ASSESSMENT OF THE EFFECT OF POLLEN GRAINS OF MAIZE (*ZEA MAYS L.*) ON *CERCOSPORA ARACHIDICOLA* HORI. AND ON INFECTION OF LEAVES OF GROUNDNUT (*ARACHIS HYPOGEA L.*) CAUSED BY THE FUNGUS.

A Thesis presented by

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Dedication

To Nora Terlabie, my lovely and dependable wife and to my mum who denied herself a lot of comforts to make me what I am today and to my twin sister.

DECLARATION

I hereby certify that this thesis is my own original work. All assistance and references to relevant literature have been duly acknowledged. This thesis has not been submitted, either in whole or in part for a degree or other qualification in any other university.

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(SUPERVISOR)

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ABSTRACT

Conidia of *Cercospora arachidicola* are straw-coloured or olivaceous, sub-fusiform, multicellular, 28.3-68.1µm in lengths. They contain 3-7 cells. They germinated in distilled water and at 85-100% R.H. The optimum pH for germination was 5.0-6.0; any cell of the conidium germinating produced only one germ tube. The germ tube commonly comes from the end cells with occasional germ tube emerging from one or two median cells. Pollen of *Zea mays*, glucose (1.0-8.0%) and peptone (0.1-2.0%) failed to stimulate more median cells to germinate. Maize pollen, however, stimulated germination of the conidia at pH 3 and 10.

During germination tests using conidia in water drops on sterile glass slides, the conidia germinated better and produced slightly longer germ tubes in the absence of pollen grains of *Zea mays* than in the presence of both non-sterile and autoclaved pollen grains. Particularly with the sterile pollen grains, percentage germination decreased with increasing pollen density. Maize pollen when added to solutions of glucose (1.0-8.0%) and peptone (0.1-2.0%) did not improve germination of the conidia. Conidia in pollen-free aqueous suspension drops on leaflets of groundnuts also germinated well than in suspension drops containing the pollen. Percentage germination in the presence of the pollen on leaflets with reduced fungal flora (a third of the original) and bacterial flora (a sixth of the original) was still lower than in the absence of pollen. Maize pollen at moderate densities did not alter the infection rate of groundnut by *C. arachidicola* but high density of pollen on non-sterile groundnut leaves encouraged excessive phylloplane microfloral population which suppressed germ tube growth.

Conidia stored at 0, 20, 40, 60, and 80% R.H. survived better during the initial 30 days at 20, 40 and 60% R.H. than at 0 and 80% R.H. The pattern of survival changed thereafter and conidia stored at 0% R.H. showed the highest survival on the 50th day; 35.1 percentage viability at 0% R.H. decreased with increasing relative humidity to 29.2% at 80% R.H. Maize pollen could not rejuvenate the aged conidia.

It was concluded that since maize pollen reduced percentage germination *of C. arachidicola* conidia, and high pollen density suppressed germ tube growth on non-sterile groundnut leaves, a closer spacing of maize plants in mixed farms could be recommended to encourage greater pollen deposit on the groundnut plants. This would be an environmentally friendly control measure provided the conidia of the other *Cercospora* leaf spot fungus, *Cercospora personata*, are similarly affected by maize pollen.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Pollen grains vary considerably in composition and probably also in food value. The chief constituents of pollen are carbohydrates, fat, proteins and various inorganic mineral substances. Carbohydrate content is high. It can be as high as 48.35 per cent as found in pollen of *Pinus contorta* .The next in abundance are proteins. Date Palm *(Phoenix dactylifera*) pollen is richest in protein, reaching 35.5 per cent. All have a very low fat content of only one-three per cent. Other important components are mineral elements (calcium, iron, magnesium, phosphorus and potassium) (Todd and Bretherick, 1942), plant growth substances (auxins,, ethylene, gibberellins hydroxypiridin cytokinins and steriods) (Redemann, 1949;) and vitamins (nicotinic acid, panthetic acid, pyridoxine and riboflavin) (Stanley and Liskens, 1974).

It is obvious that it is this outstanding array of compounds, shown inTable 1 which makes pollen of certain plants including *Crocus albiflorus, Papaver sp., Plantage sp.*, *Pyrus sp. Triflolium sp. and Zea mays* of particular biological value to honey bees (Maurizio, 1951). This stimulates the development of brood food glands, ovaries and fat bodies, and also prolongs the life span of honey bees (Maurizio, 1951).

Table 1.The major components of maize pollen reported in the literature.

COMPONENT	REFERENCES
Mineral element; Calcium, magnesium Nitrogen, phosphorus, sulphur	Knight et. al (1972)
Carbohydrates (34.26%)	Anderson and Kulp (1922)
Specific carbohydrates: Reducing sugar, Non-reducing sugar Starch. Galactose, Glucose, Glucosamine, Mannose, Raffinose, Stachyose	Todd and Bretherick (1942) Ueno (1954)
Protein (28.30%)	Anderson and Kulp (1922)
Amino acids Essential amino acids Proline	Tseluiko (1968) Anderson and Kulp (1922)
Lipid (1.55%)	Knight et.al.1972
Enzymes (Catalase)	Beckman,Scandalios and Brewbaker(1964)
Vitamins (Nicotinic acid, Pantothenic acid, Pyridoxine, Riboflavin)	Nielson, Grommer and Lunden (1955)
Hormones Hydroxypiridin	Redemann (1949)

Stanley and Liskens (1974) noted that upon wetting, viable pollen grains become leaky and the leachate of the pollen contains many of the compounds of the cytoplasm. A pertinent report of Hutchinson and Barron (1977) showed that the leachate could effect positive chemotropism in fungal hyphae. In successful positive chemotropism of tests carried out on Water Agar in Petri dishes, hyphae of the test fungi in most instances entered live pollen grains of *Pinus nigra* either by physical penetration or lysing of the wall of the pollen tubes. They coiled inside the pollen grain to form structures similar in morphology to pelotons of endomycorrhizal fungi. One hundred and sixty-two fungal species were tested. made up of 26 members of Ascomycota and Deuteromycota and 136 members of the Basidiomycota. In all, only 45 species were attracted by the leachate of the pollen grain of *P nigra.* Of these, 32 exhibited a consistent positive response while 13 species exhibited a weak or inconsistent positive response. The 45 species, with the exception of 2 (*Amblyosporium botrytis*, a member of the *Deuteromycota* and *Chaetomium cochliodes*, a member of the *Ascomycota*) were members of the Basidiomycota. This means that as many as 117 fungal species were not attracted by the leachates. It is therefore obvious that the relationship is not universal.

In nature, both viable and non-viable pollen grains could play a valuable role in the ecosystem. Stark (1972) observed that pollen grains falling in the temperate forest on the litter surface in the spring would move into the decomposition layer principally by water and would subsequently be attacked by filamentous fungi. Stark (op cit.) proposed that the nitrogen and phosphorus contained within the pollen grains were sufficient to allow these fungi to complete litter decomposition in environments where nitrogen and phosphorus were limiting for litter decay fungi. Lee Kenkel and Broth (1996) determined that 1.0 g of *Pinus banksiana* L. pollen yields 20 mg of nitrogen and that only 0.1 per cent of this was leached from *P. banksiana* pollen after 24 hours. Thus, 80 $kgha^{-1}y^{-1}$ of pollen recorded in a mixed pine forest in Wisconsin by Doskey and Ugosgwu (1989), for instance, would provide a potential 1.6 $kgha^{-1}y^{-1}$ of nitrogen that could be utilised by lignicolous fungi. This amount is comparable to the estimated amount of nitrogen contributed by free-living nitrogen-fixing bacteria in wood, woody debris and litter (Jurgensen, et al. 1987; Roskosti, 1980; Weber, and Sundman, 1986).

Another general natural event is that the leaves of plants are showered with pollen. This pollen deposit plays a critical role in the life of a number of specialised phyllosphere fungi (Oliver, 1978; 1983) as well as supplementing the nutritional requirements of pathogenic and saprophytic fungi in the phyllosphere (Chu-Chou and Preece, 1968). During an investigation on a group of phyllosphere fungi of evergreen trees and shrubs in South Africa, Oliver (1978) observed that the fungi *Retiarius bovicornutus* and *Retiarius superficiaris* captured pollen grains deposited on leaves by wind. This was first noticed on *Cassine peragua L.* Pollen caught by the fungal traps was visible to the naked eye as a net-like deposit at the tips and downwardly directed edges of leaves and along the veins, and all regions where rain-water and dew accumulated. In microscopic preparations the fungi could be seen to form radiating anastamosing colonies on leaf surface to which they were firmly attached by the mucilaginous outer layer of the hyphal wall. From these net-like systems a number of short pointed branches protrude vertically. When pollen grains landed on or near these mycelial traps, the short hyphae grew towards them, penetrated the pollen wall and established a mycelium within the grain.

The directional growth of the elongating hyphae appears to be a chemotropic response to a stimulus from the pollen grain. The stimulating principle seemed to come from the germinal region as attachment and penetration always took place there. It was remarkable that if a pollen grain landed on the germinal region, the approaching short hypha encircled the grain to reach the germinal region. The outer layers of the pollen grain persisted throughout the dry summer and until the rainy season is well established, then the mycelium in the grains put out short hyphae, which broke through the exine and produced the characteristic spiked mycelium and branched conidia.

Studies by Knox and Heslop-Harrison (1970) also showed that fungal attack of pollen of *Pinus banksiana* invariably took place through the dorsal region between the wings where the intine bound protein is located. No appressorium is formed but there is a swelling of the hyphal tip before the penetration of the pollen wall, apparently by mechanical pressure (Oliver and William, 1975). When the penetrating-hypha reaches the lumen of the pollen it develops into a branched moniliform mycelium, which rapidly fills the grain. The hyphal walls gradually become thickened, followed by formation of chlamydospores. The pollen coat normally remains intact for along time.

The rapidity of the parasitic reaction suggested that the attractant began to leach out of the pollen immediately on moistening. Indeed, several relevant reports indicate that when dry pollen is moistened, it releases enzymic protein within a few seconds. Thereafter, substantial amounts of other compounds will be released. For example, 10 per cent of the dry weight of *Pinus elliottii* pollen in water is lost after 60 minutes (Stanley and Search, 1971).

Studies by Chu-chou and Preece (1968) showed that pollen diffusate had been dialysed against distilled water for 24 hours, the non-dialysable portion (inside the tube) had no effect at all on *Botrytis* spore germination, whereas heat-concentrated dialysate promoted spore germination to the same extent as freshly prepared pollen diffusate. The effective component of the diffusate was, therefore, water-soluble, dialysable and heat-stable. Many workers (Deverall and Wood, 1961;Kosnge and Hewitt, 1964; Last, 1960; Purkayastha and Deverall, 1965) have demonstrated that sugars highly stimulated germination of *Botrytis cinerea* spores and infection of its host. But, it seems that the concentration of sugars in the diffusate was too low to influence germination of the spores to the extent recorded by Chu-chou and Preece (op cit). Neither glucose nor fructose detected in the diffusate by paper chromatography at 10 times the concentrations occurring in the diffusate stimulated spore germination. Evidently, other substances besides sugars must be involved in the stimulation of germination of the spores.

The percentage of *B. cinerea* spores, which germinated in distilled water, decreased sharply with the age of the culture providing the spores. Thirty-five per cent of spores from one-week old cultures germinated while only two per cent from two-week old cultures did so, and none of the spores from four-week old cultures germinated. Spores from one-, two-, three-, four- and five-week old cultures, on the other hand, showed 100, 96, 90, 85 and 55 per cent germination, respectively in the presence of strawberry pollen.

Effects on lesion development on petals of strawberry paralleled the rejuvenation effects of pollen on the germination of old spores. For example, 88 per cent of successful inoculations were obtained by adding pollen to spores from five-week old cultures, whereas only 11 per cent success was recorded in corresponding tests using distilled water only. Germination of spores in suspension droplets of 'moribund' spores of five-week old cultures in the absence of pollen on this occasion was due, most probably, to the presence of nutrients in the strawberry petal exudate.

Stimulated fungal growth has been observed near pollen grains on leaves, suggesting possible effects of the presence of pollen at the early stages of infection by plant pathogenic fungi. Ogawa and English (1960) reported pollen-stimulated *Botrytis cinerea* spore germination and germ tube growth on almond *(Prunus amygdalus)* petal. Similarly, Bachelder and Orton (1963) observed that a heavy deposit of pollen grains served as a locus of infection of flowers and other parts of American holly (*Ilex opaca*) by *B. cinerea*. Chu-chou and Preece (1968) carried out extensive relevant studies on the effect of pollen of strawberry *(Fragaria moschata)* on the germination of conidia of *B. cinerea* and the infection of petals and fruits of strawberry and leaves of broad bean by the germ tubes.

Pollen grains were added to spore suspension droplets by placing in a droplet one whole anther, which immediately released its pollen grain, approximately 2,500 in number into the droplet. Spore inoculum densities of four and eight spores per unamended aqueous droplet produced zero and 14 per cent infection respectively. Addition of pollen of to the spore suspensions resulted in improved successful infections of 63 and 93 per cent, respectively, by the two spore densities.

Grey mould attack of strawberry fruits caused by *B. cinerea* usually starts near the base where anthers still remain attached. (Jarvis, 1961; Moore, 1961; Powelson, 1960). On the premise that infection was encouraged by residual pollen, Chu-chou and Preece (op cit) compared the rate of infection of inoculated (sprayed with *B. cinerea* spores) intact fruits from which the anthers had been removed. They found out that presence of anthers markedly stimulated both the speed and severity of infection. For example, removal of the anthers of immature green inoculated fruits completely prevented infection while fruits with intact anthers showed 88 per cent infection, eight days after inoculation with *B. cinerea*. Pollen of strawberry also greatly promoted infection of leaves of broad bean by *B. cinerea* resulting in increased number of spreading lesions similar to1the symptoms of aggressive infections described by Wilson (1937).

The effect of pollen on the inoculum threshold of *B. cinerea* on strawberry petals was studied by Gaumann (1950) using different densities of spores of 16-1600 spores per suspension droplet. The results showed that the inoculum threshold was near 160 spores per droplet of unamended distilled water. Below this concentration, petals could not be infected by *B. cinerea*. At 160 spores per droplet the percentage inoculation that caused infection was only seven per cent. The figure increased with increasing inoculum density, reaching 78 per cent with 800 spores per droplet. When pollen grains were added to droplets, the inoculum threshold was reduced to 16 spores per droplet, which caused 18 per cent infection. At 160 spores per droplet, 97 per cent of inoculations with pollen added were successful, and 100 per cent success was achieved with a density of 400 spores per droplet. Mansfield and Deverall (1971) also showed later that pollen enabled *Botrytis cinerea* to overcome the inhibitory action of Wyerone acid, an antifungal product of infected bean leaves.

Besides *Botrytis sp*. pollen grains or entire anthers enhanced infections by *Alternaria* (Channon, 1970), *Fusarium* (Fokkema, 1968; Strange and Smith, 1971) *Cladoasporium* and *Helminthosporium Bipolaris* (Fokkema, 1971) and *Phoma* (Warren, 1972). Fokkema (1970) also observed a vastly improved growth of *Helminthosporium sativum* in the presence of pollen of rye.

Saprophytic fungi are also affected by pollen. Barnes (1969) showed that Scanning Electron Microscopy could make out the spots where pollen grains had been deposited on leaves after the grains had been removed. When the leaf surface of red clover *(Trifolium pratense*) was examined, 80 per cent of the germinated resident saprophytic fungus spores were found close to pollen grains or to spots where pollen had been.

The presence of pollen may not necessarily lead to greater infection. Warren (1972) observed differences in response to pollen by phylloplane microflora. He found that leaves of sugar beet *(Beta vulgaris)* showered with its own pollen supported large populations of 280-500 c.f.u.cm^{-2} of leaf surface of yeast, *Cladosporium sp.* and *Aureobasidium pullans* but spores of *Phoma betae* applied to those leaves gave only 3-5 per cent aggressive infection. On the other hand, plants that were not in bloom and, therefore, had no pollen deposits on their leaves and low phylloplane microflora population of 9,700 c.f.u.cm^{-2} of leaf surface, gave 88 per cent aggressive infection when they were inoculated with *Phoma betae* together with pollen of rye *(Secale cercale*). It was concluded that natural deposit of pollen apparently promotes growth of antagonistic microflora that inhibits pathogens. Apparently, the intensity of infection may be decided by the time of arrival of the pollen and the pathogen in the infection court, and the proximity of the propagule of the pathogen to the pollen grains.

Obomanu (1988) showed that, addition of maize *(Zea mays)* pollen grains at densities of 412-13,200 ml to aqueous conidial suspension of *Curvularia lunata* only slightly increased percentage germination but greatly improved germ tube growth, almost by a factor of two in some cases. The rate of infection of plants inoculated with conidial suspension without pollen grains was close (54.5 per cent) to rates of infection of 57.1-61.1 per cent of plants inoculated with conidial suspension with pollen grains of densities of 3.300-26400 grains ml^{-1}. Application of pollen grains to the leaves four and eight hours before conidium inoculation did not significantly increase the infection rate. Obomanu (1988) also observed that the pollen had no effect on bacterial and yeast populations on maize leaves but caused greater development of *Mucor sp*. The pollen grains also had no significant effect on the rate of maize leaf infection by *Curvularia lunata*.

Anyebuno (1990) defined the conditions, which make the pollen grains of maize stimulatory, ineffective or inhibitory. The percentage germination of conidia of *Alternaria sp.* and *Cercospora arachidicola* increased with increasing extract concentration from 1/8 dilution to the standard concentration (0.1g pollen grains in 10 ml distilled water). Germ tubes also grew longer with increasing extract concentration and more cells of the multicellular conidia in higher extract concentration, produced germ tubes.

Higher pollen extract concentration above the standard concentration became inhibitory and *C. arachidicola* were most severely depressed. With all three species, the higher concentration of the extract beyond the standard concentration, the fewer the number of germ tubes produced. The high pollen extract concentration may by itself be inhibitory to the conidia. At the same time this inhibition may be accompanied by inhibition by metabolites of the increased phyllosphere bacterial population.

The stimulation of the phyllosphere microflora by the pollen could interfere with growth of leaf pathogens and saprophytic species as well. This, however, may always not be so. For studies which have been carried out to deliberately alter the phyllosphere population level have not led to a change in the activity of the test organisms. Kinkel and Andrews (1988) reported that leaves of apple (*Malus pumila)* with the normal phyllosphere microflora, and those treated with 15 per cent hydrogen peroxide solution for 75-90 seconds to remove 99 per cent of the surface bacterial and fungi and inoculated with *Aureobasidium pullulans* or *Cladosporium cladosprioides* supported growth of these fungi to the same extent. In the final analysis, the influence of the phyllosphere population depends more on quality of the members of the population rather than on quantity.

The content of pollen of maize is very complex causing it, on one hand, to stimulate spore germination and growth of microorganisms and on the other, to inhibit, depending on circumstances.

The spreading tassel at the apex of the maize stem with hundreds of dangling anthers produces immense quantity of pollen. Pohl (1937) estimated that one maize plant produces 18,500,000 pollen grains. In addition, the maize pollen is so large (700,000 $\mu m^{3)}$) and heavy that unless a fair breeze is blowing, it falls almost vertically to the ground (Percival, 1965). Consequently, the over-towering maize plant showers any inter-planted crops below during mixed farming with abundant pollen.

Mixed farming is probably the most common farming practice in Ghana. It is a good system because it facilitates very effective use of the available soil nutrients during a growing season. The deep-rooted crops will be able to absorb nutrients that would be lost to shallow-rooted crops. Also, fibrous-rooted plants will be able to hold the soil and reduce soil erosion. If legumes are included, they improve the nitrogen status of the soil. Different crops have different plant nutrient requirement, and in this system of farming, the available plant nutrients are used more evenly.

Maize is an important and common crop in mixed farms in West Africa since it is the staple food of most of the inhabitants of this region. It is planted in mixed farms with the legumes, bambara bean *(Vigna subterranea (L) verde.)*, cowpea *(Vigna unguiculata L.)* and groundnut (*Arachis hypogea* L.), and the root tuber crop – cassava *(Manihot esculenta* Cranz.) There is bound to be an interaction between leaf fungal parasites of these crops and the pollen of maize deposited on the leaves.

Leaf spot is one of the commonest plant fungus diseases in Ghana, the greatest proportion being caused by *Cercospora species (*Clerk, 1974). Leaf spots are patches of dry and brittle dead tissue or necrotic spots, some as tiny as pinheads, without extending any further and are sharply demarcated from the surrounding healthy tissue. The cells of the dead tissue or a necrosis maintain their form because death is predominantly or even exclusively caused by toxins rather than by cell wall disintegrating enzymes. The dead leaf spot seldom exceeds 10mm in diameter and would not extend any further even under most favourable environmental conditions The lesion is either sharply demarcated from the surrounding healthy tissues or separated from them by a band of yellow tissues of dying cells. It is generally believed that the dead host cells become filled with substances that in turn either kill the parasite or prevent its further growth (Clerk, 1974). The spots may also occur on petiole and stems. Sometimes, a spot on the petiole may damage so much tissue that the entire leaf dies.

The *Cercosporas* belong to the Deuteromycota. Their conidia are, generally, long, slender and multiseptate and borne on stiff erect, oddly crooked conidoiphores, which emerge in tufts through the stomata. Bambara bean, cassava, cowpea and groundnut all suffer from serious *Cercospora* leaf spot diseases. Those of Bambara bean, cassava, and cowpea are caused by *C. canescens, C. Henningsii and C. cruenta*, respectively. Groundnut has two leaf diseases caused by *C. arachidicola* and *C. personata*, respectively. These two *Cercospora* diseases have distinctive distinguishing symptoms. *Cercospora arachidicola* produces irregular circular spots, up to 10mm in diameter, and light tan or yellow in colour when freshly formed. With age the spots become reddish-brown to black on the lower surface of the leaf and light brown on the upper surface and surrounded by a bright yellow halo (Jenkins, 1938). Observations tend to show that the halo surrounding each lesion is perhaps in some way related to the carbohydrate content of the leaf cells, since they appear best developed on leaves supposedly high in carbohydrates. Spots caused by *Cercospora personata* are circular and smaller and not more than 7mm in diameter and with no noticeable yellow halo. The spots are dark-brown or black on both surfaces of the leaf. The structures of the fungi also show differences. Haustoria are formed by *C. personata* but not by *C.arachidicola*. *C.arachidicola*, besides, has very long slender conidia up to 100μm in length while conidia of *C. personata* are shorter broader up to 60μm long (Clerk, 1974). The work reported in this thesis focussed on *C.arachidicola* because its leaf spots were far more abundant during the course of this work and provided conidia in quantities needed for the work.

The work reported in this thesis was carried out to examine the effect of pollen of maize *(Zea mays)* on conidia of *Cercospora arachidicola* and on infection of groundnut caused by the pathogen. The thesis contains mainly results of studies on:

i. The germination of conidia, pattern of germ formation and growth germ tubes of *Cercospora arachidicola* as affected by leachate of maize pollen grains only or in combination with nutrients, groundnut leaf exudates and metaboltesof phylloplane microorganisms.

ii. Longativity of conidia of *C.arachidicola* stored at different relative humidities and effect of maize pollen on germination and germ tube evelopment of aged conidia.

iii. Effect of maize pollen grains on infection of groundnut with varying phylloplane microfloral populations by *C. arachidicola.*

CHAPTER TWO

MATERIALS AND GENERAL METHODS

i. MATERIALS

a. *Cercospora arachidicola* Conidia

Conidia of *C. arachidicola* used were obtained from naturally infected leaves of groundnut (*Arachis hypogea*) plants growing in three localities:

1. Plants raised in the Teaching Garden of the Department of Botany, University of Ghana, Legon.
2. Plants in local private farms near the University of Ghana, Legon.
3. Plants in farms in the Dodowa area, about 20 kilometres from Legon.

b. Groundnut (*Arachis hypogea*) Seeds.

Seeds of the commonly cultivated 'white' variety were purchased from the store of the Ghana Seed Company in Accra.

c. Soil

Groundnut plants were raised in plastic buckets, with drainage holes at the bottom, for infection experiments. Garden loamy soil was obtained from plots previously used for cultivation of legumes for this purpose. The soil was found to contain compatible *Rhizobium* strain for nodulation of groundnut.

d. Pollen Grains of Maize (*Zea mays*)

Pollen grains of maize used throughout this study were provided by 70-80 days old 'Composite Four' variety plants raised specifically for this project in the Teaching Garden of the Department of Botany, University of Ghana, Legon. The grains were obtained from the store of the Ghana Seed Company, Accra.

e. Chemicals

Hydrogen peroxide and Glycerol were purchased from a local pharmacy. Chloral hydrate, ethanol, glucose, peptone, sulphuric acid and Tween 80 used came from the chemical store of the Department of Botany, University of Ghana, Legon. They were originally purchased from British Drug House (BDH), Poole, England, United Kingdom.

f. Tubers of Irish Potato (*Solanum tuberosum*).

Tubers of Irish Potato were purchased from a local grocery shop whenever needed.

ii. GENERAL METHODS

a. Raising of Maize Plants

Grains of the 'composite four' maize variety were soaked in distilled water for 48 hours and those showing no discolouration and looking apparently healthy were sown on plots prepared in the Teaching Garden. They were planted at a spacing of 100 x 100 cm, and were watered daily at the initial stages and once in three days from the age of 30 days until they tasselled.

b. Collection of Pollen Grains

Transparent cellophane bags were tied round the tassels just before the anthers dehisced. The pollen grains spilled out into the bags when the anthers broke open. The bags were carefully removed and taken into the laboratory where the collected pollen was transferred with a sterile micro-spatula into sterile McCartney tubes and stored in a refrigerator at 4° C until needed.

c. Raising of Groundnut Plants.

Seeds of the 'White' groundnut variety were soaked overnight in distilled water and the swollen and wholesome ones among them sown on beds prepared in the Teaching Garden. They were planted at a spacing of 30 x 30cm so that they would be sufficiently crowded as they grew to discourage the breeding of the aphid, *craccivora* that transmits the Groundnut Rosette Virus Disease. The aphid is unable to thrive under conditions of high humidity around crowded groundnut plants (Clerk, 1974) .The plants were watered daily.

d. Collection of Conidia of *C.arachidicola*

The growing plants were naturally infected, and infected leaflets with spots bearing mature conidia, were carefully detached and conveyed to the laboratory in transparent polythene bags. The conidia were harvested in different ways for different purposes and these would be described at appropriate places in the Experimental Details.

e. Culture Media

Different culture media were used for different purposes at various stages of the work. The composition and preparation of the media used are as follows:

1. Potato Dextrose Agar (PDA) (Ainsworth and Bisby, 1971).

Potato tuber (peeled)	200 g
Dextrose	10 g
Agar-Agar	15 g
Distilled water	1L

The peeled potato tubers were cut into pieces and boiled in 400ml water until they started to break up. The suspension was strained with muslin cloth and the extract collected in 500ml beaker and allowed to cool. It was then poured into a 1L measuring cylinder and topped up with distilled water to the 1L mark. The extract was then transferred into a 2L Erlenmeyer flask and the glucose and agar-agar added. The mixture was stirred and heated in a water bath to melt the agar-agar before the medium was dispensed into 250ml Erlenmeyer flasks. The flasks were plugged with non-absorbent cotton wool and autoclaved. The plugs were covered with aluminium foil before autoclaving to prevent the entry of water vapour.

2. Nutrient Agar (oxoid)

Twenty-eight grams of Nutrient Agar were dissolved in one litre of distilled water. The suspension was then heated in a water bath to melt the agar and dispensed into 250ml Erlenmeyer flasks. The flasks were plugged with non-absorbent cotton wool and then autoclaved. The plugs were covered with aluminium foil before autoclaving to prevent the entry of water vapour.

3. Water Agar

Fifteen grams of Agar-agar were dissolved in one litre of distilled water in a 1L Erlenmeyer flask and heated in a water bath to dissolve the Agar. The preparation was dispensed into 250ml Erlenmeyer Flasks and the flasks plugged with non-absorbent cotton wool, and autoclaved. The plugs were covered with aluminium foil before autoclaving to prevent the entry of water vapour.

f. Methods of Sterilization

Nutrient and Culture media, distilled water, pollen grains,McCartney tubes,beakers and measuring cylinders, were sterilized by autoclaving at 1.1Kg cm^{-2} steam pressure at 121°C for 15 minutes. Petri dishes were sterilized by heating at 160 °C for 6 hours in an electric oven.

Tips of forceps, inoculating needles and loops and micro-spatula were flamed to red heat and air-cooled before use. Glass slides thoroughly cleaned, were stored in 90 per cent ethanol and flamed just before use.

Glass lids of solid watch glasses were cleaned with 5% aqueous Dettol solution rinsed with sterile distilled water and stored in 90 per cent ethanol and then flamed just before use.

The inoculating room was sterilized by spraying heavily with 5.0 per cent aqueous Dettol solution and allowed to stand for 20 minutes before use.

Top of the working bench in the inoculating room was wiped with 70 per cent ethanol. The interior of the incubator where inoculated media were incubated was sprayed with a mixture of 1.0 per cent potassium permanganate solution and 1.0 per cent aqueous Dettol solution and the door closed for 20 minutes before the incubator was used.

g. Spore Germination Chamber- Petri Dish

Spore germination tests in suspension drops or at 100 per cent R.H. were carried out in Petri dishes. The bottom of the dishes was lined with sterile moistened filter paper. Slides carrying conidial suspension drops or dry conidia were placed on supporting V-shaped glass rods in the Petri dishes, with the 'spore side' facing upwards. The moist filter paper maintained an internal atmosphere of 100 per cent relative humidity. In prolonged tests, the filter paper was periodically re-moistened to ensure that the air in the chamber remained humid.

h. Spore Germination Chamber- Van Tieghem Cell

Solid watch glass, measuring 4x4x1.5 cm with a well 3.5 cm in diameter and 1.0 cm deep, was used as Van Tieghem Cell for the conidial germination tests under different relative humidities. An amount of 2ml of the appropriate solution (5.0g in the case of solid reagents) was put into the well of the watch glass to maintain the desired relative humidity. The top edge of the watch glass was luted with petroleum jelly to provide an airtight seal when the lid was placed on it. The conidia were dusted onto the centre of the cover glass within a circumscribed area of about 1.0 cm in diameter. The lid was then placed on the watch glass with the conidium-bearing surface facing downwards. Aqueous sulphuric acid (Tetra-oxo-sulphate VI acid) solutions used in maintaining the different relative humidities are presented in Table 2.

Table 2

Aqueous sulphuric acid (Tetra-oxo-sulphate VI) solutions for maintaining desired constant relative humidities at 25^{0}C (Extracted from data of Solomon, 1952).

%Relative humidity at 25^{0}C	Weight in gm. of H_2SO_4 per 100g of solution	Weight of water in gm. per 100g of solution
5	69.44	30.56
10	64.45	35.55
15	60.80	39.20
20	57.76	42.24
25	55.01	44.99
30	52.45	47.55
35	50.04	49.96
40	47.71	52.29
45	45.41	54.59
50	43.10	56.90
55	40.75	59.25
60	38.35	61.65
65	35.80	64.20
70	33.09	66.91
75	30.14	69.86
80	26.79	73.21
85	22.88	77.12
90	17.91	82.09
95	11.02	88.98

Distilled water provided 100% R.H.

I. Humidity Chamber for Spore Longevity Tests.

The method described in Section (h) was used in the study of survival *of C. arachidicola* conidia at different relative humidities. Conidia stored for different lengths of time were then germinated in nutrient broth to test their viability.

j. Preparation of Spore Suspension for Germination Tests.

A leaflet with a leafspot was folded across the spot with the sporulating surface outermost. The medium drop on a glass slide was touched with the lesion to transfer the conidia to the drop. The suspension drop was then quickly examined microscopically to assess the spore density. More conidia were added to suspensions of low density, while a drop of the fluid was added to dilute very dense suspension. Averagely, a spore density of 20-30 conidia in the microscope field under High Power objective- X40 was used in all the germination tests.

k. Preparation of Spore-Pollen Grain Suspension for Germination Tests

Pollen grains were transferred from the McCartney tubes with flamed inoculating pin into the spore suspension drop prepared as described in Section (j). The pin was dipped into the pollen to a marked depth and washed in the drop. The transfer was standardised so that pollen densities of 500-600, 1000-1200, 1500-1800, 2000-2400, 3000-3500, 4000 -4500, 5000 -5500 and 6000 -7000 per ml of suspending medium were obtained by adding dips of 1, 2, 3, 4, 6, 8, 10 and 12 respectively, estimated by actual haemocytometer counts.

l. Preparation of Spore Print for Germination or Storage in Viability Tests, at Different Relative Humidities

The glass lid was gently touched with the outer spore-bearing surface of the leafspot to get a spore print. The lid was then placed on the solid watch glass with the spore-bearing surface facing downwards thereby exposing the conidia to the internal atmosphere.

m. Assessment of Conidial Germination on Glass Slides and Lids of Solid Watch Glasses

At the end of the desired incubation period, 0.01 ml of 1.0 per cent Formaldehyde was added to each suspension drop or to each 'dry' spore print to arrest further spore germination and germ tube development. Percentage germination in each treatment was estimated based on not less than 300 randomly observed conidia under the High Power objective of the microscope. Any conidium with a discernible germ tube was considered as having germinated. The lengths of terminal and basal germ tubes of at least 25 germinated conidia of every treatment were measured using an eye piece graticule and the Mean Germ Tube Length calculated.

n. Assessment of Viability of Conidia Stored at Different Relative Humidities

After the desired storage period, conidial prints on the glass lids were suspended in drops of Potato Dextrose Broth and incubated at 30°C for 24 hours, after which the percentage germination (percentage viability) was determined and lengths of germ tubes measured with an eye-piece graticule.

o. Conidium Germination on the Surface of Groundnut Leaflets

This test was carried out on detached leaves placed on a sterile moist filter paper in a sterile Petri dish. A drop of sterile distilled water (about 0.01 ml) was placed on each leaflet and the conidia introduced into the drop as described for medium drops on glass slides. Pollen grains were added to the spore suspension drops of half of the preparation, while none was added to the drops of the remaining half. The preparations were then incubated at 30°C.

p. Assessment of Germination of Conidia on Surface of Leaflets

At the end of the incubation period, the leaflets were floated on a concentrated solution of Chloral hydrate for 24 hours to clear, with the conidium-bearing surface facing upwards in order to retain the conidia on the leaflets. The conidia were then stained in situ with lactophenol cotton blue. Percentage germination was then determined and the lengths of the germ tubes measured using a microscope.

q. Incubation of Conidia in Germination and Survival Tests.

The conidia for the various tests were incubated at 30°C but the period of incubation varied as indicated in Chapter III- Experimental Details

r. Buffer Solutions for Conidial Germination Tests

Buffer solutions were prepared according to the data in Tables 3 and 4 and used in Conidial germination tests.

Table 3

McILVaine's Standard Buffer Solutions Stock Solution A: 0.1M Citric acid ($C_6H_8O_7$) and Stock Solution B: 0.2M Disodium hydrogen orthophosphate (Na_2HPO_4) (Hale, 1966)

pH	Solution A (ml)	Solution B (ml)
3	15.87	4.11
4	12.29	7.71
5	9.70	10.30
6	7.37	12.63
7	3.53	16.47
8	0.55	19.45

Table 4

Boric acid: NaOH (Clark and Lubs) Buffer Solution. Stock Solution A: 0,2M Boric acid +0.2M Potassium Chloride. Stock Solution B: 0.2M NaOH (Clark and Lubs, 1958)

pH	Solution A(ml)	Solution B(ml)	De-ionised Water
8	50.0	4.0	146.0
9	50.0	21.4	128.6
10	50.0	43.9	106.1

s. Method for Testing Phytotoxicity of Hydrogen Peroxide (H_2O_2) to Groundnut Leaves

Prior to Experiment that disinfected the surface of leaflets of groundnuts with H_2O_2, the 'safe' concentration of H_2O_2 to be applied was determined. Samples of undetached leaves were immersed in H_2O_2 solutions of different concentrations for different lengths of time and rinsed with sterile distilled water. The samples were then left to stand for 24 hours and examined for bronzing. The highest concentrations of H_2O_2, which did not cause leaf bronzing, was adopted for disinfecting the leaf surface in the subsequent tests.

t. Assessment of Efficacy of Hydrogen Peroxide (H_2O_2) to Disinfect the Surface of Groundnut Leaflets.

The residual microbial population after disinfecting the leaflet surfaces with H_2O_2 was quantified using a wash-dilution plating method. Fungal populations were determined with Potato Dextrose Agar containing Ampicillin solution to suppress bacterial growth, and bacterial populations with Nutrient Agar. For each test, four leaflets were immersed in 10ml phosphate buffer solution containing 0.01 per cent Tween 80 in a McCartney tube. The Tween 80 was added to disperse the bacterial cells and fungal spores. The tubes were kept in the refrigerator at 4°C for one hour. Each McCartney tube was vigorously shaken, and for the assessment of the fungal population, 1.0 ml of the undiluted washing and

0.1ml of 1.0-% ampicillin was put in each of three Petri dishes and 20ml of melted PDA added. The dishes were gently agitated with a circular motion before the medium solidified. For bacterial population assessment, 1.0ml of the undiluted washing and 20ml of Nutrient Agar were put in each Petri dish and mixed. Plates of PDA were incubated at 30°C for five days and those of NA were kept at 37°C for 48 hours. At the end of each incubation period the colony forming units (CFU) per ml on each plate were counted and the mean calculated for each treatment.

u. Infection Tests

Because the epidermal hairs on the leaflets made spore suspension drops to roll off leaves still attached to the stem, the leaflets were detached and placed on moist sterile filter paper lining the bottom of sterile Petri dishes, and inoculated with 0.2ml *C. arachidicola* conidial suspension droplets. The inoculated leaves were kept at room temperature under constant fluorescent light to keep the leaflets green for as long as possible. The preparations were examined each morning for signs of infection.

v. Experimental Precautions

1 In setting up spore germination tests using Van Tieghem Cell, care was always taken to prevent the solution in the well from coming into contact with the spores.

2 The density of conidia in the various spore suspensions was strictly standardiized, giving 20-25 spores per High Power objective of the microscope field.

3 Care was taken to obtain uniform distribution of the conidia on the slide and host leaflets for germination tests. Leaflets with unduly crowded conidia were discarded.

4 Glassware was scrupulously cleaned with detergents,washed well with tap water and finally rinsed with distilled water and allowed to air-dry before use.

5 Glycerol-water mixtures and Tetra-oxo-sulphate (vi) acid solutions used to maintain different relative humidities were thoroughly shaken during preparation to obtain homogenous mixtures. Fresh solutions were prepared for each experiment.

CHAPTER THREE

EXPERIMENTAL DETAILS

A. Morphology and Germination Process of Conidia of *C.arachidicola*

The first exercise carried out was to study thoroughly the morphology of the conidia and the mode of development of germ tubes on germinating conidia incubated in distilled water. A conidial suspension was prepared with distilled water and drops on slides were stained with lactophenol cotton blue to make the conidia clearly visible under the microscope. The drops were covered with cover slips and observed under high power of the microscope. One hundred conidia were selected randomly from five suspension drops and the number of cells in each conidium was recorded. For each of these conidia the length was measured with an eye piece graticule. The conidia contained three, four, five, six or seven cells. From the data of measurements obtained, the mean conidial length and the range of conidial length were obtained for each category of conidia. The percentage of 100 conidia measured belonging to each of the five categories was then calculated. The data obtained are shown in Table 5. Another set of conidial suspension drops on sterile slides, but on this occasion without cover slips, was incubated at 30°C for 24 hours so that the pattern of germ tube development could be studied. The different patterns of germ tube development by the germinated conidia after 24 hours are illustrated by the Camera Lucida drawings in Fig. 1

B. Influence of Sterile and Non-sterile Maize Pollen Grains on Germination of conidia of *C. arachidicola*

A series of experiments were next carried out to study the effect of pollen grains of maize at different densities on the germination of the conidia of *C.arachidicola*

(a.) In the first experiment different aqueous pollen suspension of either non-sterile pollen or autoclaved pollen grains were used. Each set employed pollen densities of 500-600, 1000-1200, 1500-1800, and 2000-2400 pollen grains per ml estimated as described at Section k of Materials and General Methods. For each treatment of sterile and the non-sterile pollen grains, three well spaced pollen suspension drops were put on a sterile slide and sufficient conidia, about 20-30 per microscope field under X40 objective were added to each droplet to provide the desired conidial concentration as described in the Materials and Methods. The slides were placed in humid chambers and incubated at 30°C for 24 hours. Percentage germination of each treatment was determined after the incubation period and the lengths of germ tube emerging from the basal and apical cells of the conidia measured. The studies made in the preceeding experiment in A above showed five different modes of germ tube development. . Quantitative data of these details were therefore recorded to assess any possible influence of the pollen. Results obtained are presented in Tables 6 and 7.

(b.) The preceeding experiment showed a reduced percentage germination of conidia in suspension containing either sterile or non-sterile pollen at the highest densities of 2000-2400 pollen grains per ml. A subsequent test was therefore carried out to find the effect of higher pollen densities on germination of the conidia. The experiment was repeated using pollen densities of 3000-3500, 4000-4500, 5000-5500, and 6000-7000 pollen grains per ml of the suspension. The data obtained are presented in Tables8 and 9.

(c.) Indeed, these higher pollen densities depressed further the percentage germination of the conidia. An experiment was therefore carried out to find out whether externally supplied nutrient would offset the depressant effect of the higher concentrations of the pollen. The highest pollen density of 6000-7000 pollen grains per ml, which gave the lowest percentage germination, was used in this experiment. The nutrients used were glucose at concentrations of 1.0, 2.0, 4.0 and 8.0 per cent and peptone at concentrations of 0.1,0.5, 1.0 and 2.0 per cent. Since in the earlier experiments spore germination in suspension containing non-sterile pollen and that in suspension with sterile pollen was quite close only non-sterile pollen was used in this experiment. The same preparations were made and the relevant data were collected after incubation at 30°C for 24 hours. The results appear in Tables 10, 11, 12 and 13.

(d) Because addition of either glucose or peptone to conidial drops containing 6000-7000 pollen grains per ml improved germination of the conidia, this experiment was carried out as a sequel to find the combined effect of glucose and peptone. Different combinations and permutations of glucose, of concentrations- 1.0, 2.0, 4.0 and 8.0 and peptone, of concentrations of 0.1, 0.5, 1.0 and 2.0 per cent, were used as the germination media containing pollen or without pollen. The results obtained are shown in Tables 14and 15.

C. Influence of Exudates of Maize Pollen on pH of the Germination Medium

(a) The depression of percentage germination of very high densities of maize pollen could probably be due to alteration of the pH of the suspension. An experiment was designed to find the extent to which pH of the suspension might have been altered by the pollen. Pollen suspensions were prepared with either sterile or non-sterile pollen in McCartney tubes at different densities of 500-600, 1000-1200, 1500-1800, 2000-2400, 3000-3500, 4000-4500, 5000-5500, and 6000-7000 pollen grains per ml. Since the conidia normally began to germinate after 12 hours at 30°C, the McCartney tubes were kept at 30^0C and samples of each treatment were withdrawn after 3, 6, 9 and 12 hours, respectively, and the pH measured. The results obtained are presented in Table 16.

(b) Although the results obtained and tabulated in Table 16, showed very little difference among the various treatments, the leaf surface environment could possibly shift the pH values beyond the limits of the pH range provided by the different densities of the pollen. The germination of the conidia over a wide range of pH was, therefore, studied. Two buffer solutions were used for this investigation. McILvaine's Standard Buffer solutions provided pH 3, 4, 5, 6, 7 and 8 and Clark and Lubs Standard provided pH 8 and 9. The results obtained after incubation at 30^{O}C for 24 hours appear in Tables 17 and 18.

D. Studies on the influence of maize pollen grains on germination of aged conidia of *C. arachidicola*

(a) Before aged conidia could be used, it was necessary to establish the relative humidities where conidia would germinate. The conidia could then be stored below those humidities and the effect of pollen on the germination of such conidia could be tested at intervals. The conidia were dusted onto dry glass lids of solid watch glasses containing solutions of Tetra-oxo-sulphate (vi) acid of appropriate concentrations to provide humidities of 75, 80, 85, 90 and 95% R.H. Solid watch glass containing distilled water in their wells provided atmosphere of 100 per cent relative humidity. Percentage germination of the conidia was calculated after 24 hours' incubation at 30^{0}C as showed in Tables 19 and 20.

(b) Germination occurred at the upper humidities of 85, 90, 95 and 100 per cent relative humidity and the conidia as a consequence could be stored for the ageing experiment at humidities from 0 to 80 per cent R.H.. Five humidities from this range namely 0, 20, 40, 60 and 80 per cent R.H. were adopted and the conidial were stored at these humidities as described in the Materials and Methods over 50 days. Conidial samples were withdrawn at 10 days interval and germinated by suspending in sterile distilled water with or without pollen grains. The results are shown in Tables 21-30 and in Figs. 2 and 3.

E. Germination of *C. arachidicola* conidia on groundnut leaflets with normal and reduced phylloplane microflora population

In this investigation made up of four different experiments, the conidia were germinated in the presence of maize pollen grains on natural leaf surface and on leaf surface, which had been disinfected with hydrogen peroxide.

(a) Only non-sterile pollen grains were added to conidial suspension drops on the groundnut leaflets. There were five treatments: spore suspension without pollen grains, and spore suspension with pollen of four densities, 500-600, 1000-1200, 1500-1800 and 2000-2400 pollen grains per ml of medium. The preparations were incubated at 30°C in humid atmosphere for 24 hours. The test was repeated five times and the results obtained for the five are presented together on Table 31.

(b) Washing the leaves with hydrogen peroxide did the removal of the phylloplane microfloral. This, however, required the determination of the appropriate concentration of the disinfectant, which would not destroy the leaves. The leaf samples were washed with different concentrations of hydrogen peroxide (10, 20 and 30%) for different lengths of time and rinsed quickly thereafter with sterile distilled water as already described in the Materials and Methods. The observations made are shown in Table 32.

(c) The 'safe' treatments were then repeated and the plate counting method as already described assessed the degree of disinfection of the leaflets. The results are shown in Tables 33 and 34 and Plates 1-4

(d) Similar to other relevant studies in literature, the microfloral population was reduced by hydrogen peroxide but not completely eliminated. The germination of the conidia on leaflets of normal microfloral population and that on leaflets treated with 30% H_2O_2 for one minute with reduced population were compared. The results of the germination test on these two types of leaflets in the presence and absence of pollen grains are presented in Tables 35 and 36.

F. Influence of pollen of maize on the infection of groundnut leaflets by conidia of *C. arachidicola*.

Although the previous experiment showed that the germination of conidia was slightly better on leaves in the absence of pollen than in the presence of pollen subsequent infection may or may not follow the same trend. Infection tests were carried out with non-sterilized and surface-sterilized leaflets to establish the trend of infection. Unsterilized leaflets and the leaflets disinfected with 10, 20 and 30 per cent hydrogen peroxide were inoculated with conidial suspension containing different densities of pollen grains of maize. The inoculated plates were left under a continuous supply of white fluorescent light for 8 days at 30^0C and the infection rate of the different treatments recorded. The results obtained are shown in Table 37,Fig 4 and in Plate 5.

CHAPTER FOUR

RESULTS

A. Morphology and Germination Process of Conidia of *C.arachidicoda*

The conidia of *C.arachidicola* were solitary, dry, septate (multicellular) and subfusiform in shape. They were straw-coloured or olivaceous and smooth. A conidium contained 3, 4, 5, 6 or 7 cells and they ranged from 28.3 to 68.1μm in length, and approximately 10.4 μm thick in the widest part. The data in Table 5 show that the 5-celled conidium was the most abundant (36.0 per cent) while the 3 and 7-celled conidia represented only 6.0 and 9.0 per cent of the population, respectively.

Five patterns of conidial germination were identified and illustrated in Fig.1. One germ tube emerged from the distal cell only or from the basal cell only (Figs 1a and 1b). A conidium may also produce one germ tube from the distal cell and one from the basal cell at the same time (Fig 1d.). In other instances, a conidium either produced germ tube from the medium cells only (Fig. 1c) or from an end cell and a medium cell (Fig. 1e). The commonest forms are those illustrated in Fig. 1a and Fig 1d.

TABLE 5

Details of Morphology of 100 Randomly Selected Multicellular Conidia of *C.arachidicola*

No. of Cells in Conidium	Mean length of Conidia (μm)	Range of length of Conidia (μm)	Percentage of Spores in Group
3	35.8	28.4 – 48.3	6.0
4	40.3	35.2 – 52.8	33.0
5	42.6	32.4 – 55.1	36.0
6	46.3	38.8 – 65.6	16.0
7	54.5	42.1 – 68.1	9.0

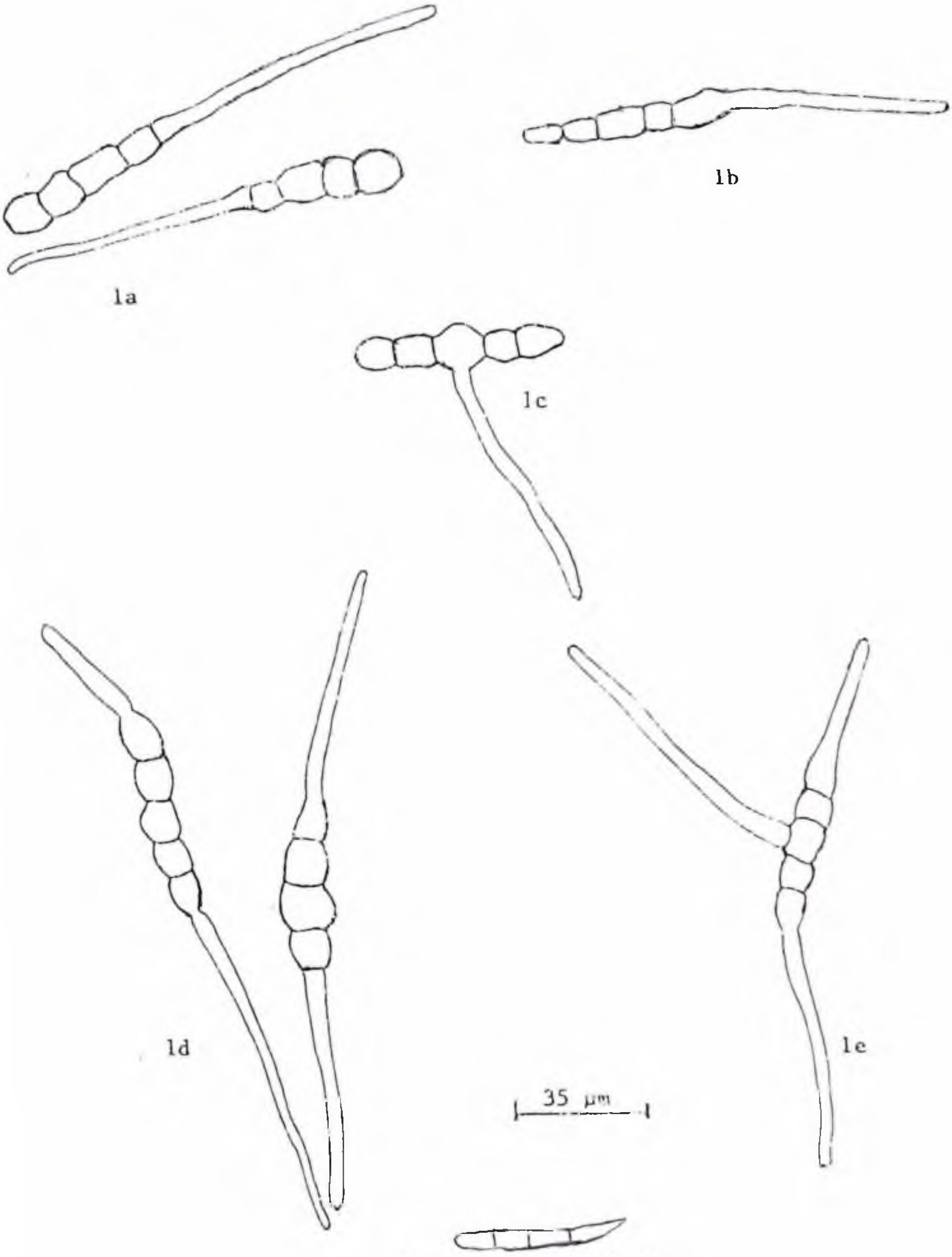

Fig.1: Camera lucida drawings of Conidia of *C. cassiicola* germinating in sterile distilled water For 24 hours at 30°C.

Note the different points of emergence of the germs tubes and the swelling of some of the cells of the multicellular conidia.

B. Influence of Sterile and Non-sterile maize pollen grains on Germination of Conidia of *C.arachidicola*.

(a) The data in Table 6 indicate that the conidia germinated very well in distilled water achieving a percentage germination of 80.5 percent. Percentage germination decreased when pollen of maize was added, in the case of both sterile and non-sterile pollen grains. Percentage germination in pollen suspensions of densities of 500 – 1800 pollen grains per ml of suspending medium was practically the same; but germination was further depressed, especially with sterile pollen in media with 2000 – 2400 pollen grains per ml. of suspension. The germ tubes in most cases were also shorter in the pollen suspensions than in distilled water without pollen, while germ tubes produced in the presence of non-sterile pollen were generally longer than those produced in suspension of sterile pollen. The germination pattern was also somehow affected by the pollen grains. The data in Table 7 revealed a number of features which can be summarised as follows:

i) The five different types of germination occurred in different proportions, with the greatest proportion being conidia producing germ tubes from both the distal and basal cells, followed closely by conidia with germ tube from the apical cell only. Very few conidia produced germ tubes from the median cells only.

ii) With both sterile and non-sterile pollen, the percentage of conidia producing germ tubes from both the distal and basal cells increased with increase in pollen density. This was more accentuated in media of non-sterile pollen.

iii) A reverse trend was observed in conidia producing germ tubes from the apical cells only.

iv) The percentages of the three other patterns of germ tube development seemed to be independent of pollen density.

(b) In the subsequent experiment in which higher pollen densities of 2000 – 7000 pollen grains ml^{-1} of suspension were used, percentage germination progressively decreased as shown in Table 8. Thus, whereas conidial germination in the pollen-free medium was 83.0 percent, it was only 58.4 per cent and 48.2 per cent, respectively in the presence of non-sterile and sterile pollen of a density of 6000 – 7000 pollen grains ml^{-1} of suspending medium. The higher pollen densities, on the other hand, uniformly supported greater production of germ tubes from both the apical and basal cells as indicated in Table 9.

(c) The results of germination tests carried out to find out whether addition of either glucose or peptone would improve conidial germination in aqueous suspension containing the highest pollen density of 6,000 – 7,000 non-sterile pollen grains ml^{-1} are presented in Tables 10, 11, 12 and 13. The already high percentage germination of the conidia in distilled water was further increased by Glucose at concentrations of 4.0 and 8.0 per cent as shown in Table 10. On addition of Glucose at concentrations ranging from 1.0 to 8.0 per cent, germination in suspensions with pollen grains increased from 67.5 percent (without Glucose) to 74.7 – 80.6 per cent. The treatment also improved the growth of the germ tubes.

It was noted that increase in Glucose concentration increased the percentage of conidia that produced germ tubes from both the apical and basal cells and from median cells and apical and/or basal cells, whereas the percentage of apical cells producing germ tubes decreased (Table 11).

Tests with peptone also showed that addition of peptone also improved the percentage germination of the conidia in the presence of maize pollen grains (Table 12) and increased the percentage of conidia producing germ tubes from both the apical cells and basal cells and those of conidia producing germ tubes from apical and/or basal cells and median cells while the percentage of conidia bearing germ tubes from the apical cells only declined (Table 13).

) Tables 14 and 15 contain data of results of tests, which used combined glucose and peptone as germination medium to which pollen grains had been added. The data in Table 14 showed that the lengths of germ tubes from both the apical and based cells showed very little variation. Percentage germination in the media on the other hand, showed clearly that, the percentage decreased when there was low concentration of both glucose (1.0 and 2.0%) and peptone (0.1 and 0.5%) but was greater at higher peptone concentrations of 1.0 and 2.0 per cent. At higher glucose concentrations of 4.0 and 8.0 per cent, very high percentage germination occurred at all concentrations of peptone.

Data in Table 15 showed that the percentage of conidia bearing germ tubes from both apical and basal cells was directly related to concentration of the peptone rather than to glucose concentration.The percentage increased with increase in peptone concentration.The percentage of conidia bearing apical germ tubes only,on the other hand,showed an inverse relationship with peptone concentration.

TABLE 6

Germination of Conidia of *C.arachidicola* in aqueous suspension of sterile and non-sterile pollen of *Zea mays* at 30°C in light intensity of 76 lux in 24 hours

Pollen Treatment	Pollen density in conidial suspension (No ml^{-1})	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from	
				Basal Cell	Apical Cell
Non-sterile	500 – 600	481	70.4	58.9	66.7
	1000 – 1200	491	73.6	61.7	60.4
	1500 – 1800	537	71.5	54.7	61.1
	2000 – 2400	456	69.7	58.2	59.3
Sterile	500 – 600	446	70.7	51.2	58.2
	1000 – 1200	589	73.5	48.9	59.6
	1500 – 1800	516	71.2	52.5	55.1
	2000 – 2400	512	61.6	53.9	57.5
Control	No pollen	563	80.5	60.5	65.5

TABLE 7

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated in aqueous suspension of pollen of *Zea mays* at 30°C in light intensity of 76 lux for 24 hours.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Pollen Treatment	Pollen density in Conidial Suspension (No. ml^{-1})	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
Non-sterile	500 – 600	26	36	16	6	16
	1000 – 1200	36	30	10	6	18
	1500 – 1800	44	24	16	4	12
	2000 – 2400	46	20	12	2	20
Sterile	500 – 600	24	44	10	2	20
	1000 – 1200	34	30	6	0	30
	1500 – 1800	30	30	14	6	20
	2000 – 2400	36	24	14	4	22
Control	No pollen	27	20	10	9	34

TABLE 8

Germination of Conidia of *C.arachidicola* in aqueous suspension of higher densities of sterile and non-sterile pollen of *Zea mays* at 30° C in light intensity of 76 lux in 24 hours.

Pollen Treatment	Pollen density in conidial suspension (No. ml^{-1})	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (µm) from	
				Basal Cell	Distal Cell
Non-sterile	2000 – 2400	676	70.5	58.22	58.22
	3000 – 3500	614	66.4	62.48	62.48
	4000 – 4500	533	64.2	55.38	63.19
	5000 – 5500	433	59.9	54.67	50.41
	6000 – 7000	445	58.4	56.8	60.35
Sterile	2000 – 2400	484	65.5	53.96	47.57
	3000 – 3500	465	63.3	59.64	61.54
	4000 – 4500	503	53.4	61.06	62.51
	5000 – 5500	540	53.2	61.06	62.24
	6000 – 7000	546	48.2	53.96	58.93
Control	No pollen	651	83.0	59.64	67.45

TABLE 9

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* incubated in aqueous suspension of higher densities of pollen of *Zea mays* at 30°C in light intensity of 76 lux for 24 hours.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Pollen Treatment	Pollen density in Conidial Suspension (No. $m1^{-1}$)	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
Non-sterile	3000 – 3500	36	32	12	4	16
	4000 – 4500	40	20	8	8	24
	5000 – 5500	44	24	8	4	20
	6000 – 7000	48	20	8	4	20
Sterile	3000 – 3500	40	24	8	8	20
	4000 – 4500	44	16	12	4	24
	5000 – 5500	40	20	8	4	28
	6000 – 7000	44	8	8	4	24
Control	No pollen	33	22	12	8	25

TABLE 10

Germination of Conidia of *C.arachidicola* in aqueous suspension of non-sterile Zea *mays* pollen of density of 6000 – 7000 grains ml^{-1} amended with Glucose at 30°C in light intensity of 76 lux in 24 hours.

Treatment	Concentration of Glucose (%)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from	
				Apical Cell	Basal Cell
Without pollen	0.0	1069	83.2	81.5	79.2
	1.0	970	79.7	80.1	79.6
	2.0	1005	81.7	80.4	80.2
	4.0	996	86.9	77.3	73.8
	8.0	1048	87.3	78.7	76.6
With pollen	0.0	1002	67.5	65.4	62.3
	1.0	800	78.8	75.6	74.0
	2.0	977	80.0	80.2	78.6
	4.0	1146	74.7	77.5	76.0
	8.0	1010	80.6	74.0	72.7

TABLE 11

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated in aqueous suspension of non-sterile *Zea mays* pollen of density of 6000 – 7000 pollen grains ml^{-1} amended with Glucose at 30°C in light intensity of 76 lux in 24 hours.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Treatment	Concentration of Glucose (%)	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
Without pollen	1.0	28	36	16	8	12
	2.0	40	28	16	4	12
	4.0	44	24	12	4	16
	8.0	40	24	12	4	20
With pollen	1.0	32	40	12	8	8
	2.0	32	32	16	4	16
	4.0	40	32	8	4	16
	8.0	44	16	12	4	24

TABLE 12

Germination of conidia of *C.arachidicola* in aqueous suspension of non-sterile *Zea mays* pollen of density of 6000 – 7000 pollen grains ml^{-1} amended with Peptone at 30°C in light intensity of 76 lux in 24 hours.

Treatment	Concentration of Peptone (%)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from Apical Cell	Mean length of Germ tubes (μm) from Basal Cell
Without pollen	0.0	1069	83.2	81.5	79.2
	1.0	1019	73.5	71.4	68.8
	5.0	949	78.8	69.2	71.6
	1.0	1004	82.1	71.6	67.4
	2.0	873	82.7	58.78	58.76
With pollen	0.0	1002	67.5	65.4	62.3
	0.1	1092	77.5	80.1	79.6
	0.5	1282	73.6	80.4	80.2
	1.0	1081	72.9	77.3	73.8
	2.0	879	75.4	78.7	76.7

TABLE 13

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated in aqueous suspension of non-sterile pollen of *Zea mays* pollen of density of 6000 – 7000 pollen grains ml^{-1} amended with Peptone at 30°C in light intensity of 76 lux in 24 hours.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Treatment	Concentration of Peptone (%)	Apical And Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
Without pollen	0.1	28	40	12	8	12
	0.5	40	32	16	0	12
	1.0	40	24	12	4	20
	2.0	48	24	12	0	16
With pollen	0.1	32	36	16	8	8
	0.5	40	28	16	4	12
	1.0	44	20	12	4	20
	2.0	44	24	8	4	20

TABLE 14

Germination of Conidia of *C.arachidicola* in aqueous suspension of non-sterile pollen of *Zea mays*, of pollen of density of 6000 – 7000 pollen grains ml^{-1} containing Glucose and Peptone in different ratios, at 30°C in light intensity of 76 lux in 24 hours.

(Each value of Percentage Germination based on a total of 400 – 500 conidia)

Concentration of Glucose (%)	Concentration of Peptone (%)	Percentage Germination In suspension		Mean length of germ tubes (μm) from apical and basal cells of conidia in suspension			
		With pollen	Without pollen	With pollen		Without pollen	
				Apical Cell	Basal Cell	Apical Cell	Basal Cell
1.0	0.1	68.0	76.3	63.1	59.7	67.6	65.5
	0.5	74.3	75.1	65.6	66.9	67.8	65.4
	1.0	77.0	78.6	66.6	61.6	64.8	64.5
	2.0	78.2	84.8	66.2	64.6	64.2	65.3
2.0	0.1	79.2	83.4	71.4	65.4	75.6	72.6
	0.5	75.5	79.0	71.5	69.3	69.9	69.3
	1.0	85.7	78.5	71.5	69.6	65.3	64.2
	2.0	86.4	83.5	72.2	70.3	73.6	70.9

Table 14 Cont'd

		Percentage Germination In suspension		Mean length of germ tubes (μm) from apical and basal cells of conidia in suspension			
				With pollen		Without pollen	
Concentration of Glucose (%)	Concentration of Peptone (%)	With pollen	Without pollen	Apical Cell	Basal Cell	Apical Cell	Basal Cell
4.0	0.1	85.6	71.6	70.7	70.4	65.1	63.4
	0.5	79.2	75.6	69.3	68.2	64.5	65.3
	1.0	82.2	77.8	65.2	63.0	65.4	63.8
	2.0	80.0	73.0	67.2	68.3	75.2	74.1
8.0	0.1	81.7	81.1	65.6	63.7	74.3	71.3
	0.5	83.9	82.8	63.3	62.2	76.2	73.1
	1.0	83.7	81.0	62.2	59.6	72.3	71.9
	2.0	85.9	87.3	61.2	60.3	69.6	68.9

TABLE 15

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* incubated in aqueous suspension of non-sterile pollen of *Zea mays* of pollen density of 6000 – 7000 pollen grains ml^{-1} containing glucose and peptone in different ratios at 30°C in light of 76 lux for 24 hours.

Germination medium (without pollen: -) (with pollen: +)	Concentration of Glucose (%)	Concentration of Peptone (%)	Percentage of Conidia with germ tubes emerging from indicated cells				
			Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
-	1.0	0.1	32	36	12	8	12
		0.5	32	32	12	4	20
		1.0	36	28	8	4	24
		2.0	44	28	8	0	20
+	1.0	0.1	36	40	12	0	12
		0.5	32	36	12	0	20
		1.0	40	24	16	4	16
		2.0	40	20	12	8	20
-	2.0	0.1	32	32	12	8	16
		0.5	36	28	12	4	20
		1.0	40	24	16	0	20
		2.0	40	20	8	4	28
+	2.0	0.1	28	36	16	8	12
		0.5	32	28	12	8	20
		1.0	44	24	12	4	20
		2.0	40	28	8	0	24

Table 15 Cont'd.

Germination medium (without pollen: -) (with pollen: +)	Concentration of Glucose (%)	Concentration of Peptone (%)	Percentage of Conidia with germ tubes emerging from indicated cells				
			Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median cell
-	4.0	0.1	24	40	16	8	12
		0.5	32	32	12	8	16
		1.0	36	28	12	4	20
		2.0	40	28	8	0	24
+	4.0	0.1	28	32	16	8	16
		0.5	40	28	8	4	20
		1.0	40	24	12	0	20
		2.0	36	24	12	4	24
-	8.0	0.1	28	36	20	4	12
		0.5	36	28	16	4	16
		1.0	40	28	12	0	20
		2.0	40	24	12	4	20
+	8.0	0.1	28	36	16	8	12
		0.5	36	32	12	4	16
		1.0	40	24	12	0	24
		2.0	40	28	8	4	20

C. Influence of Exudates of maize pollen on pH of the germination medium

(a) Irrespective of the density of maize pollen grains added to the aqueous suspension, the pH of the media showed very slight changes (Table 16). The pH of the different suspensions ranged from pH 6.0 to pH 7.0, 3 hours after the introduction of the pollen grains, and from pH 6.0 to pH 6.5, 12 hours after introduction of the pollen grains. The effects of the exudates on the pH showed no relationship with density of the pollen grains.

(b) The data in Table 17 showed that the pollen of maize could affect the response of conidia of *C.arachidicola* to pH of the medium. In the absence of the pollen grains, the conidia germinated over the pH range of pH 4.0 to pH 9.0 with an optimum at pH 5.0 – pH 6.0. On addition of the pollen, however, conidial germination also occurred at pH 3.0 and 10.0, thus extending the pH range which supported germination. Germination at pH 3.0 was, however, tardy (5.2 per cent). The optimum pH anyway remained still at pH 5.0 – pH 6.0. The respective pH's did not influence the pattern of germ tube formation (Table 18).

TABLE 16

Hydrogen ion concentration of aqueous suspension of pollen of Zea *mays* recorded 3, 6, 9 and 12 hours after introduction of the pollen into sterile distilled water of pH 6.5 and incubated at 30°C.

Pollen Density (No ml^{-1})	**Nature of Pollen**	**pH of suspension after (h)**			
		3	**6**	**9**	**12**
500 – 600	Sterile	6.5	6.5	6.5	6.5
	Non-sterile	6.5	6.5	6.0	6.0
1200 – 1200	Sterile	7.0	6.5	6.5	6.5
	Non-sterile	6.0	6.0	6.0	6.5
1500 – 1800	Sterile	6.5	6.5	6.5	6.5
	Non-sterile	7.0	7.0	6.5	6.5
2000 – 2400	Sterile	6.5	6.5	6.5	6.5
	Non-sterile	6.5	6.5	6.5	6.0
3000 – 3500	Sterile	6.5	6.5	6.5	6.0
	Non-sterile	6.5	6.5	6.0	6.0
4000 – 4500	Sterile	6.5	6.5	6.5	6.5
	Non-sterile	7.0	7.0	6.0	6.0
5000 – 5500	Sterile	6.0	7.0	6.0	6.0
	Non-sterile	6.0	7.0	6.0	6.0
6000 – 7000	Sterile	6.5	7.0	7.0	6.0
	Non-sterile	6.0	7.0	7.0	6.0

TABLE 17

Effect of pH on germination of Conidia of *C.arachidicola* at 30°C in light intensity of 76 luix in 24 hours.

Treatment	pH	Total Number of Spore observed	Percentage Germination	Mean length of Germ tubes (μm) from Apical Cell	Mean length of Germ tubes (μm) from Basal Cell
Without pollen	3.0	430	0.0	-	-
	4.0	350	68.3	55.3	51.3
	5.0	411	80.2	68.2	73.6
	6.0	418	76.6	68.6	63.5
	7.0	398	51.8	49.4	51.2
	8.0	403	42.4	37.7	33.6
	9.0	379	39.7	32.5	29.8
	10.0	382	0.0	-	-
With pollen (500 – 600)	3.0	460	5.2	28.3	25.7
	4.0	410	56.5	51.6	48.5
	5.0	380	60.7	61.2	58.2
	6.0	418	59.1	60.4	57.4
	7.0	365	56.4	58.7	54.1
	8.0	340	50.2	52.3	53.3
	9.0	392	39.4	48.5	43.6
	10.0	403	28.7	36.2	30.2

TABLE 18

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated in buffer solution of various pH at 30°C in light intensity of 76 lux in 24 hours.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Treatment	pH	Apical And Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without pollen	3	0	0	0	0	0
	4	34	30	24	4	8
	5	40	26	18	10	6
	6	36	24	20	8	12
	7	30	36	28	12	4
	8	34	40	22	0	4
	9	28	38	24	0	0
	10	0	0	0	0	0
With pollen (500-600 ml-l)	3	-*	-	-	-	-
	4	28	34	22	10	6
	5	36	30	14	6	14
	6	36	28	24	8	4
	7	32	30	28	4	6
	8	34	32	24	8	2
	9	28	34	18	8	12
	10	32	34	22	6	6

- Germinated conidia were too few to allow evaluation.

D. Studies on the influence of maize pollen on germination of aged conidia of *C.arachidicola*

(a) The conidia were incubated at 75 – 100% R.H. to establish the threshold relative humidity above which germination would take place. The percentage germinations at the different relative humidities are shown in Table 19. The conidia did not germinate at 75%and 80% RH. Over the range of 85% – 100% R.H, which supported germination, percentage germination increased with increasing humidity. Thus, percentage germination at 85% RH was 11.4 per cent and 64.0 percent at 100% R.H. Relative humidity had marked effect on the germination pattern. Table 20 indicated clear trends.

i) The percentage of conidia bearing apical and basal germ tubes declined from 56 per cent, at 100% R.H with decreasing humidity to16 percent at 85% R.H

ii) The percentage of conidia bearing germ tubes from end cells and median cells also increased with increasing relative humidity.

iii) A reverse trend was shown by conidia bearing one germ tube only from one of the end cells and the percentage of conidia involved decreased with increasing relative humidity.

TABLE 19

Germination of Conidia of *C.arachidicola* at different relative humidities at 30°C in light intensity intensity of 76 lux in 24 hours.

Relative Humidity (%)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from	
			Apical Cell	Basal Cell
100	501	64.0	69.2	64.0
95	676	57.3	61.5	61.6
90	470	46.3	62.8	57.1
85	604	11.4	52.4	51.2
80	505	0.0	-	-
75	490	0.0	-	-

TABLE 20

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated at different relative humidities at 30°C in light intensity of 76 lux in 24 hours.

Relative Humidity (%)	Percentage of conidia with germ tubes emerging from indicated cells: Apical And Basal Cell Co-jointly	Apical Cell Only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
100	56	16	4	0	24
95	32	32	12	4	20
90	24	52	16	0	12
85	16	40	20	12	12
80	-	-	-	-	-
75	-	-	-	-	-

(b) The data in Tables 21 – 30 provide sufficient information on the survival of the conidia of *C.arachidicola* and the effect of maize pollen on germination of conidia of different ages. The important details could be summarised as follows:

i) Between 17 and 35 per cent of the conidia at the different relative humidities survived 50 days storage.

ii) The conidia survived best at 20% R.H as clearly shown by data on 10, 20 and 30 days old conidia which showed 76.0 and 80.2, 65.1 and 71.3, and, 51.0 and 53.2 per cent viability, respectively.

iii) Longevity decreased appreciably at Zero and 80% R.H:

Conidia at 0% R.H. showed 46.9 and 51.8, 42.9 and 46.2, and 34.1 and 35.5 per cent viability after 10, 20 and 30 days, respectively.

Conidia at 80% R.H showed 47.2 and 50.0, 30.5 and 35.7 and, 34.7 and 36.1 per cent viability after 10, 20 and 30 days, respectively.

iv) The lengths of the germ tubes produced in 24 hours at 30°C decreased as the age of the conidia increased.

v) Conidia stored at 20% R.H consistently produced the highest percentage of conidia producing germ tubes from both the apical and basal cells.

vi) Germination of conidia of different ages was not affected by pollen grains of maize, and conidia of the same age showed closely similar percentage germination in the presence and in the absence of the pollen grains of maize.

Some of these details are depicted in Figs 2 & 3.

vii) Conidia stored at 0% R.H for 40 and 50 days then showed the highest percentage viability (Tables 27 and 29) and viability decreased with increasing relative humidity of storage.

viii) The maize pollen did still not affect germinating 40 and 50 days old conidia. In the numerous germination tests carried out in this investigation the germ tubes of *C. arachidicola* were never attracted by the pollen grains in the suspension droplets.

TABLE 21

Percentage of conidia of *C.arachidicola* able to germinate in Distilled Water and Aqueous Suspension of non-sterile Pollen of *Zea mays* of density of 500 – 600 pollen grain ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H. at 30°C in light intensity of 76 lux for 10 days.

				Mean length of Germ tubes (µm) from	
Germination medium	Storage Humidity (% R.H)	Total Number of Conidia observed	Percentage Germination	Apical Cell	Basal Cell
Without pollen	80	364	47.2	47.2-	53.8
	60	342	68.3	55.3	51.3
	40	331	80.2	68.2	73.6
	20	600	76.6	68.6	63.5
	0	488	51.8	49.4	51.2
With pollen	80	362	50.0	56.6	55.1
	60	345	56.2	53.6	54.5
	40	435	64.1	56.5	58.5
	20	618	80.2	74.3	69.9
	0	415	46.9	50.2	50.3

TABLE 22

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* germinated in distilled water and aqueous suspension of non-sterile pollen of *Zea mays* of density 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light of 76 lux after storage at 0 – 80 % R.H at 30°C in light of 76 lux for 10 days.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Germination medium	Storage Humidity (%R.H)	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without	80	16	48	20	8	8
	60	32	36	12	4	16
	40	24	20	16	8	32
	20	40	12	4	4	40
	0	20	36	20	12	12
With pollen	80	12	40	24	12	12
	60	20	28	20	16	16
	40	32	24	12	8	24
	20	52	16	12	4	16
	0	16	44	16	8	16

TABLE 23

Percentage of conidia of *C.arachidicola* able to germinate in Distilled Water and Aqueous Suspension of non-sterile Pollen of *Zea mays* of density of 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H. at 30°C in light intensity of 76 lux for 20 days.

Germination medium	Storage Humidity (% R.H)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from	
				Apical Cell	Basal Cell
Without pollen	80	397	30.5	46.8	41.9
	60	398	58.2	53.8	54.5
	40	432	63.6	59.9	56.5
	20	384	71.3	70.5	71.0
	0	396	46.2	40.0	51.3
With pollen	80	392	35.7	47.5	43.0
	60	390	55.1	56.3	57.2
	40	407	61.9	62.5	60.0
	20	381	65.1	71.0	76.6
	0	401	42.9	43.6	48.0

TABLE 24

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* germinated in distilled water and aqueous suspension of non-sterile pollen of *Zea mays* of density 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H at 30°C in light intensity of 76 lux for 20 days.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Germination medium	Storage Humidity (%R.H)	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without pollen	80	12	60	12	8	8
	60	16	48	20	12	4
	40	20	40	12	8	24
	20	56	20	4	4	20
	0	24	44	20	0	12
With pollen	80	16	60	12	8	4
	60	24	32	16	4	20
	40	20	40	24	4	12
	20	44	20	4	0	32
	0	24	28	16	4	28

TABLE 25

Percentage of conidia of *C.arachidicola* able to germinate in Distilled Water and Aqueous Suspension of non-sterile pollen of Zea mays of density of 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H. at 30°C in light intensity of 76 lux for 30 days.

Germination medium	Storage Humidity (% R.H)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from Apical Cell	Mean length of Germ tubes (μm) from Basal Cell
Without pollen	80	415	34.7	45.2	43.1
	60	432	37.3	50.3	51.4
	40	442	45.0	52.8	51.2
	20	370	53.2	62.0	60.5
	0	419	34.1	37.6	33.7
With pollen	80	413	36.1	49.8	49.2
	60	438	39.2	53.5	51.9
	40	403	44.4	54.9	50.0
	20	387	51.0	58.7	55.4
	0	414	35.5	36.9	33.2

TABLE 26

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* germinated in distilled water and aqueous suspension of non-sterile pollen of *Zea mays* of density 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80 % R.H at 30°C in light intensity of 76 lux for 30 days.

Germination medium	Storage Humidity (%R.H)	Percentage of Conidia with germ tubes emerging from indicated cells				
		Apical And Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without pollen	80	24	44	16	12	4
	60	20	36	12	4	28
	40	28	24	16	8	24
	20	48	16	20	12	4
	0	24	48	16	4	8
With pollen	80	12	52	28	0	8
	60	20	32	24	4	20
	40	24	40	20	0	16
	20	40	24	16	8	12
	0	16	56	24	4	0

TABLE 27

Percentage of conidia of *C.arachidicola* able to germinate in Distilled Water and Aqueous Suspension of non-sterile Pollen of *Zea mays* of density of 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H. at 30°C in light intensity of 76 lux for 40 days.

Germination medium	Storage Humidity (% R.H)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (μm) from	
				Apical Cell	Basal Cell
Without pollen	80	408	15.0	41.3	39.8
	60	450	17.1	44.5	41.7
	40	417	18.4	45.8	44.4
	20	405	20.9	53.0	50.8
	0	410	21.2	48.0	47.0
With pollen	80	418	13.1	40.1	37.7
	60	465	18.0	43.9	41.6
	40	399	18.0	46.2	45.4
	20	423	19.1	56.1	51.7
	0	424	20.8	48.1	47.2

TABLE 28

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* germinated in distilled water and aqueous suspension of non-sterile pollen of *Zea mays* of density 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80 % R.H at 30°C in light intensity of 76 lux for 40 days.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Germination medium	Storage Humidity (%R.H)	Apical And Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without pollen	80	20	48	8	4	20
	60	28	36	16	4	16
	40	36	40	8	0	16
	20	48	28	12	0	12
	0	24	56	4	0	16
With pollen	80	28	52	4	0	16
	60	40	40	8	4	8
	40	32	44	4	0	20
	20	44	32	8	4	12
	0	32	48	4	0	16

TABLE 29

Percentage of conidia of *C.arachidicola* able to germinate in Distilled Water and Aqueous Suspension of non-sterile Pollen of *Zea mays* of density of 500 – 600 pollen grain ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80% R.H. at 30°C in light intensity of 76 lux for 50 days.

Germination medium	Storage Humidity (% R.H)	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tube (μm) from	
				Apical Cell	Basal Cell
Without pollen	80	405	19.2	41.0	40.7
	60	440	25.4	47.7	43.1
	40	435	29.7	48.5	44.7
	20	436	34.3	51.5	45.9
	0	408	35.1	53.6	47.7
With pollen	80	430	17.3	42.2	39.0
	60	423	23.8	46.4	43.7
	40	435	26.4	48.0	43.3
	20	420	32.3	53.8	46.6
	0	434	33.7	52.4	48.6

TABLE 30

Pattern of formation of germ tubes by 50 randomly selected conidia of *C.arachidicola* germinated in distilled water and aqueous suspension of non-sterile pollen of *Zea mays* of density 500 – 600 pollen grains ml^{-1} in 24 hours at 30°C in light intensity of 76 lux after storage at 0 – 80 % R.H at 30°C in light intensity of 76 lux for 50 days.

		Percentage of Conidia with germ tubes emerging from indicated cells				
Germination medium	Storage Humidity (%R.H)	Apical And Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cell
Without pollen	80	24	44	4	4	24
	60	32	40	12	8	8
	40	40	32	16	0	12
	20	48	24	8	0	20
	0	20	52	20	4	4
With pollen	80	28	48	4	0	20
	60	36	40	8	4	12
	40	36	28	20	8	8
	20	56	20	12	0	12
	0	28	60	8	0	4

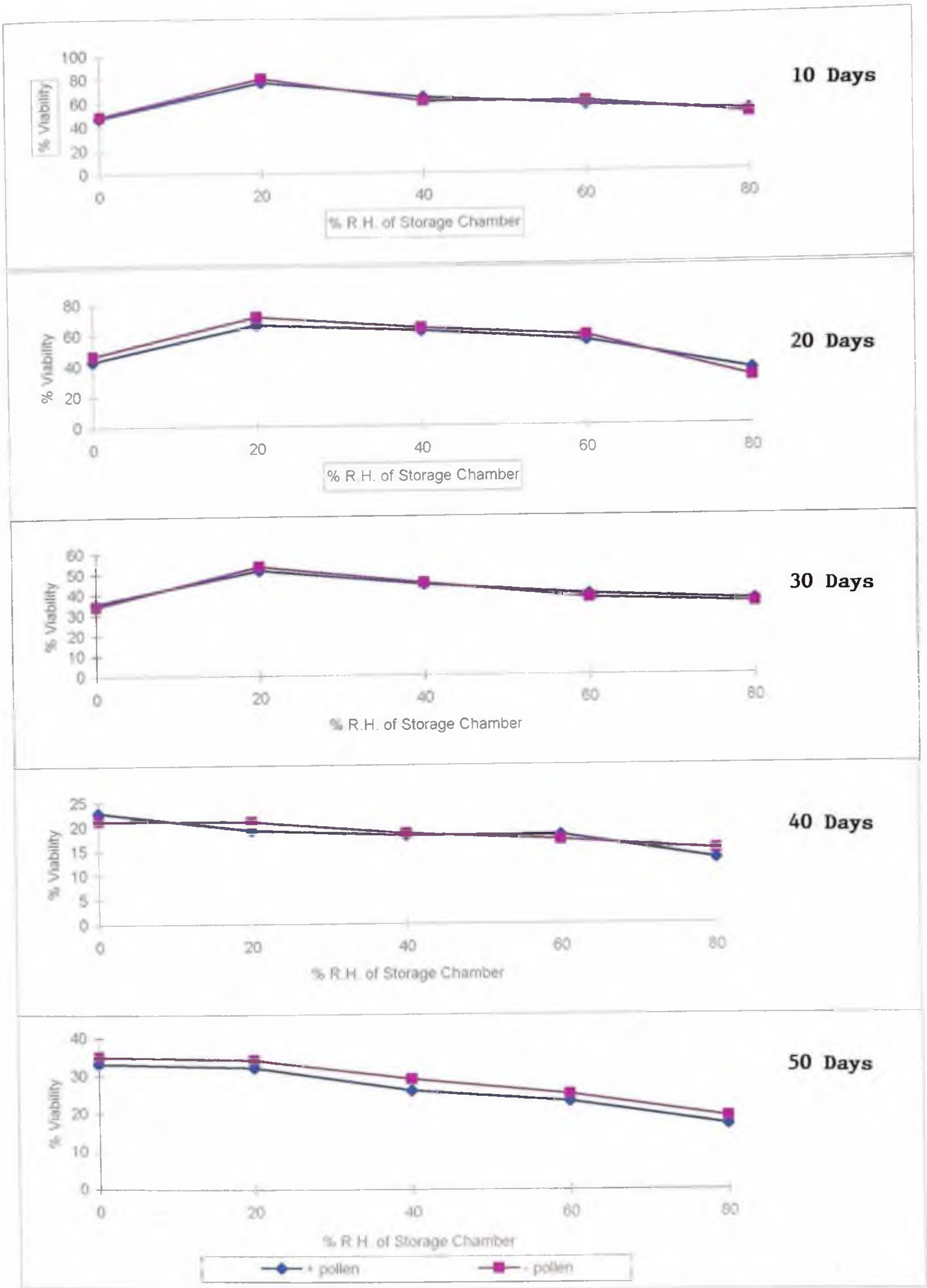

Fig.2A: Percentage viability of conidia of <u>Cercospora arachidicola</u> stored at 30°C in light of 76 lux for varying periods at different relative humidities.

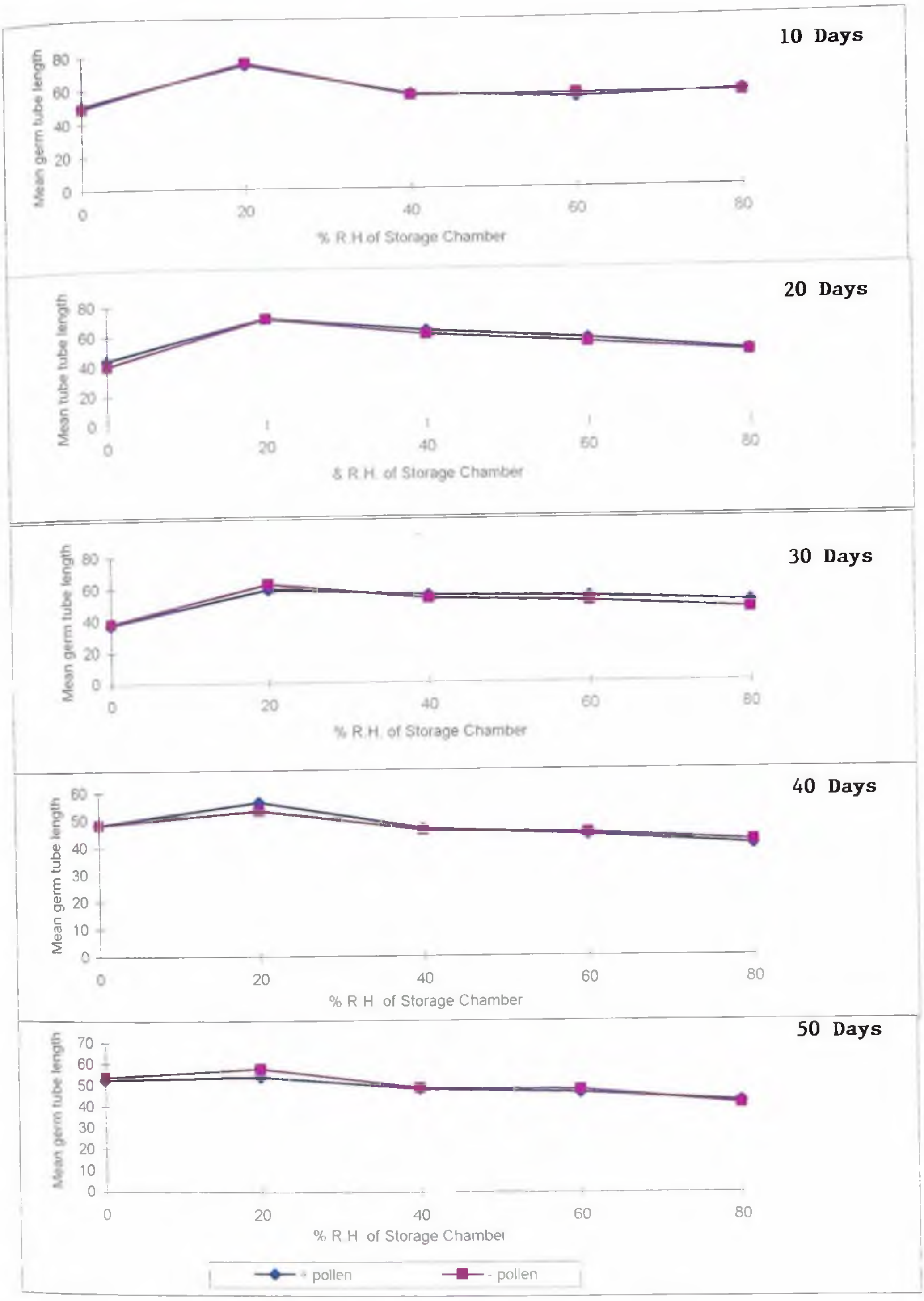

Fig.2b: Mean length of germ tubes produced by Apical cells of germinating conidia of *Cercospora arachidicola* stored at 30°C in light of 76 lux for varying periods at different relative humidities

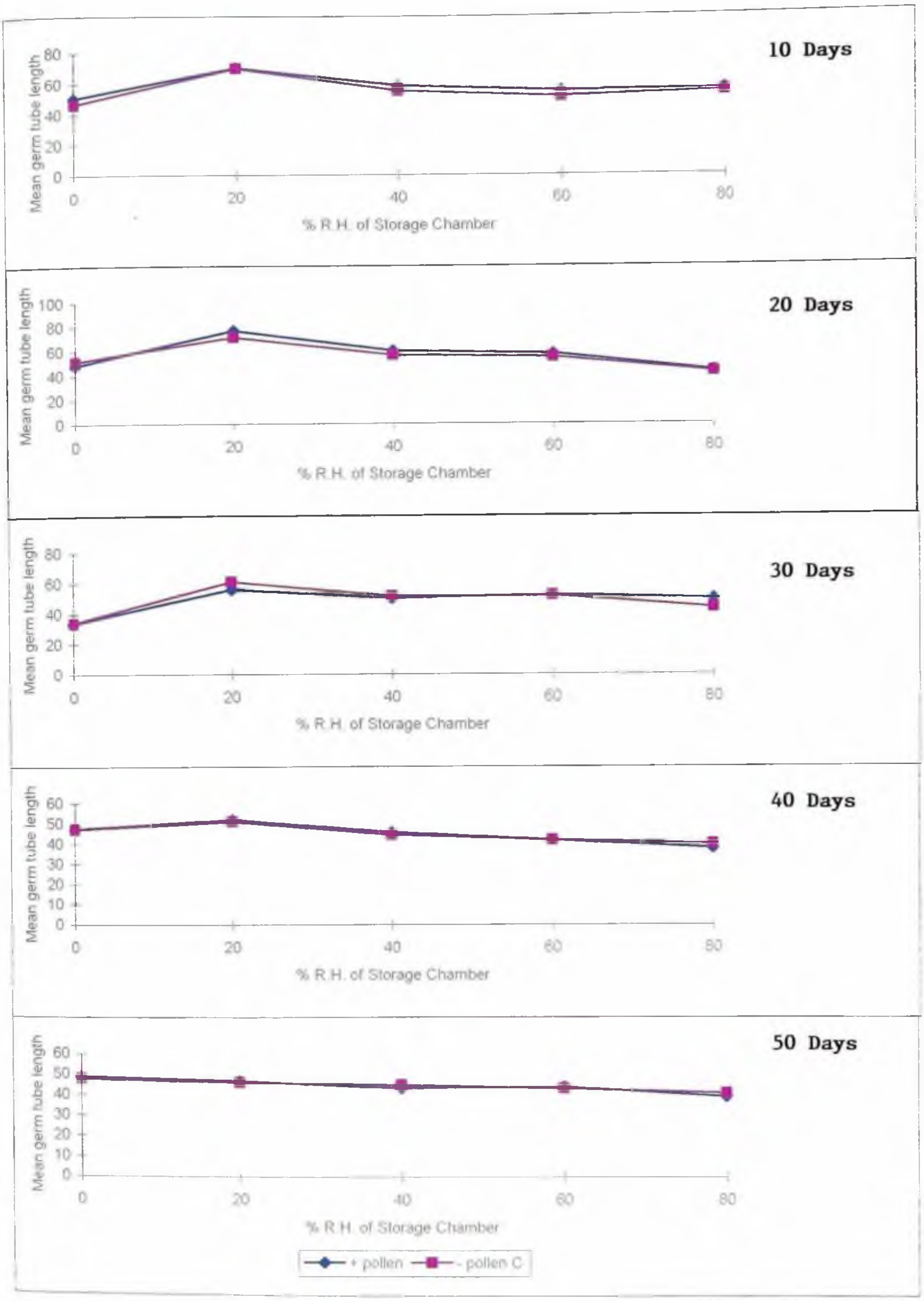

Fig.2c: Mean length of germ tubes produced by Basal cells of germinating conidia of Cercospora arachidicola stored at 30°C in light of 76 lux for varying periods at different relative humidities

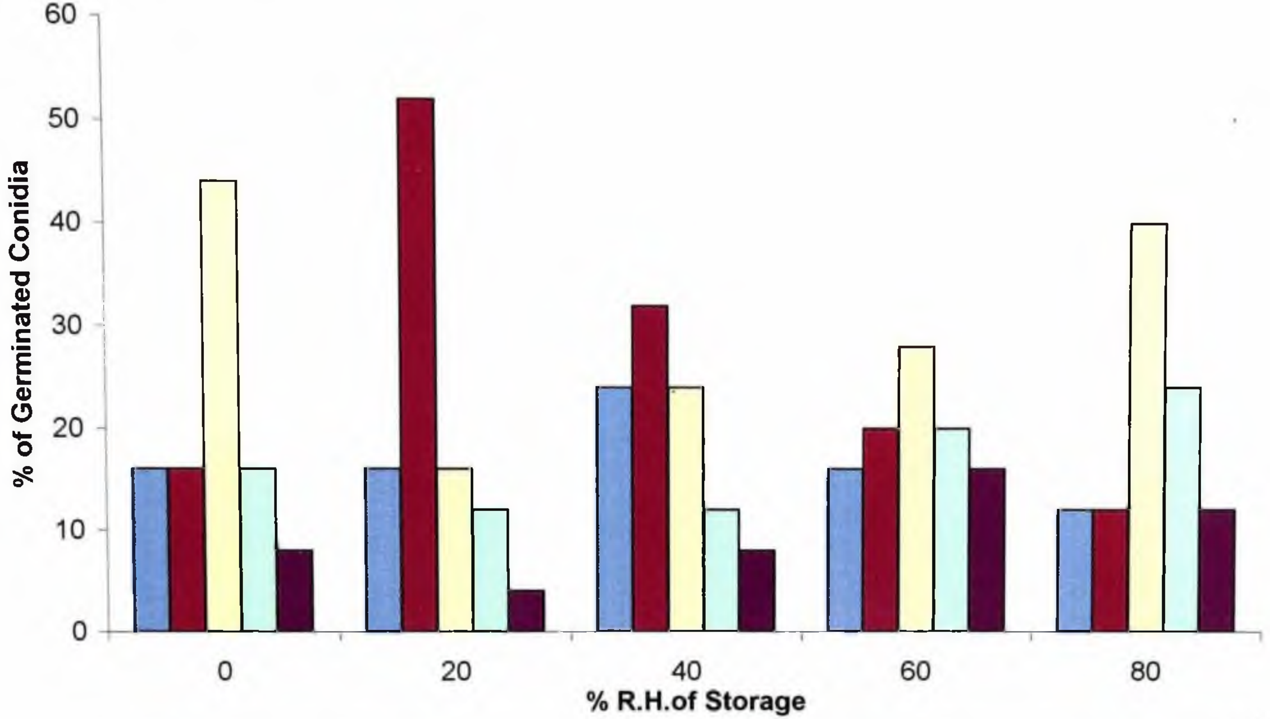

Fig. 3a Percentage of conidia of *C.arachidicola* stored at different relative humidities for 10 days and later germinated in distilled water at 30°C with maize pollen(500-600ml^{-1}) showing different patterns of germ tube development recorded after 24hrs of incubation

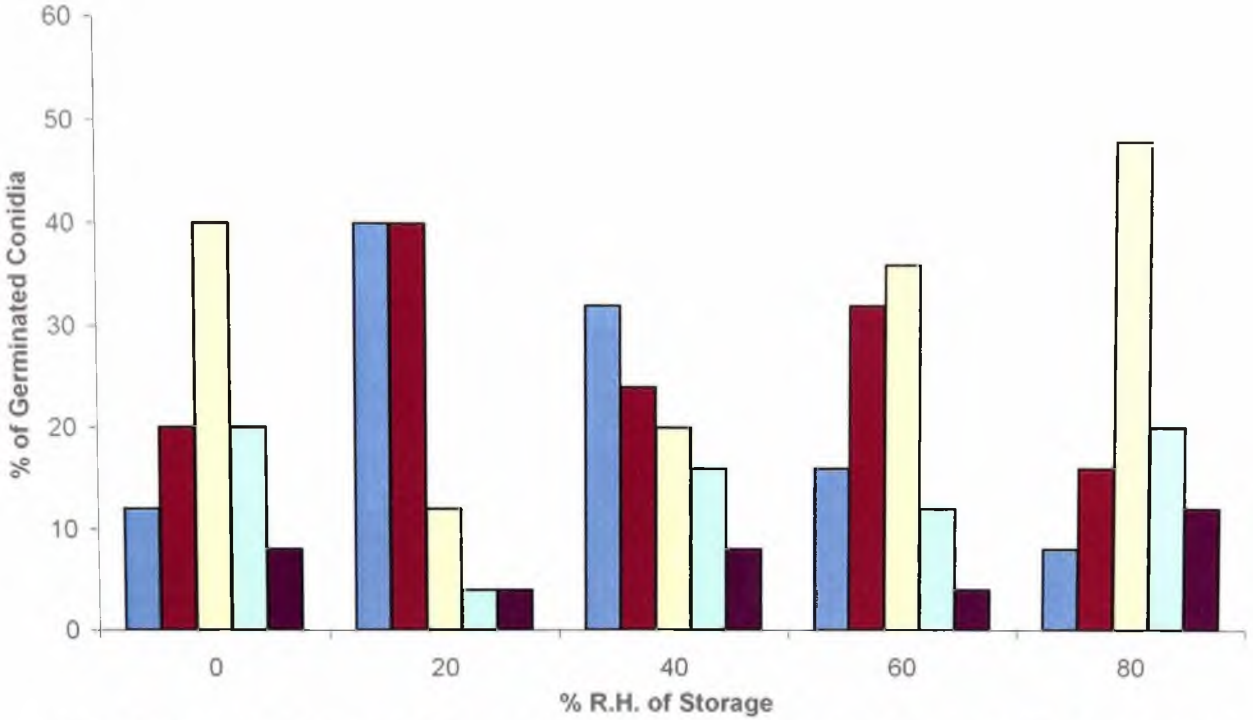

Fig.3b Percentage of conidia of *C.arachidicola* stored at different relative humidities for 10 days and later germinated in distilled water at 30°C without maize pollen showing different patterns of germ tube development recorded after 24hrs of incubation.

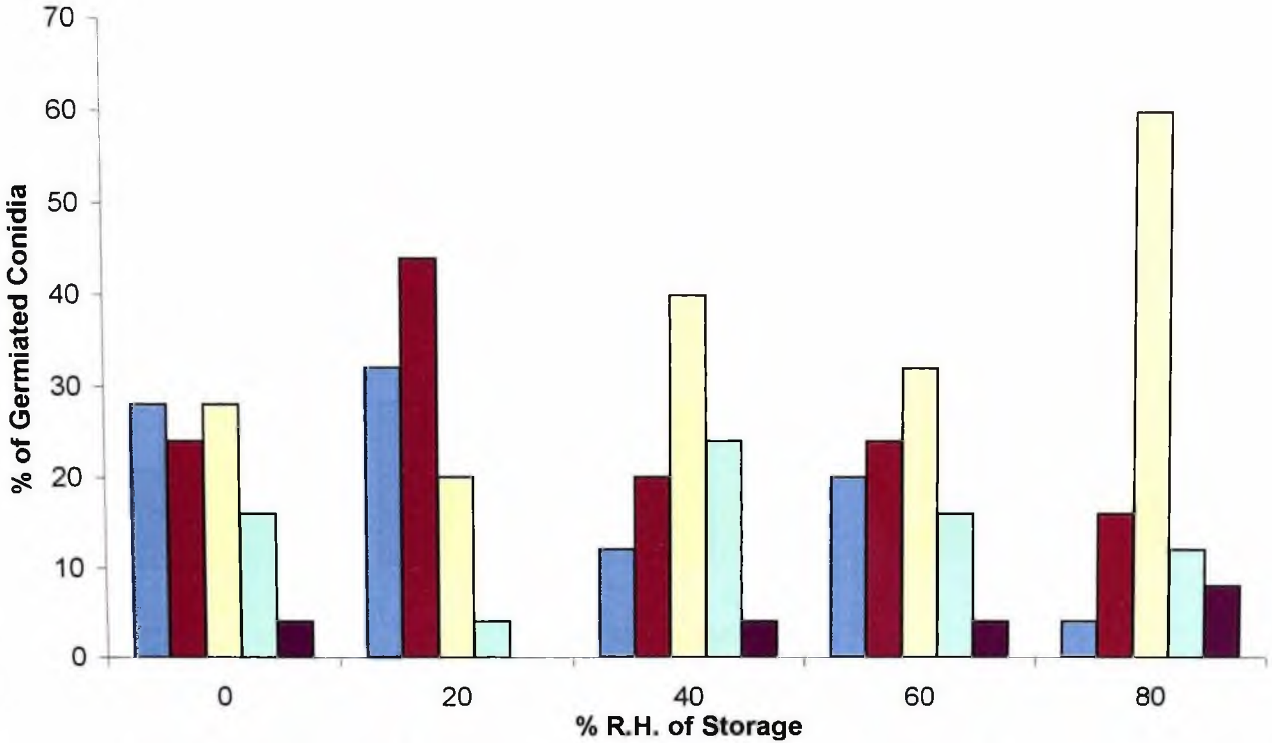

Fig. 3c Percentage of conidia of *C.arachidicola* stored at different relative humidities for 20 days and later germinated in distilled water at 30°C with maize pollen(500-600ml^{-1}) showing different patterns of germ tube development recorded after 24hrs of incubation

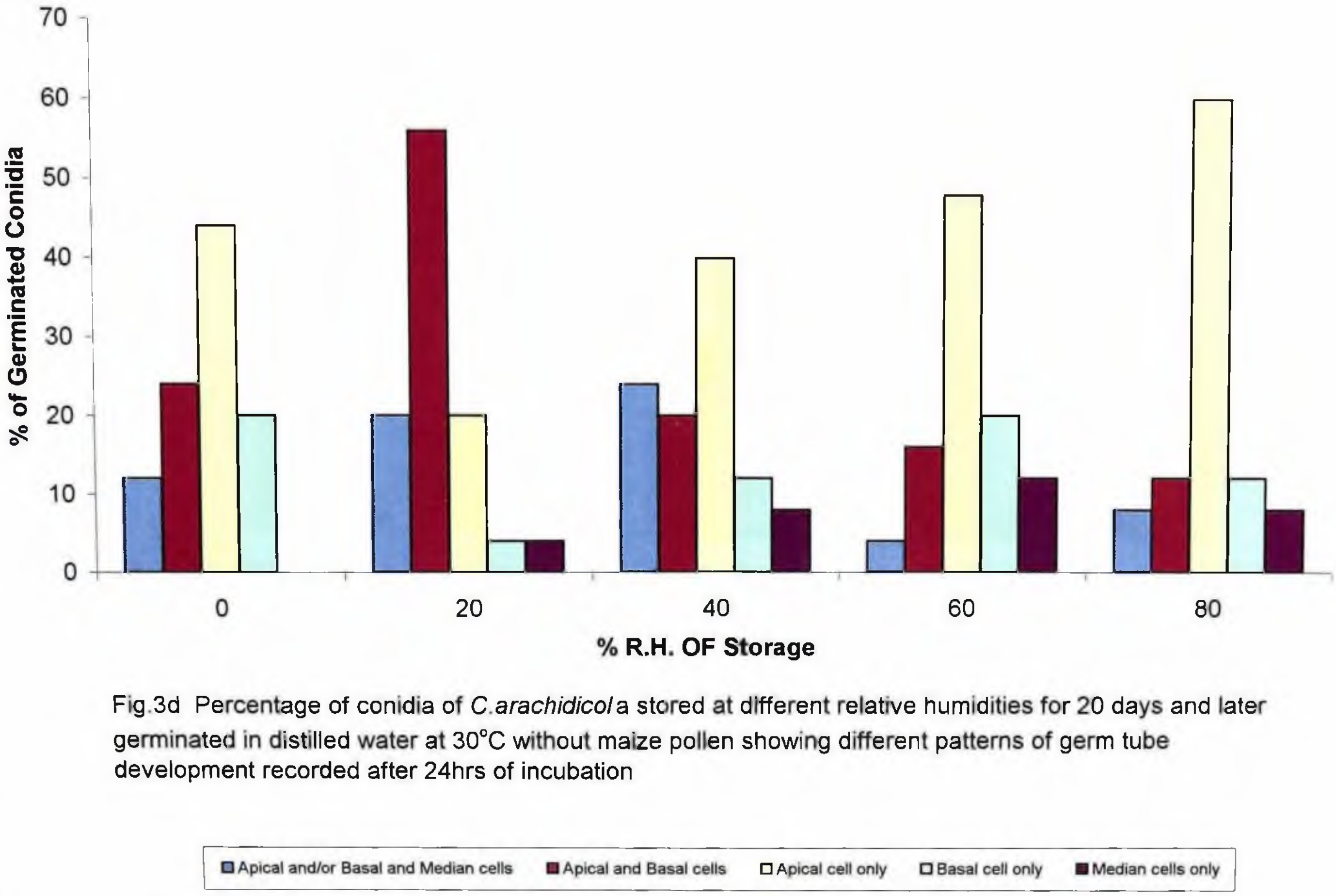

Fig.3d Percentage of conidia of *C.arachidicola* stored at different relative humidities for 20 days and later germinated in distilled water at 30°C without maize pollen showing different patterns of germ tube development recorded after 24hrs of incubation

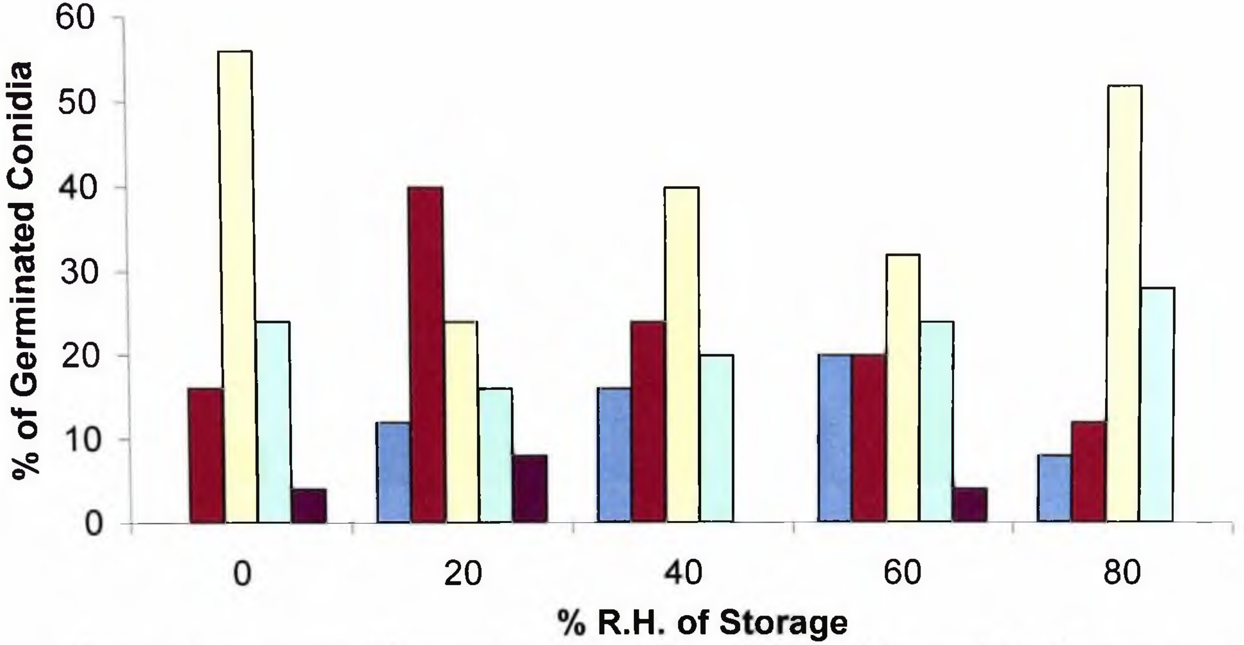

Fig. 3e Percentage of conidia of C *arachidicola* stored at different relative humidities for 30 days and later germinated in distilled water at 30°C with maize pollen(500-600m^{l-1}) showing different patterns of germ tube development recorded after 24hrs of. incubation

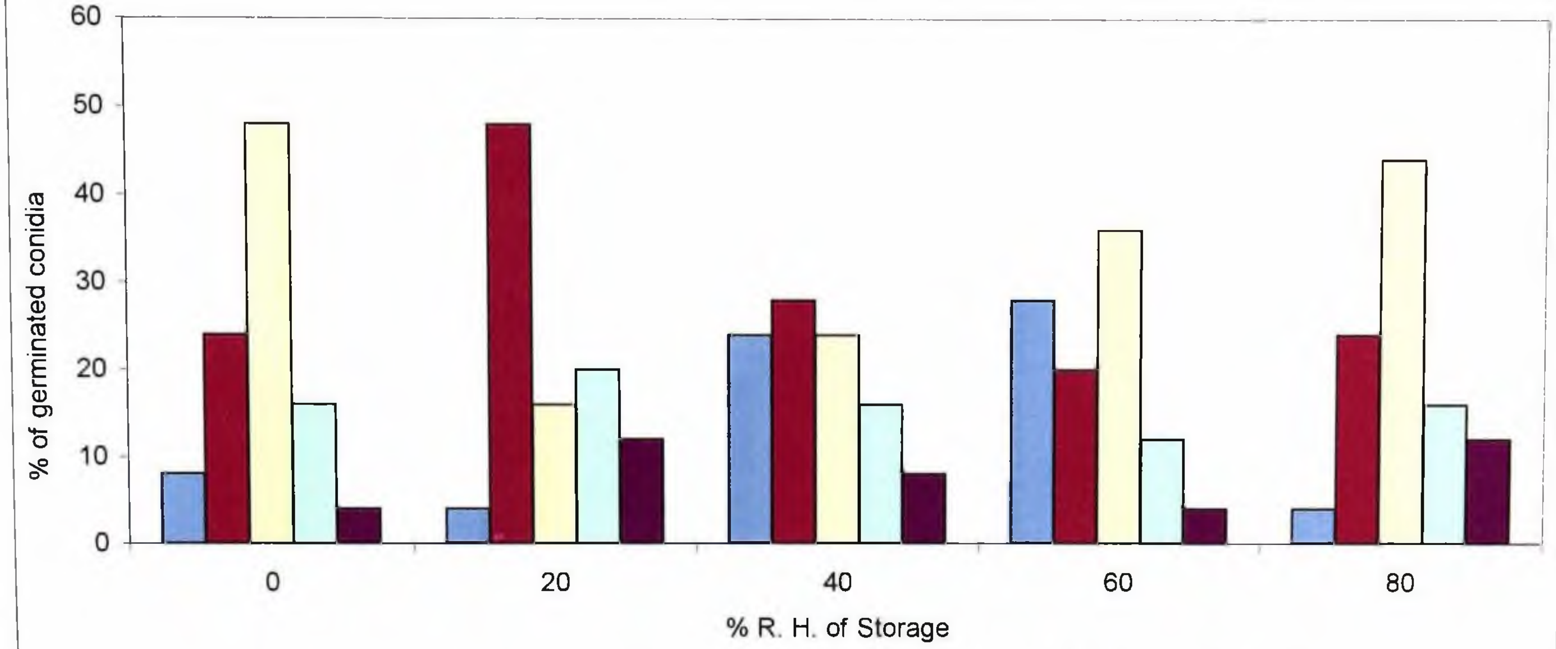

Fig. 3f:Percentage of conidia of *C.arachidicola* stored at different relative humidities for 30 days and later germinated in distilled water at 30°C without maize pollen showing different patterns of germ tube development recorded after 24hrs of incubation

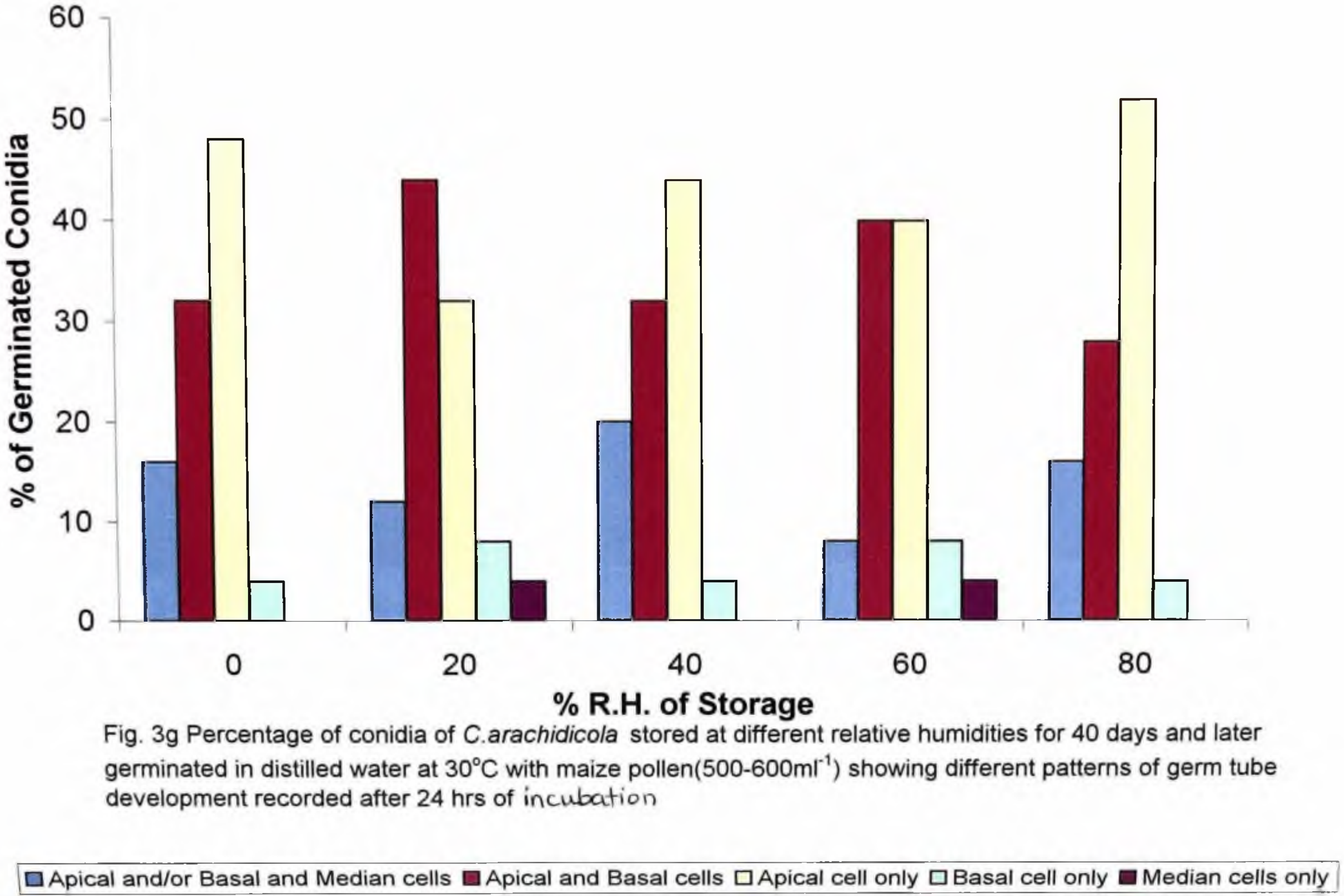

Fig. 3g Percentage of conidia of *C.arachidicola* stored at different relative humidities for 40 days and later germinated in distilled water at 30°C with maize pollen(500-600ml^{-1}) showing different patterns of germ tube development recorded after 24 hrs of incubation

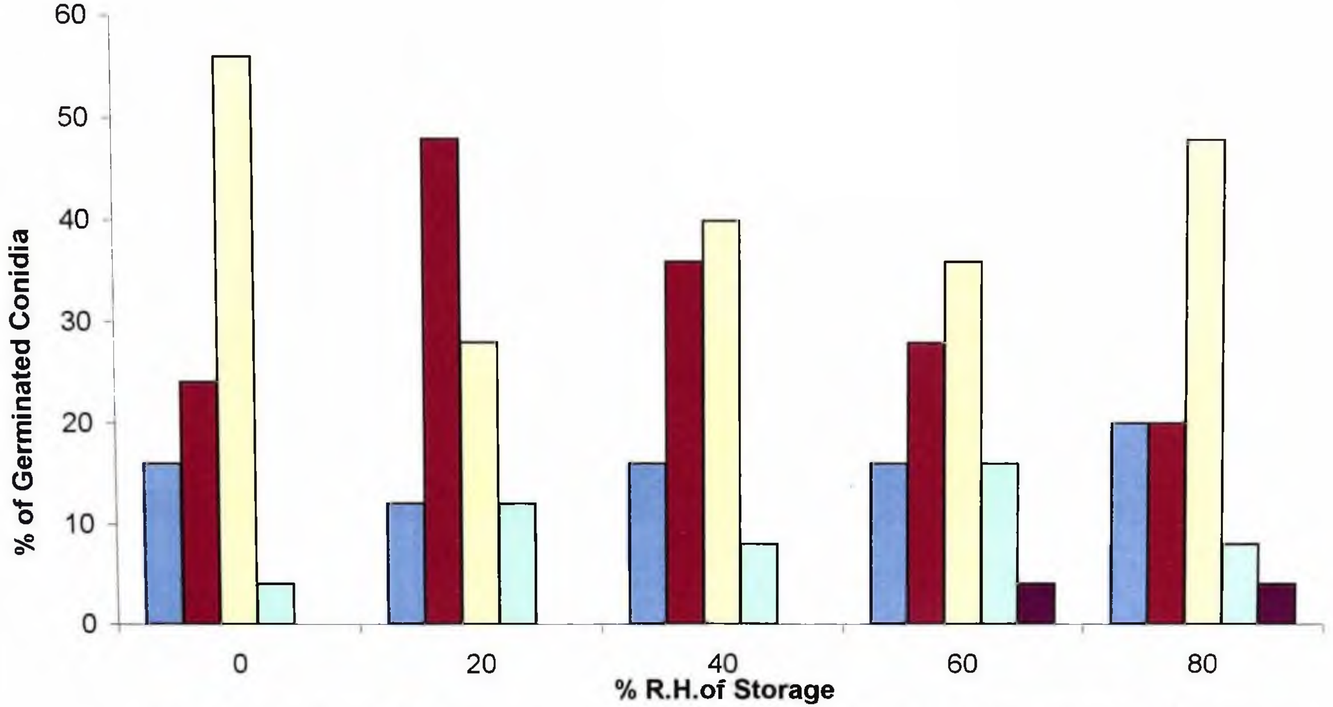

Fig. 3h Percentage of conidia of *C.arachidicola* stored at different relative humidities for 40 days and later germinated in distilled water at 30°C without maize pollen showing different patterns of germ tube development recorded after 24hrs of incubation

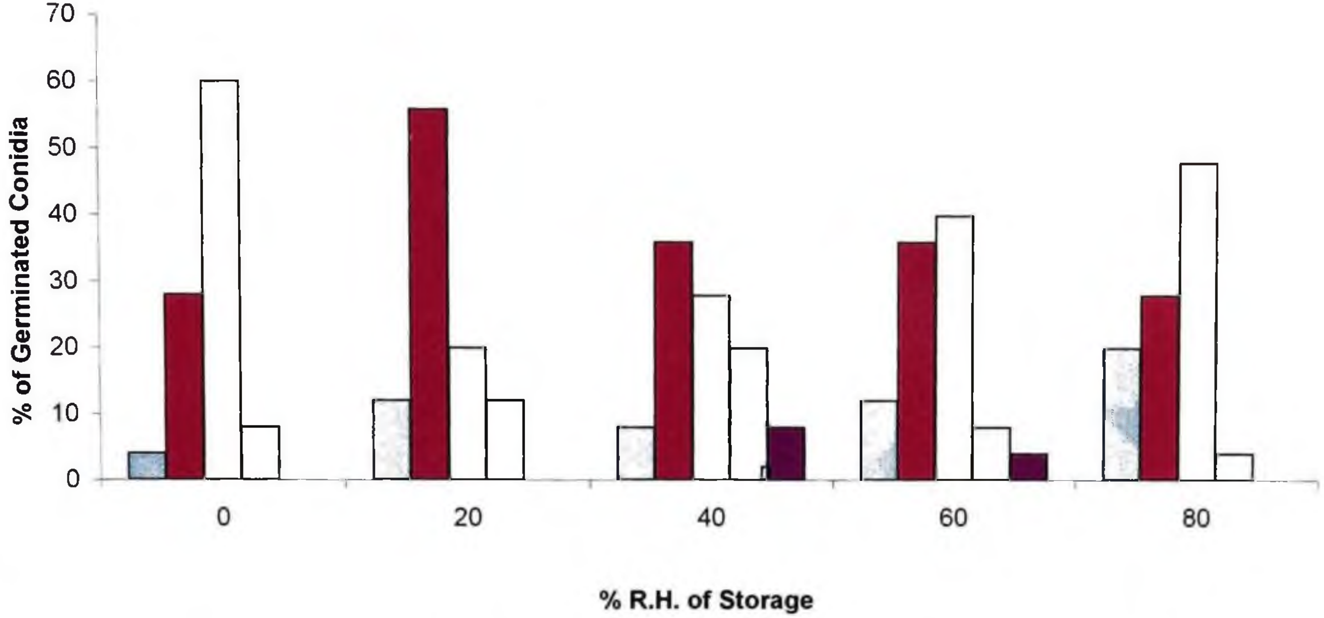

Fig. 3i Percentage of conidia of *C.arachidicola* stored at different relative humidities for 50 days and later germinated in distilled water at 30°C with maize pollen(500-600m^{l-1}) showing different patterns of germ tube development recorded after 24hrs of incubation

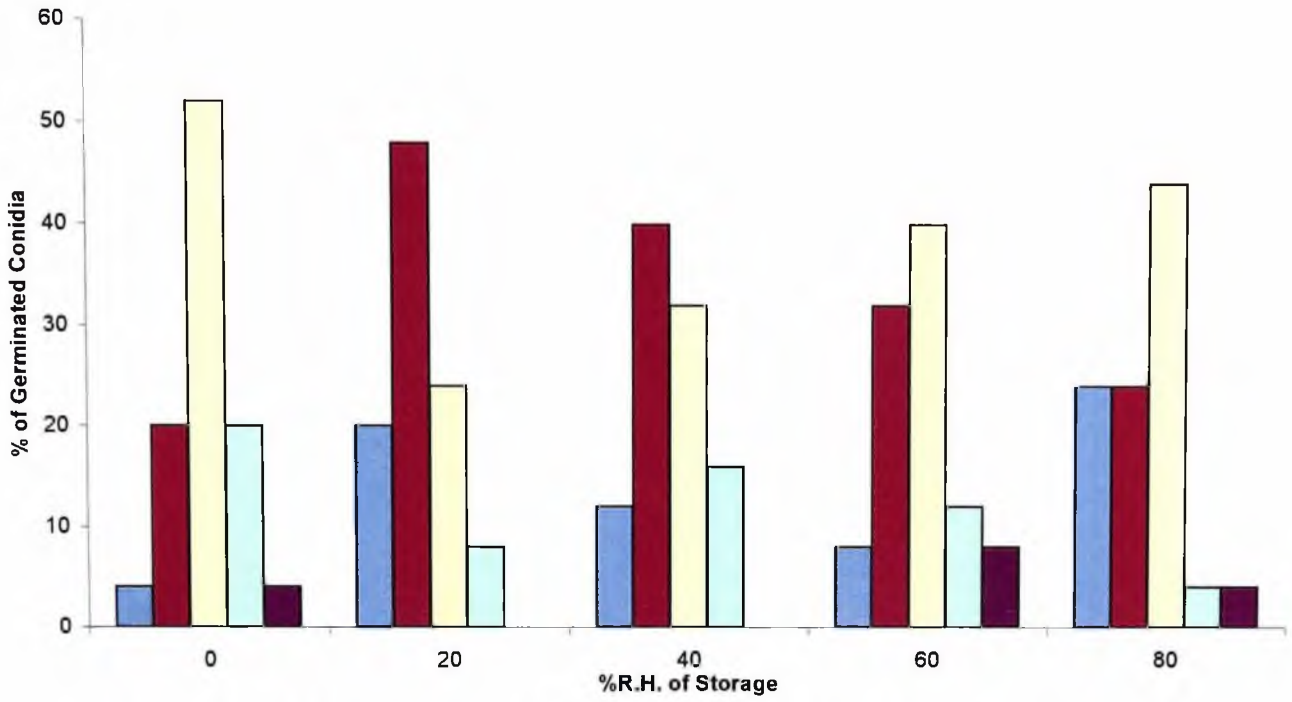

Fig.3j Percentage of conidia of *C.arachoidicola* stored at different relative humidities for 50 days and later germinated in distilled water at 30°C without maize pollen showing different patterns of germ tube development recorded after 24hrs of incubation

E. Germination of *C.arachidicola* conidia on groundnut leaflets with normal and reduced phylloplane microflora population

(a) Germination on the surface of groundnut leaflets of the conidia of *C.arachidicola* in suspension drops of different pollen densities, 0, 500 – 600, 1000 - 1200, 1500 – 1800 and 2000 – 2400 pollen grains ml^{-1} in all five tests carried out showed germination ranging from 31.0 to 52.9 per cent (Table 31). There was no recognised trend in relation to pollen density.

(b) Test of phytotoxicity of Hydrogen peroxide to the leaflets of groundnut identified the treatment, which could be tolerated by the leaflets as shown by the results tabulated in Table 32. The 'safe' treatments were application of 20% H_2O_2 and 30% H_2O_2 for only one minute and 10% H_2O_2 for two minutes.

(c) When the 'safe' treatments were used to disinfect the surface of groundnut leaflets, the population of the phylloplane bacteria (Table 33) and phylloplane fungi (Table 34) were reduced to different levels but were not totally removed (plates 1-4). The various treatments removed from 14.3 per cent (10% H_2O_2 for one minute) to 70.8 per cent (30% H_2O_2 for one minute) of bacterial flora and from 46.1 per cent (10%

H_2O_2 for one minute) to 87.5 per cent (10% H_2O_2 for two minutes and 20% H_2O_2 for one minute) of the fungal population.

(d) The data in Table 35 showed that the partial removal of the phylloplane microflora slightly improved the germination of the conidia on the leaflets of groundnut. Without pollen, germination on the unsterilized and surface-sterilized leaflets was 50.7 and 55.3 per cent, respectively and with pollen, the respective range of percentage germination was 37.4 – 48.7 and 42.5 – 51.8 percent. The pattern of germ tube development presented in Table 36 did not indicate any clear effect of surface sterilization of the leaflets.

TABLE 31

Germination of conidia of *C.arachidicola* in drops of aqueous suspension of non-sterile *Zea mays* pollen on the abaxial surface of freshly detached leaflets of groundnut lying on sterile moist filter paper at 30°C in light intensity of 76 lux in 24 hours.

Test No.	Pollen density (No. ml^{-1})	Total No. of conidia observed	Percentage Germination
1	0	492	52.4
	500 – 600	553	43.7
	1000 – 1200	499	40.0
	1500 – 1800	576	50.5
	2000 – 2400	413	49.2
2	0	486	50.2
	500 – 600	462	38.1
	1000 – 1200	452	31.0
	1500 – 1800	402	42.3
	2000 – 2400	438	44.7
3	0	390	41.5
	500 – 600	438	52.9
	1000 – 1200	422	30.3
	1500 – 1800	488	44.2
	2000 – 2400	408	33.3
4	0	418	36.2
	500 – 600	392	34.2
	1000 – 1200	488	37.0
	1500 – 1800	466	33.5
	2000 – 2400	514	36.9
5	0	428	43.9
	500 – 600	434	35.9
	1000 – 1200	470	37.2
	1500 – 1800	460	36.5
	2000 – 2400	440	47.7

TABLE 32

Reaction of leaflets of groundnut immersed for varying periods in Hydrogen peroxide (H_2O_2) solutions of different concentrations at 30°C.

Concentration of H_2O_2 (%)	Period of Immersion (minutes)	Bronzing of Leaves
10	1	-
	2	-
	4	+
	8	+
20	1	-
	2	+
	4	+
	8	+
30	1	-
	2	+
	4	+
	8	+

TABLE 33

Effectiveness of Hydrogen Peroxide (H_2O_2) solution as groundnut leaf surface sterilant against phylloplane bacteria

Test No.	Concentration of H_2O_2 (%)	Period of immersion of leaflets in H_2O_2 solution (minutes)	Mean No. of Bacterial colony – forming Units (CFU) per me of leaf washing (to the nearest whole number)
1	10	1	148
	10	2	125
	20	1	100
	30	1	70
	Untreated leaflet	-	240
2	10	1	120
	10	2	80
	20	1	73
	30	1	53
	Untreated leaflet	-	140
3	10	1	187
	10	2	124
	20	1	123
	30	1	95
	Untreated leaflet	-	226

TABLE 34

Effectiveness of Hydrogen Peroxide (H_20_2) solution as groundnut leaf surface sterilant against phylloplane fungi

Test No.	Concentration of H_2O_2 (%)	Period of immersion of leaflets in H_2O_2 solution (minutes)	Mean No. of Bacterial colony – forming Units (CFU) per me of leaf washing (to the nearest whole number)
1	10	1	11
	10	2	6
	20	1	8
	30	1	6
	Untreated leaflet	-	48
2	10	1	16
	10	2	8
	20	1	5
	30	1	4
	Untreated leaflet	-	29
3	10	1	7
	10	2	4
	20	1	3
	30	1	3
	Untreated leaflet	-	13

TABLE 35

Germination of Conidia of *C.arachidicola* in aqueous suspension of non-sterile *Zea mays* pollen placed on abaxial surface of unsterilized and 30% H_20_2 – treated surface-sterilized leaflets of groundnut at 30°C in light intensity of 76 lux in 24 hours.

Leaf Treatment	Pollen Density (No. ml^{-1})	Total Number of Conidia observed	Percentage Germination	Mean length of Germ tubes (µm) from Apical Cell	Mean length of Germ tubes (µm) from Basal Cell
Unsterilized	0	350	50.7	56.3	53.7
	500 – 600	420	48.3	54.6	55.3
	1000 – 1200	330	37.5	48.7	45.2
	1500 – 1800	310	38.2	50.4	47.0
	2000 – 2400	380	44.4	49.0	48.0
Surface-Sterilized	0	420	55.6	60.6	58.3
	500 – 600	402	51.3	54.6	53.6
	1000 – 1200	380	49.5	55.0	50.4
	1500 – 1800	350	46.2	48.2	46.2
	2000 – 2400	450	42.4	48.0	47.7

TABLE 36

Pattern of formation of germ tubes by 50 randomly selected germinated conidia of *C.arachidicola* incubated in aqueous suspension of non-sterile Zea *mays* pollen placed on abaxial surface of unsterilized and H_2O_2 treated surface-sterilized leaflets of groundnut at 30°C in light intensity of 76 lux for 24 hours

		Percentage of Conidia with germ tubes emerging from indicated cells				
Leaf Treatment	Pollen Density in conidial suspension (No. ml^{-1})	Apical and Basal Cell Co-jointly	Apical Cell only	Basal cell only	Median cell(s) only	Apical and/or Basal cell and Median Cells
Unsterilized	0	24	30	16	14	16
	500 – 600	30	30	20	6	14
	1000 – 1200	40	20	12	0	28
	1500 –1800	36	24	10	14	16
	2000 – 2400	44	28	8	8	12
Surface Sterilized	0	34	26	16	10	14
	500 – 600	40	24	18	6	12
	1000 – 1200	32	28	22	8	20
	1500 –1800	48	36	10	0	6
	2000 – 2400	36	30	14	10	10

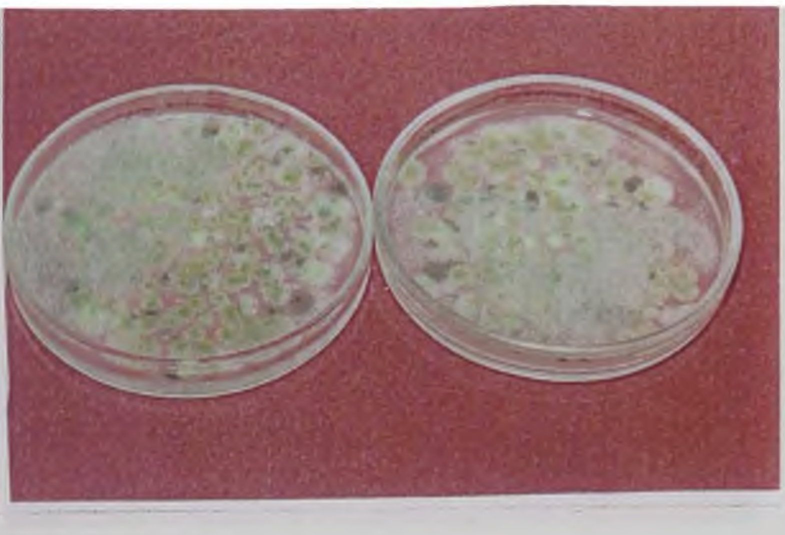

PLATE 1 Photograph showing residual phylloplane fungal Colony - Forming Units of washing of Non-sterile Groundnut leaflets on Potato Dextrose Agar after incubation for 5 days at 30°C (x 5/9)

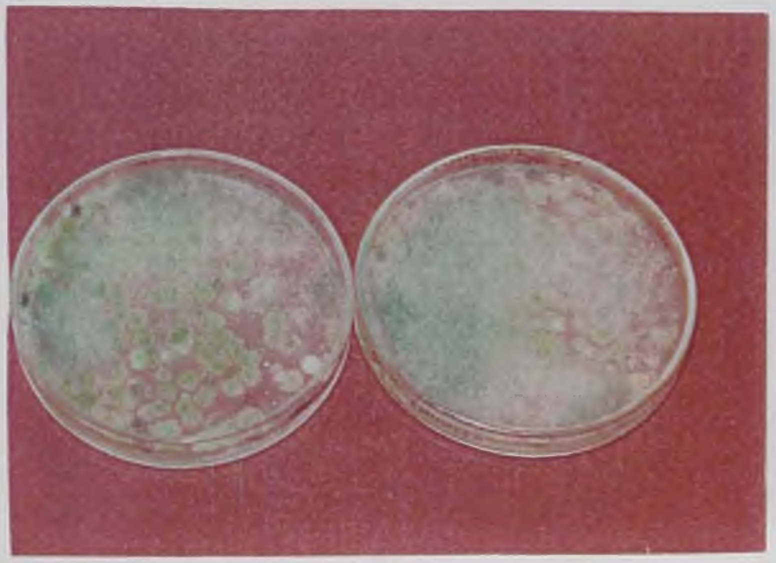

PLATE 2: Photograph showing residual phylloplane fungal Colony - Forming Units of washing of Groundut leaflets previously sterilized with 10% H_2O_2 on Potato Dextrose Agar after incubation for 5 days at 30°C (x5/9)

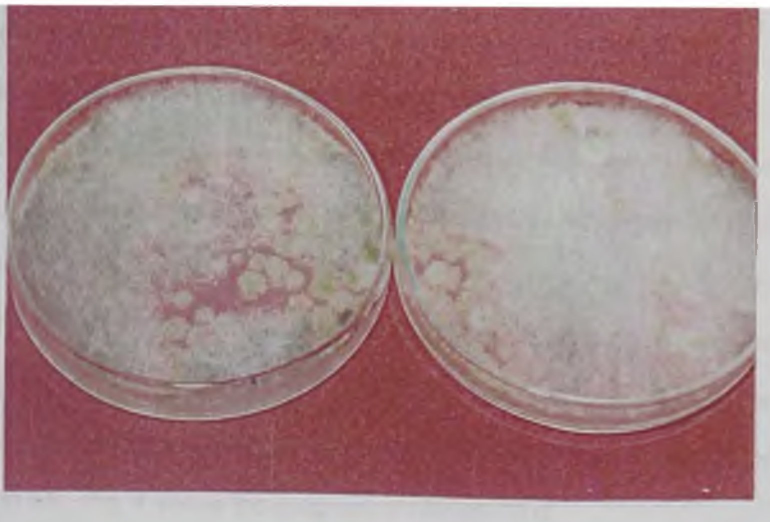

PLATE 3:

Photograph showing residual phylloplane fungal Colony - Forming Units of washing of Groundut leaflets previously sterilized with 20% H_2O_2 on Potato Dextrose Agar after incubation for 5 days at 30^{0}C (x5/9)

PLATE 4:

Photograph showing residual phylloplane fungal Colony - Forming Units of washing of Groundut leaflets previously sterilized with 30% H_2O_2 on Potato Dextrose Agar after incubation for 5 days at 30^{0}C (x5/9)

F. Influence of Non-sterile Pollen of Maize on the infection of groundnut leaflets by conidia of *C.arachidicola.*

The results of inoculation tests using non-sterilized and surface-sterilized groundnut leaflets and pollen grains at different densities are shown in Table 37. It was noteworthy that at the higher pollen densities of 4000 – 4500 and 6000 – 7000 pollen grains ml^{-1} suspending medium, C.arachidicola did not infect the unsterilized leaflets. The first leaf spots appeared in 4 – 6 days after inoculation, and with one exception the rate of infection after 8 days ranged from 2/8, to 5/8. Fig. 4 shows the progress of infection and

Plates 5 shows photographs of the inoculated leaves on the last days of incubation.

TABLE 37

Infection of eight groundnut leaflets in each set previously surface-disinfected with different concentrations of H_2O_2 before inoculation with suspension of conidia of *C.arachidicola* containing pollen of *Zea mays* and incubated under continuous white fluorescent light of 76 lux at 30°C.

Density of Pollen grains (ml^{-1})	Leaf disinfected with indicated H_2O_2 concentration (%)	Time of appearance of first leaf spots after inoculation (Days) and number of leaflets infected in parenthesis	No of leaflets infected after 8 days
0	0	4 (1)	4
	10	5 (2)	4
	20	5 (2)	5
	30	5 (1)	6
1000 – 1200	0	5 (1)	2
	10	4 (1)	4
	20	4 (2)	3
	30	4 (1)	5
2000 – 2400	0	6 (4)	4
	10	5 (2)	4
	20	4 (1)	3
	30	5 (1)	5
4000 – 4500	0	-	0
	10	6 (3)	3
	20	4 (2)	4
	30	4 (3)	6
6000 – 7000	0	-	0
	10	6 (2)	2
	20	6 (4)	4
	30	4 (1)	4

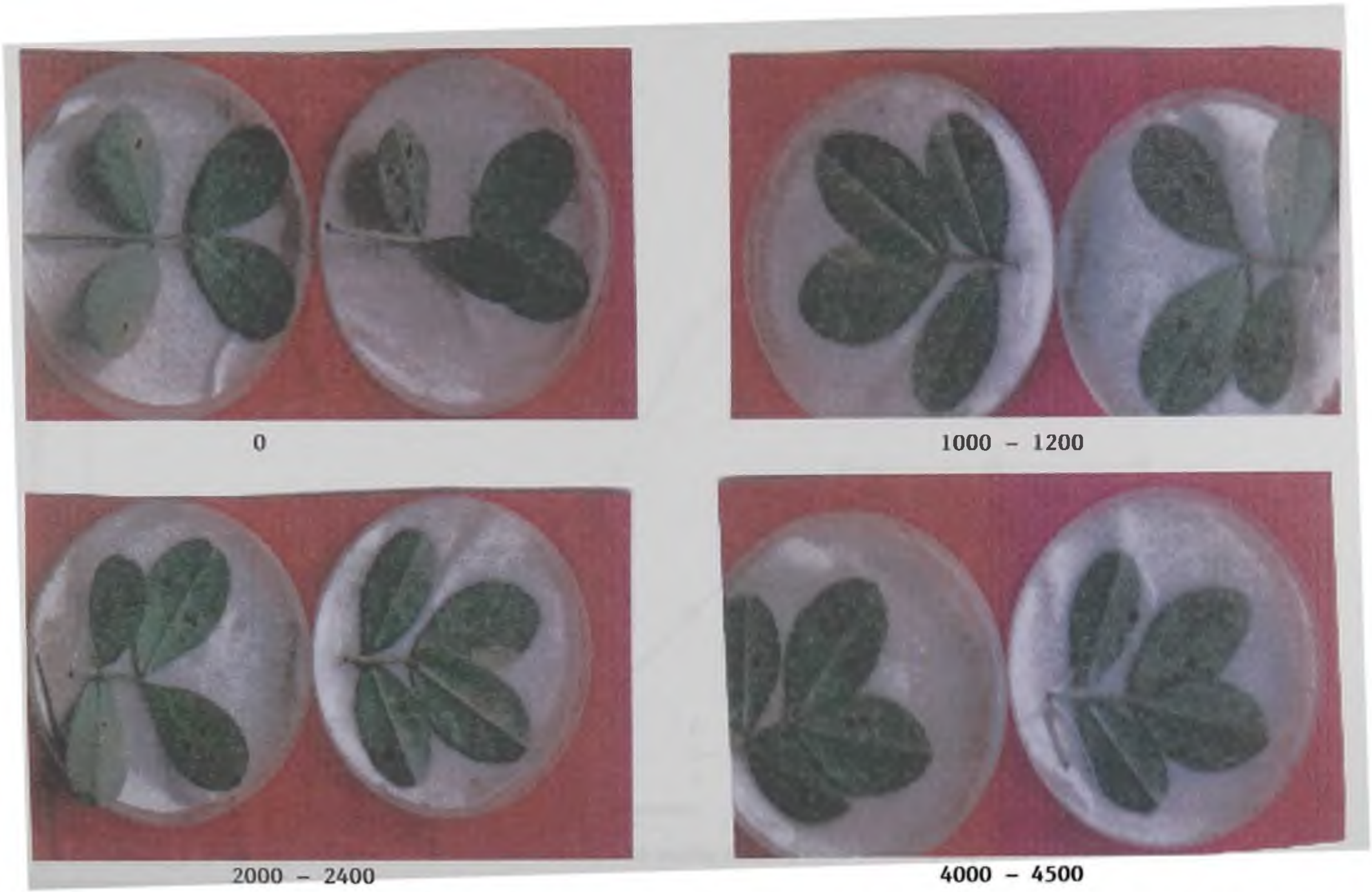

Plate 5: Photographs of previously surfaced-sterilized (treated with 30% H_2O_2 for 1 minute) Groundnut leaves showing C. arachidicola leafspots on the 8th day after inoculation with C. arachidicola conidia-maize pollen suspension and incubated under continuous fluorescent light in closed Petri dishes with humid internal atmosphere (x4/9).

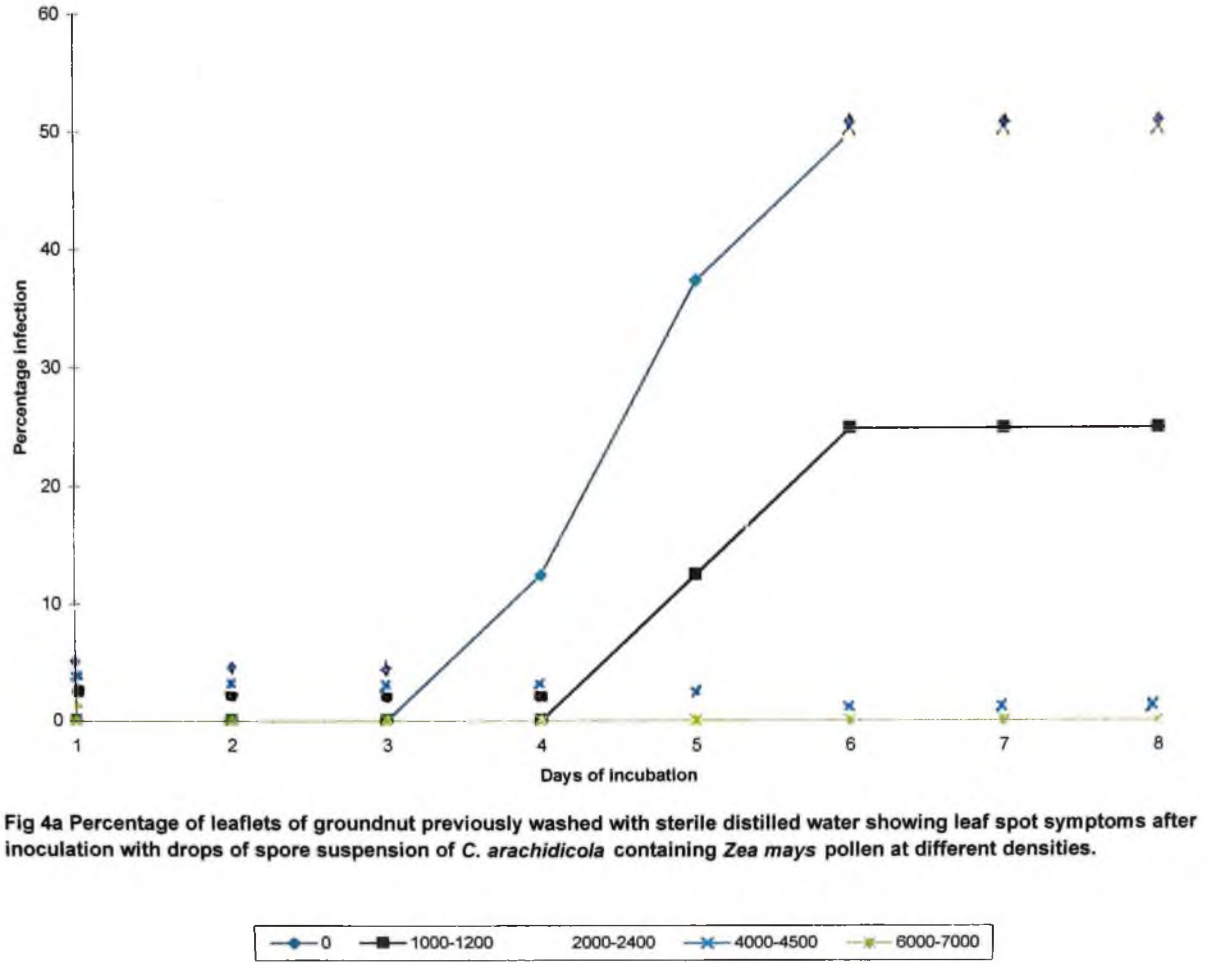

Fig 4a Percentage of leaflets of groundnut previously washed with sterile distilled water showing leaf spot symptoms after inoculation with drops of spore suspension of *C. arachidicola* containing *Zea mays* pollen at different densities.

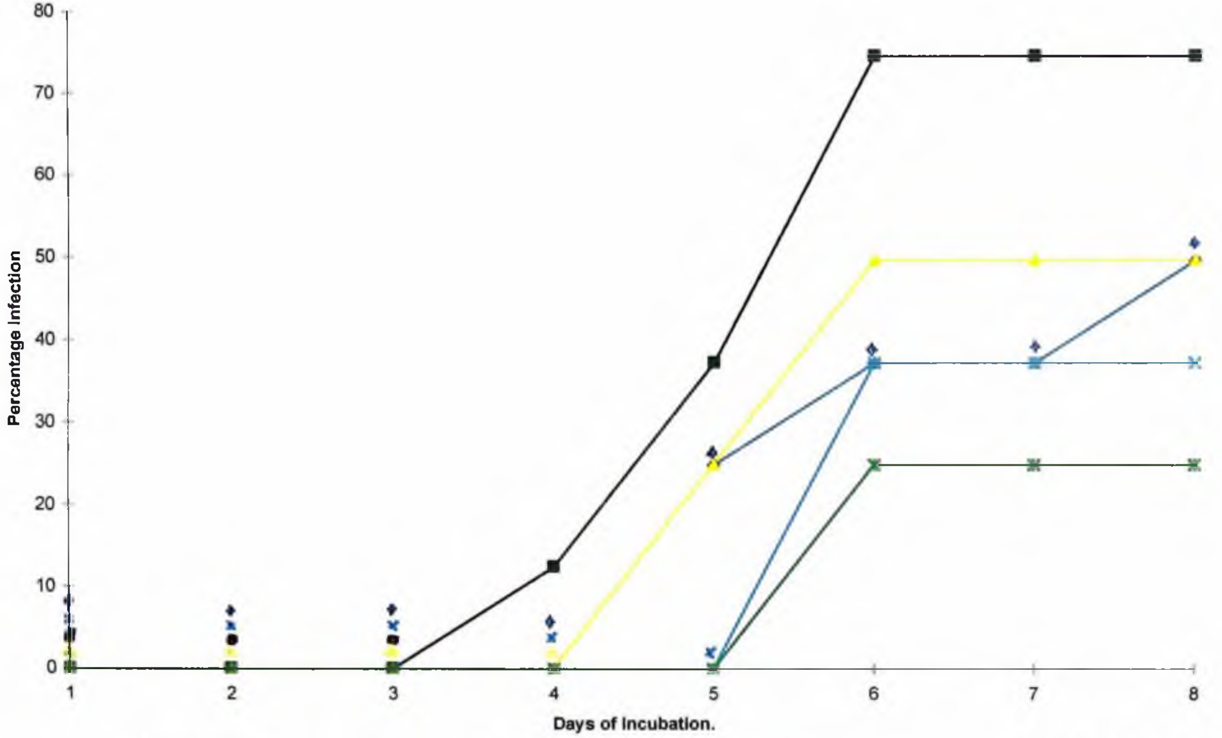

Fig 4b Percentage of leaflets of groundnut previously washed with 10% H_2O_2 solution showing leaf spot symptoms after inoculation with drops of spore suspension of *C. arachidicola* containing *Zea mays* pollen at different densities.

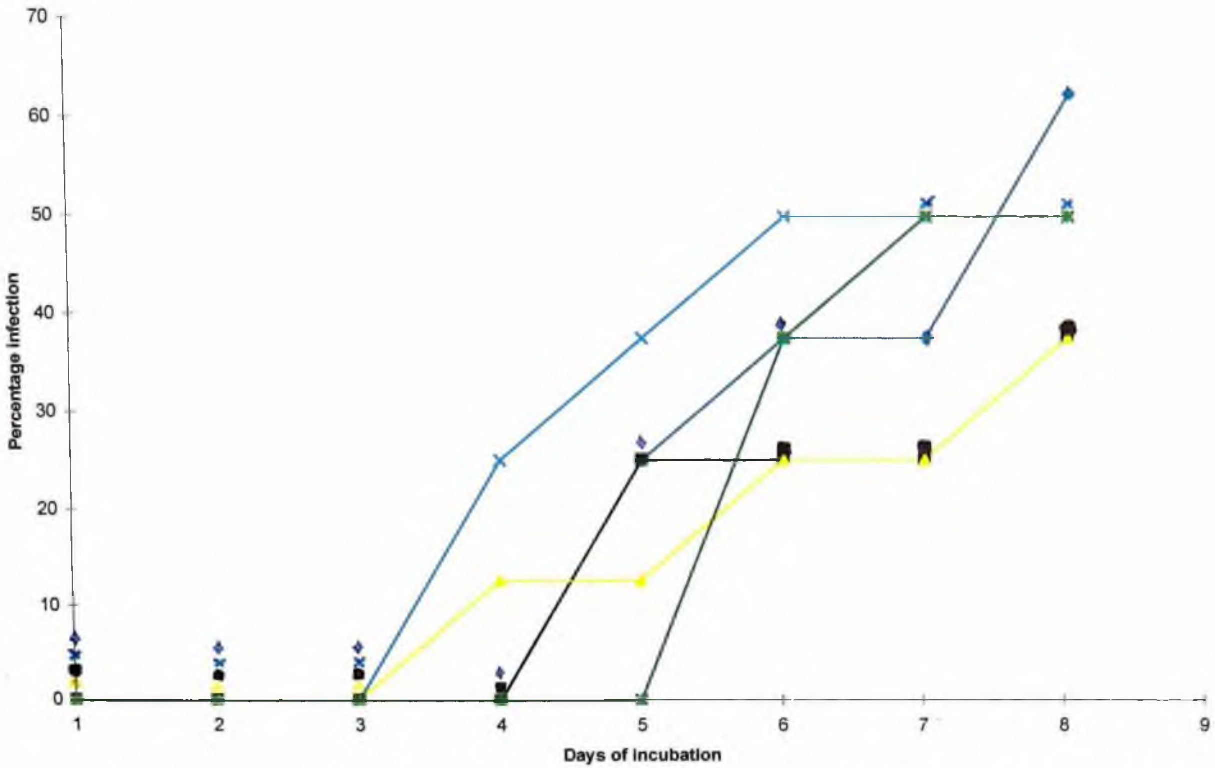

Fig 4c Percentage of leaflets of groundnut previously washed with 20% H_2O_2 solution showing leafspot symptoms after inoculation with drops of spore suspension of *C. arachidicola* containing *Zea mays* pollen at different densities.

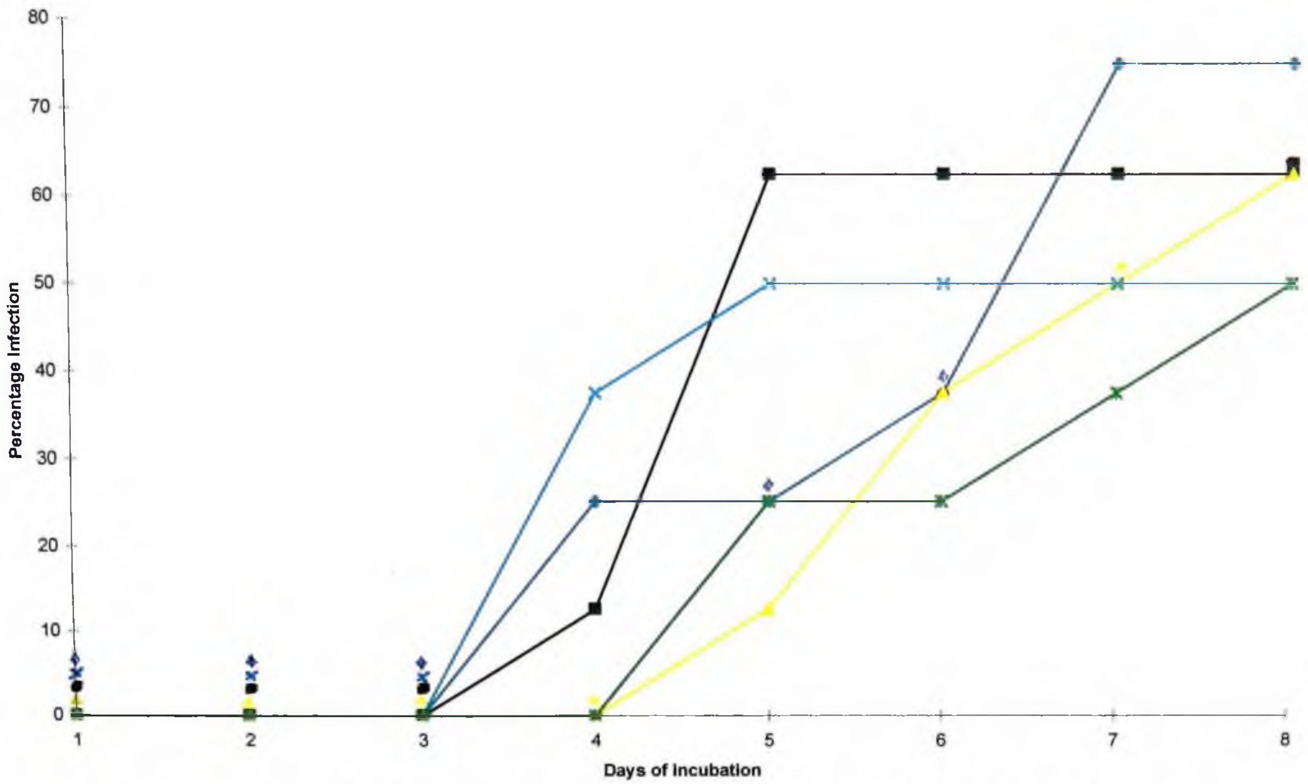

Fig 4d Percentage of leaflets of groundnut previously washed with 30% H_2O_2 solution showing leafspot symptoms after inoculation with drops of spore suspension of *C.arachidicola* containing *Zea mays* pollen at different densities.

CHAPTER FIVE

GENERAL DISCUSSION

Garrett (1956) defined inoculum potential as 'the energy of growth of a parasite available for infection of a host at the surface of the host organs to be infected. This definition embraces both the infective capacities of the inoculum, that is, its total nutrient content and especially to its content of energy substrates, and the environmental conditions under which it is operating. The inoculum potential may, therefore, vary from a maximum value to zero, as environmental conditions vary from optimal to completely inhibitory. Given environmental conditions optimal for activity of the inoculum, the most important would be the carbon compounds available to the fungus as energy substrates, whether they were carbohydrate reserves in the fungus itself or supplied externally by the immediate environment of the inoculum.

It was speculated as far back as 1908 (Brooks, 1908) that the externally supplied energy substrate play critical role in infection by fungal parasites. Precision was lent to this speculation by the observation of Last (1960) on infection of leaves of *Vicia fuba* by spores of *Botrytis fabae*. Maximum percentage germination of spores in water was maintained as cultures of *B.fabae* from which the spores were taken, aged to 40 days, whereas Infectivity to bean leaves declined to 1/10th at 25 days and 1/100th at 35 days of the parent culture, Infectivity of the ageing spores could be restored by suspending them in orange juice which contained, in addition to proteins, minerals, vitamins about 1.5% citric acid and 4.5% sucrose. Last (1960) had commented, 'Ageing conidia of *B. fabra* seem to contain adequate reserves for germination but insufficient to meet the demands for infection'.

It was presumed to begin with, that pollen of maize in mixed farms would provide external substrate for conidia of *Cercospora arachidicola* landing on leaves of groundnut for, carbohydrate content of pollen grains is usually high. It was found for example to be as high as 48.35 percent in pollen of *Pinus contorta*_(Todd and Brethrick, 1942),_and that would have been the basis for the high stimulation of infection of plants by fungi in the presence of pollen. For instance, there was an increased infection of strawberry by *Botrytis clneria*_on addition of strawberry pollen to suspension droplets from 14 percent to 93 percent (Chu-chou and Preece, 1961).The object of the present study was to determine the effect of the pollen of maize on germination of conidia of *Cercospora arachidicola*,_the major infection_units and on infection of groundnut_leaves by the conidia.

Teyegaga (1970) reported that the conidia of a local isolate of *Cercospora canescens* causing leafspot of bambara bean (*Vigna subterranea*) germinated in water best at 25, 30 and 35° C attaining 100% germination in three hours at all three temperature. Because of the absence of sufficient numbers of incubators to provide a range of temperatures, all experiments were carried out at 30°C Furthermore, the earliest germinating conidia in distilled water produced germ tubes after 12 hours of incubation and percentage germination was, therefore, assessed for all tests after 24 hours' incubation.

The high percentage germination of 80.5 per cent in sterile distilled water might be due to the presence of a large amount of endogenous substrate in the conidia (See Table 6). The considerable growth of the germ tubes would contribute to successful infection of groundnut leaves. The conidia in the presence of sufficient moisture would not only successfully germinate in the absence of nutrients in the infection court but would also produce extensive germ tubes that would also produce extensive germ tubes that would effect penetration of the host tissues.

In the absence of liquid water, the conidia would also germinate in air provided the relative humidity is sufficiently high. Percentage of the conidia germinated at 90, 95 and 100% RH was 64,0, 57.3 and 46.3% respectively (See Table 19). The germ tubes produced after 24 hours were almost as long as those formed by conidia germinating in distilled water. Even if the atmospheric humidity falls below 80% RH, it is very likely that a high enough level of humidity will be maintained at the transpiring leaf surface to support germination of the conidia. The pattern of germ tube formation in distilled water has been illustrated in fig 1. The conidia rarely produced germ tubes from the median cells only, while appreciable percentage of the conidia produced germ tubes either from apical cells only or from apical or basal cells together or from a median cell in addition to germ tube from the terminal ends. The difference between the capacities of the terminal cells and the median cell was indicative of a difference in the amount of endogenous nutrients in these cells. The behaviour of the conidia during germination shows that the cells of conidia of *C. arachidicola* were independent of each other and adjacent cells were completely separated from each other. This feature is consistent with the nature of multicellular spores as established in observations with the electron microscope. Campbell (1970) showed that the pores in the separating walls of the multicellular conidia of *Alternaria brassicola* were effectively plugged, isolating one cell from the other. Thus every cell of the spore behaved independently of the others.

The pattern of germ tubes developments observed here is similar to the observation of Woodroof (*1933*). He, however, stated that 'often several tubes emerged from a single conidium of *C arachidicola*. That was, however, not so in the isolate studied during this work, because no spore formed more than four germ tubes. Woodroof (op *cit*) also reported that, with insufficient moisture the cells might swell without developing germ tubes'. Cells of many conidia incubated in drops of sterile distilled water during this work did swell visibly without developing germ tubes. From the evidence of the present work, swelling of cells of conidia without the production of germ tube was not caused by insufficient moisture.

The greater capacity of the terminal cells than median cells for germination seems to be a common feature of the conidia of *Cercospora* species. Berger and Hanson (1963) reported that germ tubes may arise from any cell of the conidia of *Cercospora zebrina*, but the basal cell usually germinated first, while Baxter (1956) had earlier observed that the basal cell of conidia of *C. zebrina* germinated first followed by the apical cell. Teyegaga (1970) also found that the germ tubes of conidia of *Cercospora canascens* commonly emerged first from the basal cell, followed within a short time by production from the apical cell, and lastly from the median cells. But not more than three of the numerous median cells (up to 12 cells) produced germ tubes in distilled water. Anyway, the terminal cells, as a general rule, germinate so readily and produced abundant apical infective growing apices that the poor development of germ tubes by the median cells would not limit infection of the host.

More median cells of conidia of *C.canascens* were stimulated to germinate by external nutrients by Teyegaga and Clerk (1972) thereby augmenting the infective capacity of the conidia. As many as eight median cells of the conidia germinating in aqueous suspension droplets on the leaflets of the host plant, *Vigna subterranea* produced germ tubes compared to three cells that did so in droplets on glass slides. An even greater stimulation of the median cells was produced in solutions of casein hydrolysate, fructose, glucose, maltose, peptone sucrose and yeast extract. Yeast extract proved to be the most stimulating of all. Such stimulation could not be obtained when the conidia of *C. arachidicola* were incubated in solutions of glucose and peptone, (See Tables 7,11 and 13), which could be ascribed to inherent characteristics of *C.arachidicola*. Conidia of *C. arachidicola* belong to the same category as the 4-celled conidia of *Curvularia lunata*. Asomaning (1975) reported that only the two outer cells produced germ tubes in distilled water and solutions of various nutrients, and the two median cells could not be induced to produce germ tubes by fructose, glucose, haemoglobin, peptone, sucrose and yeast extract.

Many reports in the relevant literature (eg. Chu-chou and Preece, 1961), have indicated that pollen grains provide nutrients to fungal spores and pollen induces chemotropism in hyphae (eg. Oliver, 1978).It was expected that if pollen of maize would similarly affect the conidia of *C. arachidicola* it would do so in two ways, by first improving percentage germination of the conidia, and secondly, encourage production of more germ tubes by the median cells. Neither of these occurred in the presence of both autoclaved and non-sterile pollen. To summarise the events:

a. Percentage germination was not improved
b. The median cells were not induced to produce more germ tubes.
c. Pollen did not rejuvenate aged conidia (See Tables 21,23 25,27 and 29) and
d. High pollen densities of 4,000-7,000 pollen grains per ml. of suspending medium markedly reduced percentage germination of the conidia (See Table 8)
e. Germ tubes were not attracted by pollen grains in the suspension droplets.

However, the conidia in pollen suspension germinated at pH3 - pH 10, whereas those in pollen – free suspension germinated at pH 4.0 – pH 9 (see Table 17) indicating stimulation by the pollen at the extreme pH's and yet, germination at optimum pH of pH 5.0 and 6.0 in the absence of pollen was far higher, 80.2 and 70.6 percent, respectively, than the corresponding 60.7 and 59.1 percent germination in the presence maize pollen (see table 17). Be as it may, the extreme pH of 3.0 and 10.0 are unlikely to occur in the groundnut leave surface microhabitat and the stimulation by pollen of maize at these pH's would be of no practical value. It could, therefore, be concluded that, generally, pollen of maize will not increase infection of groundnuts by *C. arachidicola* and could even reduced infection if there are heavy deposits of the pollen on the leaves.

This supposition is supported by the results of the germination tests on the surfaces of the leaflets of groundnuts tabulated in Table 31.In a series of experiments, the mean percentage germination of the conidia in pollen-free aqueous suspension drops was 44.8 per cent compared to the mean percentage germination of 40.9, 35.1,43.2 and 43.1 percent, respectively in suspension drops containing 500 - 600, 1000 – 2000, 1500 – 1800 and 2000-2400 pollen grains per ml.of suspension.

Subsequent growth of the germ tubes and penetration into the host tissue will depend on the interaction among the pollen, germ tubes and the phylloplane microflora. Table 37 shows that abundant pollen grains (4000 – 7000 per ml. of the suspending medium) on non- sterile leaflets apparently stimulated prolific growth of phyloplane of bacteria and fungi, which smothered the germ tubes and prevented infection. On partially surface-sterilized leaflets with consequent microfloral population (See Tables 33 and 34) the germinating conidia caused infection. The pollen per se did not prevent infection.

Infection of new plants raised in a new cropping season depends on the capacity of the spores to survive the preceeding non-cropping period. Very great differences have been found in the survival potential of different kinds of fungi spores. The viability of all spores decreases with time and the rate of loss of vigour is dependent on inherent characteristics of the spores and upon environmental conditions, especially, temperature, humidity and light (Gottlieb, 1950;Cochrane, 1958). At any given relative humidity, increasing the temperature generally decreases the viability of the fungal spores, and lower temperatures above freezing favour longevity. The relationship between relative humidity and viability does not appear to be so simple.

Four major types have been reported. Several investigators (e.g. Anderson, Henry and Morgan, 1948; Maclaughlin and True, 1952) have found that lower relative humidity favours retention of viability because of reduced accumulation of toxic metabolites of the low rate of metabolism in the dehydrated spore. Goos and Tschirch (1962), on the other hand, reported that spores of *Gloeosporium musarum* could not withstand desiccation and survived longest at higher humidities (60-80%R.H.) than at lower humidities (0-20%R.H). Rosen and Wartman (1940) and Naqvi and Good (1957), respectively, found that uredospores of crown rust of oats and conidia of *Monilinia fructicola* retained their viability for a longer time at mid-humidities. Teyegaga and Clerk (1972) also found mid-humidities to be more favourable for survival of *Cercospora canascens* conidia than lower (0%R.H.) and higher (80%R.H) humidities. A reverse response occurs *in Aspergillus flavus*, *Metarrrhizium anisopliae* and *Rhizopus oryzae*. Teitell (1958) reported that the viability of conidia of *A. flavus* was preserved longest at zero and 85%R.H. and lost quickest at 75%R.H.while Clerk and Madelin (1965) found that conidia of *Metarrhizium anisopliae* died quickest at 45-55%R.H. and survived longest at 0-35%R.H. and 65-95%R.H..Sporangiospores of *Rhizopus oryzae* also belong to this category

(Akushie and Clerk, 1981). The true causes for the survival patterns of the third and fourth categories are yet to be determined.

In this investigation the longevity of *C.arachidicola* conidia at different relative humidities was studied but at a temperature of 30^0C and under light of 76 lux only. These temperature and light condition were adopted because local atmospheric temperatures throughout the year are close to 30^0C and because the dispersing spores are normally wind-borne for long periods exposed to light, and it is under such conditions that the longevity of the conidia becomes relevant to disease initiation. For initial 30 days of storage the conidia of *C. arachidicola* survived at 20,40 and 60% R.H than at zero and 80% (See Tables 21, 23 and 25). The high moisture content of the conidia at 80% probably supported faster metabolism with attendant accumulation of toxic metabolites leading to quicker death while desiccation at 0%R.H disrupted metabolic processes. As the storage period extended beyond 30 days, viability at the median humidities fell to levels close to that at 0% R.H. while conidia at 80% R.H. showed the least viability (See Tables 27 and 29). Probably, as storage time lengthened, the internal accumulated toxic compounds generated at 20, 40 and 60% R.H. caused rapid death reducing longevity to the level of those at 0% R.H. The storage period should be extended beyond 50 days in future investigations to establish further behaviour of the conidia at these humidities.

The results, however, indicate that after 50 days, a fair proportion of the conidia remained viable at the humidities, which the conidia were likely to encounter in the field. Since groundnut is primarily a savanna crop and cultivated almost throughout the year the conidia would readily survive the normal brief intervals between successive cropping periods.

The influence of pollen of maize on the germination of conidia of *C. arachidicola* and on rejuvenation of the aged conidia has been studied. Fortunately, pollen of maize is not likely to increase infection rate of groundnut by *C. arachidicola*. Closer spacing of the maize plants will encourage thicker pollen deposits on the groundnut plants and consequently depress germination of the conidia. This proposal can be adopted if future investigation shows that the maize pollen affects the conidia of *Cercospora personata*, the other groundnut leafspot fungus in the same way.

CHAPTER SIX

SUMMARY

1 The conidia of *Cercospora arachidicola* incubated at 30^{0}C germinated in water and at 85-100%R.H. in 24 hours.

2 Germination was better in liquid water than in saturated atmosphere,and percentage germination fell with decrease in atmospheric humidity.

3 The earliest germinating conidia in distilled water produced germ tubes in 10-12 hours.

4 The germinating multicellular conidia produced germ tubes first from the end cells and later, occasionally, from one or two median cells.

5 In distilled water a maximum of only two median cells in conidia of seven cells produced germ tubes.

6 The majority of the conidia (68%) contained 4-5 cells each.

7 There were five types of germination:

i. Germ tubes from the apical cell only.

ii. germ tube from basal cell only.

iii A germ tube each from the apical cell and basal cell together

iv. Germ tube from a terminal cell or both terminal cells and a median cell

v. Germ tube from a median cell only

8 The types of germination occurred in different proportions, with the greatest proportion of conidia producing germ tubes from both the distal and basal cell, whereas very few conidia produced germ tubes from a median cell only.

9 Germ tubes produced by the apical cell are slightly longer (measured after 24 hours' incubation) than those from the basal cell in germinating conidia producing germ tubes from both end cells.

10 The conidia germinated better in distilled water on glass slides than on Surface of groundnut leaflets.

11 Pollen grain of *Zea mays* decreased germination of the conidia.

12 Non-sterile pollen caused less reduction in percentage than sterile pollen.

13 The germ tubes of conidia germinating in suspension with non-sterile maize pollen were slightly shorter than those of conidia in suspension with sterile pollen.

14 Percentage germination in suspension with both non-sterile and sterile pollen decreased with increase in density of pollen from 500 to 7000 pollen grains per ml. of suspending medium.

15 The proportions of the different germination types in distilled water were Apical and Basal cells co-jointly> Apical cell only> Apical and / or Basal cell and Median cell> Basal cell only> Median cell(s) only.

16 This order was not altered by the presence of pollen of maize and by germinating the conidia on the surface of groundnut leaflets.

17 Buffer solutions altered this order, perhaps due to the constituents of the solutions,to: Apical and Basal cells co-jointly > Apical cell only > Basal cell only > Apical and/or Basal cell and Median = Median cell(s) only.

18 Maize pollen stimulated germination of the conidia at pH 3.0and pH 10.0.

19 Exudates of maize pollen hardly altered the pH of the suspending distilled water. The pH's of all suspensions containing 500-7000 pollen grains per ml. were between pH 6.0 and pH 7.0

20 Conidia stored for 50 days at 0, 20, 40, 60 and 80% R.H., survived to different degrees. The percentage viability after 50 days at the different humidities when germinated in the absence of maize pollen was :

0% R.H.: 35.1per-cent germination.

20% R.H.: 34.3 per cent germination

40% R.H.: 29 per cent germination

60% R.H.: 25 per cent germination

80% R.H.: 19.2 per cent germination

and in the presence of maize pollen was :

0% R.H. 33.7 per cent germination

20% R.H.: 32.3 per cent germination

40% R.H.: 26.4 per cent germination

60% R.H.: 23.8 per cent germination

80% R.H.: 17.3 per cent germination

21 Maize pollen could not re-juvinate the aged conidia

22 Leaflets of groundnut could be safely partially disinfected with 30% H_2O_2 for 1 minute, 20% H_2O_2 for 1 minute and 10% H_2O_2 for 2 minutes.

23 Treatment with :

I. 30% H_2O_2 for 1 minute removed 64.1 per cent of the bacterial flora

20% H_2O_2 for 1 minute removed 51.3 per cent of the bacterial flora

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