AETIOLOGY AND CONTROL OF CITRUS GUMMOSIS DISEASE AT THE UNIVERSITY OF GHANA AGRICULTURE RESEARCH STATION, KADE.

BY

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D E D I C A T I O N

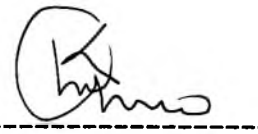
TO

MY WIFE, SON AND PARENTS.

D E C L A R A T I O N

I, Michael Kwaw Assuah, do hereby declare that the report herein presented is the outcome of my own work, and that no such work has ever been presented to this University, or any other University elsewhere in application for a degree.

Works by other authors which served as references have been duly cited.



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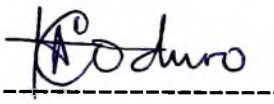

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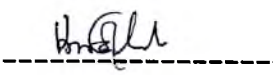

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A B S T R A C T

The citrus gummosis disease is a major problem facing the Agriculture Research Station of the University of Ghana at Kade. The disease is the cause of death of citrus plants on the station, hence this work initiated to investigate the cause(s), possible contributing factors and its control on the station.

A field survey conducted from January to June 1996 gave 36.2 -79.9% incidence (Avg. 62.6%) on six orchards of the station and an average severity index of 1.2 on a 0-5 scale. A correlation analysis proved that both disease incidence and severity increased with age (r = 0.705 and 0.876 respectively). Plants with ages above twenty years, were more severely affected by the gummosis disease.

Plants, established with rough lemon rootstock were more susceptible and severely affected by the gummosis disease than plants established with cleopatra mandarin or rangpur lime rootstocks. Infection was more frequently located on the scion (73-85%) than on the rootstock (15-27%) materials.

Termites were observed to carry soil particles which may possibly be infected with the infective units of the pathogen up the plants. Their activities probably result in wounding the surfaces of the bark by abrasion, for the pathogen in the soil to infect the plants.

Phytophthora parasitica, the hitherto known pathogen of the disease in Ghana could not be isolated, after several

attempts including the use of selective media. Rather, *Diplodia natalensis* Pole Evans, one of four fungi isolates obtained from the isolation processes, induced gummosis disease symptoms in artificially inoculated rough lemon seedlings. Koch's postulates were satisfied. *Fusarium solani* and two other unidentified fungi isolates were unable to cause the disease sysmptoms in the pathogenicity test.

In a rootstock resistance screening work, with *D. natalensis* as the test fungus, Volkameriana (*C. volkameriana* Pasq.) seedlings oozed the least amount of gum and had lesions which were smaller in size than those on the standard rough lemon seedlings. Seedlings of Swingle citrumelo (*C. paradisi* Macf.X *C. trifoliata*) and Obuasi {*C. sinensis* (L) Osbeck}, a local cultivar oozed gum profusely and Swingle in addition, developed the largest lesion as compared to rough lemon.

Ridomil (metalaxyl) at 40g and 60g ai/L, aliette (phosetyl aluminum) at 200g and 400g ai /L and phosphorous acid (Foli-r-fos) also at 150g and 300g ai/L were effective in controlling the disease on the field. Ridomil however, was more effective than the other two fungicides. Two applications of these fungicides within a year was also more effective than single application.

Bordeaux mixture (1:4) and bavistin (50% carbendazim) at 1g and 2g ai /L were ineffective against the disease.

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CHAPTER ONE

INTRODUCTION

Gummosis is an important fungal disease affecting *Citrus* and related genera in most citrus growing countries. The disease is characterised by the formation of gum-filled pockets within or beneath the bark and the exudation of gum from the cracks in the bark or skin of the trunk, limb or fruit (Gardner et al, 1952). The disease, also, causes chlorosis and die-back and eventual death of the plant when it girdles the tree trunk.

The gummosis disease of citrus was first reported in Ghana in 1925 (Bunting and Dade, 1925) and was identified (Auchinleck, 1932; Anon., 1935) as one of the two "pests" which threatened the citrus industry in the Gold Coast. A survey conducted in 1957, revealed that 40% of all seedling sweet orange trees in Ghana were infected with the disease (Leather, 1959). A recent survey conducted on sweet orange farms in the three major citrus growing regions in Ghana (Eastern, Central and Ashanti), including orchards of the University of Ghana Agriculture Research Station at Kade (ARS-Kade), gave an average of 49.5% incidence. At ARS-Kade, as high as 74% incidence was recorded for an orchard (Ofosu-Budu and Oduro, 1995).

The survey at ARS-Kade covered only two orchards (Ci. 9 & Ci. 25) established with cleopatra mandarin and rough lemon,

two of the three common rootstock materials in use in Ghana. Conclusions about the performance of rootstock materials were therefore based on two out of the three common rootstock materials. Also, the disease prevalence and intensity could not be related to the ages (an important contributory factor) of the orchards since they were of different stionic combinations. There is therefore the need for an up-to-date data on the incidence and severity of the disease with respect to the various stionic combinations and ages of the plants on the station.

The use of rootstocks resistant to the disease has long been shown as an important control measure. Yet, in practice, gummosis is considered secondary to viral diseases in rootstock breeding and selection. The reason for this consideration is that, gummosis, being a fungal disease is more manageable with appropriate fungicides than the viral diseases. Hence, the current rootstocks in use in Ghana, namely, rough lemon [*Citrus jambhiri* (Lush) Osbeck], cleopatra mandarin (*C. reticulata* Blanco.) and rangpur lime (*C. limonia* Osbeck) were selected against tristeza, a viral disease which broke out in Ghana between 1938 and 1948, rather than gummosis thus, making them susceptible to gummosis but less so to tristeza.

In some citrus growing countries however, rootstocks more resistant than those in use in Ghana are being used against the gummosis disease. Two of such rootstock materials, namely

Swingle citrumelo (*C. paradisi* Macf. X *C. trifoliata*) and Volkameriana (*C. volkameriana* Pasq.) have recently been imported into Ghana (at ARS- Kade) and need to be tested to prove their reported higher resistance to the disease under local conditions. This requires the pure culture of the reported causal organism, *Phytophthora parasitica,* of the disease in Ghana for the inoculation trials. Several attempts to isolate *P. parasitica* were unsuccessful, instead other fungi were constantly isolated, thus, prompting the need for their pathogenicity to be tested.

At ARS-Kade, field grown plants of all ages and established with the recommended rootstocks have been found infected with the gummosis disease and the application of Bordeaux mixture fungicide over the years has not provided adequate control on the station (Dr. Ofosu-Budu, K.G.; Research Officer, ARS-Kade. Personal Communication). Some systemic fungicides such as Aliette, Ridomil and Phosphorous acid which have been reported to be effective against the disease elsewhere are now available in the country and also need to be evaluated.

Based on the above information the following **objectives** were set for this work:

1. To update the incidence and severity data of the disease in the orchards of the station and to identify possible factors contributing to the disease.
2. To test the pathogenicity of the fungi isolated from the

diseased citrus plants.

3. To assess the reported higher resistance of the imported rootstock materials to the gummosis disease and
4. To screen new systemic fungicides for efficacy against the citrus gummosis disease at ARS-Kade.

C H A P T E R T W O

LITERATURE REVIEW

2.1 Incidence and importance of citrus gummosis disease

The citrus gummosis disease is present in all citrus growing countries worldwide (Hanna, 1969; Klotz and Calavan, 1969). The disease is rated second to some viral diseases such as tristeza, exocortis, psorosis and xyloporosis in importance (Bitters, 1958), in most of these countries. In other countries such as Mali, the intensity of the disease is such that it is next to none in importance (Darthenucq and Rey, 1974).

In Ghana, the disease affects seedling lemons, tangerines, grapefruits and especially sweet oranges (Bunting and Dade, 1925; Eady, 1930). The disease was reported as one of two "pests" threatening the citrus industry at the time (Auchinleck, 1932; Anon., 1935). A national survey in 1947 showed that 40% of sweet orange trees in Ghana were infected with the gummosis disease (Leather, 1959). A recent survey (Ofosu-Budu and Oduro, 1995), also revealed that an average of 49.5% of sweet orange trees in three citrus growing regions in Ghana were infected with the disease.

2.2 Aetiology

Until 1913 when Fawcett first isolated from gummosis

diseased bark and proved the pathogenicity of *Phytophthora citrophthora* in California, the disease was variously attributed to waterlogging, a bacterial pathogen, and *Fusarium spp.* (Fraser, 1942). Since then other species of *Phytophthora*, mostly *P. parasitica* and *P. palmivora* have been proved to be pathogenic to the citrus plant (Fawcett, 1913; 1923; Klotz and Fawcett, 1930; Uppal and Kamat, 1936). The prevalence of any particular *Phytophthora* species causing the disease at a time on an orchard is determined by factors such as climate, soil type and variety of stock used (Fraser, 1942). *Phytophthora parasitica* is noted to be active in warmer environments while *P. citrophthora* abounds in colder climates (Klotz and Calavan, 1969).

Due to changing cultural practices and use of wider range of rootstock materials, other fungal species such as *Botrytis cinerea, Sclerotinia sclerotiorum, Diporthe citri, Diplodia natalensis,* a *Dothiorella spp.* (Klotz and Calavan, 1969; Granada and Sanchez, 1969; Rodriguez, 1978; Mukhopadhyay, 1985) and *Macrophoma mantegazziana* (Giobelidze, 1987) have been implicated in the disease. Gumming in citrus may also be caused by physical and chemical injuries such as insects feeding on the bark and creating wounds for infection by various fungi, arsenic compounds coming into contact with the tree bark and Copper deficiency (Klotz and Calavan, 1969).

In Ghana, *Phytophthora parasitica* was reported as the causal organism of the disease (Leather, 1959; Clerk, 1974).

Available records show that Bunting and Dade (1925) first reported the disease but did not name any organism as the cause. Rather, the cause of the disease was attributed to poor edaphic conditions and bark injuries. Other reports on the disease were silent on the causal organism. Leather (1959) and Clerk (1974) reported *P. parasitica* as the causal organism for the disease. Yet, these reports did not include evidence of isolation of the pathogen. Sam-Aggrey (1971a), also reported of the incidence of a collar rot disease of citrus which was suspected to be gummosis in a nursery in Kumasi, although the causal organism involved was not isolated. Thus, evidence of the isolation of the causal organism in Ghana is lacking.

2.3 Symptoms of the gummosis disease

The most characteristic symptom of the disease is the exudation of gum from the cambial region of the infected bark (Fraser, 1942). The exuded gum flows down to the soil if the infection is close to the ground or hardens in long ridges on the surface of the bark of the trunk. The affected bark becomes necrotic, firm and darker than the surrounding healthy tissue when scraped. On removing the infected bark, the cambial surface reveals a dark-brown colour which reduces in intensity to a healthy cream surface (Klotz and Calavan, 1969). The dead bark dries, cracks and shreds (Leather, 1959; Klotz and calavan, 1969; Clerk, 1974) leaving a dark-brown and

dead wood surface. As the disease develops under favourable conditions of temperature and relative humidity it girdles a greater part of the tree trunk and eventually kills the tree. But before the tree dies, it produces a final heavy crop (Leather 1959; Clerk, 1974). Under less favourable conditions, the fungus becomes inactive and the edges of the lesions callus over (Fraser, 1942). The crown symptoms of the disease are usually not different from those of other disorders affecting the vascular system of the tree. These include chlorosis, leaf-fall and die-back of twigs of branches corresponding to the diseased part of the tree trunk (Fraser, 1942; Leather, 1959; Clerk, 1974).

2.4 Factors favouring the development of the disease

Several factors have been identified to favour the prevalence and severity of the gummosis disease. They include the following:

a. wetness of the collar of the tree (Leather, 1959; Klotz and Calavan, 1969).
b. high level of soil moisture due to inadequate internal drainage (Wong and Varghese, 1966; Clerk, 1974).
c. injuries made to the lower trunks by tools during cultivation (Leather, 1959; Klotz and Calavan, 1969).
d. temperatures near the optimum for the growth of the pathogen on the orchard (Klotz and Calavan, 1969).

e. high relative humidity around the collar of the tree due to weed growth, heaping of soil or mulch around the collar and higher plant densities (Klotz and Calavan, 1969).

f. deep planting and low budding onto the rootstock which result in bud union being at, below or closer to the soil surface, thus, exposing susceptible scion tissue for infection (Leather, 1959).

g. activities of termites recently speculated in Ghana (Ofosu-Budu and Oduro, 1995).

2.5 Preventive control measures

The preventive control measures adopted were mostly those cultural practices that tended to limit the growth and development of the disease-causing organism.

The importance of the use of resistant rootstocks had long been established, but its adoption has been hampered in areas where these rootstocks are susceptible to prevailing viral diseases. In practice therefore, resistance to gummosis is recommended to be secondary to resistance to viral diseases (Bitters, 1958).

Generally, while lemons, sweet oranges and pummelos have been found to be of lower degree of resistance, sour orange and trifoliate oranges have been observed to be of higher degree of resistance to gummosis (Klotz and Fawcett, 1930; Fraser, 1942; Klotz et al, 1958a; Klotz and Calavan, 1969).

The use of these as rootstocks against gummosis is limited because they are respectively known to be susceptible to tristeza and exocortis viral diseases. Some hybrids of trifoliate oranges, including citranges however, have been found to be torelant to both exocortis and gummosis diseases (Klotz and Fawcett, 1930; Shannon et al, 1960; Carpenter and Furr, 1962).

Resistant rootstocks, have been observed to modify the resistance of scion material to the gummosis disease (Klotz et al, 1958b; Koller et al, 1984) and vice versa (Rossetti, 1969; Vanderweyen, 1980; Feichtenberger, 1987). Therefore, various stionic combinations of rootstock and scion react differently in the presence of the disease causing organisms.

The resistance of citrus species and related genera to gummosis had been attributed to certain chemicals with fungicidal properties (Haas and Hill, 1913) whose presence or absence in significant quantities in the bark of these species modify their resistance or susceptibility to the disease (Klotz et al, 1968). These chemicals include glucosides, tannins and other materials such as gallic and protocatechuic acid (Haas and Hill, 1913). Recently, scoparone (6,7-dimethoxycoumarin), a phytoalexin has been reported to confer resistance against gummosis in citrus species (Afek et al, 1986).

Resistance to gummosis thus, vary greatly between citrus species and related genera, varieties of the same species,

individuals of the same variety and different locations of the same tree (Klotz and Fawcett, 1930). Other factors that have been observed to influence the resistance of citrus species include climatic conditions, method of inoculation and more importantly, the pathogen and its factors used in the inoculation process. For example, thirty-two selections of trifoliate orange and their hybrids (citranges and citremon) tested by planting in soils heavily infested with *P. parasitica* and *P. citrophthora* escaped the gummosis disease. However, when these were artificially inoculated by placing the fungi under the bark onto the cambium, they were all found to be susceptible in varying degrees (Klotz et al, 1967; 1968; 1969a). Volkameriana (*C. volkameriana)* was also found to be susceptible to *P. citrophthora* (Vanderweyen, 1980) but resistant when inoculated with *P. parasitica* (Fouque et al, 1977).

While screening work on the identification of resistant rootstocks to gummosis continues with *Phytophthora species* involved in the aetiology of the disease, very little work has been done with other species of fungi with proven pathogenicity. Granada and Sanchez (1969) used *Diplodia natalensis* in addition to *P. parasitica* in an inoculation test and found differences in the level of resistance of 62 cultivars, species and hybrids of citrus to both pathogens.

The use of resistant citrus species as rootstocks to control the gummosis disease was recognised in Ghana a long

time ago. The first attempt was the introduction of rough lemon and sour orange as rootstock materials because they were thought to be resistant to the gummosis disease (Anon., 1932; 1935; 1936) although the susceptibility of rough lemon to the disease was previously known (Fawcett, 1923; Klotz and Fawcett, 1930; Toxopeus, 1934). Rough lemon and sour orange were the popular rootstock materials used to rehabilitate gummosis-infected citrus farms in Ghana (Anon., 1935; 1936; Leather, 1959). The methods used included "inarch" grafting and the production of citrus buddlings for the rehabilitation of old farms and the establishment of new ones (Anon., 1932; 1935; 1936; Leather, 1959). However, it was later realised that the sour orange stock was highly susceptible to the quick decline virus (Tristeza) disease, a more devastating citrus disease which reached an epidemic proportion in Ghana from 1938 to 1948 (Lister, 1947; 1948). A report in 1944-45 showed that trees with rough lemon stocks showed greater disease (Tristeza) resistance than trees with sour orange stocks which died with no symptoms of collar rot (Urquart, 1944-45). This led to the termination of the use of sour orange as rootstock material in Ghana.

After several investigations into the tristeza malady, it was recommended that additional resistant rootstock materials should be investigated (Hughes and Lister, 1949) and led to the screening in the 1960's of some local varieties and the importation of several others for studies. The main

institutions involved in the studies were agricultural stations of the then Department of Agriculture located at Asuansi and Kumasi, the Horticulture Department of the University of Science and Technology and the University of Ghana Agriculture Research Station (ARS) at Kade (Sam-Aggrey, 1971b).

These studies emphasized on selection of rootstock materials that imparted to the scion, high yielding abilities, good fruit characteristics and tolerance to tristeza as recommended by Bitters (1958) rather than the selection for tolerance to gummosis (Sam-Aggrey, 1971b). At ARS-Kade, a plot, Ci. 14 was specifically established to test the resistance of the various rootstock materials against the common diseases (including gummosis) of citrus under field conditions (Pospisil, 1966), but no relevant records can be traced to date.

The results of these studies confirmed the conclusions of Hughes and Lister (1953) of the suitability of rough lemon, Cleopatra mandarin *(C. reticulata* Blanco.) and Rangpur lime *(C. limonia* Osbeck) as suitable rootstock materials for citrus in Ghana. With respect to the gummosis disease however, these rootstocks were known to be susceptible (Klotz and Fawcett, 1930; Fraser, 1942; Klotz et al, 1958a). Later, Darthenucq and Rey (1974) and Fouque et al, (1977) also reported the susceptibility of these rootstock materials under tropical conditions in Mali and the Cote d'Ivoire respectively. Thus,

the rootstock materials in use in Ghana have proven susceptibility to the gummosis disease.

Other cultural practices that were used to prevent and control the disease in Ghana included high budding on to the rootstock, regular brushing to avoid excessive weed growth around the trunk and avoidance of wound during weeding and suckering (Leather, 1959).

2.6 Chemical control

An earlier chemical preventive measure was the use of an impervious mixture made up of three parts sand and one part hot tar against the disease. The mixture was applied to a space cleared of vegetation round the tree base to a diameter of 45 cm and allowed to set and was reported to be effective in preventing gummosis infection after a year of application (Anon., 1939a). The Bordeaux mixture was the widely used fungicide against the citrus gummosis disease in Ghana (Anon. 1939b; Leather, 1959). The mixture was applied to the wound created by the surgical removal of the diseased bark. Reports however, indicate that the fungicide had not been effective against the disease (Sawant et al, 1990; Ofosu-Budu, Personal communication). Sawant et al (1990), found the Bordeaux mixture to be ineffective in controlling the disease when compared with other fungicides.

In some citrus growing countries however, effective

control had been achieved with the use of some systemic fungicides. The efficacy of Aliette, Ridomil and Phosphorous acid have particularly been outstanding (Davis, 1982; Fenn and Coffey, 1984; 1985; Matheron and Matejka, 1988; Sawant et al., 1990). Matheron and Matejka (1988) reported that the effectiveness of these fungicides is irrespective of the method (foliar spraying, trunk injection, trunk painting and soil drenching) used in their application. None of these fungicides has been used in the treatment of the citrus gummosis disease in Ghana.

CHAPTER THREE

MATERIALS AND METHODS

3.1 FIELD SURVEY

A survey was conducted on six selected plots or orchards at the Agriculture Research Station of the University of Ghana at Kade from February to June 1996. Three of the selected orchards had been established with the three common rootstock materials in use in Ghana namely; rough lemon, cleopatra mandarin and rangpur lime. The other three orchards, were of different ages but had been established with buddlings of the same scion-rootstock stionic combination. The selection was done with the view to evaluate the effect of age of plant and type of rootstock used in its establishment on the incidence and severity of the citrus gummosis disease. The sweet orange cultivar, Late Valencia was the scion common to all the rootstocks on the six orchards (Table 1).

Other data collected during the survey were the height at which the infections were located, part of the plant (scion/stock) infected, efficacy of Bordeaux paste on previously treated plants and possible pre-disposing factors to the disease.

3.1.1 Determination of disease incidence

The total number of plants on each plot, as well as plants showing symptoms of necrotic lesions and/or gumming

Table 1: Characteristics of sweet orange orchards at ARS-Kade selected for the field survey.

Criteria for selection	Name of orchard	Age (Yrs.)	Hecterage (H)	Rootstock	Scion
AGE	Ci. 26	6	5.2	Rough lemon	Late valencia
	Ci. 17	12	2.2	"	"
	Ci. 13	22	5.6	"	"
ROOTSTOCK	Ci. 9	23	2.2	Cleo. mandarin*	"
	Ci. 24	20	7.0	Rough lemon	"
	Ci. 24	20	7.0	Rangpur lime	"

* Cleopatra mandarin.

from any part of the plant, were counted and recorded. The results were expressed as percentage infection (incidence), for the plot and were correlated statistically to assess relationship between age and disease incidence.

3.1.2 Determination of disease severity

One hundred and fifty plants were randomly selected on each plot to determine the disease severity. The assessment was based on disease symptoms on the main trunk of the plants. The horizontal diameter of the necrotic lesion and the circumference of the trunk were measured with a measuring tape. The measurements were used to calculate the severity index (S) for the tree. The index which was devised by Ofosu-Budu and Oduro (1995) was used in assessing the portion (in degrees) of the tree trunk, in relation to the tree circumference, that was girdled by the gummosis disease. It was defined as $S = X / Y \times 360^{\circ}$, where X is the diameter (cm) of the necrotic lesion and Y is the circumference (cm) of the trunk at the centre of the necrotic lesion. The index was expressed on a 0 - 5 scale, after Ofosu-Budu and Oduro (1995) where:

0 -- $S = 0^{\circ}$ (uninfected plant)

1 -- $0^{\circ} < S \leq 90^{\circ}$

2 -- $90^{\circ} < S \leq 180^{\circ}$

3 -- $180^{\circ} < S \leq 270^{\circ}$

4 -- $270^{\circ} < S \leq 360^{\circ}$

5 -- Dead as a result of the disease.

The ages and arc-sine transformed severity indices were

correlated to assess their relationship. The severity index S (portion of the bark infected) values for the 150 selected trees were also analysed in a one-way analysis of variance for the age and rootstock orchards. The least significant difference test was used to separate the means.

3.1.3 Determination of height and part of infection

Hundred out of the 150 plants used for the severity determination were used for determining the height and part of infection on the plots used for the rootstocks studies. The height of infection was taken as the height between the soil surface and the centre of the lesion, whilst the part of infection was recorded as either the rootstock, scion or both (rootstock and scion) for each of the 100 plants on the three plots.

3.1.4 Other data collected

A visual observation was made on the orchards for clues or pre-disposing factors that could lead to the disease incidence and severity. Factors such as soil drainage and the nature of the canopy of the plants on the orchards were noted.

The effectiveness of Bordeaux mixture in controlling the disease on trees with a history of treatment was evaluated. The total number of plants that had ever been treated were counted. Plants that had been treated and were healthy or clean and those that had been treated but were re-infected

were also counted. The data collected were used to calculate the percentage re-infection for the various plots.

3.2 Isolations and pathogenicity determination of isolates

Specimens of diseased citrus bark and wood were collected from diseased plants in the selected orchards of the Agriculture Research Station of the University of Ghana at Kade and brought to the laboratory (Crop Science Department, Legon) for isolation.

The isolation procedure involved plating of about 0.5 cm^2 pieces of bark and wood excised from the leading margins of the disease on 1.5% water agar. Before plating, the tissues were surface sterilised in 1% sodium hypochlorite solution for 1 minute, rinsed with sterile distilled water and blotted on sterile filter paper. The plates were incubated at 25-28 °C and examined for mycelial growth daily. Mycelia which grew from the tissues were transferred onto Potato Dextrose Agar (PDA) plates for further growth and sporulation.

Several attempts, including the use of higher concentrations of the surface sterilant (2, 5, 10 and 20%), longer sterilising periods (2 and 5 minutes) and pawpaw agar medium were unsuccessful in isolating *Phytophthora parasitica*. Selective isolation media of 1.5% water agar + 100 ppm of nystatin (500,000 iu/5ml; 20:1 v/v) and 1.5% water agar + 100 ppm of benomyl (WP, 50% ai; 20:1 v/v) were used to suppress distracting organisms and enhance the growth of *P. parasitica*.

In the identification of the isolates, prepared slides were observed under the microscope and the characteristics of the isolates compared to those reported for the organisms.

The pathogenicity test was done using eighteen-month old potted rough lemon seedlings. The inocula consisted of 5-day old PDA cultures of the four isolates and sterile PDA for control seedlings. The surfaces of the stem of the seedlings were disinfected with 10% sodium hypochlorite solution before inoculation. The inoculation process involved inserting a small block of the inoculum of mycelium of each of the isolates under a flap of bark made by a vertical slit and covering it with a moistened sterile cotton wool. The wounds were covered with parafilm and the seedlings were arranged in a completely randomised design with two replicates (four seedlings in a replicate) and incubated in a screenhouse at 30-37 °C and 55-75% relative humidity for observation.

3.3 Rootstock Resistant Screening

The following citrus species were screened for resistance against the citrus gummosis disease: Swingle citrumelo (*C. paradisi* Macf. X *C. trifoliata*), Volkameriana (*C. volkameriana* Pasq.), Obuasi {*C. sinensis* (L) Osbeck} and rough lemon [*C. jambhiri* (Lush) Osbeck], the commonly used rootstock material in Ghana was used as the standard.

The surfaces of the stem of the eighteen-month old potted

seedlings to be inoculated were disinfected with 10% Sodium hypochlorite solution by swabbing. Discs of bark, 2 mm in diameter, were removed with a flamed cork borer from the disinfected part. Mycelia discs of similar size of the isolated fungus which had been identified as *Diplodia natalensis*, from a 5-day old PDA culture, were placed on the wood cambium surface (mycelium side to the cambium) of the seedlings. The cork borer was used to cut the discs of mycelia to ensure that seedlings were inoculated with the same amount of inoculum. The wounds were covered with moistened sterile cotton wool and wrapped with parafilm. The seedlings were arranged in a completely randomised design with single seedling replicates of five replications. Resistance was determined by measuring:

a) the diameter of necrotic lesions at the end of the incubation period of 20 days and
b) the degree of gumming.

The measured diameters were used to compute the average area of the necrotic lesions on the seedlings of the various inoculated species. The result was analysed in a one-way analysis of variance and the Least Significant Difference (LSD) used to separate means. The degree of gumming was assessed visually by estimating the volume of gum exuded by the four species.

3.4 Chemical Control

3.4.1 Field Test: - The efficacy of some fungicides were tested against the citrus gummosis disease on a twelve-year old sweet orange orchard, Ci 25 established with late valencia on rough lemon rootstock. The experiment consisted of three factors: fungicides (4), rate or dosage of application (2) and number or frequency of application (2) arranged as a 4x2x2 factorial in a completely randomised design with three replicates (3 plants in a replicate). Bordeaux mixture (36.4g Calcium oxide + 36.4g Copper sulphate in a litre of water) and a distilled water control were included. Ten of the twenty treatments were made up of eight, from two different rates of the four systemic fungicides, one from the protective fungicide (Bordeaux mixture) and one from the distilled water control (Table 2) and were applied in July, 1996. The remaining ten treatments were applied in January, 1997 and consisted of the second application of the same set of treatments in the number or frequency of application factor.

The treatments were applied by surgically removing the bark of the trees from the necrotic area to the healthy tissue and painting the wound with the fungicide using a brush. In the second application, treatments were applied to trees with re-infection at the treated site in the same manner as was done during the first application. On trees without re-infection at the treated site, treatments were applied to

Table 2: Types and rates of fungicides used for the chemical control on plot Ci. 25 at ARS-Kade.

Type of fungicide	Rate (g ai/L)	Formulation
Aliette (WP, 800g fosetyl-Al /Kg)	200	Suspension
	400	"
Ridomil (Metalaxyl MZ, 72 WP)	40	Paste
	60	"
Phosphorous acid* (Foli-r-fos)	150	Solution
	300	"
Bavistin (WP, 50% Carbendazim)	1	Suspension
	2	"
Bordeaux mixture@	1:4	"
Control	Distilled water	Water

* pH of formulations was adjusted to 5.8 with 5% KOH solution.

@ 34.6g of $CuSO_4.5H_2O$ + 34.6g $Ca(OH)_2$ per litre of water.

green surfaces of the bark after the brown outer bark had been scraped off. The following data were collected:

a. Time taken for callus to be formed at the treated site

This was recorded as the number of months taken for callus to be visible at the treated site.

b. Rate of callus formation at the treated site

The rate was assessed as the length (cm) of callus formed in a month to cover part of the wound at the treated site. The results were analysed in a one-way analysis of variance and also used to draw cummulative curves for the treatments.

c. Presence or absence of gum from the treated site

This was recorded at monthly intervals for each treated plant as positive, for plants oozing gum, and negative for plants without gum exudation at the treated site. The number of plants without gum at the treated site were used to draw a graph for the various treatments.

d. The thickness of the callus formed

This was measured by placing a piece of cardboard perpendicular to the surface of the wood at the treated site and making a mark corresponding to the thickness of the callus on the board with a sharp point. The callus thickness was then measured from the mark on the cardboard with a rule. These data were taken in November, 1996 and May, 1997 (four months after each set of treatment application) and five trees per treatment were

used. Six measurements were taken at a wound on a tree and averaged for the tree. The results were analysed in a one-way analysis of variance and means separated with the least significant difference test.

3.4.2 Laboratory test:- The fungicide treatments were tested in vitro using PDA as substrate. Fungicides were added to autoclaved and cooled test media at concentrations similar to field rates. The test fungus was the pathogenically proven isolate that had been identified as *D. natalensis*. Discs of mycelia, 2 mm in diameter, of actively growing PDA culture of the fungus were placed at the centre of the plates (mycelium side down) and presence or absence of growth was determined to the fifth day after plating.

CHAPTER FOUR

RESULTS

4.1 FIELD SURVEY

4.1.1 Field symptoms of the citrus gummosis disease

The citrus gummosis disease was observed on field grown plants than on seedlings in the nursery of the University of Ghana Agriculture Research Station at Kade. In the nursery, seedlings of various rootstocks at different ages were found to be virtually free from the disease.

On the field, the observed symptoms of the gummosis disease included the following:

a. ridges of gum exuded from cracks in the infected bark on tree trunks (Fig. 1).
b. a brown colouration on wood surfaces directly under the infected bark (Fig. 2).
c. firm, dark, necrotic, dry and shredded bark of infected trunks with time (Fig. 3).
d. dark-brown-coloured dead wood of the tree trunk as a result of girdling of the bark by the disease.
e. callus formed at the margins of the necrotic lesions in the dry season.
f. a heavy crop of numerous but small-sized fruits which normally do not reach maturity before the death of the plant.

Fig. 1 *(Top) - A characteristic symptom of gummosis disease of hardened gum (arrowed) on a late valencia sweet orange budded on rough lemon rootstock.*

Fig. 2 *(Bottom) Symptom of gummosis disease on the wood of an infected citrus plant on removal of diseased bark.*

Note: a - brown colouration (arrowed) on the infected wood.

b - cream surface (arrowed) of healthy wood.

Fig. 3 *Dead, dried and shredding bark (arrowed) of valencia sweet orange on rough lemon rootstock infected with the gummosis disease.*

g. death of the entire plant as a result of greater part of the bark of the tree being girdled (Fig. 4).

4.1.2 Effect of age of orchard on disease incidence and severity The disease incidence was highest on the twenty-two-year old plot (69.8%) and lowest on the twelve-year old plot (36.2%). The incidence on the youngest plot (six years) was 49.6% (Table 3). The severity of the disease followed a pattern which was similar to the incidences on the three plots. The highest severity of 1.47 was recorded on the oldest plot and the lowest of 0.67 on the twelve-year old plot. A severity of 0.76 was recorded on the six year plot on a 0-5 scale (Table 4).

A positive correlation between age and both disease incidence and severity was realised. Coefficients of correlation (r) of 0.705 and 0.876 were computed for disease incidence and severity respectively and the coefficients of determination (r^2) were also 0.705 and 0.777 respectively. Both coefficients were not significant for both disease incidence and severity at 5% level (Appendices 1 & 2).

The severity of the disease (portion of the bark of the tree infected) was significantly greater on the twenty-two-year old plot. The severity on the twelve-year old plot was not significantly different from the severity on the six-year old plot (Table 5; Appendix 3).

Fig. 4 *Valencia sweet orange on rough lemon rootstock killed by gummosis disease.*

Note: small-sized fruits (arrowed) on the dead tree.

Table 3: **Effect of age of citrus orchard on the incidence of gummosis disease.**

Age (Yrs.)	Plot	Total No of trees/plot	No of trees infected/plot	incidence* (%)
6	Ci. 26	1,320	655	49.6 ns
12	Ci. 17	282	102	36.2 ns
22	Ci. 22	844	589	69.8 ns

* ns = not significant at 5% level

Table 4: Effect of age of citrus orchard on the severity of gummosis disease.

Age (Yrs.)	Plot	Total No. of trees/plot[&]	No. of trees classified under 0-5 disease severity scale[*]						S[@]
			0	1	2	3	4	5	
6	Ci. 26	1,320	53	86	8	1	1	1	0.76 ns
12	Ci. 17	282	77	54	11	7	1	0	0.67 ns
22	Ci. 13	844	32	46	48	19	3	2	0.47 ns

[&] 150 trees were randomly selected on each plot for the severity assessment.

[*] 0 -- S = 0° (uninfected plant) 1 -- 0°< S ≤ 90°
2 -- 90°< S ≤ 180° 3 -- 180°< S ≤ 270°
4 -- 270°< S ≤ 360° 5 -- Dead as a result of the disease.

S = diameter of necrosis ÷ circumference of plant x 360°.

[@] ns -- not significant at 5% level.

Table 5: **Effect of age and citrus rootstock material on severity (mean portion of bark of trees girdled) of gummosis disease at ARS-Kade.**

Selection criteria	Plot	Age (Yrs.)	Rootstock	Mean portion of bark infected*	$LSD_{(0.01)}$
	Ci. 26	6	R. lemon[a]	32.26	
AGE	Ci. 17	12	" "	35.68	19.85
	Ci. 13	22	" "	96.89	
	Ci. 9	23	C. mandarin[b]	81.47	
Rootstock	Ci. 24	20	R. lemon[a]	116.8	28.56
	Ci. 24	20	R_p. lime[c]	66.69	

* figures are averages of 150 plants.

[a] Rough lemon.

[b] Cleopatra mandarin.

[c] Rangpur lime.

4.1.3 **Effect of type of rootstock of plant on disease incidence and severity:** - Disease incidence were 79.9%, 74.5% and 65.8% on the plots with rangpur lime, rough lemon and cleopatra mandarin rootstocks respectively (Table 6).

Rough lemon rootstock had the highest severity index of 1.83 with rangpur lime having the lowest index of 1.19. Cleopatra mandarin was intermediate with a severity index of 1.34 (Table 7).

The mean of the portion of the bark of the trees infected (severity) by the gummosis disease was significantly greater on the plants with rough lemon rootstock than on the plants with either cleopatra mandarin or rangpur lime rootstock. No significant differences existed between plants with rangpur lime and cleopatra mandarin rootstocks when the values of the portion of the bark of the tree infected by the disease were analysed for the trees on the three plots (Table 5; Appendix 4).

4.1.4 **Height and part of plant infected with the gummosis disease:** - The height at which infection occurred was found to range from ground level (0 cm) to 90 cm above the ground. The mode of the heights at which infection were located was about 30-50 cm above ground level (Table 8). These modal classes (30-50 cm) were frequently observed close to the bud union on the scion.

Generally, infections were either on the scion,

Table 6: Effect of citrus rootstock material on incidence of gummosis disease.

Type of rootstock	Plot[@]	Total No of trees/plot	No of trees infected/plot	Incidence (%)
C. mandarin*	Ci. 9 (23)	444	292	65.8
Rough lemon	Ci. 24 (20)	864	644	74.5
Rangpur lime	Ci. 24 (20)	259	207	79.9

[@] values in parenthesis are ages (years) of the plots.

* Cleopatra mandarin.

Table 7: **Effect of the rootstock material on severity of citrus gummosis disease.**

Type of rootstock	Plot	No. of trees classified under 0-5 scale*						
		0	1	2	3	4	5	S
C. mandarin[@]	Ci. 9	36	58	34	14	6	2	1.34
R. lemon[a]	Ci. 24	22	65	21	16	10	16	1.83
R. lime[b]	Ci. 24	25	87	29	4	3	2	1.19

* 0 -- S = 0° (uninfected plant), 1 -- 0° < S ≤ 90°

2 -- 90° < S ≤ 180°, 3 -- 180° < S ≤ 270°,

4 -- 270° < S ≤ 360° 5 -- Dead as a result of the disease.

S = diameter of necrosis ÷ circumference of plant x 360°.

[@] Cleopatra mandarin.

[a] Rough lemon.

[b] Rangpur lime.

Table 8: **The effect of the type of rootstock on height of infection of gummosis disease on citrus plants at ARS-Kade.**

Height of infection	Number of plants infected/ rootstock		
	CM[a]	RL[b]	R_pL[c]
0 - 10	3	5	4
11 - 20	7	7	6
21 - 30	10	16	14
31 - 40	23	36	21
41 - 50	38	17	31
51 - 60	8	7	10
61 - 70	7	4	8
71 - 80	2	5	5
81 - 90	2	3	1
T O T A L	100	100	100
SD	1.56	3.14	2.84

[a] Cleopatra mandarin.

[b] Rough lemon.

[c] Rangpur lime.

rootstock or on both parts of the same plant. Infection was highest on the rough lemon rootstock (27%) and lowest on the cleopatra mandarin rootstock (15%), while 20% infection was observed on the rangpur lime rootstock. Averages of 89% and 21% infections were recorded on the scion and rootstock materials respectively (Table 9).

4.1.5 Possible factors contributing to the prevalence of the gummosis disease:

4.1.5.1. Source(s) of infection:

a. Mycelia and conidia of the pathogen that had been proved pathological in this work were recovered by direct isolations from dead twigs sampled from the citrus plants in the orchards. The mycelia of the pathogen were observed together with mycelia of other species of fungi growing on the dead twigs.

b. Some infection foci were observed along the path of termites' activities on the plants (Fig. 5). Termites carry soil particles up the plants in their activities. This soil might possibly be contaminated with infective units of the pathogen. Wounds could possibly result from the abrasive forces between the soil particles and bark of trees or possible eating or chewing of the bark by termites. These could serve as entry points for the suspected infective units in the soil for infection and subsequent development of the disease to occur.

Table 9: **The effect of the type of rootstock on the part of the plant infected by gummosis disease at ARS-Kade.**

Plot	Type of rootstock	Number of infections*/plant part		
		Rootstock	Scion	Both
Ci. 9	Cleo. mandarin[e]	10 (15)	85 (90)	5
Ci. 24	Rough lemon	16 (27)	73 (84)	11
Ci. 24	Rangpur lime	7 (20)	80 (93)	13
A V E R A G E		(21)	(89)	

* Values in parenthesis are total number of infections on parts.

[e] Cleopatra mandarin.

Fig. 5 *Infection apparently resulting from termites' activities on citrus plant.*

4.1.5.2. Seasonal changes in the intensity of the gummosis disease The disease was more prevalent and severe in the wet than in the dry season. It was observed that infected plants naturally formed callus at the margins of the necrotic lesion in the dry season but the callus breaks down or becomes infected at the onset of the wet season.

4.1.5.3 Other observations

a. The canopy of the plants was observed to be closed on all the selected plots except the six-year old plot (Ci. 26) which had plants with opened canopy.
b. The soil on plot Ci. 17 Ext. (the 12-year old plot) was visually observed to be better drained than soils on the other selected plots.

4.1.6 Efficacy of Bordeaux mixture in previous treatments

Bordeaux mixture was relatively effective on cleopatra mandarin rootstock plants (28.6% re-infection). The effectiveness of the fungicide was moderate on plants with rangpur lime rootstock (41% re-infection) and less effective on rough lemon rootstock plants with an average of 49.5% re-infection (Table 10).

Table 10: **Efficacy of Bordeaux mixture one year after application at ARS-Kade.**

Plot	Type of rootstock on plot	No of plants treated[z]/plot	No of re-infected plants	% re-infection
Ci. 17 Ext.	Rough lemon	68	28	41.1
Ci. 13	Rough lemon	501	248	49.5
Ci. 9	Cleopatra mandarin	255	73	28.6
Ci. 24	Rough lemon	507	294	57.9
Ci. 24	Rangpur lime	139	57	41.0

[z] treatment was applied in April, 1995.

4.2 Isolation and identification of causal organism

Mycelia grew from diseased tissues on all the isolation media except on water agar-benomyl medium.

Four fungi species were isolated. Two of the four isolates were persistent and were frequently observed in most of the plates. They also sporulated readily in culture and were identified based on the following culture characteristics: growth rate and colour, morphology of mycelia, conidia and sporulating structures. The two other isolates did not sporulate and could not be identified.

One of the two isolates was identified as *Diplodia natalensis* Pole Evans, synonymous to *Botryodiplodia theobromae* Pat. (Holliday, 1980; Derwent Publications Ltd., 1990) because of the following characteristics: it had an abundant fluffy aerial septate mycelium, grey in colour but turned black after six days in culture (Fig. 6). It filled 9 cm diameter Petri plates within two days when a five-day old inoculum of mycelia cut with 2 mm cork borer was put at the centre of the plate. On the reverse, a greyish-green colour preceded the final black colour while the culture medium remained colourless. The isolate produced several conspicuous stromata (Fig. 7) on corn meal agar (CMA). Simple and separate pycnidia were produced from the stromata (Fig. 8) which also gave rise to numerous ellipsoidal conidia (pycnidiospores) of different ages. The immature conidia were hyaline and aseptate (Fig. 9) while the mature conidia were thick-walled, dark brown, two-celled and

Fig. 6 *Potato Dextrose Agar (PDA) cultures of D. natalensis isolated from gummosis-infected sweet orange plant.*

Note: Left -- 4-day old culture with grey and fluffy aerial mycelium.
Right -- 10-day old darkened culture.

Fig. 7 *Conspicuous stromata (arrowed) of D. natalensis developed on corn meal agar (CMA).*

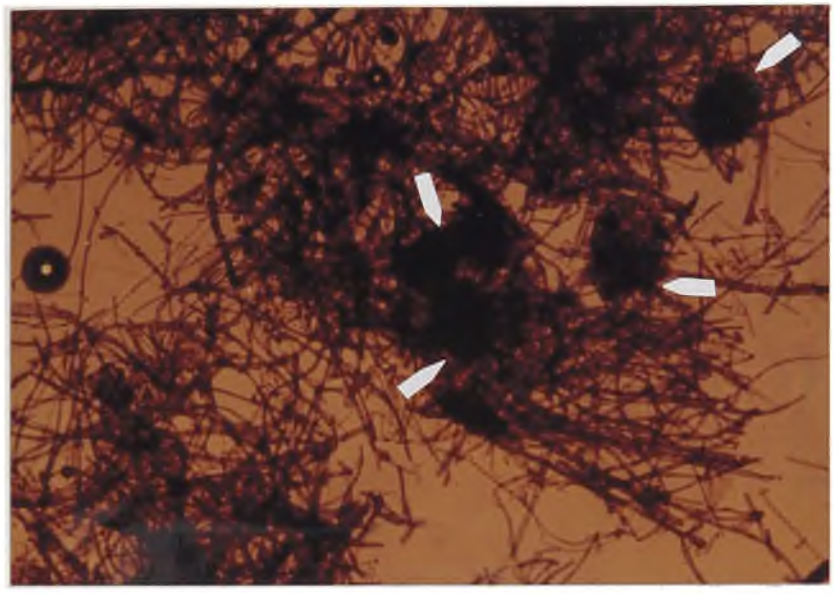


Fig. 8 *Simple and seperate pycnidia (arrowed) obtained from stromata of D. natalensis (x125).*

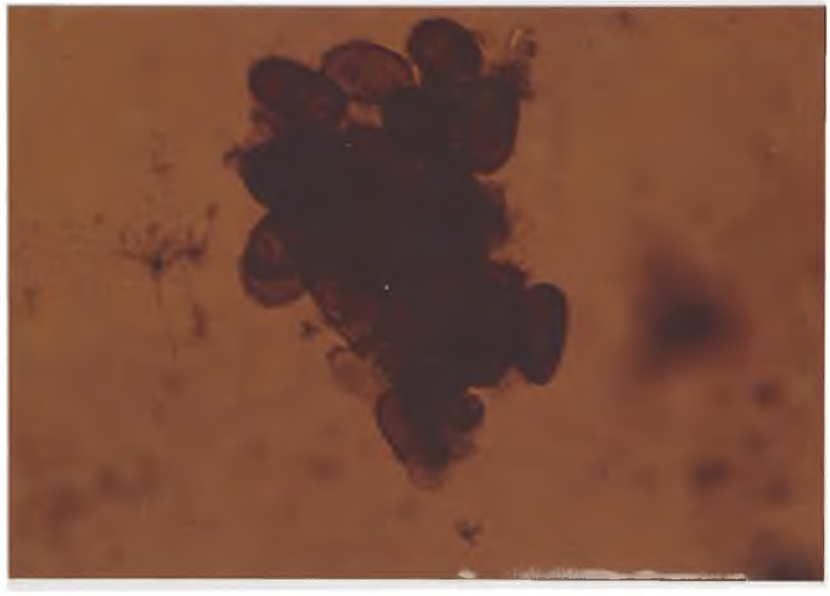


Fig. 9 *Immature and aseptate pycnidiospores of D. natalensis (x500).*

longitudinally striated (Fig. 10). A PDA culture of the organism kept under a filament bulb (60 Watts) in the laboratory resulted in a red or pink colour in the medium (Fig. 11). The observed culture and morphological characteristics were similar to those reported for *D. natalensis* by other researchers (Stevens and Wilcox, 1925; Alasoadura, 1970; Udeobo, 1974; Holliday, 1980).

The second isolate had a whitish or creamy colour and filled the PDA plates in eight days. The culture also turned brown with time. It formed chlamydospores and copious amounts of micro-conidia which were not in chains. Macro-conidia were few, boat-shaped, had one or two septation and blunt ends. These characteristics conform to those reported for *Fusarium solani* according to Snyder and Hansan classification of the genus (cited by Messiaen, 1959).

4.2.1 Pathogenicity test:

The results of the experimental inoculation test confirmed the preliminary inoculation test in which four-month old rough lemon seedlings were inoculated with the four fungi isolates. In these tests, gum oozed out of sites on seedlings inoculated with *D. natalensis* by the fourth day (Fig. 12). Gum which oozed out of the inoculated sites were absorbed in the applied cotton wool (Fig. 13). When the wounds were opened two weeks later, by removing the parafilm and the cotton wool, a canker was observed at the site. On removal of the bark below

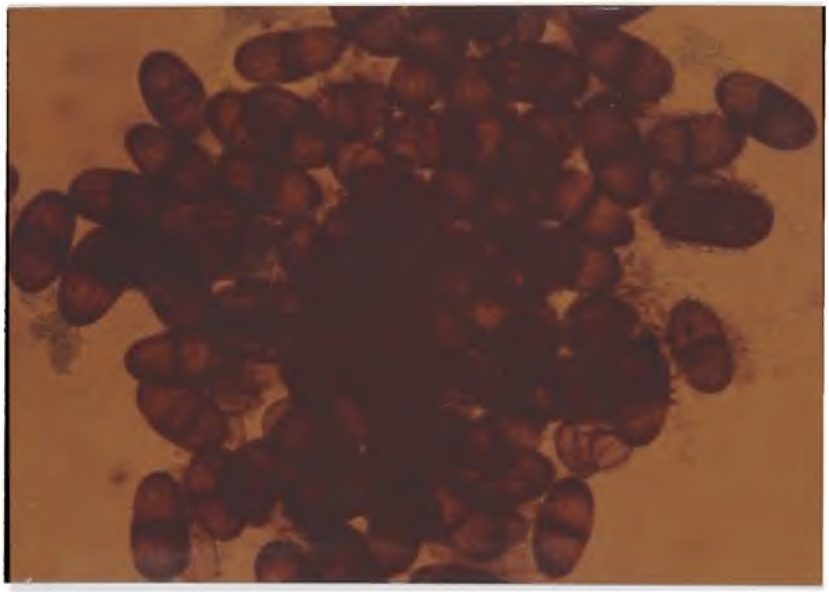


Fig. 10 *Mature and septate pycnidiospores of D. natalensis with longitudinal striations (x500).*

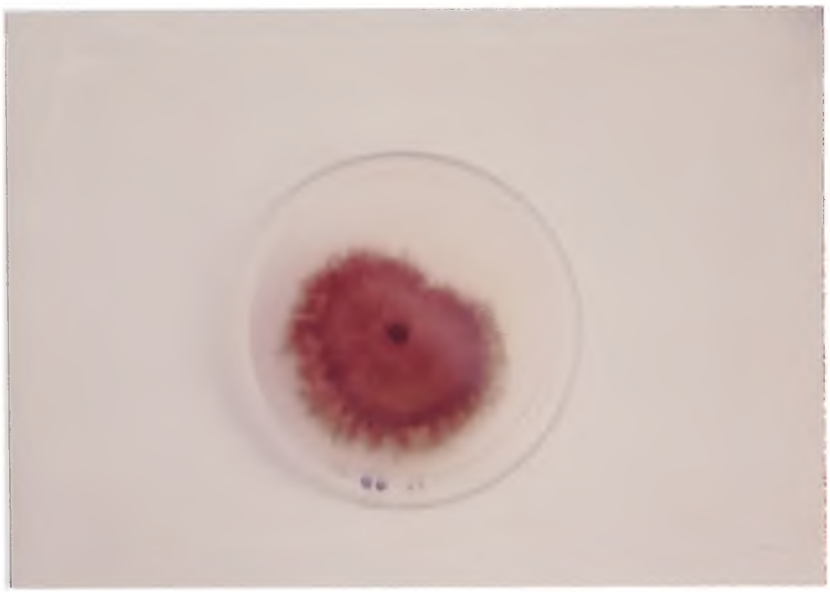


Fig. 11 *Red pigmentation developed in the PDA culturing medium under high temperatures by D. natalensis.*

Fig. 12 *(top) - Hardened gum (arrowed) exuded by four-month old rough lemon seedling inoculated with D. natalensis in a pathogenicity test.*

Fig. 13 *(Bottom) - Comparative infectivity of the fungal isolates by gum exudation.*

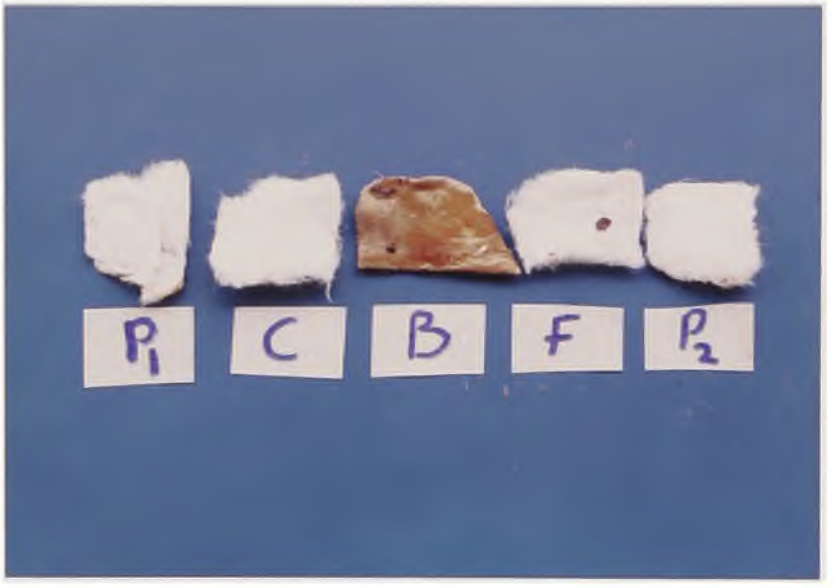
Note: P_1 *-- white (with no gum infusion) cotton wool applied to seedlings inoculated with unidentified fungus 1.*

C -- white (with no gum infusion) cotton wool applied to seedlings inoculated with sterile PDA (control).

B -- brown coloured (due to gum infusion) cotton wool applied to seedlings inoculated with D.natalensis.

F -- white (with no gum infusion) cotton wool applied to seedlings inoculated with Fusarium solani.

P_2 *-- white (with no gum infusion) cotton wool applied to seedlings inoculated with unidentified fungi 2.*


P1
C
B
F
P2

the inoculated site, the infection was observed to have developed further on the wood than on the bark. More gum oozed out and mycelia of the pathogen had developed at the wounded site by the third day after exposure (Fig. 14). *Diplodia natalensis* was re-isolated from the artificially inoculated diseased seedlings, thus, satisfying Koch's postulates.

Rough lemon seedlings inoculated with all the isolates in the preliminary paathogenicity test, resulted only in the death of seedlings inoculated with *D. natalensis*. Seedlings inoculated with *Fusarium solani* or the other two unidentified fungi and plugs of PDA (control) remained healthy from the gummosis disease (Fig. 15).

4.3 Rootstock resistance screening

4.3.1. <u>Infectibility of the citrus rootstock species</u>: - All the four citrus rootstock species, namely; swingle citrumelo, volkameriana, Obuasi sweet orange and rough lemon, on inoculation with the test fungus (*D. natalensis*), were found to be susceptible to the gummosis disease.

4.3.2. <u>Degree of gumming</u>: - The degree of gumming was approximately proportional to the area of lesion developed by the various citrus species. Gumming is the most prominent symptom of the disease and the amount of gum exuded was lowest

Fig. 14 *(Top) - Eighteen months old rough lemon seedling showing gummosis disease symptoms 17 days after inoculation with D. natalensis.*

Note : a. droplets of exuded gum (arrowed).

b. whitish mycelia of D. natalensis (arrowed).

c. flap of bark (arrowed).

Fig. 15 *(Bottom) - Preliminary pathogenicity test of the four fungi isolates.*

Note : B - death of seedling inoculated with D. natalensis 10 days after inoculation.

P_1, C, F & P_2 - healthiness of seedlings inoculated with unidentified fungus 1, sterile PDA (control), F. solani and unidentified fungus 2 respectively.


b
a
c

in volkameriana and highest in swingle citrumelo. Gumming was slight in volkameriana and rough lemon but profuse in Obuasi sweet orange and Swingle citrumelo (Table 11).

4.3.3. Development of necrotic lesion: - The area (cm^2) of necrotic lesion developed on stems of seedlings of the various citrus species was highest in swingle citrumelo and lowest in volkameriana. Rough lemon and Obuasi sweet orange were intermediate.

A one-way analysis of variance showed that the computed mean lesion area of swingle citrumelo was significantly greater than that of the other rootstock species at 1% level of significance. No significant differences were found among the mean lesion area of the other rootstock species. Interestingly, the mean area of lesion of volkameriana was found to be less than that of rough lemon (Table 11; Appendix 5).

4.4 Chemical control of citrus gummosis disease:

4.4.1 Field Test:

4.4.1.1 Time of induction and length of callus formed at the treated site: - Within the first month of fungicide application, callus had formed on some of the plants for some treatments. Bavistin at 1 and 2g ai/L and the control treatments had induced callus in all the treated plants within

Table 11: **Mean lesion area (cm^2) and degree of gumming of four citrus rootstock species.**

Rootstock species	Mean necrotic area[@] (cm^2)	Degree of gumming[•]
Volkameriana	2.83	*
Swingle citrumelo	5.03	****
Obuasi sweet orange	3.64	***
Rough lemon	3.39	**

$LSD_{(0.05;\ 0.01)}$ = 0.826; 1.139

[@] Average of five seedlings (replicates).

[•] Visual appraisal, number of asterisks corresponds to increasing amount of gum exuded.

the first month of fungicide application. Alliete at 200g ai/L and phosphorous acid at 150g ai/L induced callus in about 80% of the treated plants and 40% of plants treated with Alliete at 400g ai/L and Ridomil at 40g ai/L had formed callus within the same period. The remaining treatments, Ridomil at 60g ai/L, Phosphorous acid at 300g ai/L and the Bordeaux mixture were late at inducing callus formation during the first month. All the treatments however, had induced callus formation by the end of the second month after treatment in all the treated plants (Table 12).

The two rates of Bavistin and the distilled water control had significantly longer callus lengths at the treated sites than the other treatments within the first four months after fungicide application. Phosphorous acid at 150g ai/L and the two rates of Aliette were moderate in the length of callus induced at the treated site within the same period. Ridomil, at the two rates, Phosphorous acid at 300g ai/L and Bordeaux mixture induced significantly shorter callus lengths for the four months after fungicides were applied (Fig. 16; Appendix 6).

None of the fungicidal treatments induced further growth of callus after the fourth month till the experiment was terminated twelve months later.

4.4.1.2 Number of plants with or without gum at the treated site Although the number of plants which showed no gum

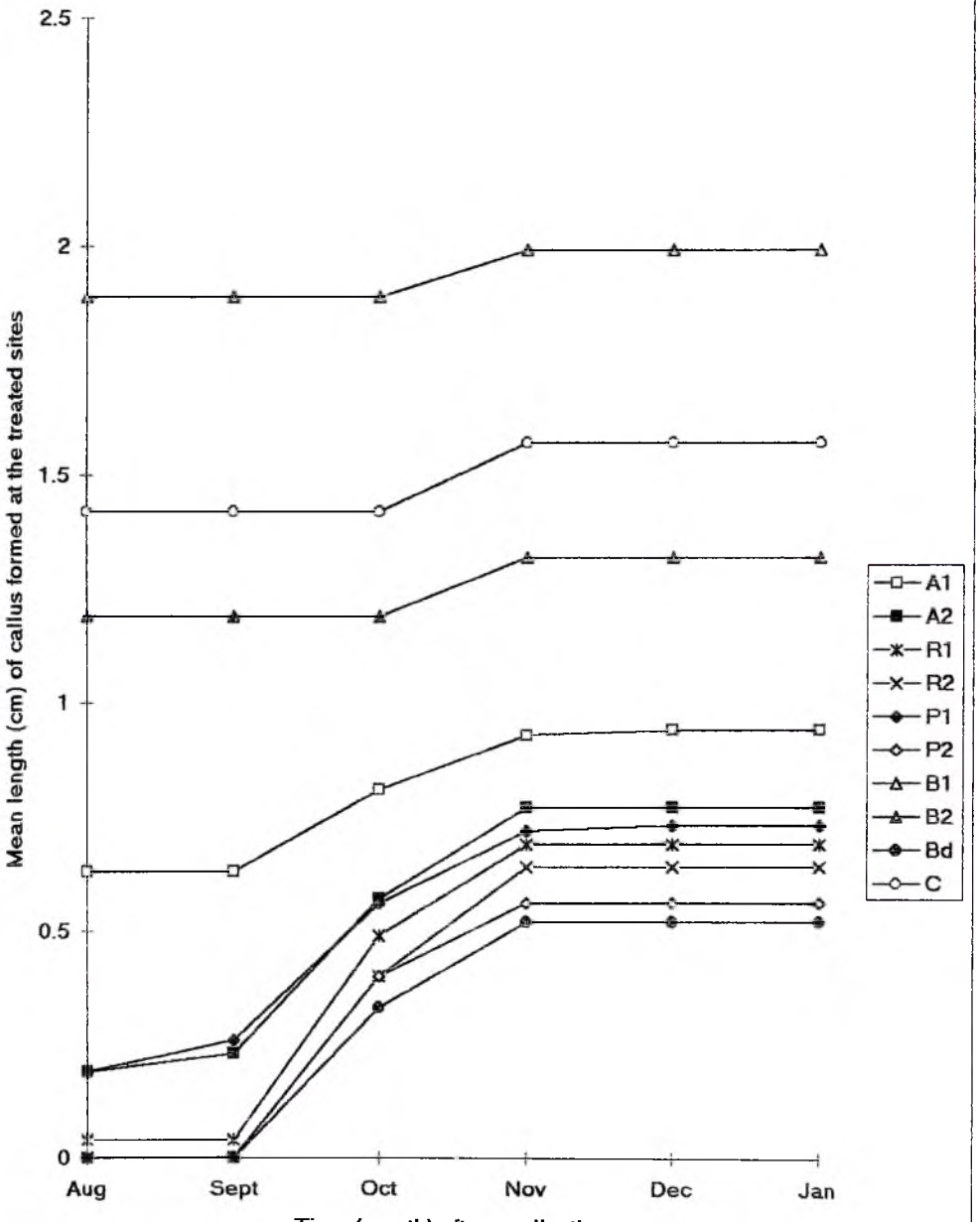
Table 12: **Effect of fungicide treatments on time of induction of callus at the treated site.**

Type of fungicide	Rate (g ai/L)	No of plants /treatment	No of plants with callus 1st month*	2nd month
Aliette	200	18	14 (78)	18
	400	"	7 (39)	"
Ridomil	40	"	8 (44)	"
	60	"	1 (6)	"
P. acid[z]	150	"	14 (78)	"
	300	"	3 (17)	"
Bavistin	1	"	18 (100)	"
	2	"	18 (100)	"
Bordeaux	1:4	"	1 (6)	17
Control	Water	"	18 (100)	18

* figures in parenthesis are percentages of treated plants with callus.

[z] Phosphorous acid.

Fig.16 Effect of fungicides on length of callus induced at the treated site six months after application



KEY: A1 = Aliette - 200; A2 = Aliette - 400; R1 = Ridomil - 40; R2 = Ridomil - 60; P1 = Phosphorous acid - 150; P2 = Phosphorous acid - 300; B1 = Bavistin - 1; B2 = Bavistin - 2g ai/L; Bd = Bordeaux mixture (1:4); C = Distilled water control.

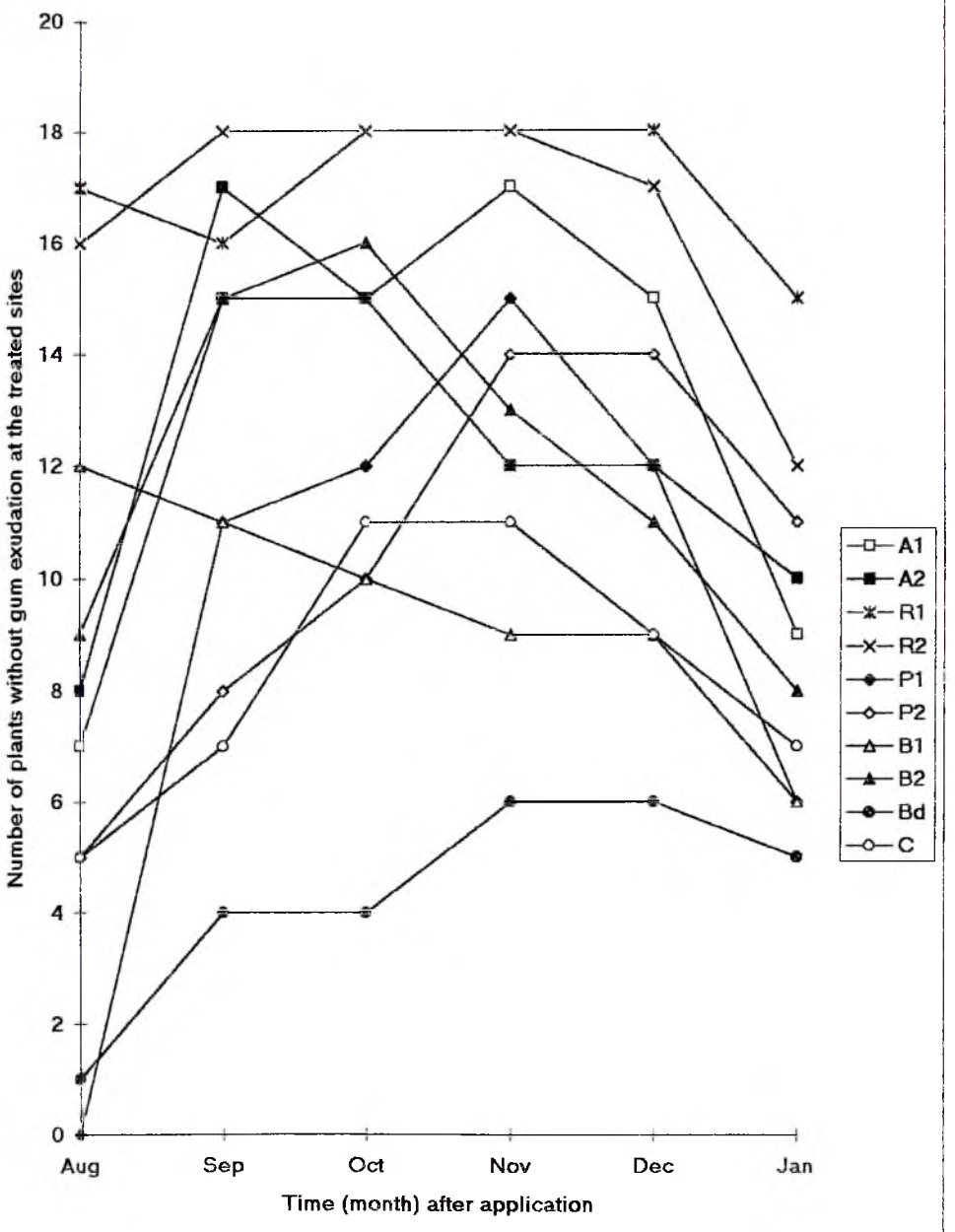
exudation at the treated site was greater for the systemic fungicides (except Bavistin at 1g ai/L) than the control, this however followed no consistent trend. Ridomil at the two rates however, gave the highest number of plants without gum oozing at the treated site within six months after treatment. The protective fungicide (Bordeaux mixture) gave the highest number of plants showing gum exudation from the treated sites for the same period (Fig. 17).

Generally, the two rates of Ridomil, phosphorous acid at 300g ai/L and Bordeaux mixture (in spite of its low activity) maintained efficacy for a longer period of five months after application. The efficacy of the remaining treatments reduced by the end of the fourth month. However, Bavistin at 1g ai/L lost efficacy in controlling gum exudation one month after application (Fig. 17).

4.4.1.3 Thickness of callus formed at the treated site

Two types of callus were observed at the treated sites on the plants. These were a thin layer of callus which later thickened with time and typical of callus induced by Aliette, Bavistin and distilled water. The other was a relatively thicker callus formed on induction, by the remaining treatments. The callus induced by Aliette at 200g ai/L, the two rates of Bavistin and the distilled water control were significantly lighter than those induced by the other treatments (Table 13; Appendix 7).

Fig. 17 Efficacy of fungicides in controlling citrus gummosis disease at ARS-Kade, six months after application



KEY: A1 = Aliette - 200; A2 = Aliette - 400; R1= Ridomil - 40; R2 = Ridomil- 60; P1 = Phosphorous acid - 150; P2 = Phosphorous acid - 300; B1 = Bavistin - 1; B2 = Bavistin - 2g ai/L; Bd = Bordeaux mixture (1:4); C = Distilled water control.

Table 13: Effect of fungicide treatments on thickness (mm) of callus formed at the treated sites.

Type of fungicide	Rate (g ai/L)	Mean callus thickness[@] (mm)	Difference from control[♦]
Aliette	200	2.6	0.2 ns
	400	3.0	0.6 **
Ridomil	40	4.2	1.8 **
	60	4.6	2.2 **
P. acid[z]	150	3.0	0.6 **
	300	3.5	1.1 **
Bavistin	1	2.6	0.2 ns
	2	2.4	0.0 ns
Bordeaux	1:4	4.3	1.9 **
Control	Water	2.4	--
LSD $_{(0.01)}$	=	0.59	

[@] Average of five replicates.

[♦] ** = significant at 1% level.
ns = not significant.

[z] Phosphorous acid.

The application of the second dose of the fungicides, did not have any effect on the rate and thickness of the callus induced at the treated sites. The number of plants exuding gum at the treated site was however affected.

Application of Ridomil or Aliette was effective in reducing the number of plants exuding gum at the treated site. Application of these fungicides for the second time was better in controlling gum exudation than single application (Table 14). Plants treated with Phosphorous acid had comparable number of plants showing no gum exudation at the treated site although the fungicde was applied once. Bavistin at the two rates, Bordeaux mixture and distilled water control gave fewer number of plants without gum exudation at the treated site irrespective of the number of applications (Table 14; Figures 18-24).

4.4.2 **Laboratory test**: - No growth (100% inhibition) was observed on the plates with the various rates of the systemic fungicides during the five-day period of observation when the pathogen was tested in vitro. The test fungus (*D. natalensis*) grew and filled (0% inhibition) the PDA control plates by the end of the second day whilst 30% of the area of the Bordeaux mixture plates was covered by the fungus also, by the end of the second day. No further growth was observed in the Bordeaux mixture plates thereafter (Table 15).

Table 14: Efficacy of fungicides against citrus gummosis disease after one year of application on Ci. 25 at ARS-Kade.

Type of fungicide	Rate (g ai/L)	No of plants treated	No of plants with(+) or without(-) gum[@] /application			
			Single		Double	
			-	+	-	+
Aliette	200	9	4	5(2)	7	2
	400	9	6	3	6	3(1)
Ridomil	40	9	4	5(3)	6	3(1)
	60	9	5	4(2)	9	0
P. acid[z]	150	18	12	6(1)	-	-
	300	18	9	9(2)	-	-
Bavistin	1	9	3	6	0	9(2)
	2	9	1	8(1)	2	7
Bordeaux[*]	1:4	9	2	5	3	6(1)
Control	Water	9	1	8	3	6

[@] Figures in parentheses are plants with infections located away from the treated site.

[*] Two plants with Bordeaux mixture single application died in the sixth month after application.

[z] Phosphorous acid was not applied for the second time.

Fig. 18 *Effectiveness of fungicides against gummosis disease on citrus plants on the field, one year after application at ARS-Kade.*

NB:- *(See close-up figures at Pgs. 59-64).*

Fig. 19 *$R_1F_2R_3T_2$ -- Gummosis-infected sweet orange plant treated with Ridomil.*

Note : a. clean and healthy callus (arrowed) at treated site.

b. infection (arrowed) at a new area away from the treated site.

Fig. 20 *$P_2F_1R_3T_3$ -- Gummosis-infected sweet orange plant treated with Phosphorous acid.*

Note : clean and healthy callus (arrowed) at treated site.

Fig. 21 *$A_2F_2R_3T_2$ -- Gummosis-infected sweet orange plant treated with Aliette.*

Note : clean and healthy callus (arrowed) at treated site.

Fig. 22 $BdF_1R_1T_2$ -- Gummosis-infected sweet orange plant treated with Bordeaux mixture.

Note : gum exudation or re-infection (arrowed) at the treated site.

Fig. 23 *$B_1F_2R_2T_2$ -- Gummosis-infected sweet orange plant treated with Bavistin.*

Note : gum exudation or re-infection (pointed at) at the treated site.

Fig. 24 *$CF_2R_1T_1$ -- Gummosis-infected sweet orange plant treated with distilled water (no fungicide control).*

Note : gum exudation or re-infection (pointed at) at the treated site.

Table 15: **Efficacy of fungicides against *Diplodia natalensis* in the laboratory.**

Type of fungicide	Rate (g ai/L)	Mean diameter (cm) of mycelial growth* day^{-1} 1	2	3	4	5
Aliette	200	-	-	-	-	-
	400	-	-	-	-	-
Ridomil	40	-	-	-	-	-
	60	-	-	-	-	-
P. acid[z]	150	-	-	-	-	-
	300	-	-	-	-	-
Bavistin	1	-	-	-	-	-
	2	-	-	-	-	-
Bordeaux	1:4	1	3	3	3	3
Control	Water	4	9	9	9	9

* Averages of four plates and measured at 24 hour intervals.

[z] Phosphorous acid.

CHAPTER FIVE

DISCUSSION

5.1 Gummosis disease as a threat to citrus production at ARS-Kade : - The citrus gummosis disease is a threat to the orchards of the Agriculture Research Station of the University of Ghana at Kade because of the high incidence of 36.2 - 79.9% (Avg. 62.6%) and the average severity index of 1.2 on a scale of 0-5 recorded on the selected plots on the station. The results confirmed an earlier report (Ofosu-Budu and Oduro, 1995), that the disease is endemic at the station and had affected trees of all ages, except seedlings at the nursery, and plants established with rough lemon, cleopatra mandarin and rangpur lime, the rootstock materials in use in Ghana.

The seemingly low average severity index of 1.2, on the 0-5 scale, becomes more threatening when viewed from the practical perspective. It implies that, on the average, all the infected plants on the station have about a quarter of the circumference of their barks killed by the disease. The obvious result is the reduction in potential yield. Also, plants in younger orchards, with smaller circumferences, could be killed prematurely ahead of their peak bearing ages.

The symptoms of the disease, observed on the field, are similar to those reported earlier for the disease in Ghana (Leather, 1959; Clerk, 1974; Sam-Aggrey and Hague, 1976) and also similar to Brown rot gummosis (Klotz and Calavan, 1969)

and citrus foot rot (Fraser,1942) for other citrus growing countries. The crown symptoms however, are usually not different from the symptoms of other disorders affecting the vascular system of trees and should not solely be used in identifying the gummosis disease.

5.2 Effect of age of citrus plant on the incidence and severity of gummosis disease: - Age could influence the incidence and severity of the gummosis disease, when orchards of different ages were assessed at ARS-Kade.

The relatively high incidence and severity recorded on the youngest plot (Ci.26) may be due to the opened nature of the canopy of the plants on this plot. The opened canopy, presumably, stimulates faster weed growth which obstruct air flow around the bases of the plants and result in higher relative humidities conducive for pathogen activities. Again, the poor drainage of the soil on this plot may be a contributing factor. Poor drainage and high relative humidity on orchards had frequently enhanced the disease prevalence and severity (Fraser, 1942; Wong and Varghese, 1966; Klotz and Calavan, 1969). Such plots need regular brushing to enhance air circulation and reduce relative humidity.

The soil of the plot of Ci 17 (Ext.), the twelve-year old plot, was visually observed to be well drained as compared to soils of the other selected plots and so could account for the

low incidence and severity figures recorded on this plot.

The highest infection percentage of 69.8 and 1.47 severity index for the oldest plot (Ci. 13) could be attributed to age. The figures compared favourably with those recorded for the rootstock trial plots which were all twenty years or above in age.

Generally, the disease was observed in orchards of the three ages studied. Infected plants were obtained in each infective class (1-5) of the severity scale implying that, plants at any age could be attacked by the pathogen. The high correlation coefficient values, although not significant statistically, obtained for both disease incidence (0.705) and severity (0.876), indicates an association between age and both incidence and severity of the gummosis disease. Furthermore, the acceptance of the null's hypothesis in the test of significance, confirms this association in the population from which the samples were taken. Thus, 50% of the incidence and 78% of the severity of the disease occurence could be attributed to the age of the plants on the orchard.

5.3 **Effect of citrus rootstock on the incidence and severity of gummosis disease**: - The rootstock material influenced the disease incidence and severity when natural infection was observed on citrus plants established with late valencia sweet orange, budded on three different rootstocks, namely;

cleopatra mandarin, rough lemon and rangpur lime, on the field.

The lowest severity index recorded for the rangpur lime rootstock plot corresponded to the highest incidence among the three rootstock plots. This statistically insignificant damage on a heavily gummosis-infected plot, may suggest that plants established with rangpur lime rootstock, probably contains relatively, higher concentrations of the fungicidally active chemicals (Haas and Hill, 1913) reported to be present in the barks of *Citrus* species. Also, the resistance of the late valencia-rangpur lime stionic combination plants, can be inferred to be high against post-penetration activities than the infection processes of the pathogen.

The resistance of cleopatra mandarin rootstock plants however, is possibly against both processes of infection and post-penetration activities due to the low infection percentage (incidence) and the insignificant damage done to the plants on this plot. Also, plants of late valencia sweet orange budded on cleopatra mandarin rootstock may probably contain appreciable quantities of the fungicidal chemicals in their barks.

The significantly severe damage and relatively high incidence recorded on the rough lemon rootstock plants, makes them the least resistant to the gummosis disease among the plants grown with the three rootstock materials. Hence, the late valencia-rough lemon stionic combination might be said to

probably possess the least amount of the fungicidally active chemicals in the bark.

On the basis of the damage done to the plants, the observed results agree with the reports of most researchers that citrus plants established on rangpur lime or cleopatra mandarin rootstocks have higher degree of resistance to the gummosis disease than plants on rough lemon rootstock (Klotz and Fawcett, 1930; Fraser, 1942; Carpenter and Furr, 1962; Klotz and Calavan, 1969; Samson, 1986). The non significant difference in severity on plants with rangpur lime and cleopatra mandarin rootstocks that was realised in this work had also been reported (Klotz and Calavan, 1969; Darthenucq and Rey, 1974; Fouque et al, 1977; Samson, 1986; Davis and Albrigo, 1994).

The very high incidence of the disease on the rangpur lime plot could therefore be an example of the rare instances where miscibility of different genotypes (rangpur lime and rough lemon) had resulted in relatively higher disease prevalence than in the individual pure stands making up the mixture reported by Chamblee (cited by Burdon, 1987). This reason may also partially explain the high incidence and severity of the disease on Ci 26, the youngest plot in the study, since it was quite close to Ci 24.

The relatively higher tendency of plants with cleopatra mandarin rootstock to resist infection better than plants with either of the other two rootstock materials is further

substantiated by the results of the study in which the part of the plant infected with the gummosis disease was studied. While infection percentage was 15, on the rootstock (area below the bud union), for the late valencia-cleopatra mandarin stionic combination, late valencia-rangpur lime and late valencia-rough lemon stionic combinations respectively had 20% and 27% of the infections located on the rootstock.

The results also showed that higher percentage of infections (73-85%) were observed on the scion. This observation confirms earlier reports that sweet orange varieties are more susceptible to the gummosis disease than other citrus species (Klotz and Fawcett, 1930; Fraser, 1942; Klotz and Calavan, 1969; Fouque, et al, 1977). The infections on the scion were more frequently observed just above the bud union, hence, increasing the height of budding to between 60-80 cm on the rootstock as suggested by some researchers, (Leather, 1959; Porto and Reck, 1984; Koller, et al., 1984), might reduce the disease prevalence. Also, the use of more resistant or highly tolerant rootstock materials could probably control the disease incidence and severity. However, improving the resistance of the scion material through breeding will possibly give a more lasting control.

The pathogen identified in this work (*D. natalensis*) is a weak parasite requiring a wound to facilitate infection (Holliday, 1980). Wounding on the host bark is therefore vital for the pathogen. Although natural breaks or openings have

been found on the barks of citrus plants, their effectiveness as infection courts have been minimal due to their superficial nature. Wounding beyond the suberised layer is required for infection to take place (Whiteside, 1971). Such wounds were observed frequently on the field and could come from two main sources: mechanical wounding during brushing and wounding due to termites' activities. The latter form of wounding seem to play a major role under local conditions because some of the infection foci were located on the pathway of these termites. Controlling these termite activities might possibly reduce the number of infections on the orchards. Furthermore, these activities of the termites seem peculiar to our local conditions as no report have been made on it anywhere to date and therefore needs special attention. Further work to investigate their role in the disease infection process might provide useful information for effective and easy control of the disease in Ghana. The termites' role, if established, will however negate the earlier suggestion that high budding onto the rootstock may reduce the incidence of the disease, since termites are not limited by height in their activities.

5.3.1 <u>The need to search for new rootstock material for citrus production in Ghana</u>: - The high prevalence and intensity values obtained for the citrus gummosis disease in this work and the observation that the rootstocks recommended for citrus production in Ghana are all susceptible to the

gummosis disease in varying degrees, makes it imperative for new rootstock materials which combine high yielding and good fruit characteristics with resistance to common field diseases such as tristeza and gummosis to be researched into in Ghana.

In citrus rootstock breeding and selection, many genetic factors have been suggested as the cause of the wide variation in resistance of *Citrus* and related genera to diseases. Whilst genetic factors have been mentioned as being dominant conditions on highly resistant (immune) species, other factors in addition to genetic constitution have been identified for less resistant species (Whiteside, 1971). These factors include age, chemical composition, succulence and vigor of inoculated tissues, water and nutrient supply, temperature and relative humidity and other environmental factors (Whiteside, 1971). These factors, as much as possible must be taken into consideration in screening rootstocks resistant to the gummosis disease.

In the rootstock resistance screening, the reported higher resistance of volkameriana rootstock was substantiated. Volkameriana was the most resistant among the four citrus rootstock species tested. It had a mean lesion area lower than that of the standard (rough lemon) and also exuded the least quantity of gum in the test. Similar reports have been given by Fouque et al (1977) and Koller et al (1984) in the Cote d'Ivoire and Brazil respectively when *P. parasitica* was used as the test fungus in a similar work. Earlier reports also

attest to the resistance of volkameriana rootstock to the gummosis disease (Carpenter and Furr, 1962). Again, Russo (1956; 1959), reported that volkameriana was resistant to *P. parasitica* and *Deuterophoma tracheiphila* Petri, the causal organism of mal secco. Volkameriana was also reported to be tolerant to tristeza, exocortis and citrus nematode (Lee, 1996. Personal communication).

The performance of volkameriana seedlings under local conditions is thus promising in the search for better rootstocks against the disease in Ghana. However, resistance in young seedling does not imply comparable resistance of the same seedling when older on the field, because infection, as was observed from the field survey can take place at any stage in the life of the plant. Field conditions are also generally different from those of the screenhouse. More investigations are therefore required to test the ability of the rootstock to resist the disease on the field vis-a-vis other requirements neccessary in the selection of resistant rootstocks.

The performance of the standard rough lemon species in the rootstock resistance screening test was encouraging. Rough lemon rated second in order of increasing degree of gumming and had lesion size not significantly different from that of volkameriana. It is also reported to be tolerant to tristeza and exocortis but susceptible to *Phytophthora* and citrus nematode (Lee, 1996. Personal communication). Its use as rootstock, however, can still be continued in Ghana.

Swingle citrumelo and Obuasi rootstock materials were highly susceptible to the disease. They cannot be good rootstock materials under local conditions although Swingle citrumelo had been reported to be tolerant to tristeza, exocortis, xyloporosis, root rot and highly tolerant to *Phytophthora* infection and citrus nematode (Rouse and Wutscher, 1985; Lee, 1996. Personal communication). Both, oozed gum profusely in the presence of the pathogen with Obuasi sweet orange seedlings oozing the gum just three days after inoculation. Swingle citrumelo rootstock developed the largest lesion and was thus, the least resistant among the four rootstock materials. The poor performance of Swingle citrumelo rootstock in this test, is in contrast with the reports of other researchers (Hutchison, 1974; Samson, 1986; Shaked and Ashkenazi, 1984) who found it to be resistant to the gummosis disease. The contrast in these results may in part be due to differences in climatic conditions and physiological states of the seedlings but more importantly, to the pathogen used for the inoculations. The result of "Obuasi" the local sweet orange cultivar which was observed on the field to be less infected with the gummosis disease by Ofosu-Budu and Oduro (1995) despite its long period of cultivation, was not surprising because it is a sweet orange cultivar. Sweet oranges are ranked among the least resistant species to the gummosis disease (Fraser, 1942; Klotz and Calavan, 1969; Samson, 1986).

Generally all the four rootstock materials were susceptible to the disease probably because of the method of inoculation used. The pathogen is a weak one which requires a wound to infect its host and the wound method used in the inoculation process might have contributed to its infection abilities. Possibly, a woundless method of inoculation could give results different from those obtained in this work, since, an unbroken bark is in itself a form of resistance against the disease (Klotz and Calavan, 1969; Whiteside, 1971).

5.4 *Diplodia natalensis*: a new pathogen of citrus gummosis disease in Ghana: - The causal organism of the citrus gummosis disease had been reported to be *Phytophthora citrophthora* and/or *P. parasitica* in most citrus growing countries such as the USA, Brazil and Israel (Fawcett, 1913; Klotz and Calavan, 1969; Rossetti, 1969). Although earlier reports (Leather, 1959; Clerk, 1974), mentioned *P. parasitica* as the causal organism of the citrus gummosis disease in Ghana, there seem to be no available literature on the isolation of the pathogen. Elsewhere, the disease has also been attributed to other fungi species such as *Botrytis cinerea, Sclerotinia sclerotiorum, Diaporthe citri, Diplodia natalensis* and a *Dothiorella spp.* (Klotz and Calavan, 1969; Rodriguez, 1978; Mukhopadhyay, 1985; Padmanabhan and

Chandhury, 1989).

Nystatin and benomyl are antimycotic antibiotic and fungicide respectively. Both chemicals have no inhibitory effect on pythiaceous fungi (Frank et al, 1958; Cremlyn, 1978) and have been used to develop selective media (Eckert and Tsao, 1962; Vaartaja, 1960) for isolating pythiaceous fungi. Therefore, the absence of *P. parasitica* on these media suggest that *P. parasitica* might not be responsible for the gummosis disease condition observed in Ghana.

The ability of *D. natalensis,* which was one of the isolated fungi, to induce the disease symptoms in the inoculated seedlings, proved the involvement of *D. natalensis* in causing the disease in Ghana. Similar reports implicating *D. natalensis* in the disease have been made in Columbia (Granada and Sanchez, 1969), Cuba (Rodriguez, 1978) and India (Mukhopadhyay, 1985). While *D. natalensis* is noted to infect the bark as well as the wood in citrus gummosis disease (Fawcett, 1936; Klotz and Calavan, 1969), *Phytophthora* gummosis is restricted to the bark (Fawcett, 1936; Fraser, 1942; Klotz and Calavan, 1969) of the citrus plant. On the field however, the bark as well as the wood were observed to be infected by the gummosis disease. Thus, emphasizing *D. natalensis* as the possible causal organism rather than *P. parasitica*. A similar report of the involvement of the wood in citrus gummosis disease in Ghana was made by Clerk (1974).

The implication of *D. natalensis* as a causal organism of

the gummosis disease in Ghana in this work, however, does not rule out the possibility of the involvement of *P. parasitica* in causing the disease. In fact, Granada and Sanchez (1969), Rodriguez (1978) and Mukhopadhyay (1985) reported that both *Phytophthora spp.* and *D. natalensis* were involved in causing the gummosis disease in their respective countries. More isolations with disease materials from other citrus growing areas in Ghana to study the involvement of *Phytophthora species* in causing the disease in Ghana is therefore recommended.

Diplodia natalensis is a common tropical pathogen (Holliday, 1980) and has been identified on several crops including yam, maize, cocoa, cola and sweet orange in Ghana. On sweet orange trees, the pathogen causes twig die-back and fruit rot (Bunting and Dade, 1925; Piening, 1962). In spite of the long list of hosts on which *D. natalesis* had been identified (Piening, 1962), this work is the first report indicating that *D. natalensis* causes the gummosis disease of citrus in Ghana.

The confirmation of *D.natalensis* as the causal organism of the citrus gummosis disease in Ghana, suggests an additional strategy in the control of the disease. The benzimidazole group of fungicides, for example, benomyl, which do not act on *Phytophthora species* but controls *Diplodia species* can be considered an additional fungicide for the control of the disease in Ghana.

5.5 Chemical control of citrus gummosis disease

The results of the study in which fungicides were applied against the citrus gummosis disease showed that Ridomil, Aliette and Phosphorous acid are effective in controlling the disease on the field. Similar results were reported by Timmer (1979), Farih et al (1981) and Matheron and Matejka (1988).

Bavistin and distilled water (control) were exceptionally good in wound healing. In contrast, Ridomil, Bordeaux mixture and Phosphorous acid (300g ai/L) were slow in healing the wound while the two rates of Aliette and Phosphorous acid at 150g ai/L were moderate.

Although fast wound healing ability of a fungicidal treatment is desirable, it is better desired if the treatment combines that ability with effectiveness. Except for Bavistin, the systemic fungicides were effective in controlling the disease than the protective (Bordeaux mixture) fungicide and the no fungicide (distilled water) treatment. This emphasizes the advantages of being able to act on internal mycelium and giving ample protection as well as being able to withstand the weathering effects of climatic conditions of systemic fungicides over the protectants.

Ridomil treatments gave higher fungitoxic effect against the disease than either Aliette or Phosphorous acid. Similar reports of higher fungitoxic activity of Ridomil over Aliette on field grown citrus plants had been made (Farih et al, 1981; Timmer and Castle, 1985; Sandler et al, 1988).

The two rates of Aliette and Phosphorous acid were comparatively similar in their fungitoxic activities in this study. The similarity in activity of Aliette and Phosphorous acid might confirm the assertion that Aliette is degraded to Phosphorous acid in plant tissues (Williams et al, 1977). The initial lack of effectiveness of the higher rates of these fungicides, (Aliette and Phosphorous acid at 400 and 300g ai/L respectively), may be due to their higher concentrations in the plant tissues, thus, making them fungistatic rather than fungicidal in action (Afek and Sztejnberg, 1989). With time however, the effectiveness of Aliette as fungicide increased, and may probably be due to its conversion to Phosphorous acid and its subsequent translocation from the point of application. In contrast, the lack of fungitoxicity of Bavistin at 2g ai/L was due to low concentration in the plant tissues. It effectiveness reduced three months after application. Bavistin at 1g ai/L was too low a concentration for effective fungicidal activity. It effectiveness dropped a month after application. The differences in the effectiveness of the fungicides could also be attributed to their different modes of action.

Generally, infection foci developed at other parts of the plants, away from the treated sites for both the systemic and protective fungicides. This observation is unexpected and cannot easily be explained as both acropetal (Timmer, 1979; Davis, 1981; 1982; Farih, et al., 1981) and basipetal (Timmer

and Castle, 1985; Matheron and Matejka, 1988) movements of Ridomil within the plant have been reported.

The erratic nature of the fungitoxic activities of the systemic fungicides on the field as compared to the complete inhibition of the growth of the pathogen in the laboratory bioassay could be due to the differences in the growth substrate of the fungus under the two conditions and also weathering effect of climatic conditions on the field. Similar erratic results had been reported for Aliette on field grown citrus plants (Davis, 1981; 1982; Timmer and Castle, 1985).

Bordeaux mixture, the protective fungicide, generally had very low fungitoxic effect (lower than the control) in the first six months after application against the disease. Two of the plants which were severely affected by the disease and were treated with the fungicide died in the sixth month of the experiment. Its fungitoxic effect however, improved later and performed relatively better than the Bavistin and the distilled water treatments by the end of the experiment. The low fungitoxic activity of the Bordeaux mixture, in the first six months, may be due to rainfall in the month of application, which might have diluted or washed away the fungicide. This may also explain the lack of effective control of the disease by the fungicide in previous applications on the station and elsewhere in India (Sawant, et al, 1990). Similar lack of effectiveness had been observed on the station for other protective fungicides such as Kocide and Potassium

permanganate (Ofosu-Budu, Personal communication).

The cure, although not significant, achieved in some of the plants treated with distilled water (control) might probably be due to dehydration at the wounded sites.

The observation during the field survey that the plants became more susceptible in the wet than in the dry season and that the plants formed callus naturally in the dry season to resist further invasion by the pathogen might be important in the search for effective control for the disease on the field. Rossetti (1969), Hartman and Nienhaus (1974) and Matheron and Matejka (1989) also reported on similar seasonal influence. Rossetti (1969) however, proved the influence of the climatic elements to be indirect and that the physiological state of the plant was directly responsible for the observed seasonal changes of the disease. Feichtenberger (1990), also observed this seasonal influence and went further to determine the best time of application of Ridomil and Aliette to control the gummosis disease on citrus in Brazil. A similar study is thus suggested for Ghana to determine the most appropriate time of application of these fungicides, noting that, the period of formation of natural callus (dry season) is the exact opposite of the period required for the formation of induced callus over created wounds. Hence the question as to whether it will be profitable to enhance the natural callus forming process of the plant by applying chemical fungicides during the dry season to induce thicker and effective callus or apply the

fungicides just before, or in the wet season to induce artificial callus to prevent and control the disease can best be answered after an extensive and elaborate investigation.

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A P P E N D I X 1

DETERMINATION OF CORRELATION BETWEEN AGE OF ORCHARD (X) AND DISEASE INCIDENCE (Y)

X	Y					
6	49.6	$(\Sigma X)^2$	=	1600	$(\Sigma Y)^2$	= 24211.36
12	36.2	ΣX^2	=	664	ΣY^2	= 8642.64
22	69.8	ΣXY	=	2267.6	n	= 3

$\Sigma X = 40 \quad \Sigma Y = 155.6$

$$\text{Correlation coefficient (r)} = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\sqrt{\Sigma X^2 - \frac{(\Sigma X)^2}{n} \cdot \Sigma Y^2 - \frac{(\Sigma Y)^2}{n}}}$$

$$r = \frac{2267.6 - \frac{40 \times 155.6}{3}}{\sqrt{664 - \frac{1600}{3} \times 8642.64 - \frac{24211.36}{3}}}$$

$$r = \frac{192.9}{273.4}$$

$$= 0.705$$

TEST OF SIGNIFICANCE FOR CORRELATION

Ho : $\Gamma \leq 0$

H_A : $\Gamma > 0$

$t_{cal.} = \frac{r}{S_r}$ Where $S_r = \sqrt{\frac{1 - r^2}{n - 2}} = \sqrt{\frac{1 - .705^2}{3 - 2}} = 0.709$

$t_{cal.} = \frac{0.705}{0.709} = 0.9944$

Decision rule: Accept Ho if $t_{(tab.)}$ is greater than $t_{(cal.)}$.
Reject Ho when otherwise.

Tabular $t_{0.05(1)1} = 6.314$

Conclusion: Since tabulated t is greater than calculated t, Ho is accepted with no significant correlation between age of orchard and disease incidence.

COEFFICIENT OF DETERMINATION (r^2) = 0.705^2 = 0.497

A P P E N D I X 2

DETERMINATION OF CORRELATION BETWEEN AGE OF ORCHARD (X) AND ARC-SINE TRANSFORMED DISEASE SEVERITY (Y)

X	Y	Y^1				
			ΣX	= 40	ΣY^1	= 16.73
6	0.76	5.00	$(\Sigma X)^2$	= 1600	$(\Sigma Y^1)^2$	= 279.89
12	0.67	4.69	ΣX^2	= 664	$\Sigma Y^{1\,2}$	= 96.56
22	1.47	7.04	ΣXY^1	= 241.16	n	= 3
ΣX= 40		ΣY^1=16.73				

$$\text{Correlation coefficient (r)} = \frac{\Sigma XY^1 - \frac{\Sigma X \Sigma Y^1}{n}}{\sqrt{\Sigma X^2 - \frac{(\Sigma X)^2}{n} \cdot \Sigma Y^2 - \frac{(\Sigma Y^1)^2}{n}}}$$

$$r = \frac{18.09}{20.64}$$

$$= 0.876$$

TEST OF SIGNIFICANCE FOR CORRELATION

Ho : $\Gamma \leq 0$

H_A : $\Gamma > 0$

$t_{cal.} = \frac{r}{S_r}$ Where $S_r = \sqrt{\frac{1 - r^2}{n - 2}} = \sqrt{\frac{1 - .876^2}{3 - 2}} = 0.482$

$t_{cal.} = \frac{0.876}{0.482} = 1.8174$

Decision rule: Accept Ho if $t_{(tab.)}$ is greater than $t_{(cal.)}$.

Reject Ho when otherwise.

Tabular $t_{0.05(1)1} = 6.314$

Conclusion: Since tabulated t is greater than calculated t, Ho is accepted with no significant correlation between age of orchard and disease incidence.

COEFFICIENT OF DETERMINATION (r^2) = 0.876^2 = 0.777

A P P E N D I X 3

ANALYSIS OF VARIANCE FOR MEAN PORTION (°) OF BARK OF TREES OF DIEFFERENT AGES GIRDLED BY THE GUMMOSIS DISEASE

Sources of variation	Degree of freedom	Sum of squares	Mean square	$F_{cal.}$	$F_{tab.}$
Total	449	2369532.0			1%
Bark girdled	2	396392.4	198196.2	44.9**	4.66
Error	447	1973139.6	4414.2		

** significant at 1% level.

A P P E N D I X 4

ANALYSIS OF VARIANCE FOR MEAN PORTION (°) OF BARK OF TREES ON DIFFERENT ROOTSTOCKS GIRDLED BY THE GUMMOSIS DISEASE

Sources of variation	Degree of freedom	Sum of squares	Mean square	$F_{cal.}$	$F_{tab.}$
Total	449	4284455.3			1%
Bark girdled	2	199196.9	99598.4	10.9**	4.66
Error	447	4085258.4	9139.3		

** significant at 1% level.

A P P E N D I X 5

ANALYSIS OF VARIANCE FOR MEAN NECROTIC LESION AREA (cm^2)

DEVELOPED ON STEMS OF INOCULATED SEEDLINGS

Sources of variation	Degree of freedom	Sum of squares	Mean square	$F_{cal.}$	$F_{tab.}$	
Total	19	19.18			5%	1%
Citrus species	3	13.09	4.36	11.47**	3.24	5.29
Error	16	6.09	0.38			

** Significant at 1% level.

A P P E N D I X 6

ANALYSIS OF VARIANCE AND MEAN SEPARATION FOR MEAN LENGTH (cm) OF
CALLUS FORMED AT THE TREATED SITES

Sources of variation	Degree of freedom	Sum of squares	Mean square	$F_{cal.}$	$F_{tab.}$	
Total	719	483.82			5%	1%
Fungicides	9	234.90	26.1	80.41**	1.89	2.43
Month	3	21.47	7.16	22.05**	2.61	3.80
F X M	27	6.73	0.25	0.77		
Error	680	220.72	0.33			

** Significant at 1% level.

MEAN SEPARATION:

Fungicides	Rates (g ai/L)	Mean length of callus /month 1st	2nd	3rd	4th
Aliette	200	0.633	0.633	0.811	0.933
	400	0.189	0.228	0.567	0.767
Ridomil	40	0.044	0.044	0.489	0.689
	60	0.000	0.000	0.400	0.639
P.acid[z]	150	0.194	0.261	0.561	0.722
	300	0.000	0.000	0.400	0.556
Bavistin	1	1.894	1.894	1.894	1.989
	2	1.194	1.194	1.194	1.317
Bordeaux	1:4	0.000	0.000	0.328	0.517
Control	water	1.422	1.422	1.422	1.567

$LSD_{(0.01)}$ = 0.491

[z] Phosphorous acid.

A P P E N D I X 7

ANALYSIS OF VARIANCE FOR MEAN THICKNESS (mm) OF CALLUS FORMED AT THE TREATED SITES

Sources of variation	Degree of freedom	Sum of squares	Mean square	$F_{cal.}$	$F_{tab.}$	
					5%	1%
Total	49	36.02				
Callus thickness	9	31.11	3.46	28.83**	2.15	2.88
Error	40	4.91	0.12			

** Significant at 1% level.