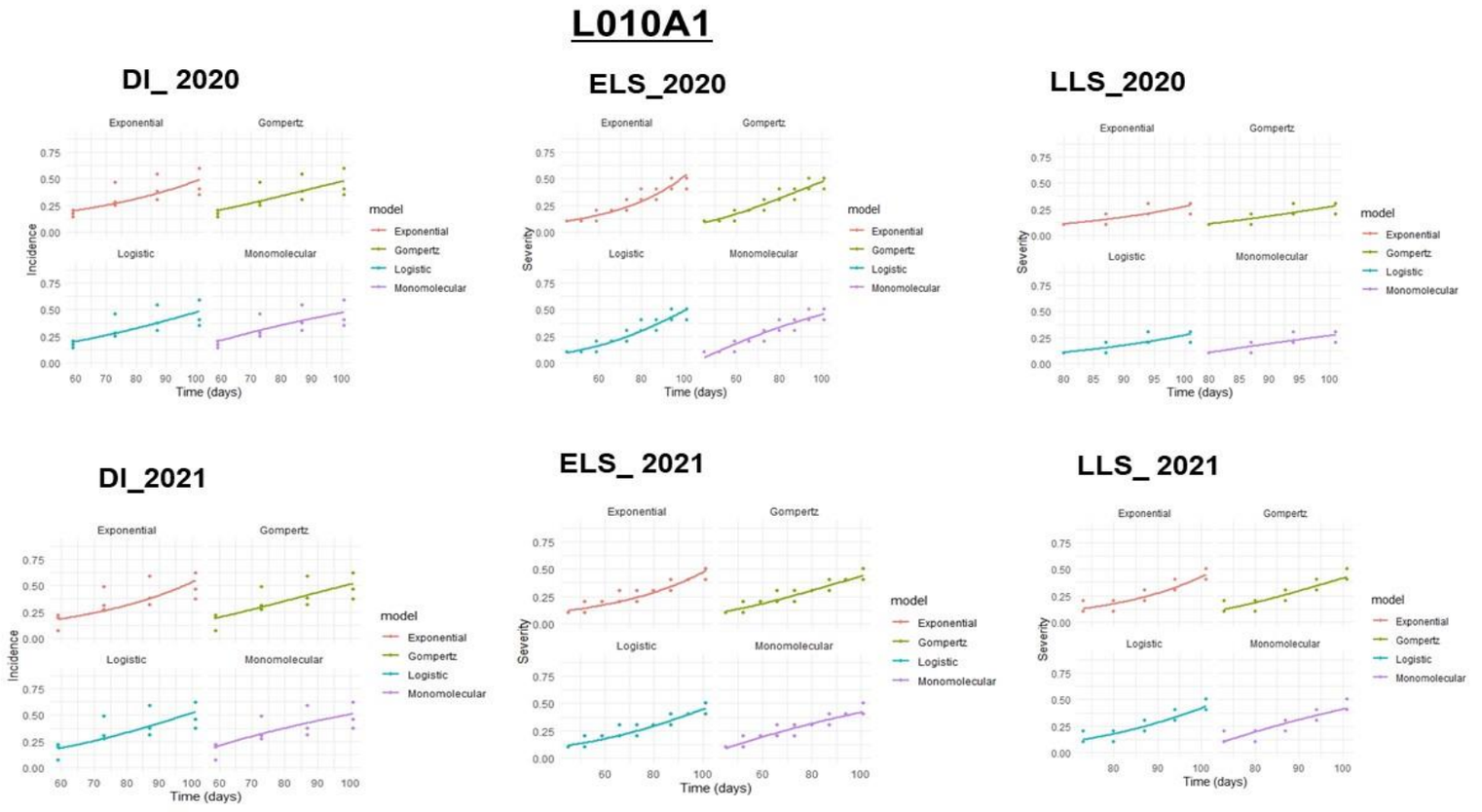
APPENDICES

Appendix 1: Symptoms of the early and late leafspots.

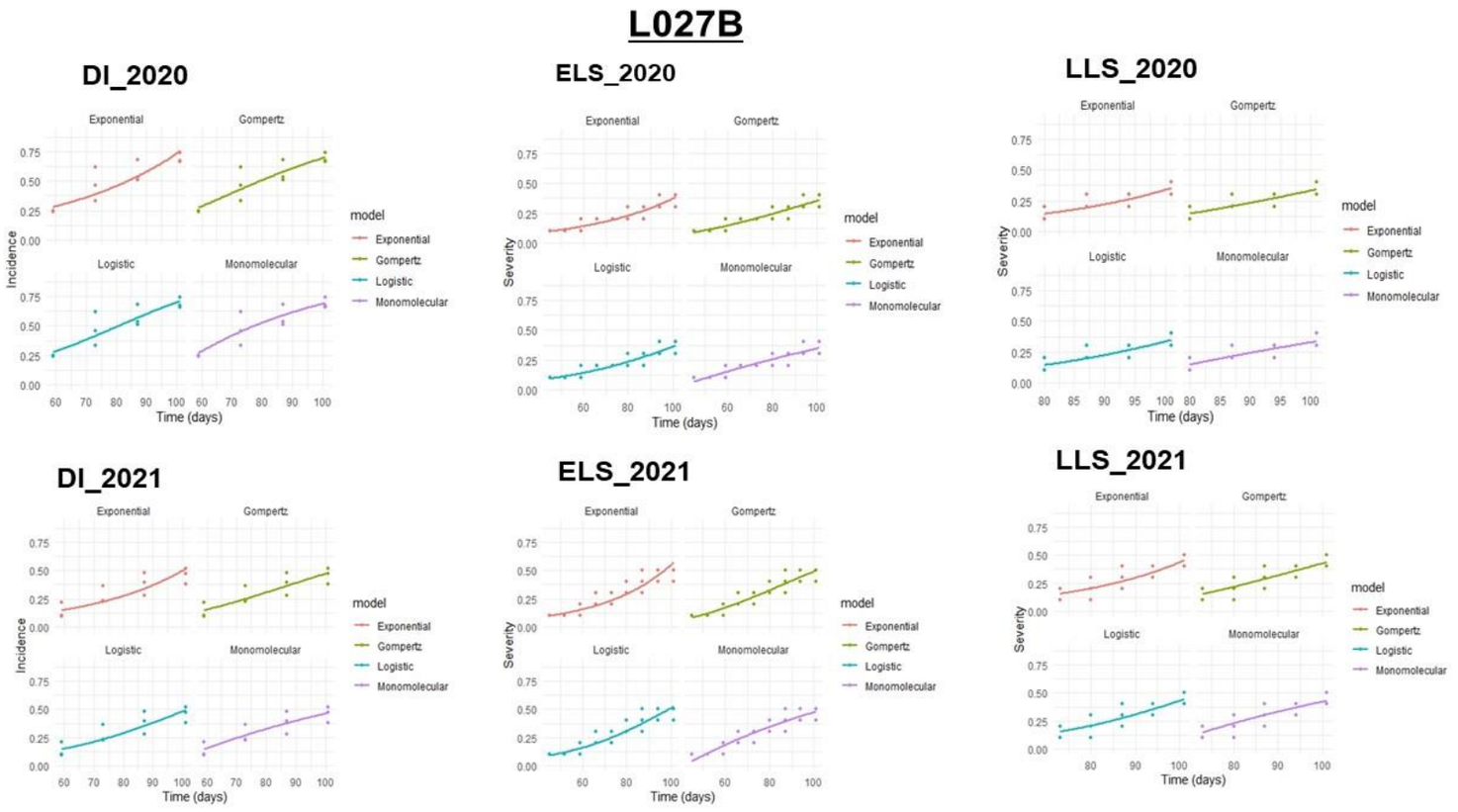
Early leafspot *(Cercospora arachidicola)*

Late leafspot *(Cercosporidium personatum)*

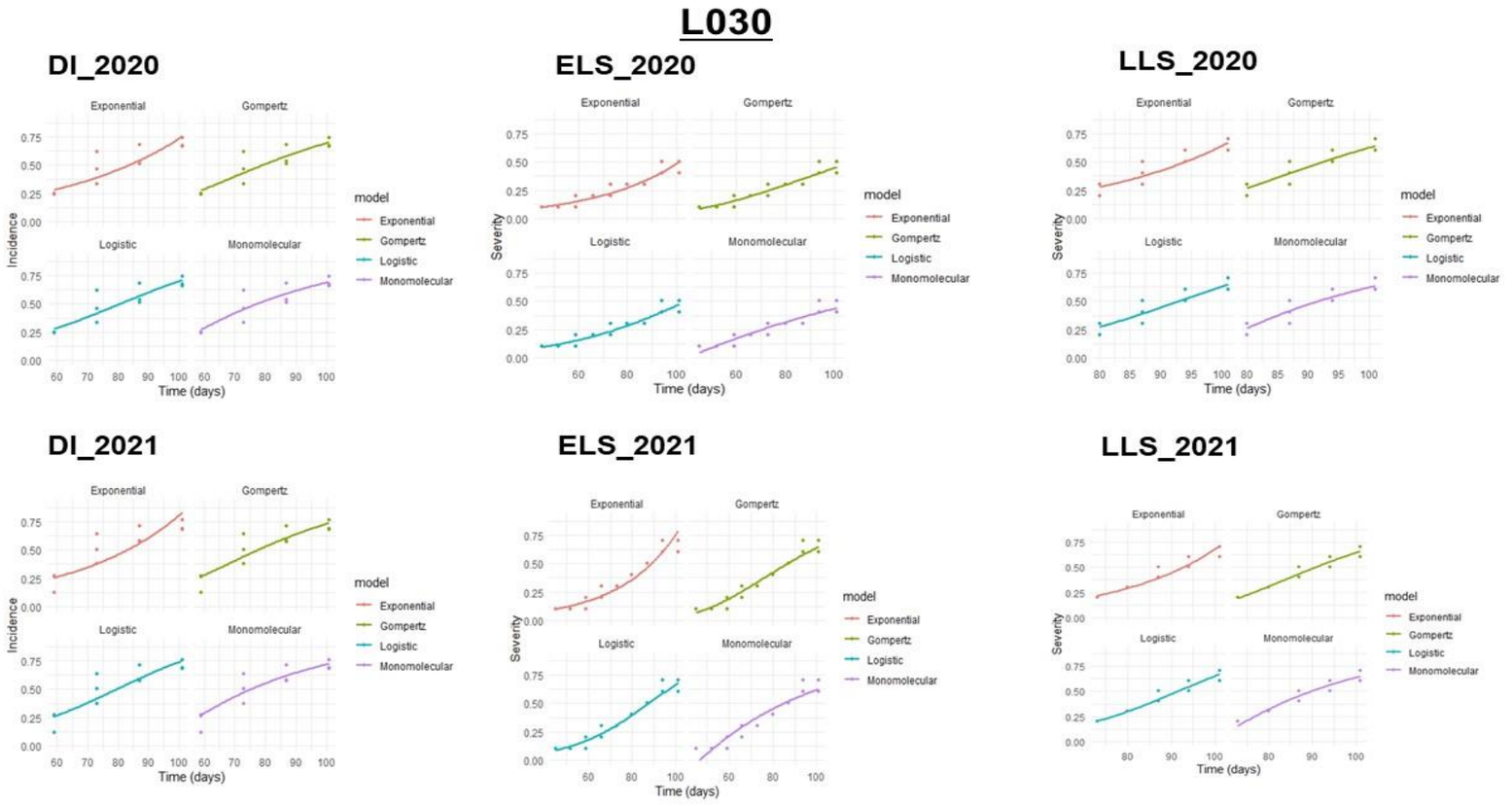
Appendix 2:Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L010A1 in two years



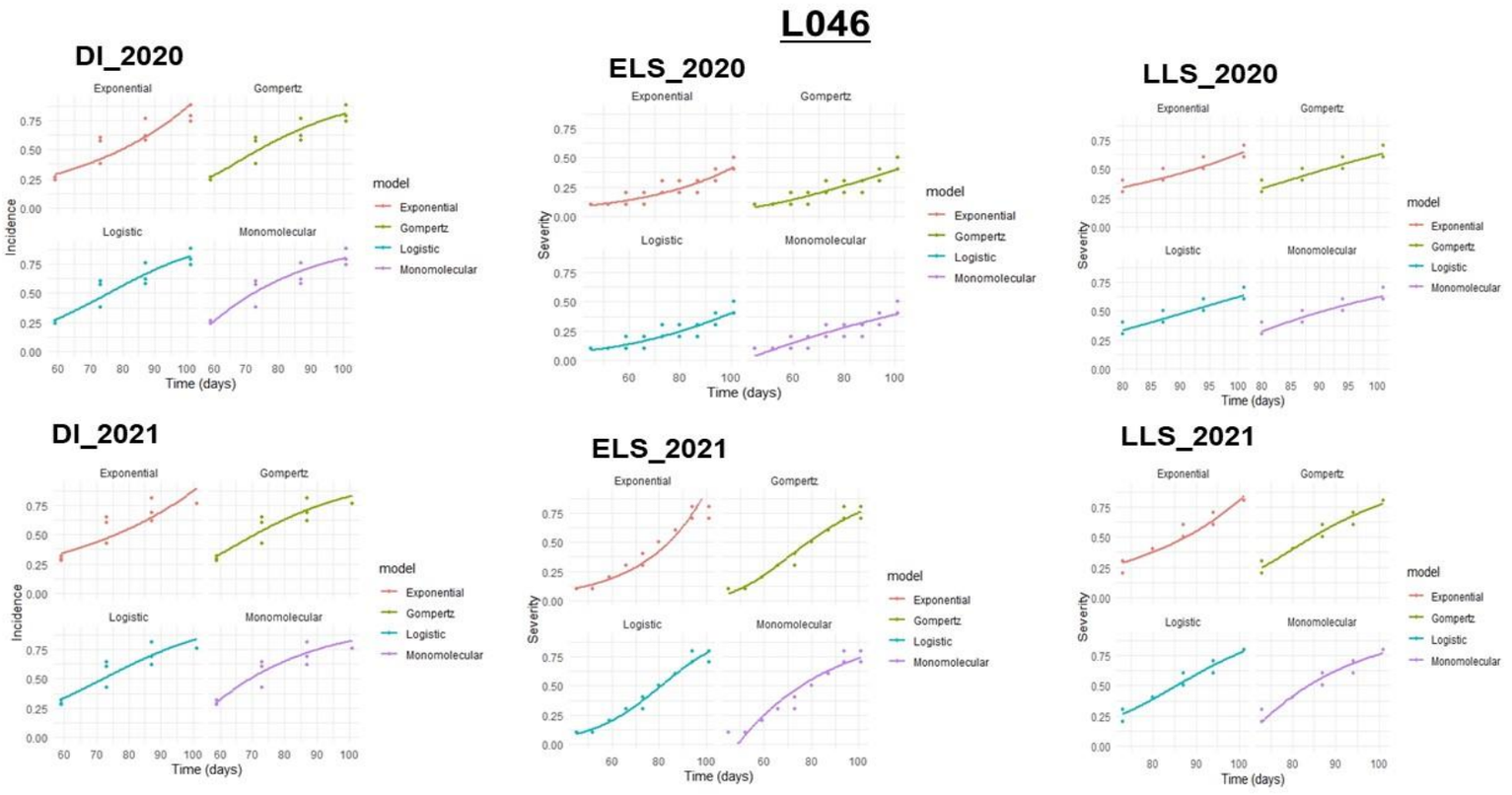
Appendix 3:Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L027B in two years



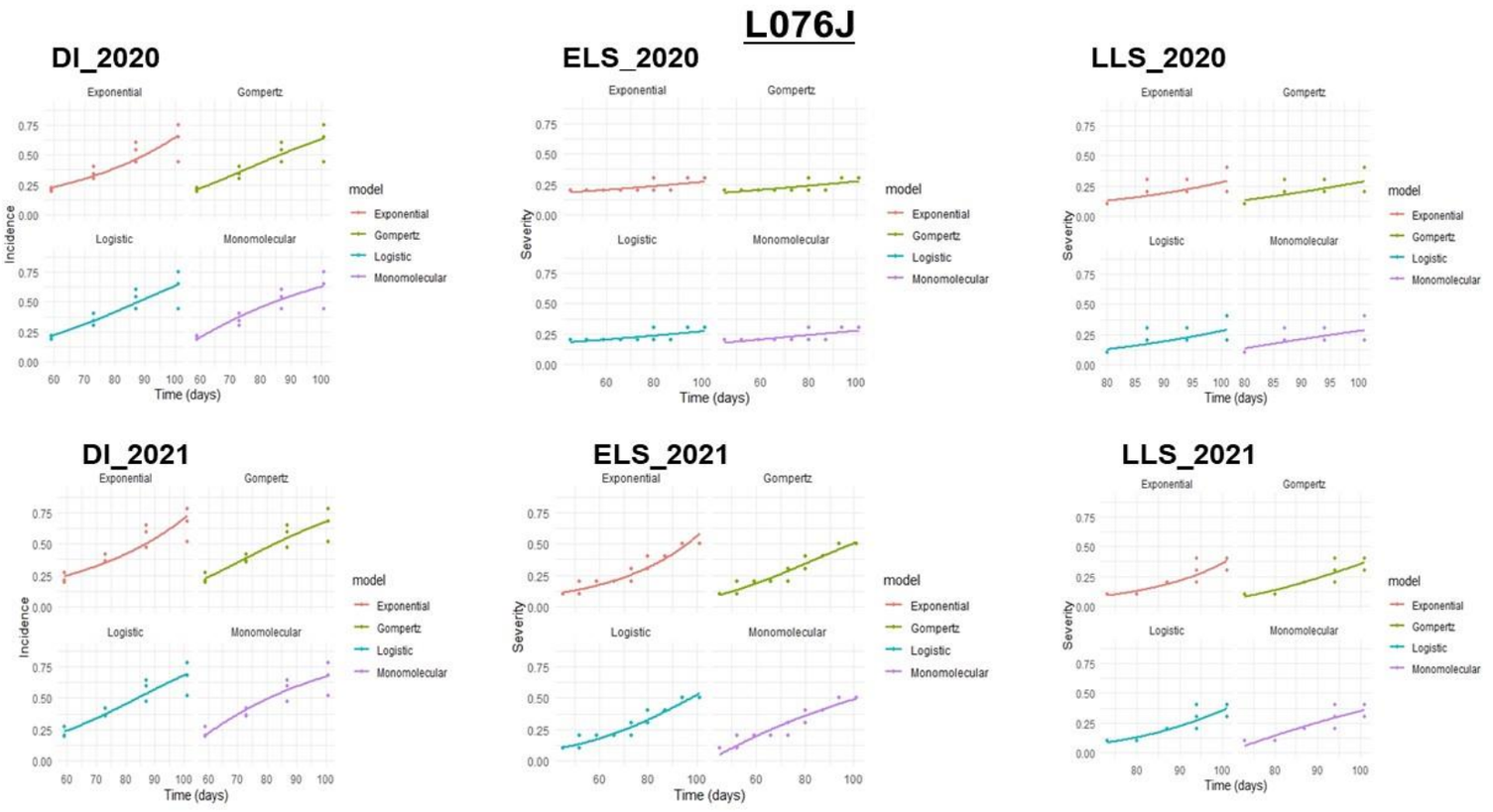
Appendix 4: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L030 in two years



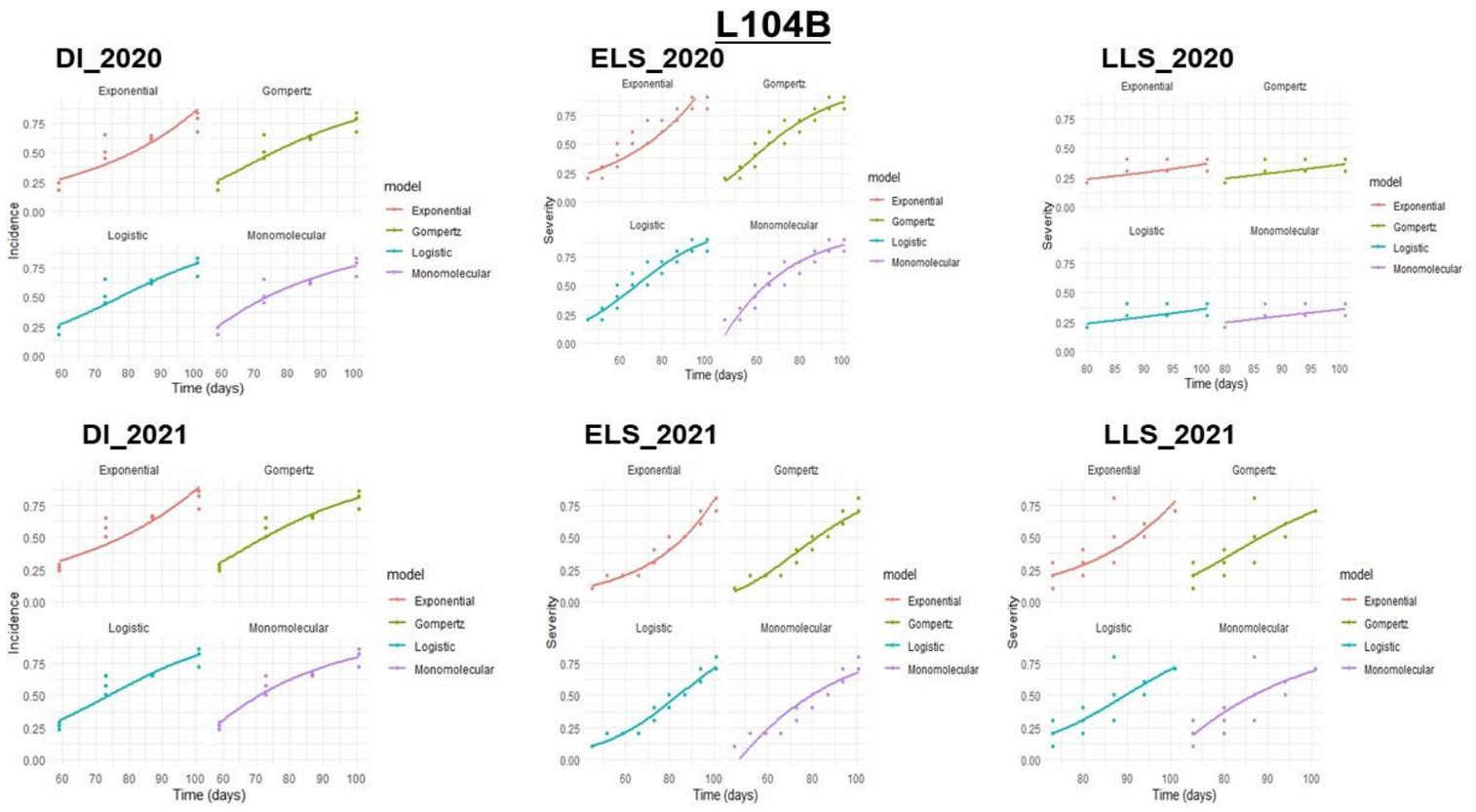
Appendix 5: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L046 in two years



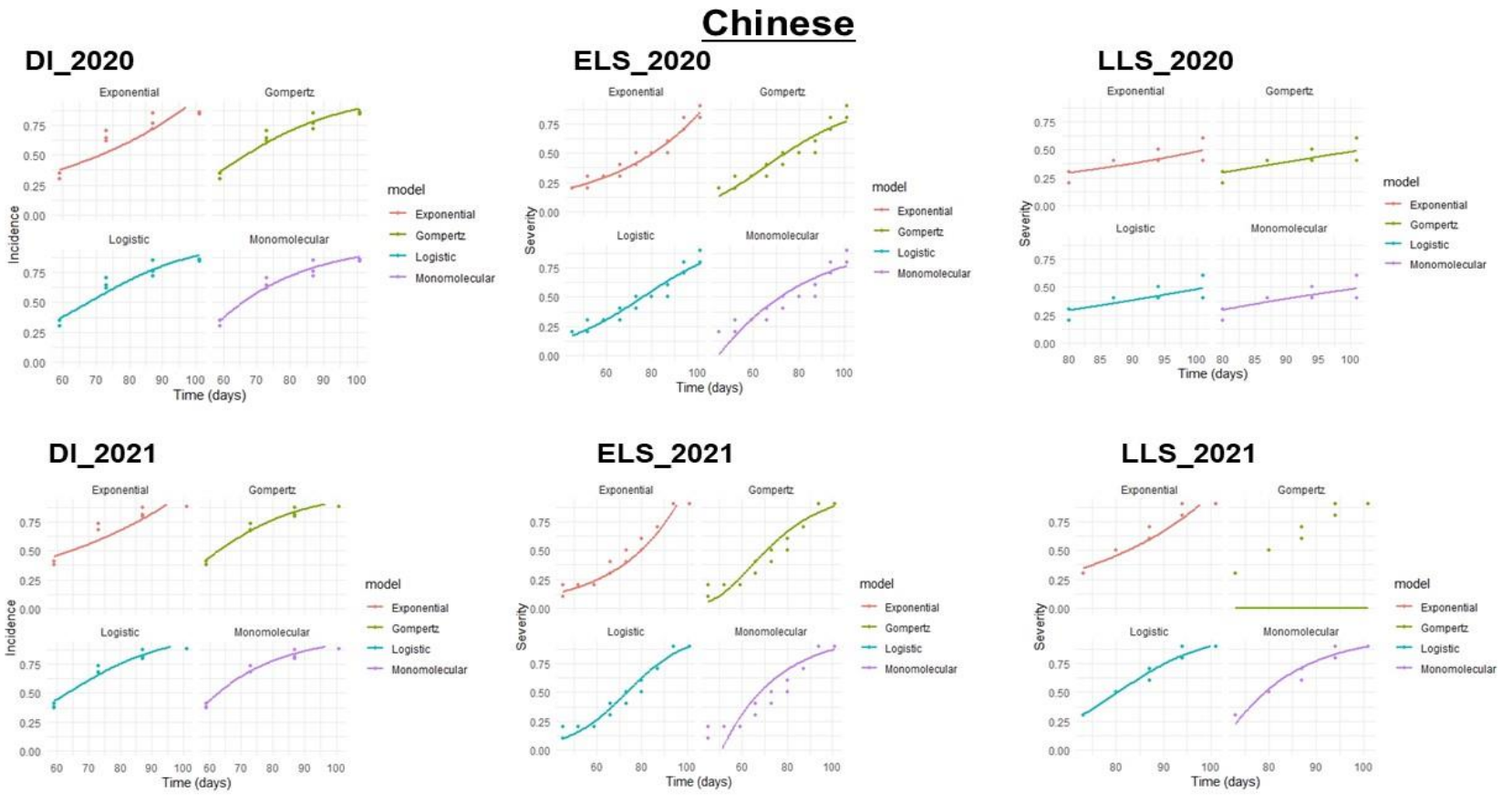
Appendix 6: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L076J in two years



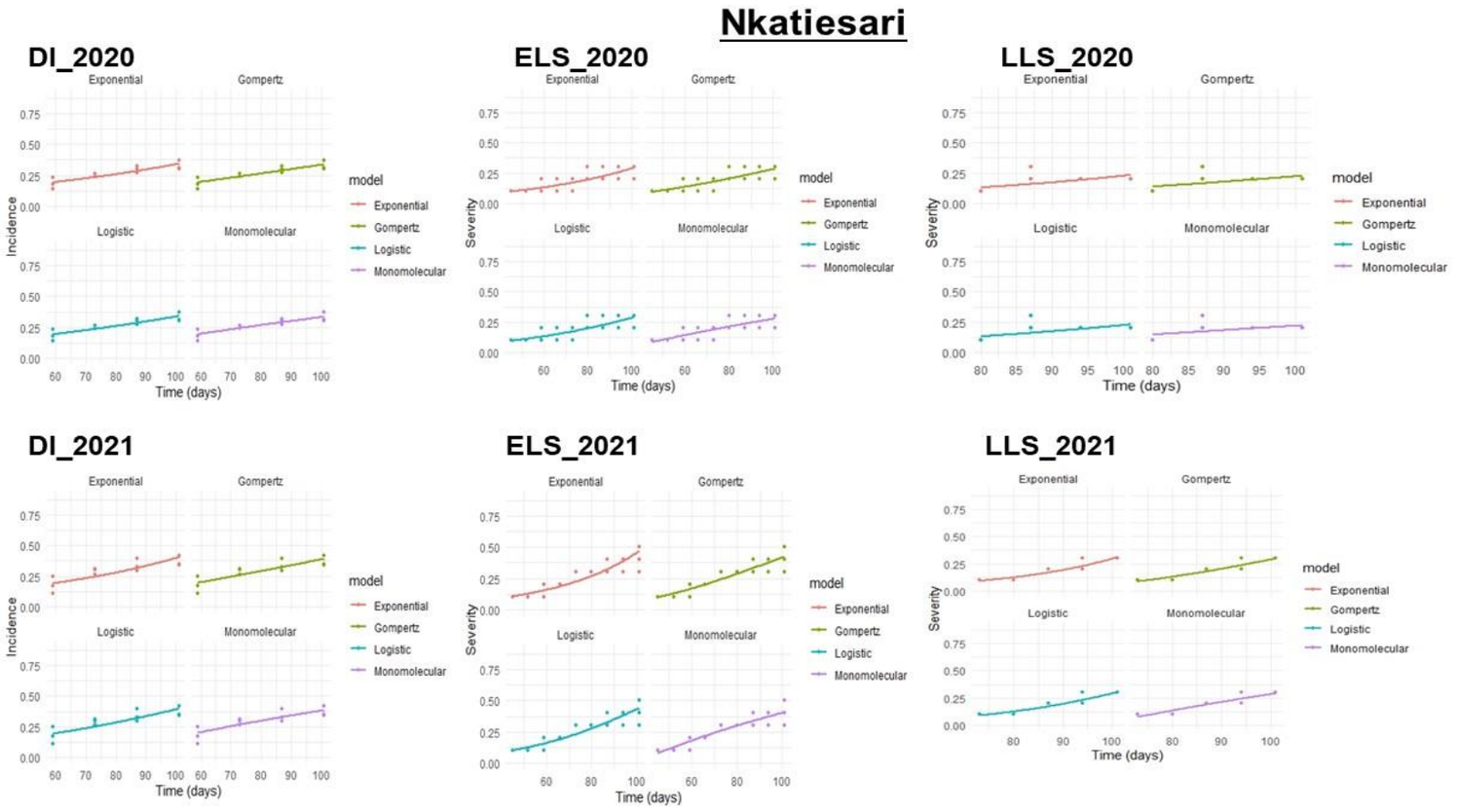
Appendix 7: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for L104B in two years



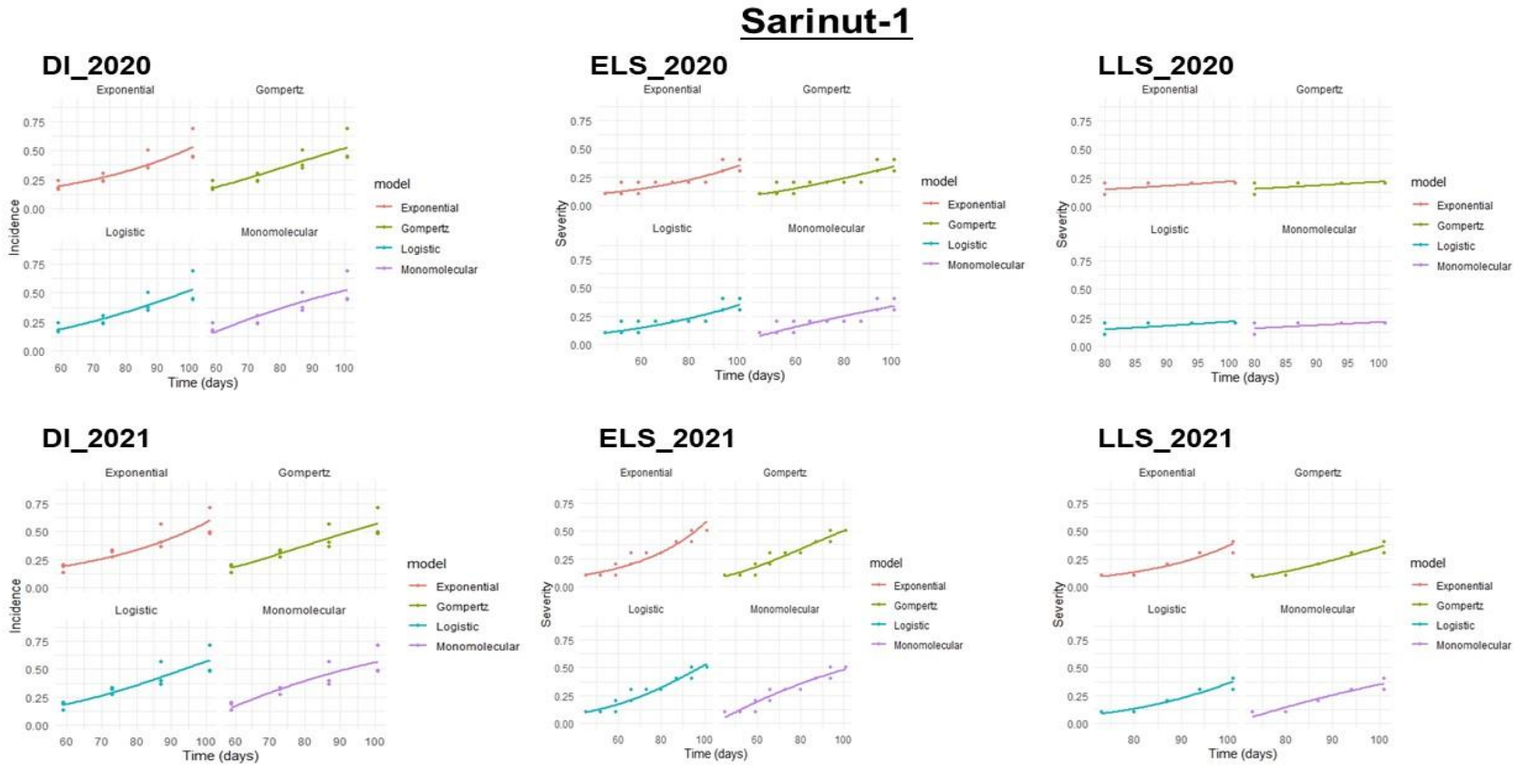
Appendix 8: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for Chinese in two years



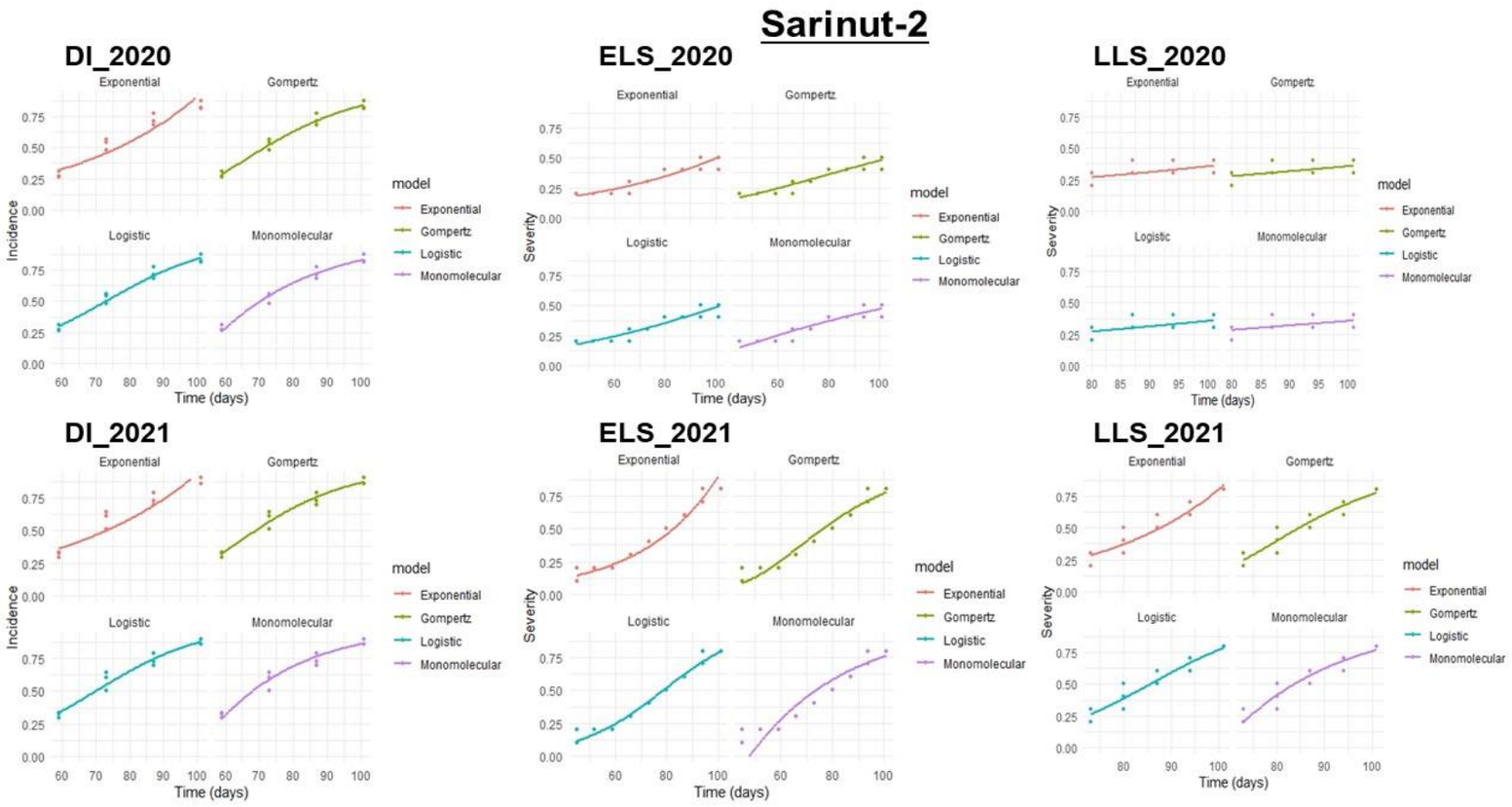
Appendix 9: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for Nkatiesari in two years



Appendix 10: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for Sarinut-1 in two years



Appendix 11: Observed fitted curves in relation to leafspot disease incidence, and early and late leafspot severity for Sarinut-2 in two years



LEAFSPOT DISEASE EPIDEMICS AND COMPONENTS OF RESISTANCE OF SOME SELECTED GROUNDNUT (Arachis hypogaea L.) GENOTYPES IN GHANA

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