

AETIOLOGY AND IMPORTANCE OF FOLIAGE DISEASES AFFECTING CITRUS
IN THE NURSERY AT THE AGRICULTURAL RESEARCH STATION (A.R.S.).

KADE.

BY

JOSEPH OKANI HONGER



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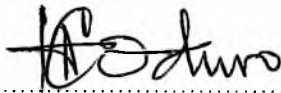
DECLARATION

I hereby declare that except for reference to other peoples work which have been duly cited, this work is the result of my original research and that this thesis has neither in whole nor part been presented for another degree elsewhere.



.....
JOSEPH HONGER

(STUDENT)



.....
PROF. K.A. ODURO.

(SUPERVISOR)



DEDICATION.

This work is dedicated to

Mrs. Irene Komley Dan-Okine of The Holy Spirit Cathedral, Accra.

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ABSTRACT

A research work was carried out at the Agricultural Research Station to determine the disease status of the seedlings and budlings in the nursery. Field surveys were carried out on three separate occasions between October and December 2003 during the dry season and repeated in March to May 2004 in the rainy season.

Four diseases were detected. These were citrus scab, citrus canker, greasy spot-like disease and brown leaf spot of rough lemon. The causal agent for brown leaf spot was confirmed to be *Alternaria citri*, and that for citrus canker was confirmed to be *Xanthomonas campestris* pv. *citri*, a bacterium that has not been previously reported in Ghana. Pathogens for scab and greasy spot-like disease could not be isolated and identified.



Brown leaf spot and scab were found to be restricted to rough lemon, which is used as rootstock in the nursery and therefore could not be transferred to the field. Comparatively, citrus canker and greasy spot-like disease were found to have a wide host range and have infected the following citrus varieties in the nursery: late valencia (sweet orange), walters (grapefruit) *Cleopatra mandarin*, *satsuma mandarin* and ponkan (*mandarins*), king disemis and ortanique (hybrids); and the rough lemon. The two diseases have a potential of being transferred to the field as they infect the scion as well.

Both the incidence and severity of the diseases in all the species and varieties of the citrus studied were higher in the rainy season than in the dry season.

The use of blunt syringe in inoculation studies of the canker disease gives quick results as compared to the spraying method.

Studies on the effect of the diseases on yield, to complete their importance were not feasible within the period of the study.

CHAPTER ONE

INTRODUCTION

The different species of cultivated citrus constitute major fruits of subtropical regions (Rice *et al.*, 1986) and is the most important fruit crop in West Africa (Tweneboah, 2000). Primary species of cultivated citrus are the sweet orange (*C. sinensis*), lemon (*C. limon*), grape fruit (*C. paradisi*), lime (*C. aurantifolia*) and mandarin (*C. reticulata*). It also includes hybrids such as tanger and tangelo (Rice *et al.*, 1986). They belong to the order, Geraniales and to the family, Rutacea. They are evergreen trees or small shrubs and vary in size from 3-5m tall (Rice *et al.*, 1986; Albrigo and Davies, 1994; Tweneboah 2000).

The crop is used mainly as human food either in the raw state or in the processed state. The crop serves as an important source of vitamin C and was used extensively in the past to prevent the occurrence of scurvy in ships (Albrigo, and Davies, 1994). In the processing industry the crop, especially sour orange is processed into orange marmalade and other products. By- products of the juice extraction are important in soft drink production where the oils and juice are used for flavours, the juice pulp cloud and the peel and rag extracts are used for pectin production (Albrigo and Davies, 1994). Large amounts of oils in the peels are extracted for the making of perfumes (Tweneboah, 2000), sections of the fruit are canned and exported from most producing countries to other countries, while dried peel pellets are used in the processing of feed for cattle (Albrigo and Davies, 1994). In India, the dry peel pellets are milled and used in cleaning metals (Morton, 1987).

In Ghana, citrus is cultivated in most parts of the country and is gradually gaining popularity among farmers in certain areas in Ashanti, Central and Eastern regions. In some of these areas the crop is gradually replacing cocoa as the main cash crop of the people (Ofosu-Budu; Personal communication). The crop also serves as source of income to the farmers. Earnings from the crop amounted to Two hundred and fifty thousand dollars to Ghana in 2002 (Ghana Export Promotion Council, 2002).

Despite the growing popularity of the crop in Ghana, its production is hampered by the incidence of diseases. Currently not less than fifty different diseases of the crop have been reported attacking the crop in Ghana (Oduro, 2000). These diseases affect all the parts of the crop, including roots, leaves and fruits (Clerk, 1974). Almost all the major pathogens of plants, including fungi, bacteria and viruses, have been reported attacking the crop in Ghana, predominated by fungi diseases (Clerk, 1974; Oduro, 2000).

Though citrus can reproduce sexually through seeds, most commercial plantings in Ghana are done vegetatively. This is partly due to the fact that the crop exhibits polyembryology and as such desirable characteristics of parent trees would not be exhibited by sexually propagated offsprings (Tweneboah, 2000). Furthermore there are some advantages offered by certain rootstocks such as the ability to withstand certain diseases endemic in an area, which are good for the establishment of the crop in the field (Clerk, 1974)

The commonest propagation method is by grafting or budding of nursery plants before they are planted on the field (Tweneboah, 2000). At the nursery, budlings showing undesirable characteristics are destroyed to ensure that plants on the field are only the desirable ones.

The nursery is therefore very important in the cultivation of citrus in Ghana. Commercial growers of the crop either depend on people specialized in the raising of seedlings for sale or have their own nursery where seedlings are raised for propagation (Tweneboah, 2000). Others with large plantations have their own nurseries where planting materials are raised for the orchards and at the same time to be supplied to other farmers. One of the last group of cultivators of the crop is the Agricultural Research Station of the University of Ghana.



The farm is located at Kade in the Eastern Region of Ghana and carries out research into the citrus crop. The station has large plantations of the crop and a large nursery that feed the orchards with planting materials and also serve as source of planting materials for most commercial farmers of the crop in the country.

For a very long time, very few leaf spot diseases had been recorded in the nursery. These diseases had been managed successfully with cultural practices such as proper fertilization and general farm sanitation. Also when deemed necessary, the use of copper based fungicides have also been successful in controlling these diseases. Due to this, the outbreak of diseases at epidemic levels were rarely encountered in the nursery. Also

most of these diseases were restricted to rough lemon, which was used mainly as a rootstock, and as such, its spread to the farmer's field was not much of a problem. Effect of diseases on foliage of scion, which could result in poor quality planting materials were rarely encountered, hence incidence of foliage diseases in the nursery was not of much concern to the station. (Brentu; Personal comm.)

In recent times however, there appear to be an upsurge of foliage diseases in the nursery. Presently all the different varieties of the crop in the nursery, including both the scions and rootstocks are diseased. The alarming rate of spread of these diseases in the nursery and the rapid destruction of the foliage of the seedlings has become a great concern to the station. The most disturbing observation about these diseases is that the routine disease control measures in the nursery appear to be ineffective against these diseases. With the exception of funguran, which appear to suppress these diseases, all the other fungicides have proved ineffective against these diseases (Brentu; personal comm.). With the diseases affecting foliage of scions, there is high risk of disseminating these diseases to the field of farmers, who depend on the station for planting materials. This would ultimately prove detrimental to the citrus industry in the country as a whole, if these diseases prove to be destructive in the farmer's field.

Since the station carries out a lot of research into the crop, different varieties and planting materials of the crop from different places are taken there for study. In such a case, accidental introduction of new and destructive diseases to the nursery may be

encountered. With the important role played by leaves in the survival of trees especially, of budded materials, urgent steps need to be taken to control destruction of the foliage of the young trees in the nursery. This therefore calls for urgent steps aimed at identifying the various diseases in the nursery, with the view to ensuring that highly destructive ones, which may be present, are quickly eradicated from the nursery and also to ensure that measures are put in place to prevent the occurrence of such diseases in future.

Therefore a study in the nursery to identify these diseases and their causal agents would yield very important information, such as, which disease or diseases are most damaging in the nursery and therefore require urgent attention. It would also determine whether some of the cultural practices and other disease controlling measures in the nursery need to be changed completely or modified.



The objectives of this project work were to identify the various diseases and their causal agents in the nursery. The relative incidence and severities of the diseases were also ascertained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and spread of citrus.

Cultivated species of citrus are believed to be indigenous to south-east Asia (Tweneboah, 2000), particularly the subtropical regions (Rice *et al.*, 1986). Recent evidence suggests that, the Yunmen province in south central China, may be an important source of origin of the crop due to the diversity of the citrus species found there and the system of rivers that could have provided dispersal of the fruit to the south (Gmitter and Hu, 1990). Many types of the citrus species are believed to have moved to various Arabian areas such as Oman, Persia, Macedonia and Palestine long before Christ (Tolkowsky, 1938) and had been playing important role in the Jewish religion as early as 50 AD-150 AD (Webber *et al.*, 1967).

Limes apparently originated in the East Archipelago and were probably brought across the sea of Oman by Arabian sailors and subsequently to Egypt and Europe (Albrigo and Davies, 1994). Sweet orange was introduced to Europe during the last half of the 15th century by Arab traders and finally into West Africa by the Portuguese and Europeans (Tweneboah, 2000).

Citrus was believed to be cultivated in their natural habitat before introduction into Europe by the Portuguese (Tweneboah, 2000). Currently the crop is cultivated primarily between latitude 40° N and 40° S. More northern and southern locations of commercial production exist where temperatures are moderated by ocean winds (Albrigo and Davies,

1994). The main centers of production in the world are southern Africa, Israel, The United States of America, Brazil, Spain, Japan and Italy, with the United States being the largest producer. In Africa, large-scale production occurs in the Mediterranean coast and Zimbabwe, Mozambique, South Africa and Swaziland. In the other areas cultivation of the crop is on small scale and mainly for auto-consumption (Albrigo and Davies, 1994; Tweneboah 2000; Rice *et al.*, 1986).

2.2 Importance of nurseries in the propagation of citrus.

Citrus can be propagated sexually through seeds, but commercial growers of the crop prefer to use vegetative propagation. The use of vegetative propagation offers many advantages to the grower. It enables the grower to enjoy the benefits of a particular citrus cultivar, such as abundant fruiting (Tweneboah, 2000). The method also widens the choice of site for the establishment of orchards, as cultivars, which would normally not perform well in a particular environment when grown sexually, can be grown profitably in the same environment with the choice of the right type of rootstock. Another advantage of this method is the early fruiting due to reduction in juvenile period of growth.

Before transplanting the budded materials, they are nursed in the nursery to ensure that the union of the stock and scion had been obtained. Secondly the young trees are observed carefully and diseased or weak trees are destroyed. This ensures that only good and healthy planting materials are transplanted on the field (Albrigo and Davies, 1994), because the availability of healthy planting materials is essential if the grower is to have

any surety of the continued health and productivity of the orchard (Fraser, 1967). The importance of nursery in the cultivation of the crop cannot be overemphasized and the success or failure of an orchard is largely dependent on how well the propagating and growing of the crop has been done in the nursery.

2.3 Diseases as constraints to citrus production.

Worldwide, the most serious limitation to the profitable production of citrus in otherwise suitable environment is the diseases caused by bacteria, mycoplasma, viruses and fungi (Albrigo and Davies, 1994). As with other diseases, symptoms observed on leaves can either be as a result of direct attack on the foliage or as an infection on other parts of the plant (Agrios, 1997).

Diseases that affect leaves of plants can be very devastating due to their ability to reduce drastically, the photosynthetic ability of leaves, which could reach as far as 40% reduction. (Wood *et al.*, 1988). This could be very devastating when it occurs in the nursery, where proper development of the foliage is very important for the success of the propagation of the crop. Currently, pathogens recorded causing diseases of citrus leaves include fungi, bacteria and other graft transmissible pathogens (Agrios, 1997).

Symptoms induced by these pathogens are varied. These include leaf distortion (Clerk, 1974), destruction of leaf chlorophyll (Albrigo and Davies, 1994), presence of materials that mask the leaf surface (Wood *et al.*, 1988), and puncturing of holes in the leaves. In other cases there is premature defoliation and the plant loses almost all the diseased

leaves (Amador, 2003). Though these symptoms vary, their end effect is always inimical to the growth of the plant as they hamper the leaves ability to photosynthesise (Albrigo and Davies, 1994).

2.4 Importance and aetiology of some foliage disease of citrus

2.4.1 Greasy spot.

This disease was first reported in Cuba and Florida in 1915. Currently the disease has spread to Central and South America, The Carribeans and some Asian countries (Timmer *et al.*, 1988).

The disease is reported to be of economic importance in areas of citrus cultivation with relative humidity almost up to 100%, which occurs simultaneously with high temperatures (Timmer *et al.*, 1988). Morton (1987) has reported that high humidity in excess of 90%, which occur after heavy infestation of the rust mite, can also lead to the rapid development of the disease.

The most serious consequences of the disease are defoliation. Losses up to 25% in fruit yield in sweet orange and about 45% in grapefruit have been recorded in Florida (Timmer *et al.*, 1988). It has been reported to cause yield loss in humid climates in America and Asia (Albrigo and Davies, 1994). The disease is responsible for premature leaf drop, which usually occur at the end of the first growing season. When leaves drop prematurely, it results in shortage of carbohydrates and their redistribution in the tree to

stimulate leaf replacement at the expense of fruit development, culminating in low yields (Albrigo and Davies, 1994).

Greasy spot is a fungal disease caused by *Mycosphaerella citri* and its anamorph, *Cercospora citri-grisea* (Fisher) (Whiteside, 1972). The organism does not produce any fruiting structures on living leaves. However after leaves have fallen and decomposed, ascocarps containing ascospores are produced in abundance (Timmer *et al.*, 1980).

Symptoms of the disease first appear as a mottle on the upper side of the leaf with a slightly raised pale orange to yellowish brown blister on the lower leaf surface. Later affected areas of the leaf become darker brown or black and greasy in appearance, and much of the chlorosis disappear. Leaves often drop before the lesions develop the dark greasy symptom that gives the disease its name (Timmer *et al.*, 1988).

Certain diseases with similar symptoms as the greasy spot have been recorded in many parts of the world. The aetiological agents of these diseases are not known though certain fungi have been implicated. They are believed to be fungal disease mainly because they could be controlled by copper fungicides (Timmer *et al.*, 1988). An example of such a disease is reported in Japan and is known as psuedo-greasy spot (Koizumi, 1986). Another one has been reported in Australia (Timmer *et al.*, 1988), and in Argentina (Marco and Whiteside, 1986).

2.4.2. Melanose.

Melanose is reported of being present in most citrus growing countries in the world, but important only where inoculum is abundant and rainfall occurs during the period of early fruit development (Albrigo and Davies, 1994).

The disease is reported to be of economic importance in the eastern and central parts of the Texas valley where humidity is high, and attacks all the varieties of citrus (Albrigo and Davies, 1994). Injury caused to fruit rind is superficial and may not be important if the crop is to be processed (Timmer *et al.*, 1988).

Symptoms appear as small, brown, discrete or confluent sunken spots, which later develop into pustules. The pustules on the leaves are at first surrounded by a yellow halo, which later regreens, leaving only the corky pustules. Pustules are also produced on fruits and can be confused with citrus rust mite injury, though in the case of the rust mite injury, the pustules are not raised (Timmer *et al.*, 1988). Infections of leaves occur after severe stress (Albrigo and Davies, 1994).

2.4.3 Citrus scab

The disease referred to as scab occur in three forms. These include, citrus scab, the commonest form; sweet orange scab and Tryons scab (Timmer *et al.*, 1988). The disease is reported to be a problem in raising rootstock in nurseries and seedbeds, particularly on rough lemon and sour orange. Sweet orange scab is also reported as causing infections on fruits as well resulting in reduction in fresh fruit production (Timmer *et al.*, 1988).

The causal agent attacks both sides of the leaf, but more frequently, the under surface of the leaf producing numerous corky pustules (Clerk, 1974). Symptoms appear first as translucent dots that later becomes pustules. As the disease progresses, the pustules turn into warts consisting of a mass of corky tissues, pale tan in colour. The leaves become twisted and distorted (Amador, 2003). When the disease appears at a time of leaf resistance pustules become smaller and little or no leaf distortion occurs (Timmer *et al.*, 1988).

The three different forms of disease, namely citrus scab, sweet orange scab and Tryons scab are caused by *Elsinoe fawcetti* (Bitancourt and Jenkins) anamorph, *Sphaceloma fawcetti* (Jenkins), *Elsinoe australis* (Bitancourt and Jenkins) anamorph, *Sphaceloma fawcetti* (Bitancourt and Jenkins) and *Sphaceloma fawcetti* var *Scabiosa* (MacAlp and Tryon), respectively (Timmer *et al.*, 1988). The form of the disease caused by *Elsinoe fawcetti* is a leaf spot disease and citrus species are only susceptible to the disease when young and is thus confined to new growth and may attack all citrus species except sweet and sour orange (Timmer *et al.*, 1988).

Elsinoe fawcetti produces nonseptate or one to two septate conidiophores that are closely compacted. Conidia are hyaline one celled and ellipsoidal. Being a slow growing organism, its isolation in the laboratory becomes difficult as it is easily overshadowed by other organisms present as contaminants (Timmer *et al.*, 1988).

2.4.3 Lime anthracnose

This disease, also known as wither tip, is reported of being prevalent in areas where rainfall becomes frequent during shoot emergence, bloom or early stages of fruit development. It has been reported only on key limes and never on any other citrus species (Wheeler, 1963). The disease can be a serious limiting factor to the production of the key limes and has been reported of being particularly troublesome in the wetter climates of the Caribbean (Timmer *et al.*, 1988). It was reported to be the cause of great destruction of limes in Florida (Amador, 2003).

The disease is characterized by localized necrosis of different shapes and sizes. As the disease progresses the necrotic areas dry up and fall out, forming what is known as shot holes (Albrigo and Davies, 1994). Only young tissues are susceptible to attack. Severe infection can cause the tips of affected shoot to shrivel for several centimeters, hence the name, wither tip. The disease also causes blossom blight and affected fruits may shed. Infected fruits may later develop corky lesions, which may be confused with those of canker, but unlike canker they have no yellowish halo (Timmer *et al.*, 1988).

Lime anthracnose is caused by the fungus *Gloesporium limetticola* Clausen. The organism produces conidia that are small but numerous and salmon pink coloured. The mycelium in potato dextrose agar is white and the medium becomes orange, without any setae on the acervuli (Timmer *et al.*, 1988).

2.4.4 *Alternaria* brown leaf spot of mandarins

The disease was first described in 1903 on emperor mandarin in Australia, causing blemishes on both the leaves and fruits (Pegg, 1966). It appeared in Florida where currently is a major problem on Dancy tangerine and Minneola tangelo (Whiteside, 1976).

This disease is characterized by very large, round, spots on the leaf surfaces, causing loss of chlorophyll. The spots usually occur in concentric rings with a characteristic target appearance (Albrigo and Davies, 1994). The extension of necrosis into veins is a characteristic of the disease (Timmer *et al.*, 1988). Symptoms also develop on fruit as small slightly depressed black spots. Older fruit when infected may drop. They form periderm, creating corky eruptions on the rind surface similar to those caused by melanose. However these pustules can be much larger than those caused by melanose (Timmer *et al.*, 1988).

The causal organism of the disease is *Alternaria citri*, a pathogen bearing the same name as the one causing *Alternaria* leaf spot of rough lemon but differ pathologically, in that toxins produced by the former is not effective on rough lemon. (Timmer *et al.*, 1988). Spores produced by the organism are easily recognized by the presence of both cross and longitudinal septa (Barnett and Hunter, 1972).

2.4.5 *Alternaria* leaf spot of rough lemon

The disease was first described in South Africa in 1929 (Timmer *et al.*, 1988). It has also been reported in many areas including Florida where it was erroneously referred to as anthracnose. It can be a major problem in the raising of rough lemon seedlings for rootstock (Timmer *et al.*, 1988).

Lesions vary from large, necrotic, blighted areas to small, circular spots. Spores of *Alternaria citri*, are often present. Necrotic areas are usually surrounded by an extensive chlorotic halo. Lesions tend to extend out along leaf veins. Stem infection and defoliation that commonly follows infection of the leaf blade cause die back of the shoot apices. Severe attacks lead to the development of plants with small internodes and excessive branching, which are difficult to bud (Timmer *et al.*, 1988).

The causal organism is the fungi, *Alternaria citri*, a pathogen similar to the one causing *Alternaria* brown rot of mandarin, but differing from it, pathologically. Toxins produced by this causal agent are only pathogenic to rough lemon and rangpur lime, but not on mandarins (Whiteside, 1978).

2.4.6 Sooty mould and sooty blotch

The causal organisms of these diseases produce a black velvety membranous fungal coating which appear on the surfaces of the leaves. This limits light interception and

consequently, photosynthesis. This greatly hampers the formation of sugars and thereby resulting in retarded growth and flowering (Tweneboah, 2000).

These diseases are not regarded as true diseases because the fungi associated with them do not feed on the tissues of the citrus leaves, but rather on the rich honeydew, secreted by insects, such as aphids. However, the high economic loss, as high as 40% reduction in photosynthesis, associated with these diseases, justifies their inclusion among disease of citrus (Wood *et al.*, 1988). Sooty mould is caused by the fungi, *Capnodium citri* (Tweneboah, 2000), while sooty blotch a more intense form of sooty mould is caused by *Gloeodes pomigena* (Amador, 2003)

2.4.7 Areolate Leaf Spot

This disease occurs in the humid, tropical areas of South America. The causal organism is the fungus, *Pellicularia filamentosa*. The disease can become serious in seedbeds and nurseries, particularly on sour orange seedlings (Stahel, 1940).

The disease is characterized by light coloured areas with dark concentric rings on the leaves. Lesions cease to expand under unfavourable conditions and hence vary considerably in size. Chlorotic halos form around the lesions and severely affected leaves drop off (Whiteside *et al.*, 1988).

2.4.8. *Cercospora* Leaf Spot

This disease is caused by *Cercospora angolensis*, and seems to be restricted to sub-Saharan Africa. Most varieties of citrus are susceptible to the disease (Timmer *et al.*, 1988).

On leaves, the fungus causes circular, mostly solitary spots, up to 4mm in diameter. The centers of these spots are usually grayish brown and shriveled and are surrounded by a prominent yellow halo. When the disease becomes severe a major part of the leaf blade may be chlorotic. Leaf abscission is also common. Sometimes, the necrotic area falls out creating a shot-hole effect. Lesions resemble those of canker, but differ in being flat or shrunken (Timmer *et al.*, 1988).

2.4.9 Bacterial blast

The disease occurs under cool wet conditions and may affect twigs as well. Grape fruit and oranges are the most susceptible varieties of citrus. Symptoms of the disease commonly appear on wings and petioles of leaves as water soaked lesion or reddish brown to black lesions, which quickly move in both directions, up the mid vein of the leaves and down to twigs. Once the phloem in the petiole is seriously damaged, the leaves wither, curl, and eventually drop. Infection occurs through wounds created during windstorms and heavy rains (Timmer *et al.*, 1988).

The causal organism is the bacterium, *Pseudomonas syringae* (Albirigo and Davies, 1994). The bacteria is a normal inhabitant of citrus leaves, becoming abundant on leaf surfaces only after conditions of prolonged wetting by rains at relatively low

temperatures. The disease rarely progresses when temperature is above 20° C (Timmer *et al.*, 1988).

2.4.10 Citrus Canker

Citrus canker has been described as the most serious bacteria disease of citrus (Civerelo, 1981; Graham *et al.*, 1992;). The disease causes necrotic lesions on fruit, leaves and twigs. Losses caused by the disease are reduced fruit quality and quantity and premature fruit drop (Agrios, 1997).

The disease is reported as having a devastating socio-economic and political impact because of the market standards for fresh fruits and the perception of possible inoculum transfer on fresh fruit product (Gottwald *et al.*, 2002).

Citrus canker can be endemic (Gottwald *et al.*, 2002) and hence has a greater tendency to limit greatly, a grower's choice of cultivars that have been grown profitably in other parts of the world. In certain parts of the world, the cultivation of certain cultivars of the crop that are highly susceptible to the disease has been abandoned. For example, the cultivation of grape fruit (*citrus paradisi*), has been stopped in Japan (Albrigo and Davies, 1994). Gottwald *et al.*, (2002) also reported that the cultivation of the same cultivar in Florida is almost non-profitable because of requirement for multiple bactericidal sprays per year along with other canker management schemes to maintain yield and quality. The control of the disease is also very expensive and cost as much as

70 million dollars and destruction of close to a quarter of million trees to control it in Florida, in the late 1980's (Gabriel, 2002).

Citrus canker is reported to have originated from South East Asia (Peltier *et al.*, 1926) and Japan (Agrios, 1997). Currently the disease has widened its geographical range and appears to be found worldwide, particularly in areas where rainfall and temperature increase simultaneously (Gabriel, 2002). The disease can be found in all Gulf States of America (Stall and Seymour, 1983). It has been reported in the Middle East, Africa, the Americas (Gabriel, 2002) and Australia (Agrios, 1997).

Spread of canker disease across geographical barriers has been attributed mainly to the movement of diseased propagating materials such as budwood, rootstocks and seedlings or budded trees (Agrios, 1997).



Citrus canker is caused by different strains of *Xanthomonas campestris* pathovar citri (Hasse) Dye (Civerolo, 1981). Currently the organism has been elevated to the species level and is now known as *Xanthomonas citri* ex Hasse (Gabriel *et al.*, 1989). Another organism known as *Xanthomonas campestris* pathovar *aurantifolli* has been discovered as another causal agent of the same disease (Gabriel, 2002). The two organisms have the same cultural characteristics. However the latter appears to grow well on most common media used in the laboratory, only after being cultured in a special media, while the former grows freely on most common media (Gabriel, 2002). Differentiating between the two has been reported as being possible using serological means (Bradbury, 1986),

The two organisms are reported to be pathogenic to almost all cultivated species of Rutacea, particularly, *Citrus spp*, *Fortunella spp*, and *Poncirus spp*. and though different types of the disease have been named including Asiatic canker, false citrus canker and Mexican lime cancrrosis, based on the difference in strains that caused each disease, the symptoms elicited on susceptible host are the same in all cases of the disease (Gabriel, 2002).

Symptoms on the leaf first appear as oily looking about 2-10mm circular spots, usually on the abaxial leaf surfaces. Later both epidermal surfaces may become ruptured. On Leaves, stems and fruits, the circular lesions become raised and blister like, growing into white or yellow spongy pustules. These pustules then darken and thicken into a light tan to brown corky canker, which is rough to touch. Older lesions tend to have more elevated margins and are at times surrounded by yellow chlorotic halo, which may disappear later on, and a sunken center. Defoliation and premature abscission of affected fruits may occur on heavily affected trees (Gabriel, 2002). On affected leaves, the necrotic center may fall out leaving a scattering of holes in the affected leaves (Anon, 1997).

In the past, certain diseases have been misdiagnosed as citrus canker due to the similarities in disease lesions. One of such diseases was mancha foliar, a disease caused by *Alternaria limicola* (Lopez, 1988). This disease was found in Mexico in 1982 and misdiagnosed as citrus bacteriosis, a form of citrus canker. In contrast to citrus canker experiments, results from experiments carried out on the disease show that the disease

rather develops strongly in the dry season, and that bacterial oozing was not observed when the diseased lesion was cut (Stapleton and Garza-lopez, 1988). Currently there are no *Xanthomonas* strains known to cause the disease known as citrus bacteriosis (Gabriel, 2002).

In 1910, when the citrus scab disease was introduced into the Gulf area of the United States on nursery stock from Japan, it was initially misidentified as citrus canker (Maloy, 1993)

Another opportunistic leaf spotting disease caused by *Xanthomonas campestris* pathovar *citrumelo*, known as citrus bacterial leaf spot or nursery leaf spot was found in Florida in 1984 and was misdiagnosed as citrus canker (Gabriel, 2002). In contrast to citrus canker older lesions of the disease did not show bacteria streaming, and matured trees in groves were never affected by the disease (Gabriel, 2002). Currently, due to the tissue hyperplasia associated with the disease and the extensive damage associated with the disease in nurseries (Gottwald *et al.*, 1991), most researchers treat the disease just like citrus canker (Mavriodieva *et al.*, 2004)

Two characteristic symptoms of the disease has been described as very reliable either separately or combined. The first one is the presence of water soaked margin around the diseased lesion (Timmer *et al.*, 1988) and the second is the tissue hyperplasia that results in canker on the affected parts (Gabriel, 2002).

The causal agent resides on leaf surfaces and can survive for sometime but the erupting pustules on the leaf surface are a major source of inoculum for infection. Infection of fruits and leaves are associated with leaf injury and also stomatal opening (Graham *et al.*, 1992).

2.4.11 Tristeza

This is a foliage disease caused by the *citrus tristeza virus*. The virus is usually debilitating and is one of the most destructive diseases of citrus. It suppresses new growth in the affected plant, and causes stem pitting and death of the whole plant.

Symptoms on the foliage include leaf deformities such as vein clearing, chlorosis and leaf cupping. In the advanced stages of the disease, leaf bronzing or yellowing occur followed by gradual defoliation (Roistacher, 1991).

2.4.12 Citrus exocortis

This is caused by a viroid known as the citrus exocortis viroid. The disease is present in almost all the citrus growing areas in the world, although some plants may not show any symptoms (Roistacher, 1991).

Symptoms on the leaf include the presence of brown, necrotic and cracked veins, especially in the mid vein. Leaf petiole may be severely wrinkled (Roistacher, 1991).

2.5. Control of foliage diseases of citrus

Control of foliage diseases of citrus is specific by diseases, but some general concepts are applicable (Albrigo and Davies, 1994). These concepts can be classified as regulatory, cultural, chemical or an integration of two or more methods (Agrios, 1997).

2.5.1 Regulatory

This refers to the control measure aimed at excluding a pathogen from a certain geographical area in which it was not previously known. The methods include the use of quarantine measures, use of pathogen-free planting materials and siting of farms far away from contaminated fields (Agrios, 1997). This method has been used to control the spread of many foliage diseases of citrus, especially, those that are debilitating, and whose introduction into an area in which they were previously unknown, could cause devastating loss to the grower. These include those diseases induced by the graft transmissible pathogens, such as greening, tristeza and vein enation (Roistacher, 1991), diseases induced by bacteria, such as canker (Amador, 2003), and certain fungi that are difficult to control with chemicals (Timmer *et al.*, 1988).

2.5.2 Cultural methods

These are methods that depend primarily on certain actions of the grower that creates an unfavourable conditions for the pathogen to survive, thereby, reducing the amount of pathogens present in an area, a plant or plant parts (Agrios, 1997).

One cultural method is the removal and burning of all infected plant parts that might harbour the pathogen. This method has been used to control bacterial canker in Florida (Agrios, 1997). This is also recommended for the control of mal secco disease, where diseased shoots and branches are pruned out and burned immediately (Salerno and Cutuli, 1977). Another cultural practice, the avoidance of overhead irrigation, has been recommended for the control of citrus scab (Timmer *et al.*, 1988) while Salerno and Cutuli, (1977) reported that the same method was effective in the control of mal secco disease. General farm sanitation such as washing the soil off farm equipment before moving it from one field to the other can effectively help to control certain diseases such as bacterial canker (Agrios, 1997).

2.5.3. Chemical control

Chemical control is the most commonly known method of controlling plant diseases, where chemicals that are toxic to the pathogen are used. Such chemicals either inhibit germination, growth and multiplication of pathogen or are outright lethal to the pathogen (Agrios, 1997). Chemical control is a faster way of effecting the control of plant diseases wherever plants are cultivated. The method is fairly easy to use and sometimes is the only alternative method of disease control. Most of these chemicals are copper based, with Bordeaux mixture, being the commonest one (Agrios, 1997). Other non-copper based fungicides that have been used to control foliage disease of citrus include Bendamidazole fungicides, which have been used to control mal secco, a fungal disease (Salerno and Cutuli, 1977) and sulphur dust which has been used to control citrus powdery mildew

(Timmer *et al.*, 1988). In some cases, oil has been used to control some fungal diseases such as greasy spot of citrus (Timmer *et al.*, 1988).



CHAPTER THREE

MATERIALS AND METHODS

3.1 Characteristics of area of study

The survey and sampling of diseases were done at the citrus nursery at the University of Ghana Agricultural Research Station located at Okumaning near Kade, about 120km North West of Accra. An annual rainfall of 1600mm, with a bimodal distribution pattern, characterizes the climate of the area. The temperature ranges between 25-38°, with a humidity of between 60-90%. The peaks of the rainy season occur in May/ June and September/October. A short dry spell occurs in July/August, while the major dry season stretches from November to February.

3.2 Area, location and some practices in the nursery.

The nursery covers an area of about 3200m² and is found closer to the main offices of the station. Both seedlings (raised from seeds) and budlings (raised from rootstock and buds) were found in the nursery during the period of the study. The budlings and seedlings were raised in seedling bags and have been arranged in rectangular batches referred to as beds, with each bed made up of seedlings or budlings of the same variety of the crop. There was an average number of 200 seedlings or budlings per bed. Ages of the budlings and seedlings ranged from one to two years. These beds were scattered in the nursery such that beds of the same variety could be found at different locations in the nursery (Plate I).

Budding of the seedlings is carried out in the nursery using buds obtained from the citrus orchards of the station. Overhead irrigation is the major form of irrigation practiced in the nursery and is carried out at three days intervals. Proper fertilization, general farm

sanitation and the occasional use of copper based fungicides constitute the major disease control measures in the nursery. There was no evidence of tool sterilization of any form by the workers in the nursery.

Apart from the main nursery where this study was carried out, three other smaller nurseries belonging to private individuals at the station were found to be present during the period of this study.





Plate 1. A section of the nursery showing the arrangement of seedling and budlings in beds. Mg. X 0.7

3.3 Field survey

3.3.1 Determination of field symptoms of the diseases

Field surveys were conducted on three separate in November 8th, 22nd and December 20th representing the dry season and another three occasions in May 8th, 22nd and June 19th representing the rainy season. Survey was done by random picking of beds. Seedlings and the budlings of the different varieties of citrus were examined for characteristic citrus diseases and the different symptoms recorded and named according to Timmer *et al*, (1988). Samples of the diseased leaves were collected during field inspections. Samples were cut using scalpel that was sterilized by dipping into 1% sodium hypochlorite and stored in labeled polythene bags and sent to the Crop Science Department laboratory for the isolation of the causal agents of the diseases.

3.3.2 Determination of disease incidence and severity

The determination of disease incidence and assessment of severity were done during the last survey of each season. Three beds representing three replicates of each of the eight varieties of the crop were selected at random. The number of plants that were affected by a particular disease was expressed as percentage of the total number of plants on the bed. The mean incidence from the three replicates was found and recorded as the incidence of the disease on the variety. Where the same disease was found on different varieties, the means obtained were subjected to the analysis of variance and separated by the Duncans Multiple Range Test at 5%.

Severity of diseases was assessed on twenty-four young plants per selected bed. The severity for the different diseases were found as follows:

a) Severity for greasy spot-like disease was found by determining the percentage of the leaf area affected by the disease. This was done by comparing the diseased leaf to standard leaf area diagrams developed by Gottwald (2002). The scale was modified by assigning the following indices to the various percentages;

0=No spot

1=Traces to 5% of leaf area covered

2=6% to 10% of leaf area covered

3=11% to 20% of leaf area covered.

4=>20% of leaf area covered.

In the rainy season, the mean severity figures for the different varieties were subjected to the Analysis of Variance and separated by the Duncans Multiple Range test at 5%.

b) Severity for citrus canker was determined by counting the number of spots developed on the surface of the leaf. Therefore the severity was calculated as the average number of spots per leaf. In the rainy season, the mean severity figures for the different varieties were subjected to the Analysis of Variance and separated by the Duncans Multiple Range test at 5%.

c) Severity for citrus scab and brown leaf spot on rough lemon were estimated by determining the area of the diseased leaf covered by the diseased lesions and expressing it as a percentage of the total leaf area, using the formula;

$s=t/T$, where s =severity, t =area of diseased leaf covered by the lesion and T =total area of the leaf.

3.3.3 Effect of diseases on matured citrus trees in the nursery

During the period of the study 18 matured citrus plants, which had grown out of seedlings and budlings that were not transplanted on the field, were also inspected to determine the types of diseases on them. Samples of diseased leaves from these trees were taken to the laboratory and the causal agents isolated.

3.3.4 Incidence of leaf miners

Leaves of some of the plants showing extensive curling and damages were inspected to identify the pest responsible for the damage. Two hundred plants in the nursery were picked at random and the percentage of plants with such leaves was recorded.

3.3.5 Incidence of foliage diseases in the other nurseries at the station

After the surveys in the main nursery were completed, it was extended to the other nurseries belonging to private individuals working at the station. The foliage of the seedlings and budlings in those nurseries were inspected and the types of diseases

observed were recorded. Samples of the diseased materials were also taken to the laboratory to determine their causal agents.

3.4 Laboratory work

3.4.1 Isolation and confirmation of causal agents of diseased leaves

Two methods of isolation were used. These were direct isolation and induced mycelial growth and possible sporulation (Tuite, 1969). In the case where the two methods did not yield enough results for diagnosis, other alternative methods used by other workers for isolation of causal agents of diseases similar to what was observed in the nursery, were also used.

a) Direct isolation

In this method, samples of the diseased leaves were taken and a sterile razor blade was used to scrape the surface of the diseased lesion onto slides. The scrapping was mounted in a drop of water and observed under the compound microscope to determine signs of the pathogen.

b) Induced mycelia growth and possible sporulation.

The isolation of fungi from the diseased symptoms was done first on water agar (WA) and then on potato dextrose agar (PDA). Agar agar and dehydrated PDA (Oxoid) were purchased commercially and prepared separately at the rate of 20 g/L and 39 g/L, respectively using clean distilled water. The mixtures were autoclaved At a temperature of 121°C for 15 minutes. These were poured into clean and heat sterilized plates. Tissue

segments from the advancing margins of the disease lesions of the leaves were excised with a flamed scalpel; surface sterilized in 1% sodium hypochlorite solution for 45 seconds and plated on water agar plates and covered (3 pieces per plate). The covered plates were enclosed in clean polythene bags and incubated under ambient conditions on a bench in the laboratory. Fungi, which grew out of the plated tissues, were sub-cultured on plates of potato dextrose agar (PDA), using flamed inoculating pins and cockborer. The plates were incubated in the laboratory until sufficient growth was observed.

Morphological features of the organism were observed by mounting mycelia bits and spores in water and observing with a compound microscope. Identification of the fungal isolates was based on the following culture characteristics on PDA: morphology of mycelia; growth rate and nature of spores as described by Whiteside *et al.*, (1988)

The checklist of plant diseases in Ghana (Oduro, 2001) and America (United States Department of Agriculture, 1970) were used to ascertain the status of the pathogen in Ghana and elsewhere.

3.4.2 Alternative methods for the isolation of causal agents of the diseases

a) Isolation of causal agent of scab.

The alternative method used for the isolation of the organism of citrus scab was that of Timmer *et al.*, (1988). A sterile razor blade was used to scrape some of the dried pustular growth on the diseased sample and cultured on water agar. These were incubated under ambient conditions on benches in the laboratory. Three days after incubation, growths

that were observed on the water agar were sub-cultured onto potato dextrose agar in plates and incubated until sufficient growth was observed. The morphological features were then observed under the compound microscope to identify the organism.

b) Isolation of the causal agent of greasy spot-like disease.

The alternative methods employed for the isolation were those of Timmer *et al.*, (2003). Samples of detached diseased leaves were moistened for 60 min. with water and incubated at a temperature of 28°C, for one week. The diseased specimen was soaked in water for 60 minutes every other day, during the incubation period. After the seventh day, growths on the leaves were scrapped onto a slide, fixed with lactophenol and observed under the compound microscope to examine the pathogen.

The second alternative method involved the scrapping of the surface of fallen and rotten diseased leaves onto a slide, and examining the scrapings for the conidia of the pathogen.

c) Isolation of causal agent of canker.

The method employed for the isolation of the causal agent of citrus canker was that of Gabriel (2002). Bacteria infection was first confirmed by placing a cut water-soaked margin of the disease lesion in sterile distilled water in the depression of a concave slide and covering it with a cover slip. The preparation was observed under the compound microscope using oil immersion to determine the oozing of bacterium cells.

The bacterium isolation on media was done by first taking samples of the diseased leaves washing them in running sterile distilled water. Then lesions with water soaked margins were excised using sterile scalpel and sterilized in 10% dilution of household bleach. Using a sterile razor blade, the water soaked margins were carefully removed, sectioned and the pieces dragged on the surface of yeast nutrient agar contained in plates. The shiny yellowish bacterium colonies that grew were re-streaked onto nutrient media to obtain pure cultures. The isolated bacterium was preserved on slants for pathogenicity test and characterization.

3.4.3 Pathogenicity test of isolates

Suspensions of fungal isolates were prepared by suspending the mycelium and spores from PDA, in sterile distilled water. These were used to spray three (3) months old healthy seedlings of the citrus varieties from which they were isolated. The seedlings were then covered with clean polythene bags to create a humid condition around them, and arranged in Completely Randomised Design (C.R.D), with three replicates tests of each isolate. Control was a seedling sprayed with sterile distilled water, which was also tied in polythene bags. An isolate was confirmed pathogenic to the plant if it caused the disease as observed on the original diseased leaf from the field.

Pathogenicity test of isolated bacteria was done using the inoculation method described by Gabriel (2002), called the blunt syringe method. In this method, a one-week-old preserved culture of the organism was cultured in glucose nutrient broth for two days and the bacterium together with the medium was drawn into a syringe without a needle. The

syringe containing the inoculum was then pressed gently, but firmly to the lower leaf surface and the slurry forced into the leaf through the stomata until about 2cm² of the leaf became water congested. Then some few minutes after the congestion had cleared, the seedlings were arranged in a completely randomized design in the green house. The treatment was replicated three times with a control made up of seedling inoculated with the nutrient broth without the bacteria. The bacterium was confirmed as the causal agent of the disease when it was able to cause the disease in the test plants.

3.4.4 Characterisation of isolated bacteria

a) Cultural growth on glucose media.

A glucose nutrient agar media was prepared by combining 2.5 g of glucose with 23 g of nutrient agar and autoclaved at 121° C for 15 minutes and allowed to cool. The molten media was then poured into sterile plates and allowed to set. Using a sterile loop, cells of the preserved bacteria were streaked onto the surface of the media in the plates, inverted and incubated for two days under ambient conditions in the laboratory and the cultural characteristics of the bacterium recorded to aid in its characterisation.

b) Gram staining.

The Gram stain reaction (Bradbury, 1970) was done using freshly prepared reagents.

A thinly spread film of the bacteria was made on a clean slide, air-dried and the lower side of the slide lightly flamed to fix the bacteria on the slide. The smear was then flooded with crystal violet solution for 1 minute. This was then washed in tap water for 10 seconds and excess water drained off. It was carefully blotted dry using a paper towel and

flooded with iodine solution for 1min., washed in tap water for 15 sec. and blotted dry. The smear was then decolourised with ethyl alcohol for 30 sec. and rinsed in tap water for 2 sec. Safranin solution was then used to counterstain for 10 sec., washed briefly in water and blotted dry. The smear was then observed under oil immersion under the compound microscope. The results were recorded to aid in the characterization of the bacteria. A culture of *Escherichia coli* obtained from the Noguchi Memorial Institute for Medical Research served as the reference bacterium and was given the same treatment as the test bacterium.

c) Motility test

The motility test was conducted to examine whether the isolated bacterium was flagellated. A thin layer of vaseline was applied around the edge of the well on a concave slide. A loopful of the isolated bacteria suspension was placed in the center of a cover slip and the concave slide was inverted and placed on the slip so the drop of the bacteria suspension, was enclosed within the concave depression. The preparation was then turned right up again and observed under the microscope under oil immersion.

d) Catalase test.

Yeast nutrient agar was prepared by combining 5 g of yeast extract with 23 g of nutrient agar. The mixture was dissolved in 1L of distilled water, dispensed into test tubes and autoclaved at 121 °C for 15 minutes and allowed to set as slants. Three of the slants were inoculated with the bacteria and one, which served, as a control was not inoculated with the bacteria. The slants were incubated for two days under ambient conditions in the

laboratory. Few drops of 3% hydrogen peroxide was poured gently along the walls of the tube onto the culture and the effect of the chemical on the organism was recorded. The slants that were not inoculated with the bacterium also received the same treatment.

e) Starch hydrolysis test

The method employed was that of Lelliot and Stead (1987). The media for the test was prepared by combining 28 g of nutrient agar and 2 g of soluble starch. The mixture was dissolved in 1 litre of distilled water and autoclaved at 121°C for 15 minutes. The media was poured into sterilized plates and allowed to set. An inoculating pin was used to stab the media in the plates, with a two-day-old culture of the bacteria. Each plate was stabbed at four different points in the plate. The plates were incubated in the laboratory for seven days. After the incubation, the plates were flooded with Lugol's iodine solution and the colour development was recorded.

3.4.5. Further studies on citrus canker

Investigating the inoculation method on number of days to disease expression and the stages of disease development.

Results from the pathogenicity test indicated that aetiology of the canker diseased condition could be due to the bacterial that causes citrus canker. Consequently some more experiments were designed to further conduct some detailed studies on the disease and the causal organism

A suspension of the bacterium was prepared by streaking colonies of the bacterium taken from a two week old culture. This was first streaked onto nutrient agar and after two days, the colonies were suspended in sterile tap water (Gabriel, 2002).

Two methods of inoculation were evaluated in the study. These were the blunt syringe method as described in section 3.3.3 and the spraying method. Each inoculation method was tested on a sweet orange seedling with the inoculum prepared. The controls were sweet orange seedlings inoculated with sterile tap water using each of the two inoculating procedures. Each treatment was replicated ten times and the number of days to first disease expression were recorded and subjected to the 't' test at 5%

Temperature and humidity were monitored during the period of the experiment in the screen house in both the first and repeated inoculation trials, using the Tiny plus electronic data logger.

CHAPTER FOUR

RESULTS

4.1 Varieties of citrus grown in the nursery

Eight different citrus varieties were used for this study (Table 1). Six of these varieties were from four different species of the crop while the other two were hybrids made up of the combination of different genotypes from different species of the citrus crop. Six of them were budlings while the other two namely Cleopatra mandarin and a variety of rough lemon, were seedlings. These two varieties were used mainly as rootstocks. The scions on the budded materials were obtained from the orchards on the station.

Table 1. Varieties of citrus identified in the nursery.

Variety	Species	Genetics	Use	Seedling/Budling
Late valencia	Sweet orange (<i>C.sinensis</i>)	pure line	scion	Budlings
Walters	Grapefruit (<i>C.paradisi</i>)	pure line	scion	Budlings
Satsuma	Tangerine (<i>C. reticulata</i>)	pure line	scion	Budlings
Ponkan	Tangerine (<i>C. reticulata</i>)	pure line	scion	Budlings
Cleopatra	Mandarin (<i>C. reticulata</i>)	pure line	rootstock	Seedlings
King disemis	Rough lemonx Tangerine	hybrid	scion	Budlings
Ortanique	Sweet. orangex Tangerine	hybrid	scion	Budlings
Rough lemon	Rough lemon (<i>C. limon</i>)	Pure line	rootstock	Seedlings

4.2 Diseases identified in the nursery

4.2.1 Brown leaf spot

a) Disease symptoms:

This was a leaf spot disease made up of large, round, and mostly, solitary spots. The spots were dark brown to black in colour and appeared in concentric rings with a pinhead center, giving a characteristic target appearance. Most of these spots appeared at leaf margins. Black dots, which were spores of *Alternaria sp.* were present in concentric rings in most of the diseased lesions (Plate 2).

b) Host range, incidence and severity.

The disease was found to be restricted to rough lemon. (Table 2). Disease incidence and severity on the rough lemon variety appeared to be higher in the dry season than in the rainy season_(Table 2). In the rainy season an incidence of 89% was recorded on the variety compared to 45% in the dry season. Similarly a severity of 16% was recorded in the wet season as compared to 6.3% in the dry season.

c) Identification of the causal agent.

Alternaria citri was isolated. The signs of the organism were observed when the scrapings from the surface of the diseased lesions were examined under the microscope. Conidia observed were muriform with vertical septations that ranged from four to six in number, with slight constrictions at the septa. They were also divided by one or two horizontal septa. Some of the conidia were catenulated and they vary greatly in shape and

size. Most of them were short, clavate, and oblong and appeared dark olive brown (Plate 3).

Cultural characteristics of the organism on potato dextrose agar showed a loosely interwoven, slender mycelium. The organism grew and filled the entire plate within ten days after culturing on potato dextrose agar (Plate 4). When the organism was observed under the microscopes, the spores of the organism as described above were found scattered among the mycelium (Plate 5).

The pathogen had been listed in the checklist of plant diseases both in the United States and that of Ghana as the causal agent of the disease, thereby confirming the organism as the causal agent of the disease. The pathogen was able to induce the disease when it was inoculated onto the healthy rough lemon seedlings further confirming it as the causal agent of the disease (Plate 6).



Plate 2. Characteristic symptoms of brown leaf spot on the variety of rough lemon.
Mg. X 0.7

Table 2. Host range, incidence and severity of brown leaf spot in the nursery.

Variety	Dry season			Rainy season		
	Host	Incidence/%	Severity/%	Host	Incidence/%	Severity/%
Cleopatra		0	0	-	0	0
Walters	-	0	0	-	0	0
King disemis	-	0	0	-	0	0
Ortanique	-	0	0	-	0	0
Ponkan	-	0	0	-	0	0
Satwuma	-	0	0	-	0	0
Late valencia	-	0	0	-	0	0
of Rough lemon	+	45	6.3	+	89	16

- = absent. + present



Plate 3. Muriform spores of *Alternaria citri* scrapped directly from the diseased lesion
Note the presence of catenulated spores (arrowed) at the lower left of the figure.
Mg. X 800

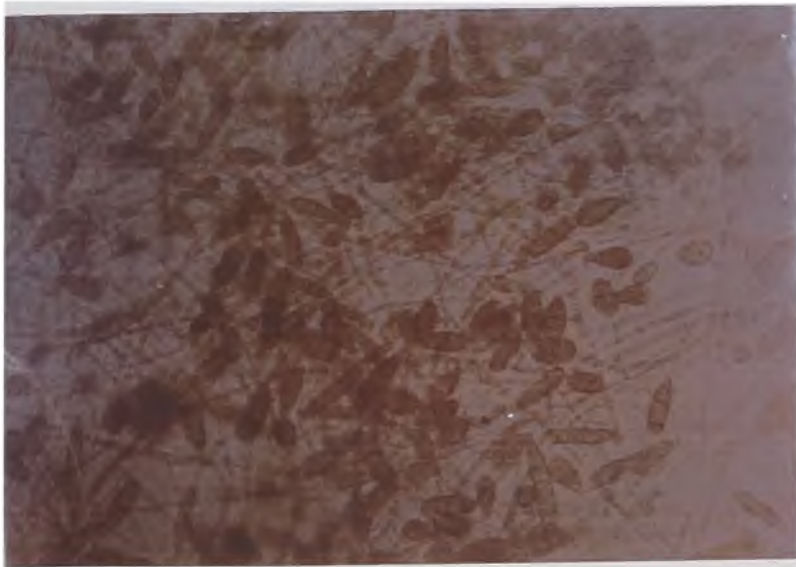


Plate 4. Muriform spores of *Alternaria citri* entangled in mass of mycelia on P.D.A.
Mg. X 800



Plate 5. Cultural characteristics of *Alternaria citri* on Potato Dextrose Agar. Mg. X 0.8



Plate 6. Brown leaf spot caused by *Alternaria citri* on the variety of rough lemon test seedlings. Note the absence of disease lesions on the control. Mg. X 0.7

4.2.2 Citrus scab

a) Disease symptoms.

The disease was characterized by protuberances present at the lower surface of the affected leaves, with a corresponding depression on the opposite side of the leaf. The depressions were surrounded by large yellow halo. The protuberances were brown in colour and in most cases appeared as pustules. Scrapings from the pustules revealed the presence of pieces of mycelia of the pathogen under the microscope. The pustules in most cases were spread on the surface of the affected leaf, resulting in distorted leaves (Plate 7).

b) Host range, incidence and severity

The scab disease was found restricted to the variety of the rough lemon in the nursery (Table 3). The disease was also found in two out of the three other nurseries inspected.

In the rainy season, an incidence of 78% was recorded on the variety as compared to 48% in the dry season. Similarly, severity of 55% was recorded on the variety in the wet season as compared to 25% in the dry season, an indication that the disease was more in the rainy season than in the dry season (Table 3).

c) Identification of the causal agent.

All the attempts at isolating *Elsinoe fawcetti* and its anamorph, *Spaceloma fawcetti* proved futile. Instead *Fusarium sp.* was consistently isolated when the isolation method proposed by both Tiute (1969) and Timmer *et al.* (1988) were used.



Plate 7. Characteristic symptom of citrus scab in the nursery. Mg. X 0.7

Table 3. Host range, incidence and severity of citrus scab in the nursery

Variety/species	Dry season			Rainy season		
	Host	Incidence/%	Severity/%	Host	Incidence/%	Severity/%
Cleopatra		0	0		0	0
Walters	-	0	0		0	0
King disemis	-	0	0		0	0
Ortanique	-	0	0		0	0
Ponkan	-	0	0		0	0
Satsuma	-	0	0		0	0
Late valencia	-	0	0		0	0
Rough lemon	+	48	25	+	78	55

- = disease is absent. + = disease is present.

4.2.3 Greasy spot

a) Disease symptoms

The disease symptom was characterized by the presence of yellow mottle on the upper part of some of the affected leaf surfaces, with a matching, slightly raised, pale orange to yellowish brown blisters on the lower surface. On some of the leaves, affected areas of the leaves appeared darker brown and greasy in appearance, with a yellow halo surrounding some of the spots on the upper surface (Plate 8).

b) Host range, incidence and severity.

Greasy spot was found to have a wide host range, affecting all the varieties of the crop in the nursery. In the surveys conducted during the dry season, the disease was found to have affected only sweet orange and *Cleopatra mandarin*. During this period, the disease was also recorded on 10 out of the 18 mature citrus trees in the nursery. The disease was not found in the other nurseries in the station and hence confined to the main nursery. In the rainy season the disease was found on all the varieties of the crop in the nursery.

Disease incidence on the different varieties of the crop in the rainy season varies significantly, ranging from 37%, which was recorded on walters (*C. paradisi*) to 6.7 on the variety of rough lemon (*C. limon*). Severity figures also varied among the various varieties with the highest of 3.8, recorded on late valencia (*C. sinensis*) and the lowest of 1.7 recorded on walters (*C. paradisi*), king disemis, ortanique, ponkan (*C. reticulata*) and satwuma mandarin (*C. reticulata*).

c) Identification of the causal agent.

Mycosphaerella citri, the causal organism of the disease known as greasy spot in other citrus growing area was not isolated in this study. The repeated wetting and incubation method used did not result in the production of the ascospores of the organism. Also scrapings from fallen and diseased leaves did not show any sign of the organism. The anamorphic stage of the organism was also not isolated on media.



Plate 8 Characteristic symptoms of greasy spot-like disease in the nursery. Mg. X 0.7

Table 4. Host range, incidence and severity of greasy spot-like disease in the nursery.

Variety	Dry season			Rainy season		
	Host	Incidence/%	Severity	Host	Incidence/%	Severity
Cleopatra	+	27	2.9	+	35.7 ^{bc}	3.7 ^{bc}
Walters	-	0	0.0	+	6.7 ^b	1.7 ^a
King disemis	-	0	0.0	+	5.0 ^a	1.7 ^a
Ortanique	-	0	0.0	+	37.3 ^{bc}	1.7 ^a
Ponkan	-	0	0.0	+	34.3 ^{bc}	1.7 ^a
Of R. lemon	-	0	0.0	+	10.0 ^b	2.7 ^b
Satsuma	-	0	0.0	+	31.7 ^{bc}	1.7 ^a
Late valencia	+	35	3.3	+	37.0 ^{bc}	3.8 ^{bc}

- = disease is absent, + = disease is present

Means followed by the same alphabets in a column are the same.

4.2.4 Citrus canker

a) Disease symptoms.

The disease symptom was a complex one made up of three stages based on the stage of development of the spots, with each stage showing distinguishing features, but retaining some of the features of the stages preceding it.

i) In the first stage, the spots appeared as very tiny but visible spots or as translucent dots surrounded by enlarged, yellow chlorotic areas. The translucent dots appear visible only when held against a source of light (Plate. 9).

ii) The second stage as seen in Plate 10, was made up of clearly visible spots, which were brown and sunken on the upper surface of the affected leaves and were surrounded by yellow chlorotic areas. At the lower surfaces of the leaves, the spots appeared as round, raised corky growths, surrounded by yellow halo. Some of the larger spots appeared irregular in shape as dry warty pustules that were sunken at the upper surface of the leaves. Tissues found at the center of the spots appeared papery and overstretched. On the lower surface of the leaves, the warty growth appear raised, rough to touch and were bordered by a water soaked area.

iii) Plate 11 showed the final stage of the disease which was a 'shot' hole effect produced on the affected leaves, with the internal margins of the holes showing traces of the overstretched tissues associated with the second stage.



Plate 9. Characteristic symptom of the first stage of citrus canker.
Note the pinpoint spots surrounded by yellow chlorotic halo. Mg. X 0.7



Plate 10 Characteristic symptoms of the second stage of the citrus canker disease.
Note the shapes of the yellow chlorotic halo surrounding the spots. Mg. X 0.7



Plate 11. Characteristic symptoms of the last stage of citrus canker. Note the presence of shot holes. Mg. X 0.7

b) Host range, incidence and severity.

Citrus canker was found to have a wide host range, affecting all the varieties of the crop in the nursery. In the dry season survey, the disease was found on only sweet orange and Cleopatra. However all the varieties of citrus were found infected with the disease within the rainy season survey (Table 5). Three out of the 18 matured citrus trees found in the nursery were found with the disease in the wet season. The disease was also present in all the nurseries on the station.

Incidence of the canker disease on the citrus varieties of the crop ranged from 38.3% on rough lemon to 100% on grapefruit in the rainy season. With the exception of ponkan, all the budlings showed more than 50% incidence in the rainy season. In the dry season, only two of the varieties, namely late valencia and Cleopatra mandarin were diseased. Late Valencia showed a high incidence of 80% while Cleopatra showed an incidence of 68% (Table 5).

Results from the severity determination tests showed higher severity of the disease on the budlings as compared to the seedlings. Severity score of 18.2 in late Valencia was highest followed by walters, with a severity figure of 8.5 (Table 5).

Table 5. Host range, incidence and severity of citrus canker disease in the nursery.

Variety	Dry season			Rainy season		
	Host	Incidence/%	Severity	Host	Incidence/%	Severity
Cleopatra	+	68	3.5	+	92.3 ^{bcd}	5.6
Walters	-	0	0.0	+	100 ^{bcd}	8.5
King disemis	-	0	0.0	+	47.7 ^b	6.2
Ortanique	-	0	0.0	+	38.3 ^{bac}	6.4
Ponkan	-	0	0.0	+	83.3 ^{bc}	6.8
Of R. lemon	-	0	0.0	+	94.0 ^{bcd}	3.9
Satsuma	-	0	0.0	+	83.0 ^{bc}	2.1
Late valencia	+	80	6.5	+	94.0 ^{bcd}	18.2

- = disease is absent, + = disease is present

Means followed by the same alphabets in a column are the same.

c) Identification of the causal agent.

Xanthomonas campestris pv citri was isolated from both young and old lesions of the disease. The bacterium cells were observed to be oozing out when pieces of the water soaked margins of the disease were put into a drop of water. On glucose nutrient agar, the colonies of the bacterium appeared shiny yellow, mucoid and convex (Plate 12). In the motility test, the bacterium cells were observed to be motile. In the Gram stain test, the organism retained the red colour indicating that it was a Gram-negative bacterium. The stained bacterium cells were short rods. The bacterium was also catalase positive producing gas bubbles in few seconds after the introduction of the hydrogen peroxide. In the starch hydrolysis test, the area immediately surrounding the bacterium cleared while the other area showed the black colouration, showing the bacterium to be a starch-hydrolyzing organism.

Xanthomona scampestris pv. citri has not been listed as a causal agent of the disease in Ghana. The organism induced similar characteristics symptoms of citrus canker when it was inoculated onto healthy seedlings of sweet orange.

The first symptom of the disease was evident seven days after inoculation. It consisted of a yellow area on upper leaf surface. Then fifty (50) days after inoculation, scab like outgrowth, characteristic of the disease started to appear at the lower part of the inoculated leaves, surrounded by large yellow chlorotic area. Small solitary canker spots with raised margins, which turned slightly warty, developed on the upper leaf surface

(Plate 13). Further expansion of the disease lesion was not observed until leaves began to senesce.



Plate 12. Cultural characteristics of *Xanthomonas citri* isolated from the citrus canker disease in the nursery. Mg. X 0.7



Plate 13 Development of young canker disease symptoms on inoculated test plants.

Mg. X 0.7

4.3 The influence of method of inoculation on the development of citrus canker disease on seedlings

Results from the inoculation showed that number of days for the first stage of the canker symptoms to develop varied significantly between the two inoculation methods. Whereas symptoms developed within an average of 7.8 days following inoculation, it took 52.6 days after inoculation for the disease to develop using the spraying method (Table 6).

b) Stages of disease development

i) Using the blunt syringe inoculation method.

First disease symptoms on the plants inoculated with the inoculum was evident seven days after inoculation. It consisted of a yellow area on upper leaf surface with a slight bulged area on the upper surface with the presence of black spots at lower side of the leaf (zone of inoculation).

The second stage of the disease, characterized by the presence of scab like outgrowth, started to appear at the lower part of the disease leaves, twenty-one days after the emergence of the first stage of the disease (Plate 14). On the upper surface of the affected leaves, small, solitary canker spots with raised rough warty margins developed. There was a gradual expansion of the lesions until the leaves begin to senesce.

ii) Using the spraying method.

The first stage of the disease was observed about fifty three (53) days after inoculation. The symptoms first appeared as tiny solitary spots surrounded by yellow halos. These

spots underwent very little expansion and the scab like growths surrounded by water soaked margins on the lower surface also begins to form (Plate 15).



Plate 14. Citrus leaves showing young canker spots following inoculation with the bacterium using the blunt syringe method (Arrowed). Mg. X 0.7



Plate 15. Citrus leaves showing young canker spots (Arrowed) following inoculation with the bacterium using the spraying method. Mg. X 0.7

Table 6. Influence of method of inoculation on canker disease development.

Method of inoculation	Mean number of days
Blunt syringe	7.8
Spraying	52.6

4.4 Incidence of leaf miners at the nursery

Leaf miners were found to cause the curling and leaf destruction of citrus seedlings observed in the nursery. The pest was identified by the presence of meandering serpentine larva mines, which were present vertically on the ventral side of affected leaves (Plate 16). The larvae appeared as very minute and translucent greenish yellowish worms, located inside the leaf mines. The mines were covered with a silvery film of the leaf epidermis. A high incidence of the pest infestation was recorded on 125 of the 200 trees inspected being affected, representing an incidence of 62.5%.

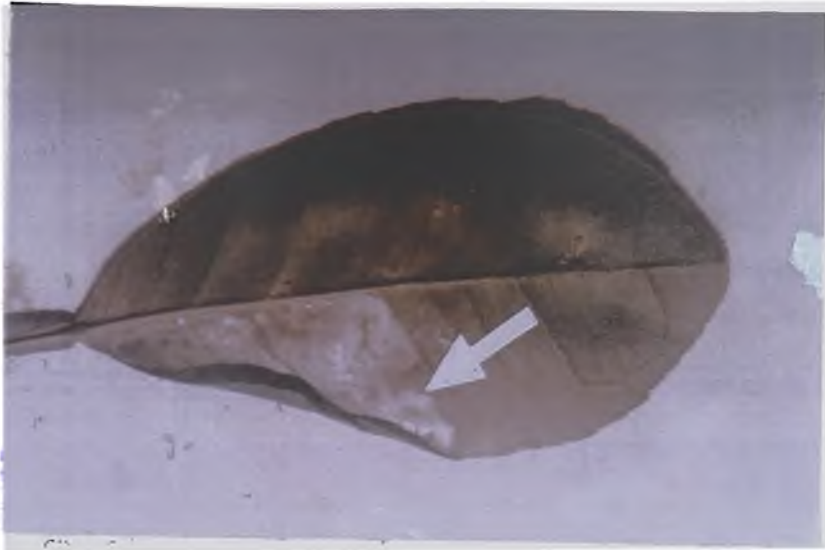


Plate 16. Citrus leaf attacked by citrus leaf miner (*Phyllocnistis citrella*) in the nursery. Note the serpentine nature of the mines. Mg. X 0.7

CHAPTER FIVE

DISCUSSIONS

Four different kinds of disease were found in the nursery. These were citrus canker, brown leaf spot of rough lemon, citrus scab and greasy- spot like disease of citrus. Citrus canker was characterized by water soaked margins and a yellow halo surrounding the disease lesion which has been found as a reliable diagnostic symptom (Gabriel, 2000; Stall, 1988). *Xanthomonas campestris pv. citri*, which was isolated and confirmed in this study has also been reported as the causal agent of the disease (Civerolo, 1981). The disease has also been reported in many citrus nurseries (Agrios. 1997).

The second disease, brown leaf spot has also been reported as very prevalent in citrus nurseries. The target appearance of the disease lesion and the presence of the spores of the causal agent on the surface of the disease lesion as well as the restriction of the disease to the rough lemon variety of the citrus crop have been reported as being diagnostic features of the disease (Timmer *et al.*, 1988). The causal agent of the disease, *Alternaria citri* was also isolated and confirmed in this study.

The third disease found in the nursery was the greasy spot like disease, with the characteristic greasy nature of the disease lesion (Timmer *et al.*, 1988). *Mycosphaerella citri* and its anamorph, *Cercospora citri*, reported to be the causal agents could not be isolated

The fourth disease found in the nursery was the citrus scab. The cocky, and pustular nature of the spots and the distortion of diseased leaf had been reported as a characteristic symptom of the citrus scab (Clerk, 1974). *Elsinoe fawcetti*, the causal agent of scab could not be isolated.

Not all the four diseases were of equal importance. *Alternaria* leaf spot and citrus scab were found not be of much importance to the station. Excessive branching and shorter nodes of affected trees, associated with the brown leaf spot on rough lemon that makes the plants difficult to bud, was not observed in this study. Similarly, stunted growth of affected trees associated with scab disease was also not observed. Also, disease severity in both the dry season and the wet season were not very high. Secondly, the two diseases were found to be restricted to the rough lemon variety, which was utilized mainly in the nursery as a rootstock. This means there is a less likelihood of the diseases being transferred into the field, when proper pruning of the budded material is carried out before transplanting. This also makes the diseases of less importance to the nursery.

On the other hand, greasy spot like disease and citrus canker could be important diseases at the station. All the varieties of the crop in the nursery were found to be susceptible to these two diseases and this could make the control of such diseases very difficult and expensive. Being found on the foliages of the scions as well, these diseases have a potential of being transferred onto the farmers field and the orchards of the station. The infection of matured trees in the nursery is an indication that these diseases may not be restricted to nurseries only but can establish well on matured trees in the field as well.

Between the two diseases, greasy spot- like disease may not be the most threatening disease in the nursery. Unlike the citrus canker, greasy spot -like disease showed very low incidence figures in both the wet and dry seasons. Also with the exception of late valencia, all the budlings had low severity figures. Cleopatra mandarin and rough lemon, which had high severity figures were used mainly as rootstock and therefore might not affect the quality of planting materials produced.

Determination of the effects of the diseases on yields to complete the studies on their importance was not feasible because the seedlings and budlings in the nursery had not reached the stage of fruit bearing during the period in which the study was carried out.

Citrus canker was found to be a very devastating disease in the nursery. The wide host range, high incidences and the physical destruction of foliage of the seedlings and budlings make it a disease that demands rapid attention. The disease remained the only one with a 100% incidence on a walters (*C. paradisi*) within the period of the experiment. The damage caused to the foliage was rapid resulting in production of poor quality planting material. The high severities showed on the hybrids, namely ortanique and king disemis, indicates that combination of different genotypes to control the disease may not be possible, and thus confirms the assertion by Lelliot and Stead (1987), that all hybrids of the citrus crop are susceptible to the disease. The high incidence and severity on walters (*C. paradisi*) indicate that the raising of planting materials of the variety could be very unprofitable in the nursery.

The cultural practices being adopted in the nursery could favour the development of the disease. Overhead irrigation, just like rainfall, wet the leaf surfaces and thereby induces

the oozing out of bacterium cells from diseased lesion, thereby aiding the spread of the disease among the seedlings and budlings in the nursery. Secondly, budding and pruning of leaves with unsterilised tools could contribute to the spread of the bacterium in the nursery. Also the arrangements of the seedling and budlings in crowded rows and columns can facilitate the spread of the disease in the nursery.

From the on going discussions it could be inferred that with the emergence of citrus canker in the nursery there is the need for a modification of certain practices in the nursery. For example the station needs to explore other alternative methods of irrigation, since the practice of overhead irrigation would continue to accelerate the development of the disease. Secondly, tools sterilisation must be taken as one of the disease prevention measures in the nursery to prevent further spread of not only the canker, but also any other disease that could spread through the use of infected working tools and equipment. Also with the disease being found in all the nurseries at the station, any control measure instituted against the disease must not be restricted to the main nursery alone, but must be extended to the other nurseries as well to ensure its total eradication from the station.

Apart from cultural practices, the climate in the area is favourable for the development of the disease. Frequent rainfalls and high temperatures that characterise the climate of the area are known to exacerbate the disease leading to serious damage and tree death (Anon., 1997; Gottwald *et al.*, 2000; Roistacher, 1988) and this may account for the high incidence of the disease recorded in the rainy season.

Another possible factor contributing to the high incidence of the disease was the incidence of the Asian leaf miner (*Phyllocnistis citrella*) in the nursery. The association

of the pest with citrus species and related Rutacea species has been reported (Kalshoven, 1981). Its presence in West Africa has long been suspected (Heppner, 1998). The larvae mines observed on the affected leaf surfaces in the nursery might serve as entering points of the bacterium in the nursery (Hill, 1918; Ando *et al.*, 1985). In its burrowing activities, the larva of the pest may bore holes into the canker spots, which may result in the spread of the bacterium (Mavrodieva *et al.*, 2004). Though the pest was highly suspected as a contributory factor in the spread of the disease in the nursery this was not investigated in this study and therefore further studies in this area is recommended.

Most of the budded materials recorded higher severity figures. This observation could be attributed to the type of rootstocks used in the nursery. From the survey it was realised that the commonest rootstocks used in the nursery were rough lemon (*C. limon*), cleopatra mandarin (*C. reticulata*) and *Citrus volkameriana*. These three rootstocks are known to promote vigorous growth in scions resulting in rapid emergence of young growths on the plant (Hearn and Hutchinson, 1977; Castle, 1988) and hence predisposing the scion to the canker disease, which affects young growths more than matured tissues (Albrigo and Davies, 1994). This observation was not investigated and therefore further work in this area is recommended.

Results from the screen house experiment in which different inoculation methods were used further confirmed the disease as citrus canker. The various stages of the disease as recorded in this study have been reported by earlier researchers (Gabriel, 2000; Gottwald *et al.*, 2000) and thus confirm the disease as canker. The result also showed that factors

that accelerate the entrance of the bacterium in to the leaves could greatly accelerate the development of the disease, since the syringe places the bacterium directly into the leaves. Thus in pathogenicity studies on the disease, the blunt syringe method is highly recommended as compared to the spraying method.

Canker disease is a threat not only to the station, but also to the citrus industry in the country as a whole. The spread of the disease in Ghana can have a very devastating effect on the citrus industry in the country. Currently citrus is gaining popularity in Ashanti, Eastern and Western regions, which have high humidities and temperatures and frequent rainfalls in most parts of the year. Such environmental characteristics have been reported as very favourable for the disease (Whiteside *et al.*, 1988; Anon., 1997). Serious damage and death of trees affected by the diseases have also been reported as being accelerated by severe rains and winds and so with occurrence of the disease in such areas its effect would be very devastating.

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APPENDIX

ANOVA for incidence of greasy spot-like disease in the nursery

SV	DF	SS	MS	V. r.	Fpr.
Treatment	7	4530.50 (2835.87)	647.21 (404.84)	28.29 (19.17)	<0.001
Residual	16	3660.00 (22.88)			
Total	23	4896.50 (3171.84)			

Transformed data in brackets.

ANOVA for severity of greasy spot-like disease in the nursery

SV	DF	SS	MS	V. r.	Fpr.
Treatment	7	18.374062	2.624866	335.98	<0.001
Residual	16	0.125000	0.007812		
Total	23	18.499062			

Transformed data in brackets.

ANOVA for severity of canker disease in the nursery

SV	DF	SS	MS	V. r.	Fpr.
Treatment	7	12249.96 (6786.118)	1749.99 (969.445)	147.57 (115.63)	<0.001
Residual	16	190.00 (134.147)			
Total	23	12439.96 (6920.265)			

Transformed data in brackets.

ANOVA for severity of canker disease in the nursery

SV	DF	SS	MS	V. r.	Fpr.
Treatment	7	490.49167 (12.994762)	70.07024 (1.85639)	6468.12 (4013.83)	<0.001
Residual	16	0.17333 (0.0074000)	0.1083 (0.0004625)		
Total	23	490.66500 (13.002162)			

Transformed data in brackets.