

**CAUSES AND CONTROL OF TUBER ROTS OF WHITE YAM
(*DIOSCOREA ROTUNDATA* POIR VARIETIES ARABA,
ASANA AND PUNA)**

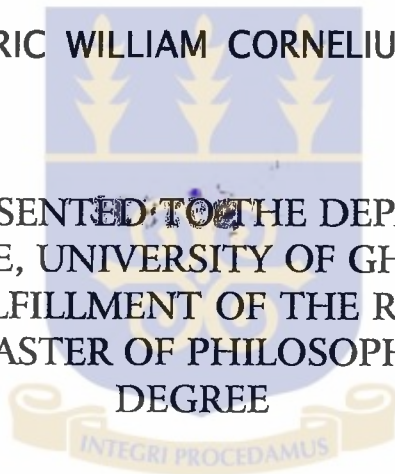
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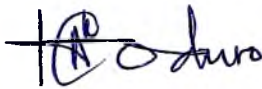
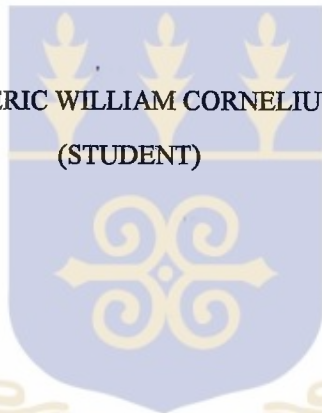
DECLARATION

I declare that the work embodied in this thesis represents my original work and has not been submitted to another university for the award of a degree.

Any help received in writing this thesis and all sources used have been duly acknowledged.



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DEDICATION

To the Glory of God and to my children, Nana Banafu, Panyin and Kaakra.



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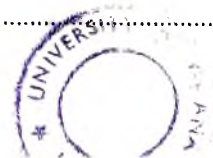
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ABSTRACT

Studies were undertaken to identify the pathogens associated with rotting of white yam (*Dioscorea rotundata* var. Araba, Asana and Puna) in Ghana, to identify exportable white yam varieties with superior shelf life and to evaluate the effectiveness of lime and neem wood-ash in controlling rots in wounded yam tubers.

A field survey was conducted among yam retailers and exporters in Accra. The results revealed that: a major constraint to yam production and marketing in Ghana is the high percentage of tuber rot of 10-30% for retailers and 10-40% for exporters; Puna is the most preferred white yam variety for export and local consumption; Some handling practices predispose tubers to rot diseases; traditional rot control measures is not widely practiced; yams shipped abroad are kept in poorly ventilated containers.

Identification of the three yam varieties based on tuber characteristics, showed that the furrow markings on the corky periderm (skin) of the three white yam varieties are consistent with variety and therefore a reliable index for their identification.

Isolations made from tubers of the three white yam varieties obtained from three yam markets in Accra and a yam barn at the Crop Science Department, University of Ghana Legon, and subsequent pathogenicity studies with the isolates revealed that storage rots were caused by *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Botryodiplodia theobromae*, *Erwinia carotovora*, *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium sp* *Penicillium brevi-compactum*, *Penicillium sp.*, *Rhizopus stolonifer* and *Scutellonema bradys*. This is the first record of the ability of *A. oryzae* and *F. culmorum* to cause rot in yam. Symptoms aiding the identification of *A. niger*, *E. carotovora*, *P. brevi-compactum*, *R. stolonifer* and *S. bradys* are described. Puna had the highest mean frequency of

infection by pathogens (41.9% and 41.3%), followed by Asana (34.4% and 30.7%) and then Araba (23.7% and 28.0%) in the market and barn respectively. After ten months of storage Araba had the lowest weight loss of 39.31%, and a lower rotting percentage of 27.5% ($P = 0.05$). Even though at the 16th week all the yam tubers had sprouted, the rate of sprouting of Araba was significantly lower ($P = 0.05$). Lime was significantly more efficacious in suppressing decay in wounded tubers of the three yam varieties than neem wood-ash ($P = 0.05$). Lime also inhibited growth of all the fungal isolates *in vitro*.

CHAPTER ONE

INTRODUCTION

Yam (*Dioscorea spp.*) is a very important carbohydrate staple food in West Africa, the Caribbean area and parts of South-East Asia. The tuber, which is the only edible part, has a tremendous capacity to store food reserves. Yam is important in the social and religious lives of West African peoples and is gaining prominence as an export crop. The most important yam species grown in Ghana is the white guinea yam, *D. rotundata* Poir (ICRA, 1996).

Of the estimated yearly global yam production of 30.3 million metric tons, some 95-96% is believed to originate from West Africa (FAO, 1994) where possibly 60 million people are involved in its production and marketing (Nweke *et al.*, 1991). Estimates by the Food and Agriculture Organization (FAO) suggest that over the past 15 years world production of yams has been rising and that this is almost entirely due to increased production in Africa (FAO, 1994). Recent statistics show that this trend is also true of Ghana with national production figures of 2.26 million metric tons in 1996 up from 1.7 million metric tones in 1994 (PPMED, Ghana, 1996).

Notwithstanding the importance of yam to both the national and regional economies, in comparison with other crops, little attention has been paid to the constraints that hinder it's efficient production in Africa. In 1994, the National Agricultural Research Strategy Plan for Ghana identified yam as a priority crop for research and development and specifically identified the need to reduce post-harvest losses. These losses are mainly due to tuber dehydration, respiratory loss of carbohydrates, tuber sprouting and microbial decay.

The highest losses in yam are thought to be caused by rots resulting from microbial attack (Coursey, 1967) but there is little information on pathogens of yam rots in Ghana. Bunting (1924), Dade and Wright (1931) and Piening (1962) listed *Botrydiplochia theobromae* as the only pathogen associated with post-harvest yam decay in Ghana and subsequently four other pathogens were named by Bediako (1978) and Abeka (1989). But a number of micro-organisms have been isolated and reported elsewhere (Noon, 1978). There is therefore the need for a comprehensive study of the biotic causes of yam tuber decay, to provide the basis for planning control strategies.

Losses have also been known to vary within species and varieties of yam (Coursey, 1967). Therefore, comparative study on the shelf life of important white yam varieties would be useful in identifying varieties with longer shelf life which can be utilized in breeding programs.

Control methods for rots of yam tubers include curing, refrigeration, irradiation and the use of chemical pesticides. Although some of these methods are effective, they are not practicable in the rural areas where most of the yam is produced. Because yam in Ghana is produced by peasant farmers, simple easy to apply and cheap storage techniques are needed. Such storage techniques, preferably should include use of natural materials. In the West Indies and parts of South-East Asia, cheap and non-toxic alkaline materials such as commercially prepared lime and wood-ash are used for controlling rots on wounded yam tubers (Coursey, 1967). In Ghana, however, there is no report on the use of these materials for controlling yam tuber rots. Their effectiveness is therefore worth investigating.

The objectives for the study therefore were:

- (i) to obtain from yam retailers and exporters, through a field survey, information on important white yam varieties (especially those in the export trade) extent of rot diseases, factors believed to influence the diseases and traditional control measures used.
- (ii) to identify the pathogens involved in the rotting of the white yam varieties, Araba, Asana and Puna.
- (iii) to compare the shelf lives of the three yam varieties.
- (iv) to evaluate the effectiveness of lime and neem wood-ash in the control of rots on wounded tubers
- (v) to determine *in vitro* the effect of these two materials on the growth of the rot pathogens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany of yam and its importance in West Africa

Yam plants are monocots and members of the genus *Dioscorea*, family *Dioscoreaceae* and order *Dioscorales* (Ayensu and Coursey, 1972). Some degree of controversy has centred on the nature of the yam tuber (Njoku, 1963; Lawton and Lawton, 1969; Degras, 1993) which is the edible part. The currently accepted view is that the yam tuber is neither a stem structure nor a root structure. Rather it has its origins as a hypocotyl structure (Degras, 1993).

Although more than 600 yam species exist, ten of them are used extensively for food and are therefore called yams (Onwueme, 1982). These are the white Guinea yam (*D. rotundata* Poir), yellow yam (*D. cayenensis* Lam), water yam (*D. alata* L.), trifoliolate yam [*D. dumetorum* (Kunth) Pax], aerial yam (*D. bulbifera*) Chinese yam [*D. esculenta* (Lour) Burk], cush-cush yam (*D. trifida* L.), cinnamon yam (*D. opposita* Thunb), *D. japonica* Thunb and *D. hispida*. Some yam species are only eaten occasionally while others are not used for food at all because they are toxic (Degras, 1993). Species such as *D. composita*, *D. floribunda* and *D. mexicana* are grown for the manufacture of pharmaceuticals because they contain the steroidal saponins (Osagie, 1992).

In West Africa the domestication of yam was associated with the people of the 'yam zone' stretching from the Cameroon mountains to the Bandama river in central Côte d'Ivoire (Hahn *et al.*, 1995). This zone produces 95-96% of the world's total production estimated at 30.3 million tons per year and about 60 million people are involved in its production and marketing (FAO,

1994). In West Africa, yam plays a vital role in traditional culture, rituals and religious as well as local commerce.

2.2. White yam varieties grown in Ghana

The white yam, *D. rotundata* is a native of West Africa and is grown on a greater acreage than any other species in Ghana (ICRA, 1996) and in the world (Onwueme, 1982). White yam varieties are therefore the most important yams in Ghana.

Few crops have been known to have as many varieties as yam and much confusion exist among the cultivated forms, which in most parts of the world have never been properly scientifically classified (Ayensu, 1972). There are about 26 varieties of white yam grown in Ghana (Tetteh and Saakwa, 1994). Some of these are shown in Table 1.

The taxonomic position of these varieties are somewhat confused because there are no universally accepted names and detailed description of the distinguishing features are lacking (Ayensu, 1972; Onwueme, 1982). Varieties are given local names which more or less describe certain attributes they have or are named after whoever introduced them to the area (Tetteh and Saakwa, 1994). Some varieties are sometimes given different names in different localities and others have slight differences generally recognized only by farmers (Ayensu, 1972). Some of the different varieties named may therefore be the same.

Table 1: Local names of some white yam varieties grown in Ghana.

<u>Local Name</u>	<u>Language from which name was obtained</u>
Alorndoh	Konkomba
Araba	Akan
Asana	Konkomba
Asobayere	Akan
Bayara	Konkomba
Blouse	English
Chamba	Konkomba
Daloba	Konkomba
Denteh-pruka	Konkomba
Firk/Forick	Konkomba
Inkrokota (Mensah)	Akan
Puna (Kpene)	Konkomba
Punjo	Konkomba
Satiba	Konkomba
Serwa	Akan
Kasaati	Konkomba
Kpaajol	Konkomba
Kaligmankata	Konkomba
Kpasajo	Konkomba
Kiliga	Konkomba
Krokropa	Akan



Source: Anon., 1993 : Ghana Standards Board, specification for yam export G506 (unpublished).

Farmers and yam dealers in Ghana usually identify various varieties by their tubers characteristics or their shoot characteristics while growing in the field. Identification may be based on tuber shape, tuber-skin colour and structure, tuber flesh colour and tuber flesh texture. The colour of sprout and shoot tips, quantity and distribution of spines, and bloom on the stem, and leaf shape, size, and colour are other characteristics on which varietal classification may be based. There are also differences in flowering and tuber dormancy behaviour (Onwueme, 1982). These characteristics, especially those related to the tuber should be properly studied to aid in the easy identification of the white yam varieties by non-farmers such as yam retailers, exporters and housewives. Efforts should also be made to scientifically characterise the traditional yam varieties in Ghana by their isozymic patterns.

2.3 Methods of traditional storage of yams in Ghana

In many countries yams are stored without any special precautions in ordinary store-rooms, sheds or huts specially constructed for this purpose (Osagie, 1992). Whatever the system, the essential requirements are adequate ventilation, shade and protection from direct sunlight (Hahn, *et al.*, 1995).

In Ghana, various systems of storage for yams have been in use since time immemorial without substantial changes. The underground storage technique is practiced in two forms. Some farmers leave the mature tuber in the soil where it was grown and harvest them when required particularly, a few days before the main market day. Other farmers dig a circular trench and line the sides with dried leaves. The yam tubers are arranged horizontally in the pit and covered with mulch (GTZ/MoFA, 1995). While underground, tubers are exposed to attack by pre-harvest pest such as beetles, termites and/or mice. Deterioration of tubers is also enhanced due to the high temperature and humidity underground and contact among the tubers (Osagie, 1992). This method is becoming less common in Ghana.

The heap/platform storage is also practiced in Ghana by farmers and sellers. The tubers may be heaped on the floor, in huts or houses or arranged on wooden platforms and laterite. Fires are lit weekly and the smoke produced used to fumigate the tubers which are accessible to rodents, insects and other pests. Storage losses are high (GTZ/MoFA, 1994).

The commonest method of yam storage in Ghana is storage in the traditional yam barn (GTZ/MoFA, 1995). A yam barn consists of a vertical framework to which the yam tubers are tied with local materials such as raffia. The framework is made from vertical poles, preferably live, 5-10cm in diameter and set 1m apart. The height of the poles vary from 1-2m. Cross members are

usually of bamboo or raffia palm-leaf mid-rib (Igbeka, 1985). There are variations on this basic pattern of the barn (Coursey, 1967). Barns are effective for yam storage through the dry season but once the rainy season starts tubers tend to deteriorate rapidly (Degras, 1993). IITA has developed an improvement upon the traditional barn method by using simple open-work shelving inside the barn. Its advantages include placing tubers on shelves with less time and labour. Also, damaged tubers can be more easily spotted and be removed (Hahn *et al.*, 1995).

2.4 Factors affecting the shelf life of yams

Shelf life of yams is the period of time during which they can be stored and remain suitable for use. Consumers normally prefer tubers that are fresh.

While it is frequently believed that yam stores well as compared to other root and tuber crops (Booth, 1974) others hold a contrary view (Coursey, 1967; Rawnsley, 1969; NRCRI, 1982; Ikotun, 1989; GTZ/MoFA, 1995). Coursey (1966) revealed that storage losses of carbohydrates and water from yam tubers vary considerably across countries and regions and within species and varieties. The yam tuber is a perennating organ containing a high percentage of water (60-70%) and carbohydrates. It actively respire, transpires, sprouts and is also an excellent host for micro-organisms and pests. The shelf life problems of yam tubers have therefore been attributed mainly to:

- (a) respiratory loss of carbohydrates
- (b) dehydration of yam tubers
- (c) tuber sprouting
- (d) losses due to insect and rodent attacks
- (e) microbial mediated rots.

Respiration of the living yam tuber uses the stored carbohydrates as substrate and constitute an invisible source of storage loss (Onwueme, 1982). Studies

on dry matter losses through respiration showed that tubers stored for five months may lose over 10% of their dry matter through respiration (Osagie, 1992).

Dehydration of yam tubers with high moisture content is the result of water loss through transpiration and culminates in reduction of tuber fresh weight without loss of dry matter (Onwueme, 1982). The loss under any given situation is restricted by the tuber periderm which is comparatively impermeable to water vapour and removal of which increases the rate of evaporation. Water loss arising from dehydration of yam tubers in five months storage may be as much as 20% of the original weight (Coursey and Walker, 1960). Mozie (1982) found that tubers lost more than 50% of their original weight during the first four months in storage under continuous air flow. Desiccated tubers may lose viability, become unpalatable and rot easily (Onwueme, 1982). Dehydration of tubers is different from tissue hardening which makes the tuber flesh difficult to chew after cooking. It affects only *D. dumetorum* and occurs within a few days after harvesting (Chinsman and Fiagan, 1986).

When yam tubers sprout in storage, they mobilize food reserves, and use it to produce inedible sprouts (Onwueme, 1982). In principle, storage is possible as long as the tuber remains physiologically dormant (Passam, 1977). However, once dormancy is terminated and sprouting begins, the tubers senesce and can no longer be stored effectively (Passam and Noon, 1977). This state of the tuber, according to Passam and Noon (1977), renders it susceptible to invasion by micro-organisms. Coursey (1961) showed that different varieties of yams vary in their length of dormancy period.



Loss of tuber carbohydrates to insect and rodent attack is substantial especially under traditional storage of yams. The activities of these pests also create wounds on tubers which act as infection courts for rot-causing pathogens (Miege, 1957; Panis *et al.*, 1974; Emehute, 1996). Insect pests on yams include yam moths, coffee bean weevil, yam beetle and termites (Degras, 1993).

Of the five storage problems outlined, rot diseases are considered to be the most important (Coursey, 1967; Ikotun, 1989). This is because rot diseases lead to total loss of tuber carbohydrates by transforming it into inedible coloured mass. The microbes involved in this are mainly fungi and to a lesser extent bacteria, nematodes and viruses. (Théberge, 1985).

2.5 Pathogens of yam rot diseases

2.5.1 Role of fungi in yam tuber rots: - The most widely reported fungus of post-harvest yam rot appears to be the ubiquitous *Botryodiplodia theobromae* which was first reported in Ghana by Bunting (1924) and later by many other workers including Dade and Wright (1931), Anon. (1962), Piening (1962), Okafor (1966), Ogundana *et al.* (1970), Noon and Colhoun (1979), Adimora *et al.* (1990) and Iloba (1996).

Probably of greater significance in terms of magnitude of losses are species of *Penicillium* which cause serious decay of yam tubers. Some of these are *P. oxalicum* (Adeniji, 1970a), *Penicillium spp.* (Burton, 1970; Noon and Colhoun, 1979; Abeka, 1989; Adimora *et al.*, 1990), *P. brevi-compactum* (Burton, 1970) and *P. crustosum* (Coursey and Russell, 1969).

Fusarium spp. are significant post-harvest pathogens of yams. *F. oxysporum* and *F. solani* have been reported (Okafor, 1966; Burton, 1970; Ogundana *et al.*, 1971; McNamara and Acholo, 1996).

Aspergillus niger is the most widely reported and important yam rot organism in the genus *Aspergillus* (Adeniji, 1970a; Ogundana *et al.*, 1970; Noon and Colhoun, 1979; Adimora *et al.*, 1990; Otunsanya, 1996). Other species reported include *A. flavus* (Noon and Colhoun, 1979), *A. tamarii* and *A. flavipes* (Adeniji, 1970a).

Rhizopus stolonifer has been reported by Adeniji (1970a), Noon and Colhoun (1979), Abeka (1989) and Adimora *et al.* (1990) to be a highly pathogenic fast rotter but some people consider it as a secondary invader (Noon, 1978).

Other fungi reported on yam tubers are *Cladosporium spp.* (Burton, 1970; Adeniji, 1970a), *Colletotrichum spp.* (Burton, 1970; Iloba, 1996), *Sclerotium rolfsii* (Baudin, 1956), *Rhizoctonia spp.* (Burton, 1970) and *Roselinia spp.* (Lartey and Martyn, 1943). The inventory compiled by Noon (1978) listed over 50 fungi although their degree of pathogenicity were not always studied.

There is a limited information on the causal agents/pathogens responsible for rotting of yams in Ghana. For example, only one fungus *Botryodiplodia theobromae* was listed to be the pathogen isolated from rotten stored yam in Ghana (Bunting, 1924; Dade and Wright, 1931; Piening, 1962) and was cited by the United States National Academy of Science (USNAS) in their authoritative book (USNAS, 1981) which also listed six and seven fungi in Nigeria and Côte d'Ivoire respectively. Subsequently, *Rhizopus stolonifer*, *Penicillium spp.*, *Aspergillus spp.* and a bacterium have been reported in

Ghana (Bediako, 1978; Abeka, 1989) as against the numerous pathogens reported elsewhere.

2.5.2 Bacteria isolated from rotting yams: - Bacteria have often been observed when soft rot of yam develops into wet rot. Coursey (1967) advanced the argument that internal bacteria with latent infectibility could contribute to tuber rot. However, *Erwinia spp.* has been widely reported to be responsible for watery soft rot of yams (Burton, 1970; Noon and Colhoun, 1979; Théberge, 1985). Noon (1978) also found a dry rot caused by *Corynebacterium sp.* Other bacteria associated with soft rots of yams include *Serratia spp.* (Okafor, 1966; Burton, 1970; Noon and Colhoun, 1979), *Pseudomonas spp.* and *Proteus spp.* (Ezieke, 1989), *Clostridium*, *Vibrio* and *Bacillus tenus* (Obi, 1981).

2.5.3 Nematodes isolated from rotting yams: - Some plant parasitic nematodes attack the tuber in storage and cause lesions which facilitate invasion by fungi and other micro-organisms (Osagie, 1992). *Hoplolaimus*, *Pratylenchus* and *Scutellonema spp.* give rise to dark necrotic lesions which are seen as skin cracking and corky appearance (Smith, 1967, Ekundayo and Naqvi, 1972; Adesiyani *et al.*, 1975; Théberge, 1985). The carbohydrate constituents of *D. rotundata* tuber infected by *Scutellonema bradys* were compared with those of the an uninfected tuber (Adesiyani *et al.*, 1975). The increase in monosaccharides recorded in the nematode-infected yam tuber explains one of the ways by which the nematodes may predispose yams to infection by secondary invaders. The simple sugars have been shown by Ogundana *et al.* (1970) to support the growth of fungi which invade the yam tissue.

2.5.4 Virus suspected in yam rot: - The internal brown spotting (IBS) of yam tubers which consists of spherical or elliptical nodules of necrotic cells distributed

within the main parenchyma of the tuber tissue is a serious pathological problem (Degras, 1993). Its etiology has been suspected to be viral.

2.5.5 Methods of infection and symptoms produced: - The pathogens associated with rot diseases of yams are usually weak parasites which enter yam tubers through injury. Tuber injury may result from growth cracks, as well as attack by insects and nematodes (Booth, 1974) and rodents (Booth and Proctor, 1972). Tuber injury also occurs during handling and transportation. If these injuries could be minimized, the incidence of yam decay could be substantially reduced.

The characteristic symptoms produced by tuber infection are soft rots (where the tissue is slightly softened and turns pink in colour), wet rot [where the tuber tissue disintegrates into a watery mass (Okafor, 1966)] and dry rot [where the tissue becomes brown, dries up and finally crumbles away as a brown powder (Théberge, 1985)]. If these symptoms could be related to the pathogen(s) involved, it will help in planning effective control measures.

2.6 Control of yam rot diseases

Work on the control of yam tuber rot diseases has focused on prolongation of dormancy, direct prevention or elimination of rot-causing organisms by various treatments and by manipulating storage conditions usually temperature and humidity.

2.6.1 Delayed sprouting: - Various chemical sprout suppressants have been tested (Hayward and Walker, 1961; Campbell *et al.*, 1962; Rivera *et al.*, 1974; Olurunda *et al.*, 1974; Passam, 1977).

Passam (1977) observed that naturally occurring plant hormones (gibberellic acid, 3-indole-acetic acid and kinetin) had little or no effect on the dormancy of yam tubers. This was attributed in part to inefficient penetration of the chemicals through the thick skin of the tuber (Passam, 1977). Another reason is that these chemical compounds can only prolong dormancy in yams if they exert their effect on the meristematic cells at the sprouting loci (Passam *et al.*, 1982). These loci are formed only just prior to breakage of dormancy and arise from beneath the periderm (Onwueme, 1973). Therefore the time for applying the chemicals is critical.

Chemicals such as methylalphanaphthalene acetic acid have long been found to inhibit sprouting but shows phytotoxicity and inhibit periderm formation. (Campbell *et al.*, 1962). Martin (1977) and Mantell *et al.* (1977) have also shown that Gibberellic acid (GA) prolongs the dormancy of yam tubers. Wilson and Wickham (1982) reported that yam tubers treated with GA were fresh in appearance and did not sprout six months after harvest, while untreated yam tubers were shrivelled, sprouting and unmarketable after the same period. The concentrations of GA ranged from 10^{-5} M to 10^{-6} M.

Gamma radiation of 7.5 krad has also been successfully used to delay sprouting of tubers for over eight months after wounds on the yam tubers had healed (Adesuyi and Mackenzie, 1973; Adesiyi, 1977; Adesuyi, 1978; Demeaux *et al.*, 1982). However, a dose of 20.0 krad and above induced damage and browning of the internal tissue of the yams. Though it has no phytotoxicity or inhibition of periderm, commercial application of gamma irradiation to yam tubers poses technical and economic difficulties because of the facilities required as well as cost of operation.



2.6.2 Use of chemical pesticides: - Post-harvest fungicide treatments of yam tubers often give variable results. Rots caused by *P. oxalicum* were controlled by thiabendazole dip at a concentration of 250ppm active ingredient for a period of ten minutes and benomyl at 100 ppm active ingredient (Ricci, 1973). While accepting that thiabendazole and benomyl generally reduced levels of fungal infection and fresh weight losses in yam tubers, Thompson *et al.* (1977) observed that the fungicides interacted with storage conditions, having less or no effects in ambient storage conditions but with large consistent effects in cold storage. However, Coursey (1966) is of the opinion that external application of fungicides on yam tubers could not effectively prevent fungal rot where the rotting was partly attributed to pre-harvest infection by nematodes. Roth and Richardson (1965) also reported that fumigation with methylbromide was generally effective in the control of pest on yam tubers including nematodes.

Other pesticides used include lime wash, borax, Bordeaux mixture (Coursey, 1961, 1967), sodium O-phenylphenate plus 2-6-dichloro-4-nitroaniline (Burton, 1970), lime and local gin (Ogali *et al.*, 1991) and melaic hydrazide (Santoh and Tanbe, 1971). The use of pesticides to control rot diseases on yam tubers has not been widely accepted for fear of toxic residue. Therefore most workers recommend curing technique which does not involve the use of chemicals.

2.6.3 Curing: - One of the most effective and simple means of reducing post-harvest water and pathological losses of several root crops has been curing. This process involves the post-harvest exposure of yam tubers to high temperatures and humidities for a short period. The process promotes suberization and wound periderm formation to prevent infection by wound pathogens and reduce the rate of physiological weight loss. The germination capability of the

tuber is not affected by this process so the tubers which have been treated can be used for vegetative propagation.

Wilson and Wickham (1982) found out that yam tubers of *D. alata* and *D. rotundata* could be cured satisfactorily at ambient temperature of 28-32°C and 78% r.h. within 5 days. The effectiveness of curing for reducing losses due to rots in yams has also been reported by Booth (1972), Thompson (1972), and Adesuyi (1973b). However, Passam *et al.* (1976) concluded that when bisected or deeply cut by knife, tubers of *D. rotundata* undergo wound repair by suberization and wound periderm formation but abrasions or superficial cuts are not sealed by a layer of periderm, resulting in prolonged loss of moisture and increased susceptibility to rotting. All wounds, squashed areas and other injuries should consequently be cut clean (GTZ, 1993). This contrasts with the report of Martin (1974) which suggests that periderm formation does not generally occur during curing of yams. Cured yams meant for storage should be handled carefully because subsequent bruises predisposes them to deterioration just like uncured yams (Noon, 1978). The optimum curing conditions is influenced amongst others by the type of yam, the type of wound and the degree of tuber maturity (Booth, 1978).

- 2.6.4 Temperature and humidity regulation: - The manipulation of yam storage atmosphere has been used to prolong shelf life of tubers. Adeniji (1970b) studied the extent of decay caused by *P. oxalicum*, *A. niger* and *B. theobromae* in tubers at different temperatures and observed a negligible decay for four weeks at 15°C. The results were confirmed by Adesuyi (1973a), Ogundana *et al.* (1970) and Noon and Colhoun (1979). The control of nematodes in stored yams has also been achieved at temperatures of 12 -13°C (Thompson *et al.*, 1973). Cold storage of yam tubers is limited by chilling injury at temperatures

below 12-13°C (Young, 1923; Anon., 1938; Coursey, 1968; Noon and Colhoun 1981).

Humidity clearly influences the degree of disease development during storage. Noon and Colhoun (1979) demonstrated that tubers stored at 89% r.h. and 35°C for 24 days showed over 50% of rot mainly by *B. theobromae* whilst at 35°C and 30% r.h. negligible decay was observed after 95 days. A temperature of 16°C and r.h. of 70% has been recommended as optima for storing cured yam tubers (Noon, 1978). The regulation of temperature and humidity to prolong shelf life of yam tubers is however not practicable for most yam farmers who are in rural areas and may not have access to electricity.

- 2.6.5 Possible use of some fertilizers to control yam rot diseases: - Many farmers still believe that fertilizers have a deleterious effect on the yam crop either by burning it in the soil or by rendering tubers more susceptible to rot in storage (Kpeglo *et al.*, 1980). Although there has been several studies on the control of post-harvest yam decay, these have been scarcely related to fertilizer use (Coursey, 1967; Adesuyi, 1973a). Kpeglo *et al.*, (1980) indicated that nitrogen, phosphorus and potassium fertilizers do not significantly affect the weight loss of yam tubers in storage. However, high rates of nitrogen significantly increased percentage sprouting of tubers and affected storability (Kpeglo *et al.*, 1980). Determination of the levels of calcium that could confer "resistance" to storage losses in yam varieties has therefore been advocated.
- 2.6.6 Traditional control methods: - The educational standard and income levels of most peasant yam farmers and dealers in West Africa who are mainly in rural communities makes the adoption of some of the modern yam rot control

methods impossible. Such farmers, therefore, use traditional methods to control tuber rots.

Most recorded work on traditional methods of reducing storage rot diseases has been on improved storage methods. Other techniques include pre-storage treatments such as: pit curing (Nnodu, 1986); curing under jute sheet or jute sacks (FAO, 1990); keeping freshly harvested yams in shade for a few days and the application of wood ash, lime, wet earth, vegetable oils and extracts from some local plants on the wounded surfaces of the tubers. These practices have either received little or no attention from researchers.

Experiments by Adimora (1986) showed that the incidence of post-harvest decay could be reduced by treatment of all cut and wounded surfaces with an alkaline material such as lime-wash or wood-ash. In Ghana, wood-ash is applied by a few farmers, housewives and yam dealers with the same objective of reducing post-harvest rots. The method consists of sprinkling wood-ash (collected from a fire place) over the exposed and wounded surfaces of the yam tubers. In most instances only tubers to be used for planting are treated with the ash.

The sources of the wood-ash used by Anon. (1938), Adimora (1986) and others are unknown and this makes the repeatability of their studies difficult. It has been shown by Duta (1995) that the chemical composition of wood-ash depends on the type and part of plant from which it was obtained. There is therefore the need to repeat some of the works stating the kind and part of plant used. The stem of the neem plant (*Azadirachta indica* (J) Juss) which is a common fuel-wood in some areas in Ghana could be worth using.

It has also been the practice of some yam farmers in the Caribbean areas to use lime to control rots at cut and wounded surfaces of tubers (Anon., 1938). The efficacy of lime is therefore worth investigating. The white colour of lime on edible yam tuber surface will make it more acceptable than the seemingly dirty colour of wood-ash. It is likely that both wood-ash and lime treatments on wounded yam tubers perform the same function. It is however expected that because of their varying chemical composition the mechanisms could be different.



CHAPTER THREE

MATERIALS AND METHODS

3.1 Survey on tuber rots of white yam in Accra

In order to get new baseline data on the yam trade in Ghana, especially on storage and market diseases and to confirm information from limited literature and personal communication, a questionnaire-type survey among yam retailers and exporters was considered necessary. The survey was conducted from September to November, 1996.

Questionnaires were designed (Appendix 1.), pre-tested and administered to 10 yam exporters, 20 yam retailers in the Konkomba yam market and 15 retailers each in the Timber and Haatso yam markets all in Accra. The respondents were selected at random.

3.2 Identification of white yam varieties Araba, Asana and Puna used in the studies

The three white yam varieties used for the study were identified through interviews with yam exporters, retailers and consumers and by careful observation of some internal and external characteristics of the tubers. The characteristics used were tuber shape, tuber skin colour and structure, tuber flesh colour, tuber flesh texture and cooking characteristics.

3.3 Isolation and identification of micro-organisms associated with decayed yam tubers

3.3.1 Sources of rotten yam for isolation: - Diseased materials of Araba, Asana and Puna varieties of white yam from which the isolations were made were obtained from two sources. One group was obtained from the three yam markets in Accra on weekly basis and the other group from an experimental material kept in a barn on monthly basis. This was to allow for the comparison of isolates from the market and the barn.

3.3.2 Isolation and identification of fungi: - Isolation of fungi from the three white yam varieties was done first on water agar (WA) and then on potato dextrose agar (PDA). Agar agar (OXOID) and dehydrated PDA (OXOID) were purchased commercially and prepared separately by weighing 20g and 39g respectively in one litre of distilled water and autoclaved at 1.05kg/cm² pressure at 121⁰C for 15 minutes. All media for isolation were treated similarly unless otherwise stated. The method employed for the identification of spoilage fungi was that of Miles and Misra (Collins and Lyne, 1976). Tissue segments from the advancing margins of the rots in Araba, Asana and Puna were removed with flamed scalpel, surface sterilized in 1.0% sodium hypochlorite solution for two minutes and plated on water agar plates (five pieces per plate). The plates were enclosed in clean polyethylene bags and incubated under ambient condition (23-30⁰C) on a bench in the laboratory with fluorescent lighting in the day which was put off at night.

Fungi which grew out from plated tissues were sub-cultured on plates of potato-dextrose agar (PDA). If a mixed culture was encountered, it was repeatedly sub-cultured until individual pure cultures were obtained. Pure isolates were kept on PDA slants and stored in refrigerator until needed. Morphological characteristics of mycelial bits mounted in lactophenol were

observed with a compound microscope. Conclusive identification of fungal isolates was based on the following culture characteristics on PDA: growth rate and colour; morphology of mycelia, conidia and sporulating structures as described by Thom and Raper (1945), Alasoaduro (1970), Booth (1971), Barnett and Hunter (1972), Udeobo (1974), Ramirez (1982) and Samson and van Reenen-Hoekstra (1988).

3.3.3 Isolation and identification of bacterium: - The dilution method was used in the isolation of the bacterium. A small amount of tissue was removed from the advanced portion of the lesion on the three white yam varieties using a sterile scalpel. The tissue was surface-sterilized for 3 minutes in a 1:10 dilution of a household bleach (5.25% active sodium hypochlorite). After rinsing in water, the tissue was chopped up with a sterile scalpel in a droplet of water in a plastic Petri dish. After incubation for 2-3 minutes at room temperature, the macerate was streaked into nutrient agar (NA) media and yeast extract-dextrose-carbonate (YDC) agar with a wire loop. Streaks were made in four right-angled directions, flaming the loop after each directional streak and incubated for three days in the laboratory at 23-30^oC. A single colony (well spaced from other colonies) was re-streaked on the media to confirm the purity of the culture. The culture characteristics observed after the incubation period were recorded to aid proper identification of the isolates.

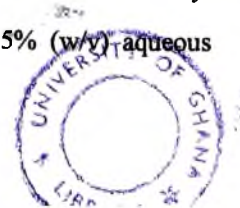
Motility test (Bradbury, 1970) was carried out to ascertain whether the bacterial cells isolated were alive. A drop of the suspension prepared from two-day old bacterial isolates were examined on microscope slides and the characteristics recorded.

The Gram stain reaction (Bradbury, 1970) was done using freshly-prepared reagents and *Escherichia coli* obtained from the Noguchi Memorial Institute

for Medical Research as a reference culture. A thinly separated bacterial film on a clean slide was dried in air without heat and the underside of the slide was slightly flamed twice to fix the bacteria to the slide. The smear was flooded with crystal violet solution for one minute, washed in tap water for a few seconds and excess water drained off. It was slightly blotted dry on a paper towel. The smear was then flooded with iodine solution for one minute, washed in tap water for a few seconds and blotted dry. It was decolourized with ethylalcohol, until the solvent flowed colourlessly from the slide (about 30 seconds), blotted dry and rinsed in tap water for two seconds. Counter-staining was done for ten seconds with safranin solution, washed briefly in tap water and blotted dry. The slides were then observed under microscope.

Anaerobic growth was tested using the following solution: Peptone (2.0g), NaCl (5.0g), KH_2PO_4 (0.3g), Agar (3.0g) and Bromothymol blue (3.0ml of 1% aqueous solution) added and made up to 1 litre with distilled water. The pH was adjusted to 7.1 and 5ml of the basal medium added to two 3cm diameter test-tubes and sterilized at 121°C for 20min. A 10% aqueous solution of glucose was prepared and sterilized by filtration. Sterile glucose, 0.5 ml, was aseptically added to each tube. Two tubes were stab-inoculated with the bacteria to be tested and one tube was covered with a layer of sterile melted petroleum jelly to a depth of about 5mm. It was replicated five times and the color change was observed and recorded.

Spore determination test was done by suspending a colony of bacteria growing on nutrient agar (NA) in a drop of water on a slide and air dried. The slide was flooded with 5.0% (w/v) aqueous malachite green and stained for 10 minutes. It was washed thoroughly under running water and dried briefly. Counter-staining was done by flooding the slide with 0.5% (w/v) aqueous



safranin for 15 seconds, rinsed thoroughly with water and blotted dry. Cells were observed under microscope.

To differentiate between *Erwina* and *Xanthomonas* the isolate was plated on Miller-Schroth (MS) medium and incubated in the laboratory to test for growth.

Pectolytic activity (Bradbury, 1970) was finally tested using potato. Two opposite surfaces of fresh Irish potato tubers were peeled and surface sterilized by wiping with cotton wool soaked in 70% alcohol. A sterile 10mm cork-borer was pushed into the tubers through the opposite ends. The cork-borer was then removed from the tuber and the cylindrical pulp pushed out by a sterile 8mm cork-borer. Four slices were then transferred into Petri-dishes with 10ml suspension of two-day old culture of the bacterium in nutrient broth. The same was done for the control experiment where the nutrient broth was not inoculated with any bacterium and the set up replicated five times. The Petri dishes were then incubated at laboratory temperature (23-30°C) for three days. The slices of potato pulp were examined daily for rot. This was done by prodding and tearing the slices with sterile needles. The manual by Schaad (1988) was relied upon in conclusive identification of the bacterium. Pure cultures of the bacterial isolate was preserved for further studies on nutrient agar slants in a refrigerator at 5°C.

- 3.3.4 Isolation and identification of nematode: - This was done by cutting thin slices (6-10mm) of yam tissue transversely at the sides on tubers along their length and then placed in glass dishes containing 20ml water. The dishes were covered and left for 24 hours. Drops of water in the glass dishes were taken and observed under the microscope for nematodes which were identified based on Jenkins and Taylor (1967).

3.3.5 Determination of pathogenicity of isolates:- Pathogenicity test were conducted by inoculating healthy yam tubers of Araba, Asana and Puna with pure cultures of each bacterial and fungal isolate according to the method described by Okafor (1966). The method was as follows: Sound yam tubers free from bruises, cuts, rots or any visible defects were washed in running tap water for 10 minutes, surface sterilized by immersion in 5% sodium hypochlorite solution for four minutes, and dried at room temperature. Each tuber was wiped with 95% ethanol prior to inoculation. A sterile 5mm diameter cork-borer was used to make 8mm deep wells on the tubers. Agar blocks of bacterial and fungal colonies were removed from the margins of three-day old cultures to be tested and placed into the wells. The mouths of the wells were wiped with 95% ethanol and the wells occluded with yam before sealing tissue plugs with melted paraffin wax (Fig. 1.). A control was set up in a similar manner but inoculated with sterile PDA and NA blocks.

There were five replicate tests of each isolate. The positions on the tubers were randomized and spaced such that two tubers were used to contain the 11 isolates and the control. This was in response to a recommendation by Adimora (1986). Inoculated tubers were stored at ambient conditions in the laboratory for 21 days with temperatures ranging between 23-30⁰C. At the end of storage the tubers were cut longitudinally across the points of inoculation and the symptoms of rot which had developed recorded and compared with those present in the original diseased tubers from where the isolates were obtained. Re-isolation and identification of the rot-causing organisms from inoculated yam tubers was also done. An isolate was confirmed pathogenic if it caused rot similar to that present on the diseased tubers from where it was isolated. The nematode was not re-inoculated into healthy yam tissue because *Scutellonema bradys* is a well known yam rot nematode (Jenkins and Taylor, 1967; Thompson *et al.*, 1973; Adesiyani, 1996; Théberge, 1985).



Fig. 1. Inoculated yam tubers stored in wooden inoculation chamber in the laboratory.

- Note:
- i. The wounds sealed with melted paraffin wax
 - ii. The random arrangement of the pathogens in the inoculated cavities covered with paraffin wax.

- (a) *Aspergillus flavus*
- (b) *Penicillium brevi-compactum*
- (c) *Aspergillus niger*
- (d) *Botryodiplodia theobromae*
- (e) *Fusarium sp.*
- (f) *Aspergillus oryzae*
- (g) *Rhizopus stolonifer*
- (h) Control
- (i) *Fusarium oxysporum*
- (j) *Erwinia carotovora*
- (k) *Penicillium sp.*
- (l) *Fusarium culmorum*

- iii. The black background in the Fig. is the floor of the inoculation chamber.

3.4 Comparison of shelf life of yam varieties

Sound yam tubers of Araba, Asana and Puna were used for the study. The tubers range in weight from 1.75 - 4.05kg. The experiment was conducted between December, 1996 and October, 1997 in a barn situated in the Sinna's garden of the Crop Science Department, University of Ghana. The barn was roofed and open-sided, measuring 3.8m x 2.0m x 2.2m. The sides were protected from rodents by surrounding it with a strong wire mesh. Forty tubers of each variety were divided into four replicate groups of ten to constitute a treatment. Tubers were labelled, weighed and stored on shelves using a randomised complete block design (RCBD) layout. The temperature range was 24-31⁰C and 48-83% r.h. taken with a thermohydrograph placed in the barn (Fig. 2.).

Tubers were examined every four weeks during a 40 week period for rots, sprouting and weight loss. Rotten yams were identified by visual inspection, the rotten ones being shriveled in appearance and/or soft to touch. Where there was doubt, the peeling off of skin at the affected site normally revealed brown rotten tissue. Tubers with rot were removed and micro-organisms associated with the rot isolated as before. Tubers were considered sprouted, when sprout buds penetrate the tuber skin. Tuber weight loss was assessed by weighing individual tubers at the beginning of the experiment (weeks) and progressively at four weekly intervals.



Fig. 2.

Yam tubers from a farmer's field stored in a barn at the Sinna's garden, University of Ghana, Legon. The thermohydrograph for recording the temperature and relative humidity in the barn (Arrowed); The randomized complete block layout of the three white yam varieties (Araba, Asana and Puna); The yam tubers arranged on the bottom and two top shelves constitute the experiment on the use of lime and neem wood-ash to control yam rot.



3.5 Treatment of wounded yam tubers with lime and neem wood-ash

Sound yam tubers of Araba, Asana and Puna were artificially wounded and the wounds treated with lime paste and neem wood-ash. The wounds were created by pushing a flamed 20mm cork borer 3mm into the ground tissue of the tubers at 'head', 'middle' and 'tail' regions. The peel and tissue in the punched area were scraped off with a flamed scalpel. There were three wounds per tuber. They were then placed together with wounded but untreated yam tubers in the Sinna's garden yam barn for storage. Five tubers from each variety constituted a treatment and each treatment was replicated three times. Sieved neem wood-ash (0.5g) and 86% lime (calcium oxide) paste (1.5g) were spread uniformly on the wounded areas using a spatula (Fig. 3). The neem wood-ash was sieved with test sieve No. 25 (0.6mm) while the lime paste was formed by soaking 86g of commercially-prepared lime in 100ml sterile distilled water overnight. The control treatment consisted of wounded but untreated yam tubers.

The tubers were stored for ten weeks (March - June 1997) at a temperature range of 24-30⁰C and 48-83% r.h. The layout was RCBD. At 2 weekly intervals, one tuber each from the replicates was selected at random and cut transversely into 'head', 'middle' and 'tail' sections. Each section was then cut longitudinally along the treated area and the rotten area estimated from a measurement with a rule.



Fig. 3. Wounded areas on yam tubers treated with lime and neem wood-ash. Left-right: Two tubers each treated with lime, untreated (control) and neem wood-ash.

3.6 Laboratory test on effect of lime and neem-wood ash on rot-causing organisms

The effectiveness of lime and neem-wood ash on radial growth of fungi isolated from diseased yam tubers were assessed in the laboratory by plating the isolates on lime and neem-wood ash amended PDA i.e. lime potato dextrose agar (LPDA) and ash potato dextrose agar (APDA) respectively. The levels of the treatments were 0.38, 0.75, 1.50 and 2.25g of lime and 0.13, 0.25, 0.50 and 0.75g of neem wood-ash per 15ml of PDA in a 90 mm Petri dish. The control experiment was unamended PDA. Fungal isolates were plated singly on the media (three replicate plates per isolate) and incubated in the laboratory at 23 - 30°C using a completely randomised design (CRD) layout. The plates were observed daily and colony diameter measured with a rule for seven days. Where there was growth, the nature of growth was also observed. The pH of neem wood-ash solution (48%) and lime solution(48%) were also determined with a pH meter.

CHAPTER FOUR

RESULTS



4.1 Survey results

4.1.1 Major yam markets in Accra: - Response to questionnaires administered revealed that the major yam markets in Accra are the Konkomba, Timber and Haatso yam markets. These big markets deal in yams only and most retailers from smaller markets obtain their supplies from there.

4.1.2 Popular yam varieties for export and the time they are available: - The white yam (*Dioscorea rotundata*) was confirmed as the most common species of yam sold and exported from Ghana. Four varieties of white yams were found to have high preference among retailers and exporters in Accra. Respondents prefer Asana (100%) and Puna (100%) followed by Araba (84%) and Punjo(76%). The least preferred yam varieties for export were Kpajol (20%) and Labrakor (12%).

Times of the year when the preferred varieties are available on the markets are August-February (Puna), November-March (Asana) and December till the arrival of new yam around August (Araba and Punjo).

Desirable qualities of exportable yams were good shape, medium size (1.5-4.0kg), uniform skin colour, no bruises, cuts and rots.

4.1.3 Sources of yam supply to retailers and exporters: Table 2 shows the sources of yam supply to the retailers and exporters interviewed. Retailers do not obtain their yam from village markets but 20% of exporters buy yams from

Table 2. Sources of yam supply for retailers and exporters in three yam markets in Accra.

Source of supply	Percent of sellers involved			Exporters
	Konkomba market	Timber market	Haatso market	
Village market	0.0	0.0	0.0	20.0
Retailers/exporters farm	60.0	13.3	13.3	10.0
Farm gate	20.0	26.7	26.7	10.0
Urban/city market	20.0	60.0	53.3	60.0
Others	0.0	0.0	6.7	0.0
Total	100.0	100.0	100.0	100.0

that source. More yam retailers from Konkomba market (60%) sell yams from their own farms as against 13% for sellers from Timber and Haatso markets. Less than 27% and 10% of sellers and exporters, respectively, buy yams at farm gate. The most popular source of supply is buying from urban markets where more than half of retailers and exporters patronize. Seventy percent of respondents indicated that the duration of storage is not as important as the condition of the tubers at the time of purchase.

4.1.4 Transportation of yam to Accra: - The transportation of tubers from producing centers to Accra is mainly by road (93% of respondents) and to a lesser extent by boat from the northern part of Ghana to Akosombo and onward by road to Accra (7 % of respondents).

All yam tubers are packed into lorries by hand throwing and catching and then stacked loosely in lorries. It takes an average of two to four days to transport yams from producing centres to Accra.

4.1.5 Storage of yams prior to selling/exporting: - Table 3 and table 4 shows the type and duration of storage respectively employed by the retailers and exporters. More than 80% of retailers and 70% of exporters store yams in open and airy places under straw mat or tarpaulin cover (Fig 4) usually leaving those for sale uncovered during the day (Fig 5).

Table 3. Methods of yam tuber storage

Method of storage	Percentage of sellers involved			
	Konkomba market	Timber market	Haatso market	exporters
Roofed and open sided house	15.0	13.3	0.0	30.0
Heaped in airy open place	85.0	86.7	100.0	70.0
Total	100.0	100.0	100.0	100.0

Table 4. Duration of yam tuber storage

Duration of storage (days)	Percentage of sellers involved			
	Konkomba market	Timber market	Haatso market	exporters
1 – 2	15.0	26.7	20.0	10.0
3 – 4	70.0	46.7	53.3	50.0
5 – 7	10.0	20.0	20.0	30.0
14 and above	5.0	6.6	6.7	10.0
Total	100.0	100.0	100.0	100.0



Fig. 4. Yam tubers stored in heaps and covered with straw mats and tarpaulin.



Fig. 5. Wounded tubers exposed to the elements at the Konkomba yam market, Accra. Wounds (Arrowed) occurred during handling and transportation.



In all 70% of respondents inspect their yams periodically while in storage. Yam storage pest are not a major problem but rats, mice, scale insects, mealy bugs and yam moths are the pest encountered.

4.1.6 Annual loss of yams to rots: - When 50 sellers from the three yam markets and 10 exporters responded to the questionnaire, the percentage of tubers rotten per annum ranged from 10-30% for sellers and 10-40% for exporters. Table 5 shows their distribution among exporters and retailers in the yam markets.

Table 5: Annual percentage yam tuber rot observed by retailers and exporters in Accra

Annual tuber rot (%)	Percentage of sellers involved			
	Konkomba market	Timber market	Haatso market	Exporters
1 – 10	10.0	13.3	20.0	0.0
10 – 20	80.0	66.7	80.0	60.0
20 – 30	5.0	20.0	0.0	30.0
30 – 40	5.0	0.0	0.0	10.0
Total	100.0	100.0	100.0	100.0

More than 60% of all retailers and exporters experience 10-20% tuber rot and above per annum with all yam exporters being in this group. Degree of rotting above 20% is encountered more by exporters (40%) than sellers (10%, 20% and 0% in Konkomba, Timber and Haatso markets respectively).

4.1.7 Susceptibility of yam varieties to rots and sprouting: - Puna was identified as the most susceptible variety to rots by 80% of the retailers and exporters. The least susceptible varieties named are Araba (60%) and Punjo (40%). A good number (60%) of the respondents could detect rot in tubers with no external

symptoms by pressing tubers with their fingers and the scent produced. Puna was identified as the fastest sprouting yam (80%) while Araba and Punjo are considered the slowest sprouting yams.

- 4.1.8 Control of yam rot diseases: - Table 6 shows the methods of yam rot disease control adopted by respondents. Most of them (88.3%) do not apply any control measure. Seed yams cut for minisett production are treated with lime or wood-ash by only 3.3% of respondents while pre-storage curing by keeping yams in shade for a few days is even less popular (1.7%). There was no information on the efficacy of the lime and wood-ash used.

Table 6: Methods of yam rot disease control by retailers and exporters

Method of control	Percentage respondents involved
Pre-storage curing	1.7
Application of wood-ash to wounded tubers	3.3
Application of lime to wounded tubers	5.0
Disinfection of yam store and means of transport	1.7
No control measure taken	88.3
Total	100.0

- 4.1.9 Shipment of yam by exporters: - Exporters transport their tubers to export destinations mainly by sea (90%) and it takes 2-3 weeks to reach (80%). Yams in ships are stored together with cocoa and other export products in the same compartment of the ship with poor ventilation.

4.2 Identification of the three varieties of white yam

It was observed from the survey that naming of local yam varieties is one in which each locality had unique names for the different cultivars. In Accra, the names of the three white yam varieties used in the studies are the same

among exporters, retailers and consumers. These are Asana, Araba and Puna (Fig. 6) They have the following distinguishing features.

Asana has a cylindrical shape and usually rounded at the tail end, with a variable skin colour but usually light-brown and with lighter and thinner skin. Ridging on its cork covering is faint with short faint furrows running longitudinally along the tuber length making the tuber surface almost smooth. The tuber flesh colour is either white or light-yellow when fresh, with a low frequency of oxidative browning when cut. The tuber flesh texture is smooth and firm when fresh but soft when cooked and more palatable than Araba but less palatable than Puna.

Araba is more or less cylindrical in shape with both ends flattened although a few have variable shapes. The tuber skin usually has dark-brown colour with relatively thicker and tougher skin in comparison to Asana and Puna.

The ridging on the thick layer of cork covering the tubers is deep and almost circular in pattern. At the head and middle regions of the tuber, the edges of the circular cork covering are a bit lifted, exposing the white outer layer of the cortex and making the tuber surface rough. The tuber flesh colour is white and tends to undergo oxidative browning when cut especially at the sites of wounds. The flesh colour appears white with colourless taints when cooked. Tuber flesh texture is slightly smooth with a firm consistency. It is the least palatable of the three varieties.



A.



B.



Fig. 6. Some characteristics of the three white yam varieties used for the studies. Note: A, Asana (As) being cylindrical in shape with both ends flattened, light-brown with short faint furrows running longitudinally along its length giving it an almost smooth surface. Araba (Ar) being cylindrical in shape with both ends flattened, dark-brown with deep and circular ridging pattern on the cork covering of the tubers. Puna (P) being cylindrical and tapering from head to a point with long deep furrows running longitudinally along its length. B, Oxidative browning at wounded sites in cut tubers.

Puna generally has a cylindrical shape up to the middle and from there tapers to a point although a few vary in shape. Tuber skin is usually light-brown in colour with lighter and thinner skin when felt by the hand. Long and deep furrows on its cork covering run longitudinally along its length. Tuber flesh has white or cream colour when fresh or cooked. The flesh of most tubers tends to brown when cut. **Tuber-flesh texture** is slightly smooth and firm when fresh. It is softer and more palatable than Asana when cooked.

4.3 Isolation and identification of yam rotting micro-organisms

When full and parts of 294 partially-rotten yam tubers (174 from yam barn and 120 from yam markets) were sampled from December, 1996 to May, 1997, ten fungal species, a bacterium and a nematode were consistently isolated from them. The ten fungi were, *Aspergillus flavus* Link; *Aspergillus niger* van Tieghem, *Aspergillus oryzae* (Ahlburg) Cohn, *Botryodiplodia theobromae* Pat., *Fusarium culmorum* (W. G. Smith) Sacc., *Fusarium oxysporum* Schlecht *Fusarium sp.*, *Penicillium brevi-compactum* Dierckx., *Penicillium sp.* and *Rhizopus stolonifer* (Ehrenb) Lind. The identifications were based on the following:

Aspergillus flavus

Culture on PDA consisted of dense felt of yellow-green colour (Fig. 7) attaining a diameter of 3-5cm within 7 days. Mycelial growth was usually in concentric rings. Conidial head usually radiated, later splitting into several loose columns, yellowish-green becoming dark-yellow-green. Conidiophores hyaline, vesicle globose with phialides borne directly on them, conidia globose to subglobose (Fig. 8) and measured 3.6µm in diameter.



Fig. 7. Seven-day old culture of *Aspergillus flavus* on potato dextrose agar plate (x0.8). Note the concentric rings within the mycelium.



Fig. 8 Conidial head and conidia of *Aspergillus flavus* (x1600). Note the globose vesicle and conidia.

Aspergillus niger

Culture on PDA attained a diameter of 4-5cm within 7 days and consisted of a compact yellow basal felt which changed to a dense layer of brownish-black to black culture (Fig. 9). Conidial heads radiated, tending to split into loose columns with age. Conidiophores were hyaline with vesicles globose to subglobose and phialides borne on hyaline metulae holding globose to subglobose conidia (Fig. 10). Brown and subglobose conidia measured about 3.5-5.0 μ m.

Aspergillus oryzae

Colonies on PDA attained a diameter of 3-5cm within 7 days, consisting of a greenish- yellow and dull-brown felt of long conidiophore intermixed with aerial mycelium and having concentric rings within the mycelium(Fig. 11). Conidial heads radiated, conidiophores hyaline mostly rough-walled with subglobose vesicle (Fig. 12). Phialides were borne on metulae with globose to subglobose conidia which measured about 4.5-8.0 μ m.

Botryodiplodia theobromae

Culture on PDA filled a 9cm diameter plate within 2 days, consisting of fluffy aerial mycelium, grey, but turned black after 5 days (Fig. 13). When two-day old culture was kept under light from a filament bulb (60W) in the laboratory for six days, the culture became light red and the colour deepened as the days increased (Fig. 14). Culture on cornmeal agar (CMA) produced pycnidia (Fig.15) from stromata which also produced ellipsoidal conidia (pycnidiospores). The spores were hyaline and aseptate (Fig. 16) when immature and dark-brown with two cells when mature (Fig. 17).



Fig. 9. Seven-day old culture of *Aspergillus niger* on potato dextrose agar (PDA) plate (x0.8). Note the yellow marginal coloration of the black culture.

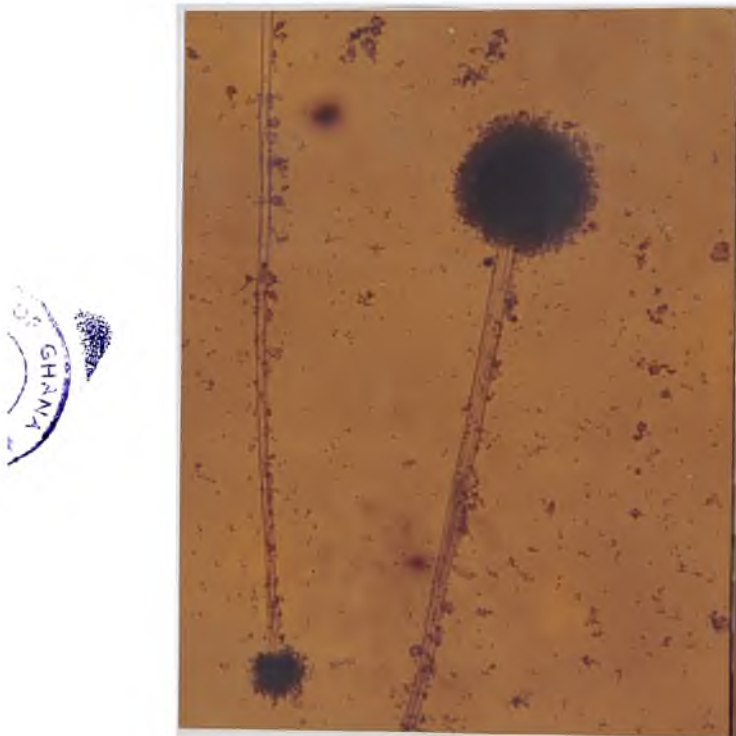


Fig. 10. Conidial head and conidia of *Aspergillus niger* (x800)



Fig. 11. Ten-day old culture of *Aspergillus oryzae* on potato dextrose agar (PDA) plate (x0.8). Note the concentric rings within the mycelium and the dark-green colour of the culture

YNV

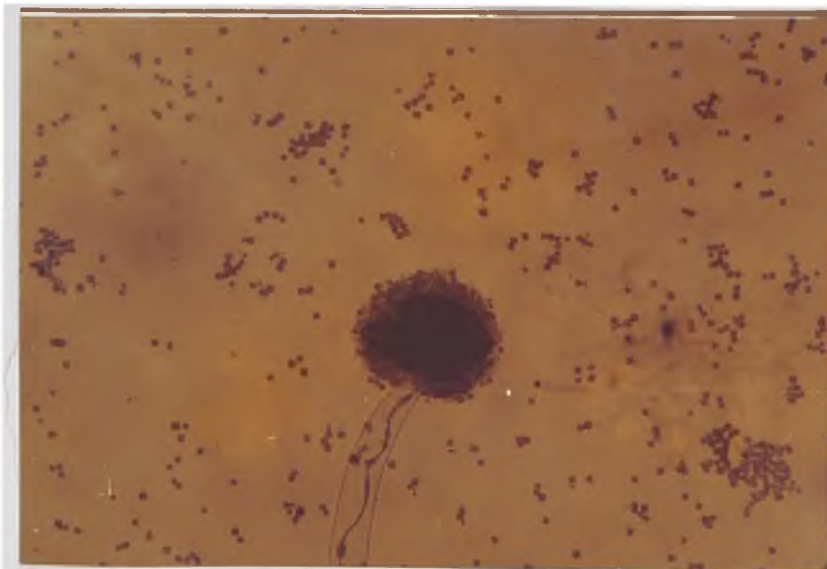


Fig. 12. Conidial head and conidia of *Aspergillus oryzae* (x800)



Fig. 13. Two and eight-day old cultures of *Botryodiplodia theobromae* on potato dextrose agar plates (x0.6). Left , Two-day old culture with grey aerial mycelium; Right ,Eight-day old culture with black aerial mycelium.

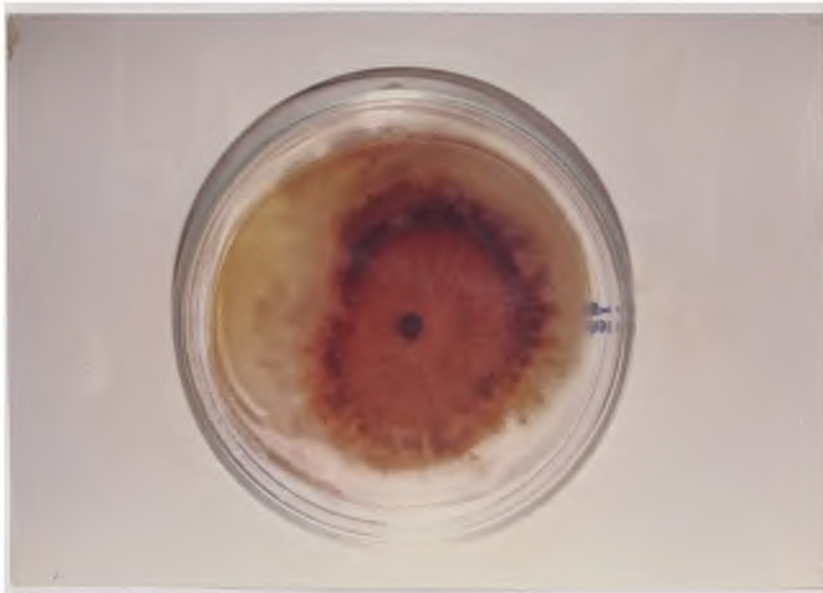


Fig. 14. Two-day old culture of *Botryodiplodia theobromae* on potato dextrose agar plate kept under light from filament bulb (60W) for eight days (x0.8).

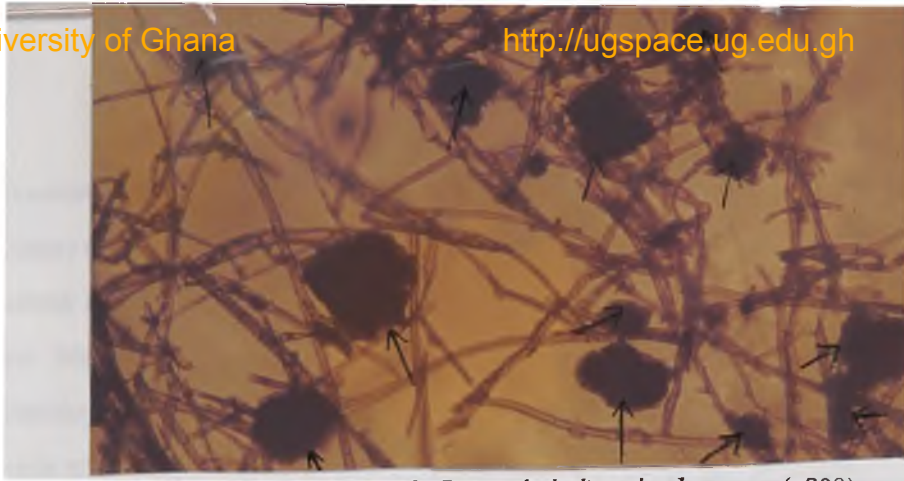


Fig. 15 Pycnidia of *Botryodiplodia theobromae* (x800). Pycnidia (Arrowed) were observed on a fourteen-day old culture on cornmeal agar (CMA).



Fig. 16. Immature conidia of *Botryodiplodia theobromae* (x800) observed on a 22-day old culture on cornmeal agar (CMA). Note the hyaline and simple-celled immature conidia



Fig. 17. Mature conidia of *Botryodiplodia theobromae* (x 800) on a 30-day old Culture on cornmeal agar. Note the dark-brown and two-celled mature conidia.

Fusarium culmorum

Culture on PDA attained a diameter of 8.5cm in 5 days. Aerial mycelium was whitish at first changing to cream (Fig. 18) and later becoming brownish-red and felty (Fig. 19) with a pale-cream pigmentation of the underside. Conidiophores were branched with short phialides. Microconidia were absent while macroconidia were septate and sickle-shaped having pointed apex (Fig. 20). Ovoid chlamydospores were present and measured 9-14 μ m in diameter.

Fusarium oxysporum

Colonies attained a diameter of 4.5cm in 4 days. Aerial mycelium was sparse and became light-orange (Fig. 21) with a light-purple tinge developing later at the older parts of the culture. The underside of the culture was a light shade of purple. Micro-conidia were elliptical and borne on lateral, simple phialides and not in chains. Macro-conidia which were elongated and pointed at both ends were borne on phialides on branched conidiophores (Fig. 22).

Fusarium sp.

Culture on PDA attained a diameter of 4.6 cm in four days, with pale cream colour (Fig. 23). Aerial mycelium was dense with powdery appearance but the underside was creamish. Conidiophores were unbranched with elongated phialides. Micro-conidia were abundant with scarce macroconidia being slender (Fig. 24), 3-7-septate, straight or slightly bent. Chlamydospores were absent.



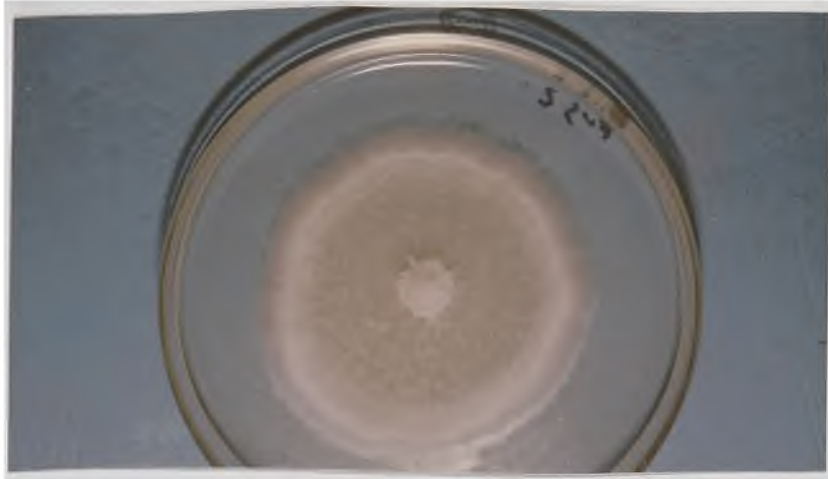


Fig. 18. Seven-day old culture of *Fusarium culmorum* on potato-dextrose agar plate (x1.0).

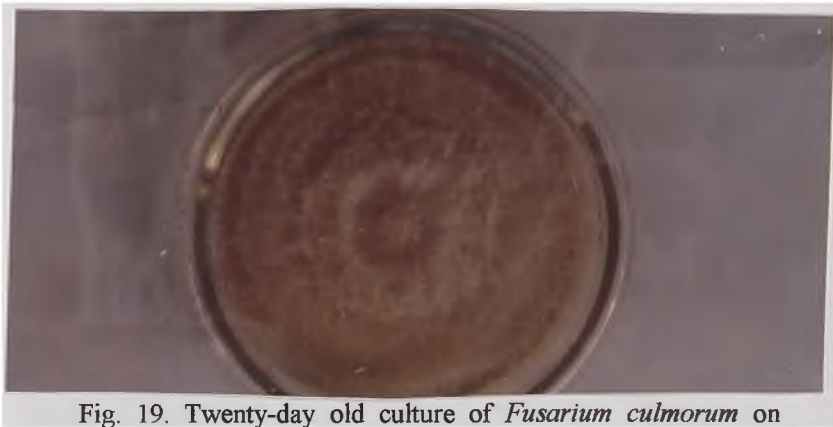


Fig. 19. Twenty-day old culture of *Fusarium culmorum* on potato dextrose agar plate (x0.8).



Fig. 20. Macroconidia of *Fusarium culmorum* (x800).



Fig. 21. Eight-day old culture of colonies of *Fusarium oxysporum* on potato dextrose agar plate (x 0.8).



Fig. 22. Spores of *Fusarium oxysporum* (x800). (a) microconidia (b) macro-conidia (c) conidiophores on which macroconidia are borne.

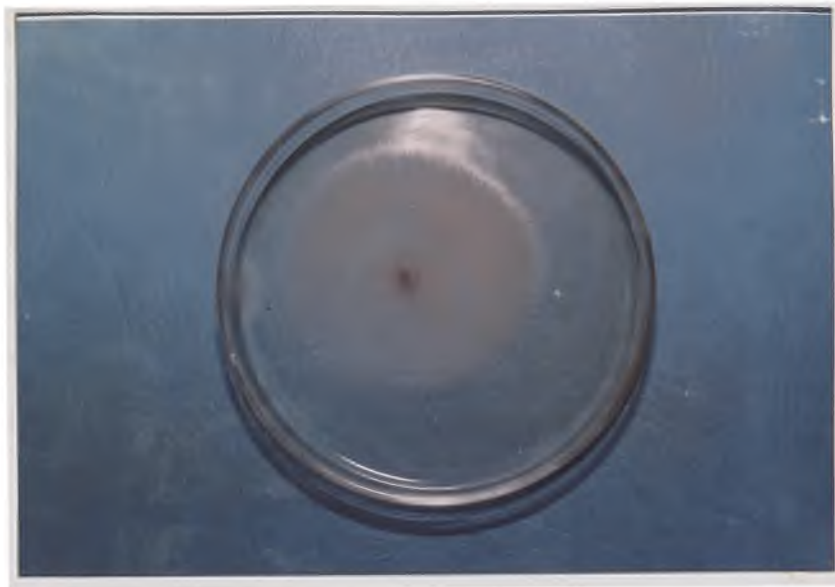


Fig. 23 Five-day old culture of *Fusarium sp.* on potato dextrose agar plate (x0.8).



Fig. 24. Structure of *Fusarium sp.* observed under light microscope (x800). Note the slender microconidia (Arrowed).

Penicillium brevi-compactum

Culture on PDA attained a diameter of 1.5cm within seven days and consisted of dense felt of large compact conidiophores, 300-500µm long and spores. The colour of the culture was grey-green (Fig. 25). Conidiophores terminated in a compact penicillus which was terverticillate (Fig. 26). The branches and metulae were usually inflated with flask-shaped phialides. Conidia were globose and smooth with diameter between 3-4µm.

Penicillium sp.

Culture attained a diameter of 4.2cm in 10 days. The colour was pale-yellow (Fig. 27) or white and yellow at the underside of the culture. Conidiophores terminated in a penicillus structure which was terverticillate. Strands of hyphae with long phialides were from aerial mycelium. Phialides were narrow, flask-shaped at the base and terminated in long slender-bearing tips. Conidia were one-celled and cylindrical with both ends rounded (Fig. 28).

Rhizopus stolonifer

Culture filled a 9 cm-diameter plate in 2 days. The fluffy aerial mycelium was whitish and became greyish-brown with age (Fig. 29). Smooth-walled and aseptate sporangiophores were in groups arising from stolons opposite the branched rhizoids. Sporangia measured about 150µm in diameter, globose and brownish at maturity with globose columella (Fig. 30).





Fig. 25. Fourteen-day old culture of *Penicillium brevi-compactum* on potato-dextrose agar plate (x0.8).

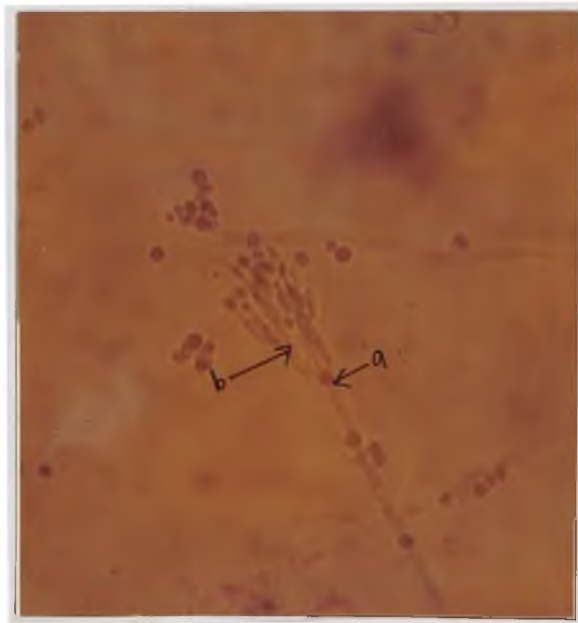


Fig. 26 Structure of *Penicillium brevi-compactum* under light microscope (x1600). (a) the inflated branch of conidiophore, (b) metulae (c) flask-shaped phialides.

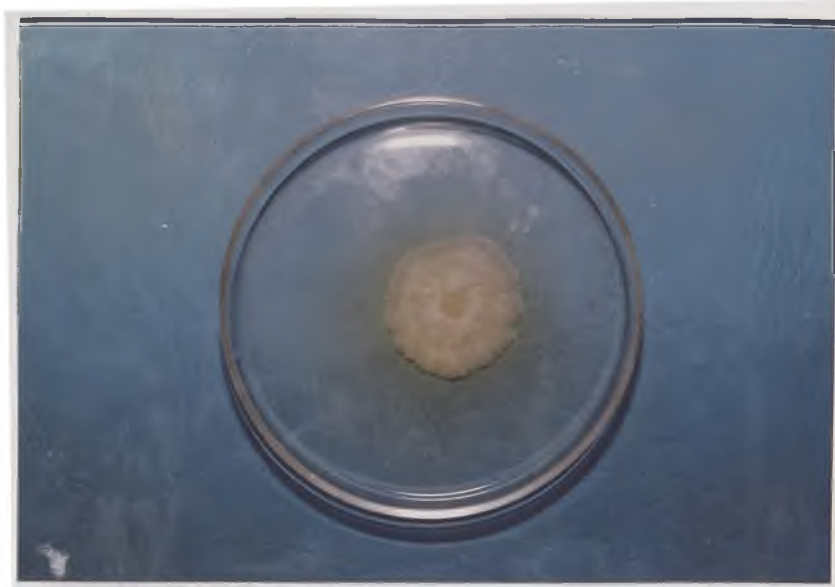


Fig. 27. Seven-day old culture of a *Penicillium sp.* on potato dextrose agar plate (x0.8).

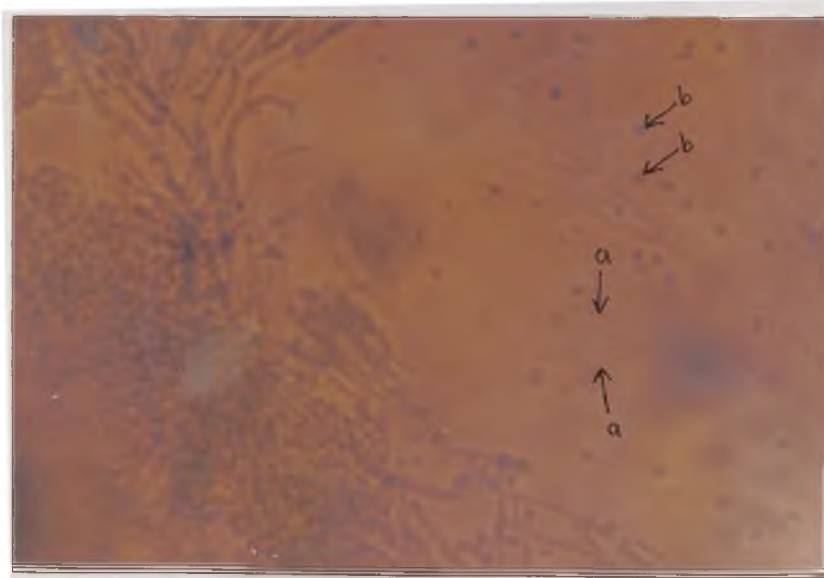


Fig. 28. Structure of *Penicillium sp.* under light microscope (x800). Note (a) the narrow phialides (b) the cylindrical conidia with both ends rounded.

Fig. 29. Four-day old culture of *Rhizopus stolonifer* on potato dextrose agar plate ($\times 0.8$).

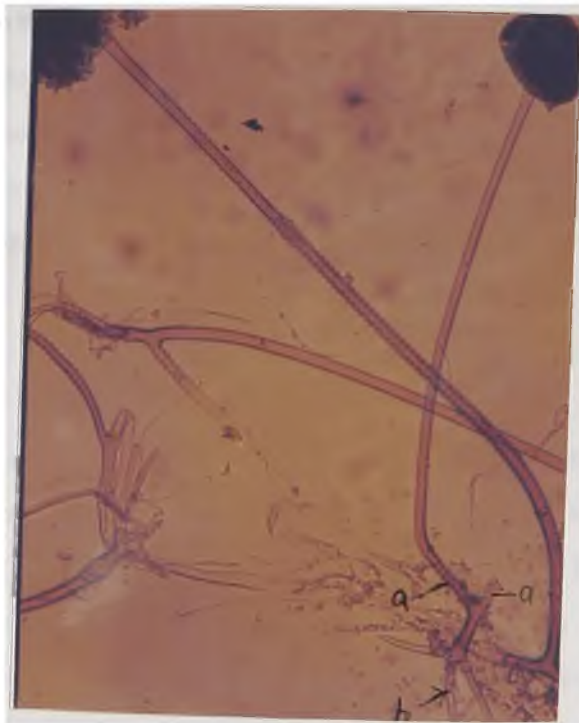


Fig. 30. Structure of *Rhizopus stolonifer* under light microscope ($\times 800$). Note (a) the group of Sporangiohores arising from a common point on a stolon (b) rhizoids opposite the sporangiophores.

Erwinia carotovora

This bacterium was isolated from watery soft rots. Discrete colonies grew on both nutrient agar (NA) and yeast extract-dextrose-carbonate (YDC) agar with light-yellow colour on NA and yellow on YDC. The Bacterial cells were motile and Gram-stained slides revealed rod-shaped bacteria stained with red colour of the counter stain (gram-negative). The *E. coli* used as a reference culture was also gram-negative. The colour of the uninoculated and inoculated basal medium in all the test tubes used for the anaerobic test changed from blue to yellow showing the presence of an anaerobic bacterium.

In the spore determination test, rod-shaped bacterial cells were stained red with no green stain in their structure. This showed that the bacterium was not a spore-producing type. Discrete colonies of the bacterium also grew on MS agar to confirm *Erwinia* and exclude *Xanthomonas*. In the pectolytic test, the potato slices in all the test tubes containing nutrient broth inoculated with the bacteria started rotting by the third day while the slices in the control did not rot.

Scutellonema bradys

Nematodes were extracted from the diseased tubers of all the three white yam varieties used for the study. However, no nematode was obtained from the sound yam tubers. The nematodes were all identified as the yam lesion nematode (*Scutellonema bradys*) based on the following characteristics. The adult was medium-sized (0.6-0.8mm long) and usually slightly curled; its motion was slow, the tail region was rounded; heat relaxed shape was coiled or open C; the heavy and coiled tylenchoid stylet was 25µm-50µm in length, heavily sclerotised and had prominent knobs (Fig. 31).



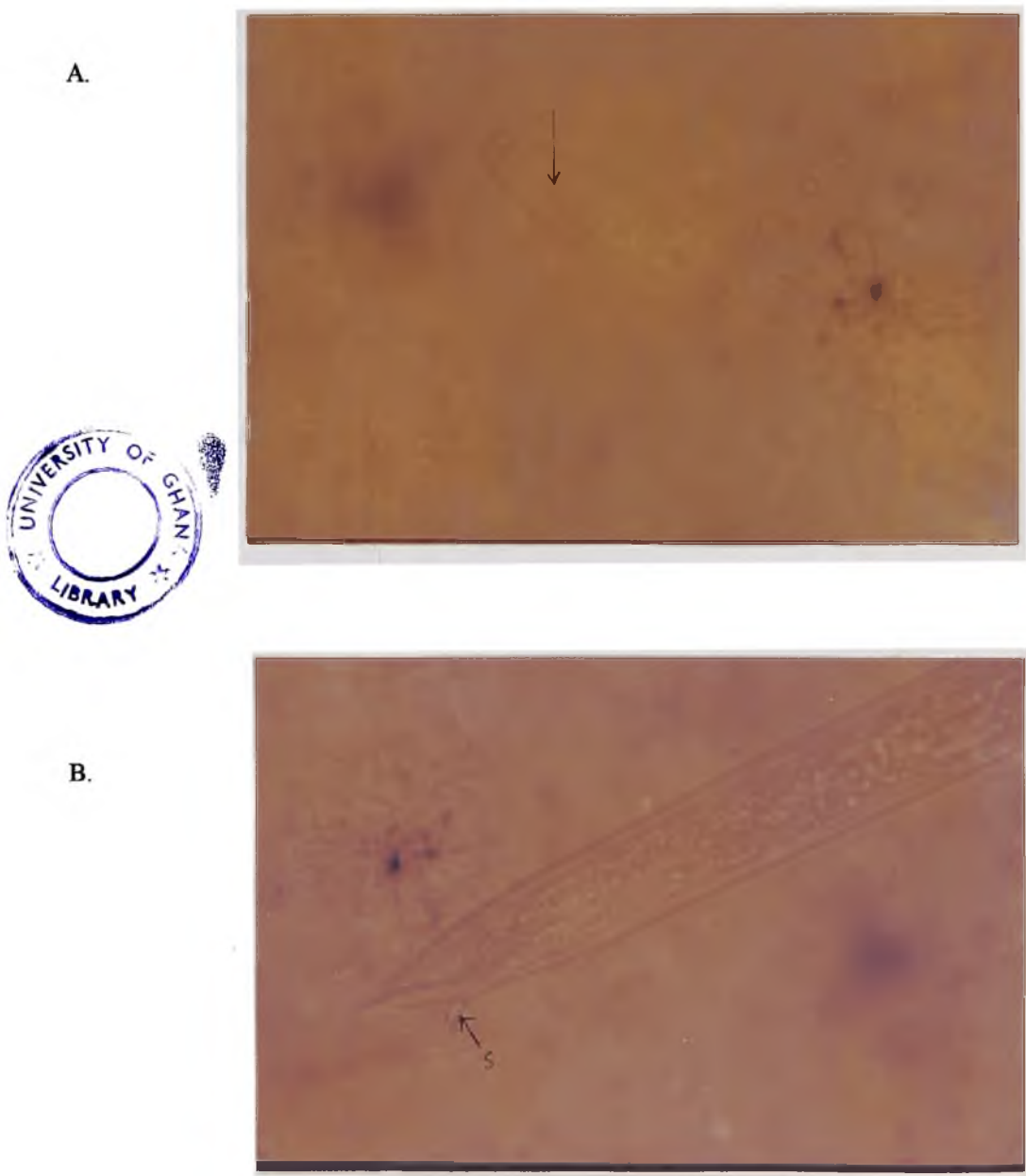


Fig. 31. *Scutellonema bradys* from diseased *D. rotundata* tuber (x1600). Note A, head region showing stylet. B, hind end of adult male showing specule(s)

4.4 Frequency of occurrence of micro-organisms in decayed yam tubers

Table 7 presents the frequency of occurrence of yam-rotting organisms isolated from forty tubers each of Araba, Asana and Puna obtained from the three yam markets in Accra between September, 1996 and May, 1997. Ten fungal species were isolated from all the three yam varieties. The bacterium *Erwinia carotovora* was, however, not isolated from Araba tubers but proved pathogenic to Araba in pathogenicity tests. There was high frequency of occurrence of the eleven pathogens in Puna with mean frequency of occurrence being (23.4%) followed by Asana (19.2%) and then Araba (13.3%). *Rhizopus stolonifer* was more frequent in Puna (63.0%) and Asana (50.0%) and was the second frequently occurring pathogen in Araba (23.0%). *Rhizopus stolonifer* also had the highest mean frequency of occurrence (45.3%) among the yam-rotting pathogens in the yam tubers, followed by *Aspergillus niger* (30.0%), *Botryodiplodia theobromae* (27.7%), *Aspergillus oryzae* (20.0%), *Penicillium brevi-compactum* (19.0%), *Fusarium oxysporum* (17.6%), *Penicillium sp.* (12.7%), *Fusarium culmorum* (11.0%), *Aspergillus flavus* (9.3%), *Fusarium sp* (7.7%) and *Erwinia carotovora* (4.3%).

The frequency of occurrence of isolates on 174 decayed tubers consisting of 58 tubers each of Araba, Asana and Puna stored in the yam barn in Sinna's garden, Legon, between December, 1996 and September 1997 are represented in Table 8.

Table 7 Frequency of occurrence of micro-organisms in decayed tubers of Araba, Asana and Puna obtained from yam markets in Accra.

Micro-organisms	Araba	Asana	Puna	
<i>Aspergillus flavus</i>	10 ^a	10 ^a	8 ^a	9.3 ^b
<i>Aspergillus niger</i>	30	35	25	30.0
<i>Aspergillus oryzae</i>	15	15	30	20.0
<i>Botryodiplodia theobromae</i>	20	23	40	27.7
<i>Erwinia carotovora</i>	0	8	5	4.3
<i>Fusarium culmorum</i>	15	5	13	11.0
<i>Fusarium sp.</i>	5	10	8	7.7
<i>Fusarium oxysporum</i>	10	15	28	17.6
<i>P. Brevi-compactum</i>	15	20	22	19.0
<i>Penicillium sp.</i>	3	20	15	12.7
<i>Rhizopus stolonifer</i>	23	50	63	45.3
Total Isolations	40	40	40	
Mean	13.3 ^b	19.2	23.4	

^a Figures represent frequency of occurrence given as a percentage of the total isolations.

^b Figures represent mean frequency of occurrence.

Table 8 Frequency of occurrence of micro-organisms in decayed tubers of Araba, Asana and Puna obtained from a yam storage barn at Legon.

Micro-organisms	Araba	Asana	Puna	
<i>Aspergillus flavus</i>	0 ^a	5 ^a	6 ^a	3.7 ^b
<i>Aspergillus niger</i>	58	17	42	39.0
<i>Aspergillus oryzae</i>	0	28	17	15.0
<i>Botryodiplodia theobromae</i>	0	16	21	12.3
<i>Erwinia carotovora</i>	0	10	7	5.7
<i>Fusarium culmorum</i>	0	0	5	1.7
<i>Fusarium sp.</i>	18	3	14	11.7
<i>Fusarium oxysporum</i>	24	0	22	15.3
<i>P. Brevi-compactum</i>	24	17	20	20.3
<i>Penicillium sp.</i>	0	19	0	6.3
<i>Rhizopus stolonifer</i>	16	38	52	35.3
Total Isolations	58	58	58	
Mean	12.7 ^b	13.9	18.7	

^a Figures represent frequency of occurrence given as a percentage of the total isolations.

^b Figures represent mean frequency of occurrence.

It was revealed that the same eleven pathogens referred to above also occurred in the yam barn. *E. carotovora* was again not isolated for Araba tubers in the barn but proved pathogenic when inoculated into healthy tubers of Araba. There were ten different pathogens isolated from Puna, nine from Asana and five from Araba. Puna again had the highest mean frequency of occurrence of the eleven isolates (18.7%), followed by Asana (13.9%) and then Araba (12.7%). In the barn tubers, *Aspergillus niger* had the highest mean frequency of occurrence among the yam-rotting pathogens (39.0%), followed by *Rhizopus stolonifer* (35.3%), *Penicillium brevi-compactum* (20.3%), *Fusarium oxysporum* (15.3%), *Aspergillus oryzae* (15.0%), *Botryodiplodia theobromae* (12.3%), *Fusarium sp.* (11.7%), *Penicillium sp.* (6.3%), *Erwinia carotovora* (5.7%) *Aspergillus flavus* (3.7%) with the least being *Fusarium culmorum* (1.7%). There was generally a higher frequency of occurrence of the pathogens in the market than in the barn.

4.5 Pathogenicity test of isolates

Pathogenicity test of isolates revealed that all ten fungi and the bacterium isolated from rotten yam tubers could rot healthy yam tubers. (Figs. 32 and 33). Morphological characteristics of re-isolated micro-organisms were similar to those used for the inoculation.





Fig. 32. Longitudinal sections of yam tubers (Puna) inoculated with the test fungi and bacterium. Picture was taken 21 days after inoculation and storage at 23-30°C.

- a. *Penicillium sp.*
- b. *Rhizopus stolonifer*
- c. *Fusarium sp.*
- d. *Botryodiplodia theobromae*
- e. *Penicillium brevi-compactum*
- f. *Fusarium oxysporum*
- g. *Aspergillus niger*
- h. *Aspergillus oryzae*
- i. Control
- j. *Fusarium culmorum*
- k. *Erwinia carotovora*
- l. *Aspergillus flavus*



Fig. 33. Various symptoms produced by the fungi and bacterium following artificial inoculation. Note: Transverse sections and a closer look to give details of the colours.

- (a) *B. theobromae* with deep, dark-brown firm rot and light-brown margins.
- (b) *R. stolonifer* with light-brown soft rot.
- (c) *A. oryzae* with light-brown firm rot
- (d) *E. carotovora* with deep, dark-brown soft rot
- (e) *P. brevi-compactum* with dark-brown watery rot
- (f) *A. flavus* with a brown soft rot
- (g) *A. niger* with black firm rot
- (h) *Penicillium sp.* with light-brown to yellow soft rot
- (i-k) *F. culmorum*, *Fusarium sp.* and *F. oxysporum* with pale, dry rots.

4.6 Symptoms consistently produced by some yam-rotting pathogens in naturally-infected tubers of the three yam varieties

When isolations were made from the advancing margins of rots of yam tubers obtained from yam markets and the barn, most of the rot diseases were found to be caused by a complex of pathogens. However, some of the rot-causing pathogens occurred singly in some rots and consistently produced peculiar symptoms in all the three yam varieties. The pathogens and symptoms produced were as follows:

Aspergillus niger

The fungus consistently produced a dark-brown to black rot which was firm-textured in all the yam varieties (Fig. 34).

Penicillium brevi-compactum

The fungus was observed to be a fast rotter producing a dark-brown wet rot in all the yam varieties (Fig. 35). A grey-green mould was typically associated with cut or damaged tuber surfaces (Fig. 36).

Rhizopus Stolonifer

The fungus caused a rapid growing soft rot with diseased tissue being whitish at first and later turning pinkish. Abundant growth of white-gray mould (whiskers) was typically associated with cut or damaged tuber surface (Fig. 37).

Erwinia carotovora

The bacterium typically caused a watery soft rot with brown liquid of pungent odour oozing from infected tubers (Fig. 38).



Fig. 34. Dark-brown to black rot produced from natural infection by *Aspergillus niger* in Puna.



Fig. 35. Dark-brown wet rots produced from natural infection by *Penicillium brevi-compactum* in tubers of Asana.



Fig. 36. Grey-green mould growth of *Penicillium brevi-compactum* on cut surface of Araba.



Fig. 37. Soft rot of *D. rotundata* var. Puna caused by *Rhizopus stolonifer*. Left, infected surface of tuber with abundant mycelial growth and sporulation by *Rhizopus* ; Right, Cut surface through an advanced rotten area of tuber.



Fig. 38. Brown liquid ooze from a watery soft rot caused by natural infection by *Erwinia carotovora* in *D. rotundata* var. Asana.

Scutellonema bradys

The nematode produced deep and light cavities in the yam tuber tissue under the tuber skin, showing dark dry rot tissue (Fig. 39). In severe cases, cracks were seen in the tuber skin which flaked off.



Fig. 39. Dry rot tissue under tuber skin caused by the yam lesion nematode (*Scutellonema bradys*) in Asana. Note the light-yellow lesions under the tuber skin which usually turn dark-brown. This symptom is usually referred to as 'yellow fever' by the yam sellers in the three yam markets of Accra.

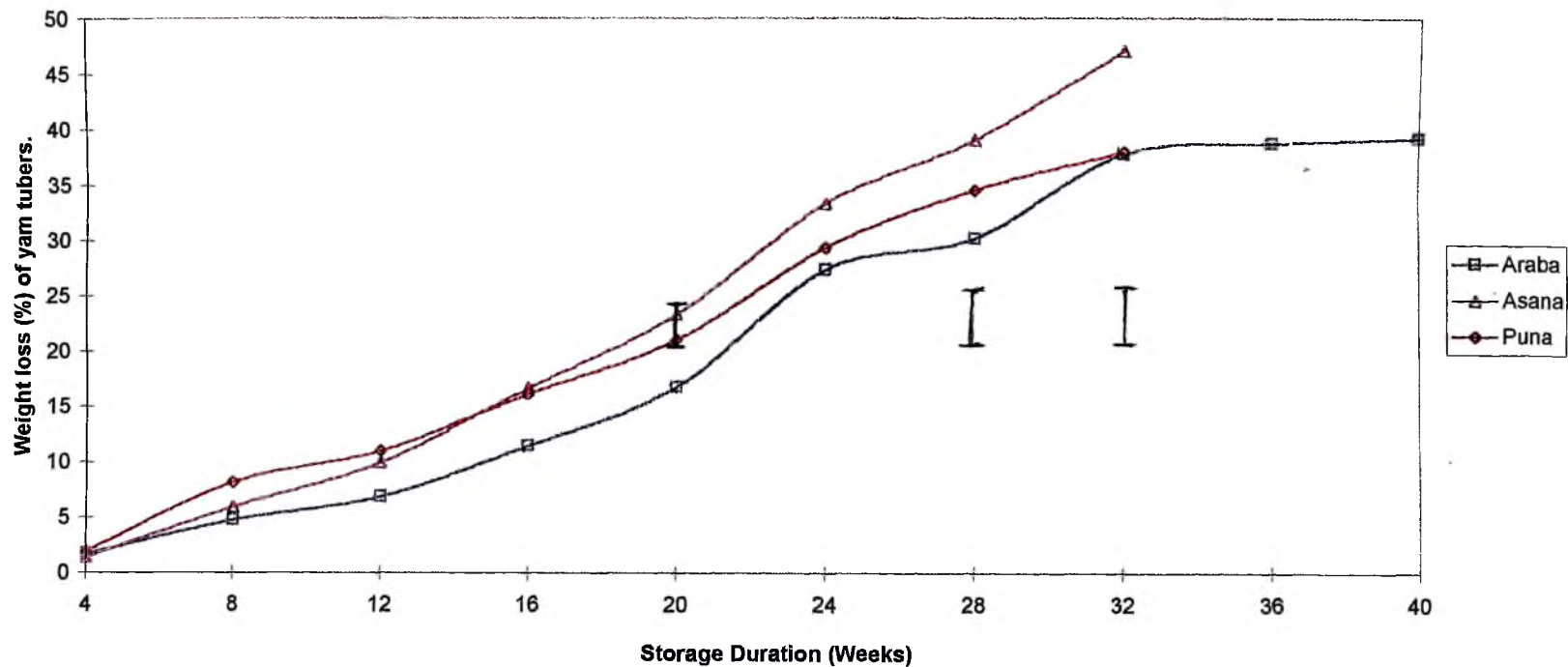
4.7 Shelf life of yam varieties

The percentage weight loss, sprouting and rotting of 40 tubers each of Araba, Asana and Puna in four replicate groups of ten tubers each were estimated every four weeks from December, 1996 to September, 1997. The estimates were based on the number of tubers that had not rotten (survivors) at the time of taking data. Temperature and relative humidity values in the barn ranged between 24-31°C and 48-83% respectively.

4.7.1 Weight loss of yam tubers: - Results of the progress in weight loss of tubers are presented in Fig. 40. During the study, there was a steady increase in the loss of fresh weight of tubers with time up to the 32nd week when all Asana and Puna tubers rotted. Generally, Araba tubers lost less weight than Asana and Puna, even though at the end of the 16th week in storage there was no significant difference in weight loss among the three yam varieties. In the 20th and 28th week in storage, however, Asana and Puna had a significantly higher weight loss compared to Araba ($P=0.05$).

4.7.2 Sprouting of yam tubers: - Results of sprouting trend of the three yam varieties are presented in Fig. 41. Araba tubers had a longer dormancy period than Asana and Puna with none of its tubers sprouting at the end of the fourth week. At the same time, 5.0% and 57.5% of Asana and Puna tubers had sprouted. Significantly lower number of Araba tubers had sprouted at the end of the 8th and 12th weeks ($P=0.05$) compared to Asana and Puna. However, all the yam tubers used for the study sprouted at the end of the 14th week.





Storage Duration (Weeks)
 Fig 40. Tuber weight loss (%) in Araba, Asana and Puna stored at 24-34 C and 48-83% r.h.
 All Asana and Puna tubers were rotten by the end of the 36th week.
 Vertical bars represent LSD (P=0.05)



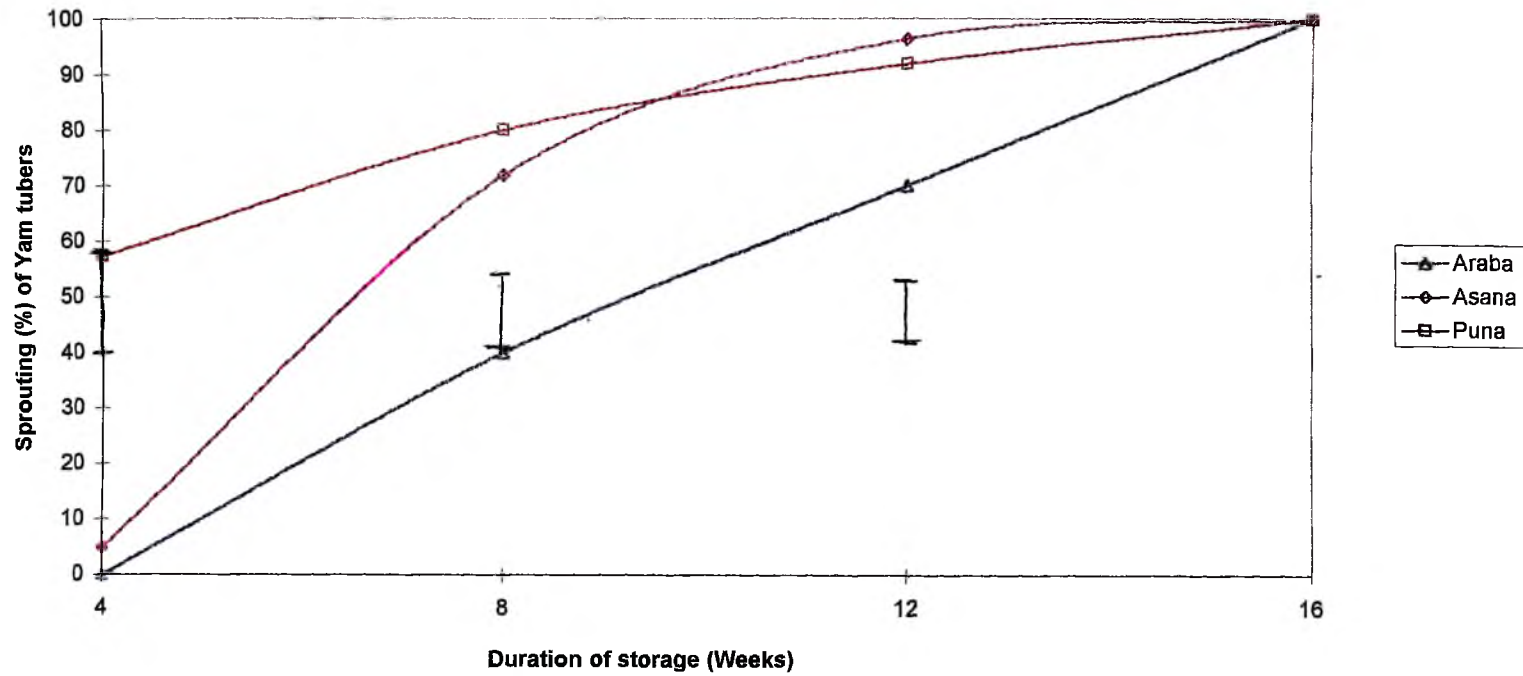


Fig 41. Sprouting (%) in tubers of Araba, Asana and Puna stored at 24-31 C and 48-83% r.h.

Vertical bars represent LSD (P=0.05)

4.7.3 **Rotting of yam tubers:** - The incidence of tuber rots among the three white yam varieties are shown in Fig. 42. Generally, Araba tubers had a significantly lower incidence of rots ($P = 0.05$) compared to Asana and Puna, even though none of the three varieties had rotten by the fourth week. There was a sharp rise in the incidence of rots for all the three varieties between the 16th and 28th week. This occurred after all the yam tubers had sprouted. By the 36th week in storage only 25.0% of the Araba tubers had rotten whereas both Asana and Puna had had 100% rot.

4.8 **Effectiveness of lime and neem wood-ash in controlling rot in wounded tubers of Araba, Asana and Puna.**

The results obtained are presented in Fig. 43. The area of rotten internal tissue in all the three yam varieties increased with the duration of storage. In all three varieties, the lime-treated areas had a significantly less internal necrosis ($P = 0.05$) than the ash-treated surface which were comparable to the control. However, Araba tubers had less internal necrosis after storage than Asana and Puna (Figs. 43 and 44).

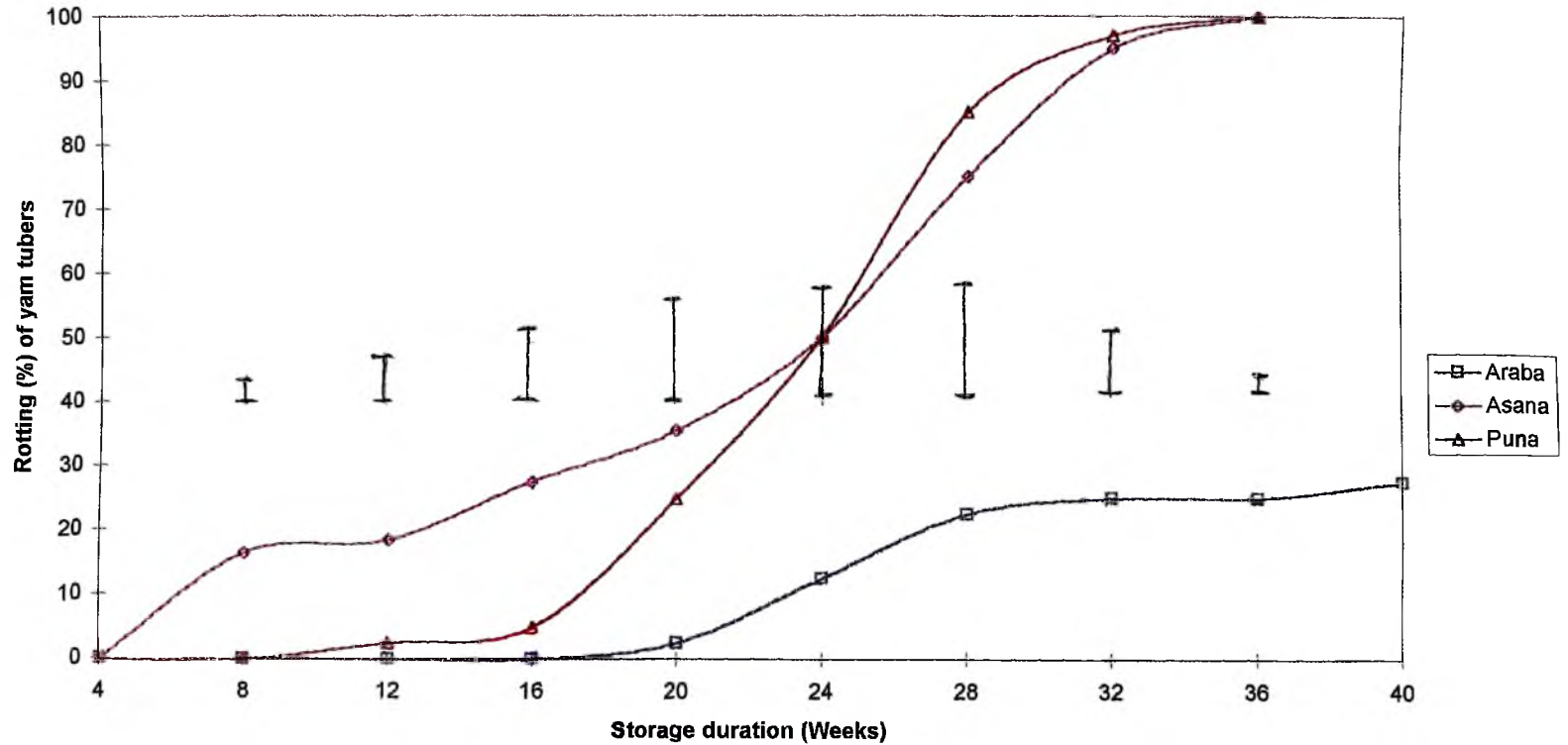


Fig 42. Rotting in tubers of Araba, Asana and Puna stored at 24-31 C and 48-83% r.h.
 All Asana and Puna tubers were rotten by the end of the 36th week
 Vertical bars represent LSD (P=0.05)

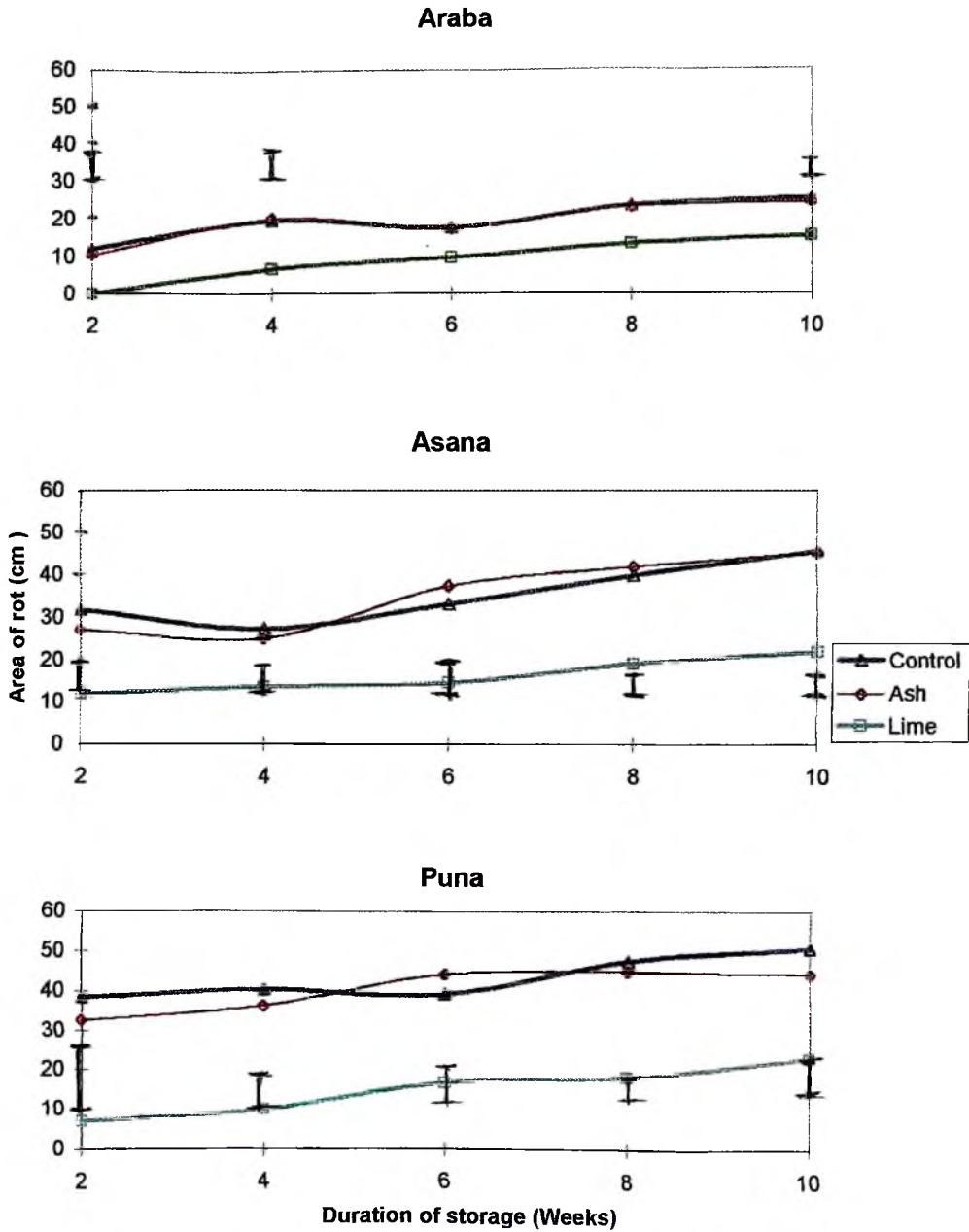


Fig. 43. Effect of lime and neem wood-ash on the area of rot in wounded tubers of three yam varieties stored at 24-31 C and 48-63% r.h. Vertical bars represent LSD (P=0.05)



Fig. 44. Effect of lime and neem wood-ash on the area of rot in wounded tubers of the varieties Araba, Asana and Puna. Tubers were stored for four weeks at 24-31°C and 48-83% r.h. in a barn at Legon.

L-R: Puna untreated, Puna with lime, Puna with ash, Asana untreated, Asana with lime, Asana with ash, Araba untreated, Araba with lime and Araba with ash.



4.9 *In vitro* test of the effect of lime and neem wood-ash on the growth of fungal isolates.

The results obtained when four levels each of lime and neem wood-ash were separately incorporated into potato dextrose agar for culturing the fungal isolates are presented in Table 9 and Figs. 45-54. There were differences in the growth rate of the isolates on the ash potato dextrose agar (APDA), lime potato dextrose agar (LPDA) and potato dextrose agar (control). The differences manifested in the luxuriance with which each fungus thrived in the different media. For example, in Fig. 48, *Botryodiplodia theobromae* is seen with fluffy mycelia to the roof of the Petri dish in the control PDA plates and the first level of neem wood-ash while the growth is subdued in the higher levels of neem wood-ash. The same trend is also noted with *Rhizopus stolonifer* (Fig. 54) and many of the fungal isolates used.

All the fungal isolates grew on all the four levels of neem wood-ash used. Growth rate, however, decreased with increasing concentration. Apart from the lowest level of lime (0.38g/15ml PDA) where there was mycelial growth at the end of the 2nd day, the fungi could not grow on the remaining rates of lime tested.

The pH of 48% neem wood-ash and 48% lime suspension were found to be 12.03 and 13.21 respectively. The two materials are all highly alkaline in nature, however, the lime is more alkaline than the neem wood-ash.

Table 9: Growth of fungi on media amended with four levels each of lime (LPDA) and neem wood-ash (APDA).

FUNGI	Colony diameter (mm) ^c								Control (PDA)
	APDA ^a				LPDA				
	L ₁ ^b	L ₂	L ₃	L ₄	L ₁	L ₂	L ₃	L ₄	
<i>A. flavus</i>	34	31	30	25	0	0	0	0	36
<i>A. oryzae</i>	37	30	16	4	14	0	0	0	38
<i>A. niger</i>	90	48	11	2	3	0	0	0	90
<i>B. theobromae</i>	90	90	32	30	0	0	0	0	90
<i>F. culmorum</i>	37	33	28	9	9	0	0	0	86
<i>Fusarium sp.</i>	25	25	21	16	19	0	0	0	40
<i>F. oxysporum</i>	24	18	3	2	3	0	0	0	49
<i>P. brevi-compactum</i>	17	16	15	2	3	0	0	0	18
<i>Penicillium sp.</i>	36	22	7	2	3	0	0	0	37
<i>R. stolonifer</i>	90	30	26	21	20	0	0	0	90

^a APDA and LPDA are potato dextrose agar amended with ash and lime respectively.

^b L₁–L₄ for APDA and LPDA are 0.13, 0.25, 0.50, 0.75 and 0.38, 0.75, 1.50, 2.25g of ash and lime respectively per 15 ml PDA in a 90mm Petri dish.

^c Colony diameters are mean values obtained from three replicates after 7 days of incubation.

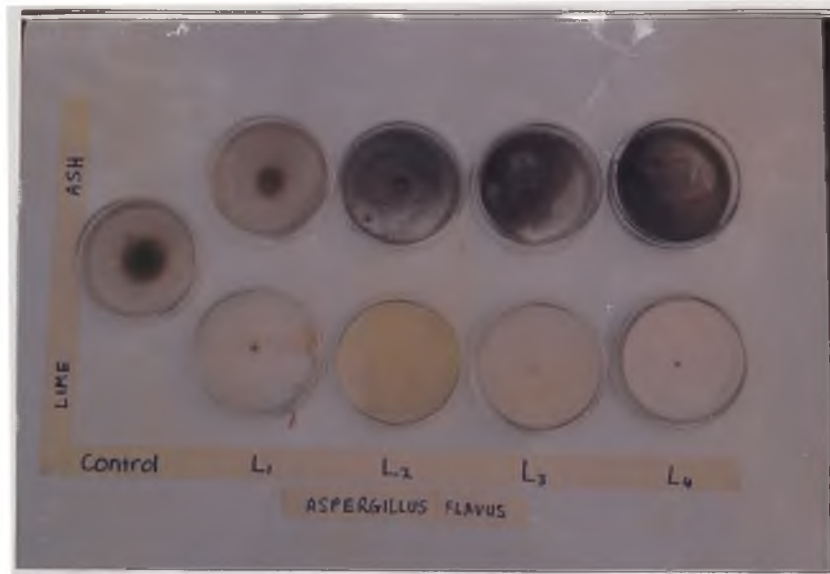


Fig. 45. Growth of *Aspergillus flavus* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



Fig. 46. Growth of *Aspergillus niger* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



Fig. 47. Growth of *Aspergillus oryzae* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



Fig. 48. Growth of *Botryodiplodia theobromae* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.

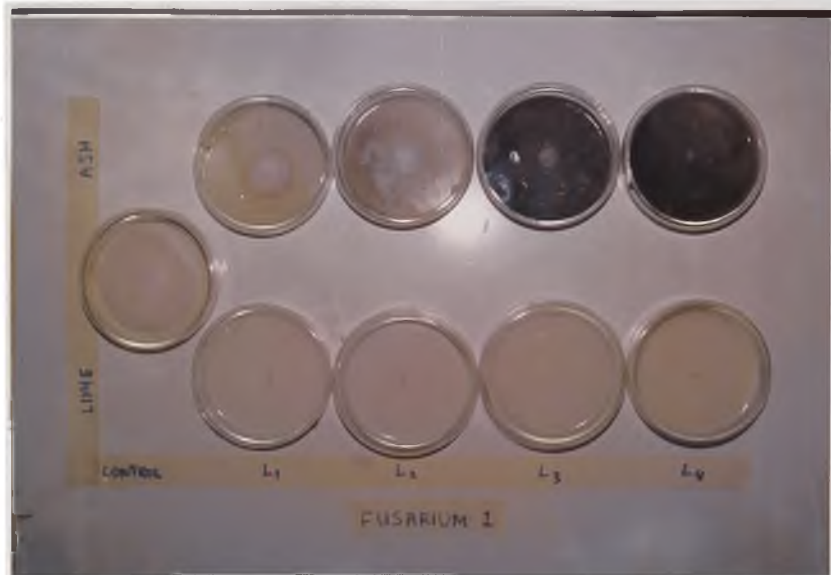


Fig. 49.

Growth of *Fusarium culmorum* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.

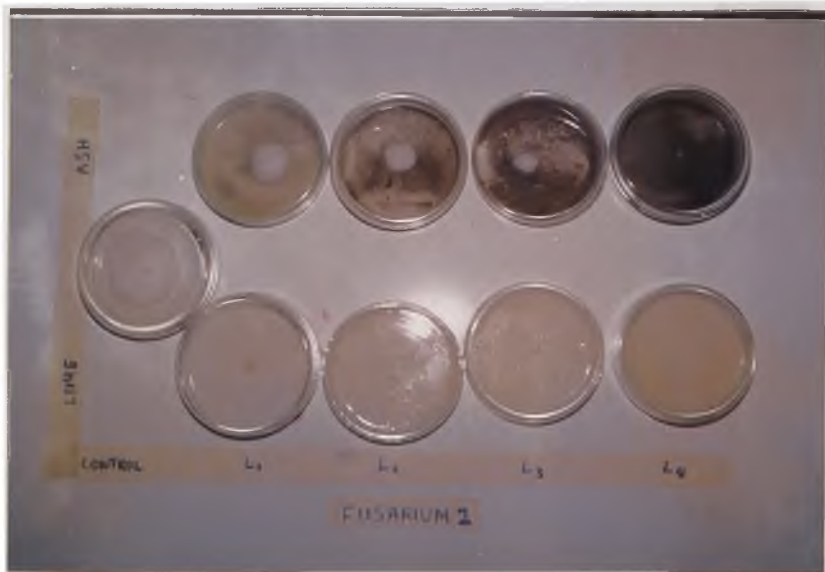


Fig. 50.

Growth of *Fusarium sp.* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.

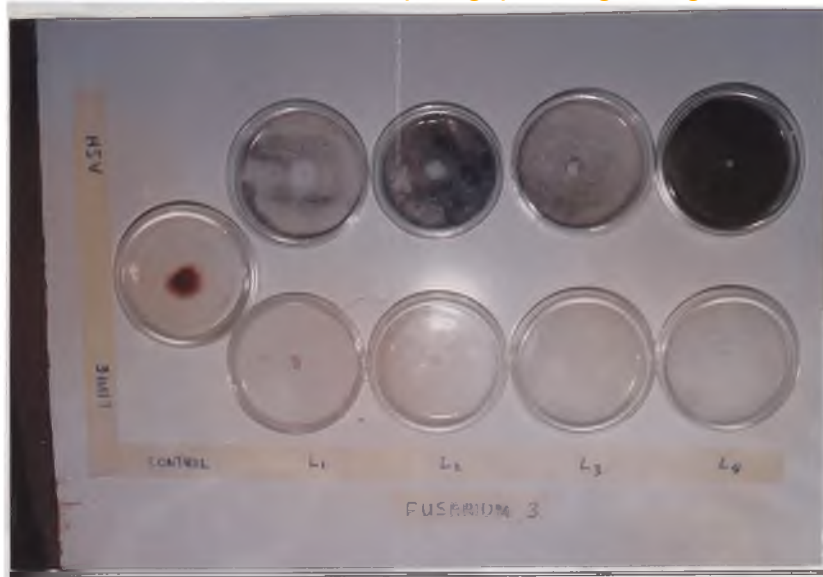


Fig. 51. Growth of *Fusarium oxysporum* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.

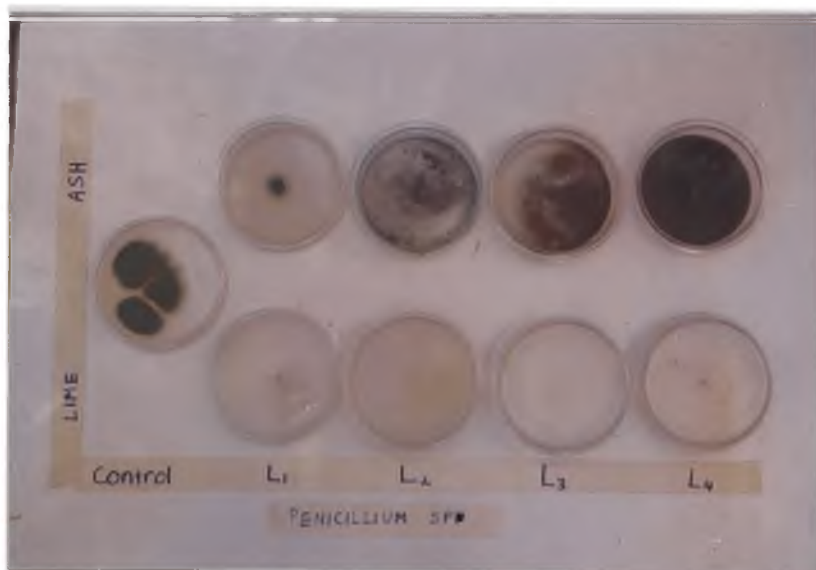


Fig. 52. Growth of *Penicillium brevi-compactum* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



Fig. 53. Growth of *Penicillium sp.* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



Fig. 54. Growth of *Rhizopus stolonifer* on media amended with four levels each of lime and neem wood-ash. Picture taken on the 7th day of incubation.



CHAPTER FIVE

DISCUSSION

5.1 Post-harvest problems of white yam in Ghana-Survey report.

Post-harvest deterioration of yam by micro-organisms is a major constraint militating against yam production and marketing in Ghana. In the current study, high percentage of rotten tubers were associated with tubers meant for retaining (10-30%) as well as those for export (10-40%). The results confirm earlier reports of high post-harvest losses of yams in Ghana (Abeka, 1989; Tetteh and Saakwa, 1994; GTZ/MoFA, 1995; Fuseini and Henkes, 1995; ICRA, 1996; Bancroft *et al.*, 1997).

Puna is the most preferred white yam variety for export and local consumption. It is also the most susceptible to rots and sprouting in storage. This has earlier been reported by Abeka (1989), ICRA (1996) and Tetteh and Saakwa (1994). If an expensive and extensively cultivated yam such as Puna forms a substantial part of tubers that decay in the local and export markets then the financial loss due to tuber decay may be under-estimated. However, two of the four most preferred yam varieties by respondents namely Araba and Punjo are known to have good storability. The high consumer preference for Puna despite its poor storability may be due to its sweet taste, aroma, early maturity and suitability for 'fufu' (Tetteh and Saakwa, 1994; ICRA, 1996). Some less preferred varieties have also been known to improve in taste with duration of storage as water content reduces (ICRA, 1996).

The popularity of the urban market as a major source of tuber supply revealed that most exporters and sellers in Accra are not involved in the production and handling of tubers from farms/village markets to wholesaling points (usually

urban markets). They therefore have to accept the physical state of the yams that reach them with a bit of sorting to remove the badly damaged ones. Exporters who need tubers that satisfy export specifications and rich retailers will have to get involved in the production, packaging and transportation of tubers to reduce the amount of damage.

Notwithstanding the decline in yam quality by the time it gets to Accra, the handling practices of the exporters and retailers themselves further increase the extent of rot diseases. Yam arrives in Accra mainly by road with tubers stacked loosely in lorries. This method of transporting yams in Ghana has been reported by Abeka (1989), GTZ/MoFA (1995), ICRA (1996) and Bancroft *et al.* (1997). A loss assessment data recovered from yams sampled at a wholesaling point (Techiman) indicated that 90% of tubers exhibited superficial grazes and 71% had their superficial tissue punctured (Bancroft *et al.*, 1997). This latter symptom appears to derive from the abrasion of yams piled against each other and the tradition of staking tubers on their distal ends which often result in gashes at these points. The above data provide a brief glimpse of the realities of post-harvest losses in yam consignments that reach Accra from more distant trading centres. Thompson (1972), showed that transporting yams in cartons as opposed to stacking them loosely in lorries, reduced the percentage rejects for export from 49.7 to 16.5.

Export yam is transported mainly by ships and take 2-3 weeks to get to their export destination depending on if they are bound for Europe or America. Exporters pack yams wrapped with sheets of paper in ventilated paper cartons. In the ship, the cartons are packed together with cocoa and other export commodities with no proper ventilation. This condition results in higher temperatures and relative humidities conducive for pathogen activity. High temperatures and humidity around stored tubers frequently enhance the

prevalence and severity of rots (Lauritzen, 1935; Tomkins 1951; Adeniji, 1970b). Such tubers need to be wrapped in pesticide-treated wrappers and kept in air-conditioned containers to reduce microbial activity, sprouting and weight loss. It has been observed that even if a pathogen is already in a tuber, the time taken to establish a rot is increased and the rate of spread decreased by lowering the temperature (Tomkins, 1951).

Although yam is stored by exporters and retailers, storage facilities are generally lacking. The practice where tubers are unloaded and stored in cone-shaped heaps in the sun and covered with straw mats and tarpaulin contributes to the high incidence of rots in the markets. These conditions have also been reported to result in the rapid curing of the tubers (FAO, 1990). Therefore, there is the need for further investigation to confirm these findings. Apart from the nightly attacks by rodents which create wounds on tubers, yam exposed to direct sunlight occasionally suffer from sun scorch during retail. Although data from Techiman suggest that heat damage is relatively minor relative to other conditions (Bancroft *et al.*, 1997), it is one of the causes of post-harvest losses that could be remedied relatively easily. The tarpaulin which is sometimes used to cover stored tubers may obstruct air flow around the tubers and result in higher temperatures and humidities from the respiring tubers.

Most exporters buy and pack their yams for export in the markets without storage. The few who convey yam to their homes and offices for packaging do so under conditions similar to those of retailers. Studies have shown that if tubers are bought cheaply during the glut period and stored well for later sale/export, it could be profitable (Fuseini and Henken, 1995). The inability of sellers and exporters to store yams for later sale may be due to the fear of tuber rots arising from lack of knowledge on proper storage methods and/or lack of capital.

The use of rot control measures is non-existent on the markets and is also not practiced by exporters and this is a reflection of the lack of extension advice on proper storage methods and rot control.

Recent attention to international trade in tropical root crops in Ghana has perhaps served to emphasise our lack of knowledge of the handling and storage behaviour of these crops. Most of the losses encountered in the local and export markets could be attributed to spoilage caused by various micro-organisms. It has been frequently pointed out how such losses may often originate in sites of mechanical damage acting as infection courts for pathogenic organisms (Ogundana *et. al.*, 1970; Booth and Proctor, 1972; Booth , 1974; Mozie, 1982).

Mechanical harvesters will perhaps help reduce drastically the amount of injury presently encountered, but this might have to wait until geneticists breed yams with similar size and shape to facilitate mechanical harvesting. So until this happens one might have to contend with harvest injury and the use of some of the traditional controls measures outlined earlier.

5.2 Identification of *D. rotundata* var. Araba, Asana and Puna by tuber characteristics.

Tuber features for the identification of the three white yam varieties have been found. In the traditional cultivation of yam in Ghana, varieties are not distinguished according to systematic and taxonomic considerations (Degras, 1993). They are evaluated in as many ways as there are ethnic groups among farmers. A predominant characteristic during the survey was that most of the cultivars had a specific name, though it did not always cover an indisputable monoclonal population. An interesting observation in this work is that the three yam varieties used in the studies were consistently named Araba, Asana

and Puna by people from different localities in Ghana dealing in yams in Accra.

Since both genotype and environment play significant roles in determining tuber characteristics (Coursey, 1967), tuber shape, tuber-skin colour, tuber-flesh colour and tuber-flesh texture are not consistently reliable. However, the furrow markings on the corky periderm (skin) of the tubers are consistent with variety and therefore a reliable index. Shoot characteristics (Onwueme, 1982) and Isozyme patterns (Hamon and Toure, 1990) have been proposed for the proper characterization of the traditional yam varieties belonging to the *Dioscorea rotundata-cayenensis* complex.

5.3 Pathogens associated with rots of yams and their frequency of occurrence in decayed yam tubers.

Twelve micro-organisms have been found to cause tuber rot in white yam. Eleven of these caused rots in all the three yam varieties. These were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Botryodiplodia theobromae*, *Fusarium culmorum*, *Fusarium sp.*, *Fusarium oxysporum*, *Penicillium brevi-compactum*, *Penicillium sp.*, *Rhizopus stolonifer* and *Scutellonema bradys*. However, *Erwinia carotovora* caused rot in Asana and Puna tubers but was mildly pathogenic when inoculated into healthy Araba tubers. Ten of the pathogens encountered in this study had been reported earlier by other workers elsewhere to be associated with yam tuber rots.

Noon and Colhoun (1979) reported *A. flavus* to be an agent causing yam decay. Some strains of this fungus are known to produce a mycotoxin (aflatoxin) which is toxic to humans. Thus the occurrence of *A. flavus* in tubers in the present study demands that further work be done to determine if the strain is aflatoxin-producing. *Aspergillus niger* which was found to be a

serious rotter of yam tubers in this study has been earlier accorded the same status by Adeniji (1970a), Ogundana *et al.* (1970), Noon and Colhoun (1979), Adimora (1986) and Otunsanya (1996).

Similarly, it has been reported that *B. theobromae* which also caused moderate rotting in this present work is a major cause of decay of yams in West Africa (Bunting, 1924; Dade and Wright, 1931; Baudin, 1956; Piening, 1962; Okafor, 1966; Adeniji, 1970a; Noon and Colhoun, 1979; Adimora, 1986; Abeka 1989). Coursey (1967) also generally considered *B. theobromae* as the most common biodeterogen of stored yams, while Ogundana *et al.* (1970) considered it among the most severe rotters of stored yams. In contrast, Adeniji (1970a) and Ricci *et al.* (1979) found this fungus to be a weak pathogen of yams when they were kept at 25°C. Ricci *et al.* (1978) found that yam decay by *B. theobromae* was severest at 35°C. The differences in the yam species, varieties and storage temperature are likely to have influenced the severity of rotting attributed to this fungus.

Erwinia carotovora has been known to be a cause of watery soft rot in yams with a pungent smell (Burton, 1970; Noon and Colhoun; 1979; Théberge, 1985). This bacterium has been suspected to attack yam tubers after prolonged storage when the cells have lost vigour and are almost at advanced stage of senescence (Bancroft, personal communication). This observation was however not made in this work.

Fusarium species have been known to be yam rotters. Noon and Colhoun (1979, 1981) have earlier established that *F. moniliforme*, *F. oxysporum* and *Fusarium sp.* are serious yam rotters.



Adeniji(1970b), Bediako (1978), Ricci *et al.* (1978) Noon and Colhoun (1979, 1981), Abeka (1989) and Adimora *et al.* (1990) reported that *Penicillium* species are serious pathogens of yam storage rot. However, *Penicillium brevicompactum* has, apart from this work, been only reported by Burton (1970) as a yam rotting fungus. This may be attributed to the inability of earlier workers to identify the many *Penicillium spp.* encountered in rotting yams to species level.

Although *R. stolonifer* has been considered as a saprophyte in yam tuber rot, its pathogenicity has been demonstrated Adeniji, 1970a; Adimora *et al.*, 1990. Noon and Colhoun(1979) also noted that its potential to cause rot increased in association with other fungi such as *F. solani* and *F. oxysporum*. *Rhizopus stolonifer* was found to be an important rotter in this study.

The yam lesion nematode (*Scutellonema bradys*) which caused dark dry rot under tuber skin in this work has been earlier observed to produce the same symptoms (Jenkins and Taylor, 1967; Thompson *et al.*, 1973; Théberge, 1985; Adesiyan, 1996) .

From available literature, it appears this study is the first record of the ability of *A. oryzae* and *F. culmorum* to cause decay of yam tubers.

The presence of all the eleven pathogens isolated from samples obtained from the yam markets in various parts of Accra as well as the barn which, in turn, come from different parts of the country may indicate that the pathogens encountered in this work constitute the pathogenic microflora responsible for the storage and market diseases of white yam in Ghana.

The pathogenic isolates had the highest frequency of occurrence in Puna and least in Araba in both the yam markets and the barn. This may be due to the varietal differences in the thickness and toughness of the corky periderm (skin) of the tubers (Abeka, 1989) and the quantity of anti-fungal phenolic compounds in the tuber 'skin' and tissue (Ogundana *et al.*, 1970; Lyon, 1972; Allen and Kue, 1974). The interplay between fungal infection of yam tuber tissue, activation of certain enzyme activity in the tissue and production of compounds with anti-fungal activity have been reported in *D. rotundata* (Ebukanson, 1989). An interesting observation in this work was that *E. carotovora* was not isolated from Araba tubers in both the market and the barn but was mildly pathogenic when inoculated into healthy tissue. *Rhizopus stolonifer* was the most frequently encountered organism in yam tubers in the market with *E. carotovora* being the least while *A. Niger* and *F. culmorum* were the highest and least encountered pathogens in the barn respectively. This differential frequencies of occurrence may reflect their abundance in the area where the yams were grown and the atmosphere of the market and barn. Pectinolytic and cellulolytic enzyme complex associated with soft rot of *D. rotundata* has been investigated by Obi and Moneke (1986). All soft rot causing fungi were characterised by high levels of hydrolase and lyase activity but some produced β - (1-4) endoglycanase in addition but its production was not indispensable. Differences in the enzymes produced by the rot-causing pathogens may therefore also contribute to their relative abundance observed.

5.4 Diagnostic symptoms of some yam rots caused by natural infection by some pathogens

Although most yam rot diseases from natural infection are usually caused by a combination of rot-causing pathogens, some are produced by a single pathogen. The consistent symptoms produced by natural infection of the three white yam varieties by *A. niger*, *E. carotovora*, *P. brevi-compactum* R.

Stolonifer and *S. bradys* in this work are noteworthy. Ogundana *et al.* (1970) had earlier reported that symptoms of diseased yam tissue depend on the yam species and the pathogen.

Natural infection by *Aspergillus niger* which resulted in a firm-textured dark-brown to black rot observed in the present study was reported by Ogundana *et al.*, (1970) to result in a black rot and by Adimora (1986) to result in a dark-brown rot with black charcoal-like margins. It is possible that the black colour of *A. niger* culture might account for its characteristic dark-brown to black rots. In contrast, the fungus has been shown to produce light-brown to pale-purple rot when artificially inoculated into *D. rotundata* tubers (Noon, 1978). The different colours produced may be influenced by environmental conditions (Burton, 1970).

The consistent isolation of *E. carotovora* from watery soft rot with brown liquid of pungent odour oozing from infected tubers confirms the observation of Théberge (1985). This symptom is attributed to the production of pectinolytic and cellulolytic enzymes by the bacteria (Agrios, 1988).

The oozing out of light-brown liquid and the production of a grey-green mould growth on cut and damaged tuber surfaces by *P. brevi-compactum* in this work is in agreement with the observation of Adeniji (1970a). High pectinolytic and cellulolytic enzyme production and an aerobic habit may explain the above symptom.

Occurrence of mycelia and sporangia of *Rhizopus* on cut and damaged surfaces of naturally contaminated yam has also been reported by Agrios (1988).



Following invasion of tubers by *S. bradys*, deep and light cavities with dark, dry rot tissue developed under tuber skin which cracked and flaked off in severe cases. This same symptom has been attributed to *S. bradys* (Jenkins and Taylor, 1967; Thompson *et al.*, 1973; Théberge, 1985; Adesiyon, 1996).

However, the other seven yam rotting pathogens produced varied symptoms in naturally infected tubers. The five symptoms considered possibly diagnostic above may therefore be used as a key to identify rot diseases of white yam caused by *A. niger*, *E. carotovora*, *P. brevis-compactum*, *R. stolonifer* and *S. bradys*. Further research to investigate the consistency of rot disease symptoms of white yam produced by the above pathogens on the other white yam varieties and the possible factors responsible for the inability of the other pathogens to produce consistent symptoms is highly recommended.

5.5 Influence of variety on weight loss, sprouting and rotting of yam tubers

Losses in yams in storage is considered to be heavy (Ogundana *et al.*, 1970; Adesuyi, 1973b; Onwueme, 1982). Storage losses are mainly due to tuber weight loss, tuber sprouting and microbial decay (Coursey, 1961). These sources of storage losses are also aspects of the tuber's shelf life which affect the nutritional value and marketability of yams (Osagie, 1992).

The fairly steady increase in the rate of weight loss in Araba, Asana and Puna in storage observed in this work confirms the reports of Campbell *et al.* (1962), Coursey (1961) and Thompson *et al.* (1977). The yam tubers used for the study were stored throughout the dry season and considerable loss of water may be expected especially in view of the high moisture content of the tubers. Weight loss is usually attributed to dehydration and respiratory losses (Coursey and Walker, 1960). The losses observed in this study most probably occurred through the same process.

Loss of water makes a large contribution to total weight loss from freshly-harvested yams which contain from 66-75% water (Onwuene, 1982). Loss of water under any given water vapour pressure deficit is restricted by the corky periderm (skin) of the tuber (Osagie, 1992). The low rate of weight loss of Araba compared to Asana and Puna in this work may be accounted for, in part, by its low rate of dehydration. This agrees with the report by Abeka (1989) that Asana and Puna have light skin whilst Araba has a thick skin and hence good keeping quality. The low rate of weight loss in Araba tubers could also be attributed to a low rate of respiratory loss of carbohydrates which reflects in its significantly longer dormancy period. Tuber respiration can account for more than 30 percent of total weight loss during harvesting and sprouting and 10 percent during dormancy (Passam *et al.*, 1978). The rate of tuber respiration is also a good indicator of metabolic activity of the tissue and thus a very useful guide to the potential storage life of the tubers.

Tuber weight loss adversely affects the appearance, texture, flavour and weight of yam tubers (factors that determine the market price). This condition can be minimized by storing tubers in locations that are not excessively dry or hot using improved and cheap storage techniques that are within the resources of peasant farmers.

The inability of yam tubers to sprout, even under suitable conditions is referred to as dormancy (Campbell *et al.*, 1962; Coursey, 1967; Onwueme, 1982). Termination of dormancy which is indicated by sprouting limits the storage life of tubers (Osagie, 1992).

The significantly lower number of tubers of Araba that sprouted in this work during the initial 12 weeks of storage compared to Asana and Puna could be due to genetic differences between the yams since all tubers were stored under

the same storage condition. Nweke and Okonkwo (1981) showed that regardless of the storage and planting conditions, tubers of *D. rotundata*, *D. alata* and *D. dumetorum* remain dormant for 14-16 weeks. Thus, the length of tuber dormancy is genetically controlled and such external factors such as availability of soil moisture suggested by Campbell *et al.* (1962) or cool temperature suggested by Coursey (1967) are inefficient triggers of sprouting. The readiness with which a yam tuber will sprout has been related to the physiological age of the tuber (Onwueme, 1982) but experiments conducted by Okoli (1980) and Passam *et al.* (1982) has shown the contrary. Indeed it is not possible to give a coherent account of the biochemistry of dormancy and sprouting in yams because the participating factors have not been completely identified (Osagie, 1992). However, the length of the dormancy period of the yam varieties studied were similar to other *D. rotundata* varieties in Nigeria (Coursey, 1961; Nweke and Okonkwo, 1981). In view of the fact that the tuber is the most economically useful part of the yam plant, more research ought to be devoted into the mechanism of sprouting as this is a key factor limiting the storage life of yam tubers.

Storage rots are the most important factors responsible for the deterioration of stored yams (Adesuyi, 1975a; Onwueme, 1982). Incidence of rots in this study varied among the yam varieties used, as was also observed by Okafor (1966). Rotting of tubers in the initial period (16 weeks) in storage was slow. The robust nature of the tubers at this relatively early stage after harvesting may account for the low number of rotten tubers encountered in all the varieties (Osagie, 1992).

The significantly high number of rotten Asana tubers observed during the early period in storage could result from infection by pathogens in the field from injury caused by nematode feeding (Ogundana *et al.*, 1971) and/or small

bruises on some of the tubers during harvesting and transportation but which were invisible to the naked eyes during selection of sound yam tubers for storage. The sharp increase in the number of rotten tubers in all the yam varieties after the 16th week in storage is interesting. The 16th week is also the period when all the tubers for the study sprouted. Passam and Noon (1977) observed that once dormancy is terminated and sprouting begins, tubers rapidly senesce, pathogenic invasion occurs and effective storage is no longer possible.

The number of rotten Araba tubers was significantly lower compared to Asana and Puna tubers. This may be due to the relatively longer dormancy period of Araba. A thicker tuber skin may have also reduced the chances of wounding which acts as infection courts for rot-causing micro-organisms (Booth and Procter, 1972; Booth, 1974; Mozie, 1982; Abeka 1989; Adimora *et al.*, 1990). A faster and thicker wound periderm formation may also contribute to the above observation (Passam *et al.*, 1976). Varietal differences in the chemical composition of the skin and tissue of tubers, especially in anti-fungal phenolic compounds, have also been known to account for differences in susceptibility of yam varieties to rot diseases. In the present work, 50% each of Araba and Puna tubers cut open showed oxidative browning especially at wounded sites as against 20% for Asana. This may explain the longer shelf life of Araba and poor storability of Asana. The short dormancy period of Puna despite the high oxidative browning action observed as compared to Araba indicates the involvement of other factors. Tomkins (1951) also showed that for a fungus to invade and spread in a tissue, the tissue must be suitable for fungal growth and must stimulate the production of the enzymes or metabolites which bring about killing and softening of the tissue. The fewer and low number of rot pathogens isolated from Araba in this work may be a reflection of the chemical composition of the tuber skin and tissue.

Further research on tuber factors responsible for the superior shelf life of Araba is suggested. If the factors are understood, they could be incorporated into breeding programmes to improve the storability of other important white yam varieties. Changes in palatability and other culinary properties of Araba with time should also be studied to determine the optimal duration of storage.

5.6 Effectiveness of lime and neem wood-ash in the control of yam rot in wounded tubers

Some of the major methods advocated for the storage of yam tubers include curing (Adesuyi, 1973b) and irradiation (Adesuyi, 1975b). Facilities for accomplishing these are expensive and unaffordable by peasant farmers. Furthermore, the results achieved with curing have varied from worker to worker (Adesuyi, 1973b). The effectiveness of lime paste in slowing down the rate of rot in wounded yam tissue of the three yam varieties over a ten-week storage period may be due to its ability to delay the onset of rot. Fungal mycelium grows best in a medium of pH ranging from 4.0-6.4. The application of 86% lime paste with high pH is likely to have raised the pH of the wounded tissue above 6.4 thus inhibiting fungal growth and reproduction as shown in the *in vitro* experiment. This gives tubers enough time to undergo wound healing. The lime paste may also have formed a physical as well as a chemical barrier to the rot pathogens in the storage environment. Cracks in the lime cover which was evident when the paste was very dry may have allowed inocula to enter the yam tissue with time. The efficacy of lime in controlling rot in yams has also been reported by Thompson *et al.* (1977) and Ogali *et al.* (1991).

The ineffectiveness of the neem wood-ash in this work despite its high pH could arise from the blowing away of the dry ash powder by wind and



continuous handling of tubers as a result of periodic inspection thereby rendering it an ineffective physical and chemical barrier. Furthermore, the pH of wounded yam tissue surface may not have been raised enough by the little neem wood-ash powder left after it had been blown off, to discourage fungal growth as seen in the *in vitro* experiment. The levels of alkali metals such as calcium, potassium and sodium among the over 40 well-known elements present in the ash of plant parts (Duta, 1995) may not be as high as the levels in the lime paste to render the wounded tissue surface sufficiently alkaline. Ash has been successfully used in controlling tuber rot on cut and wounded portions of tubers (Anon., 1938; Nnodu and Nwakiti, 1986) but the plant and the plant part used to produce the ash were not reported. The ash used was also applied as a slurry or by dipping. Variation in the chemical composition of ash from different plant species as well as from different parts of the same plant species has been reported by Duta (1995). The method of application coupled with the differences in plant part used may explain the contrasting results observed.

The ability of wood-ash to exhibit some measure of suppression of growth of yam pathogens in the *in vitro* experiment may be due to its phenolic property. Harborne (1973) had noted that phenolic polymers present in cell wall of plants are lignins and that lignins are associated with up to 30% of the organic matter of trees. The lignin yields three simple phenolic aldehydes which are related to common phenolic acids of plants. It is possible that some residual phenol exists in the wood-ash and since phenols are known to be strong disinfectants, they might be responsible for the inhibitory effects of wood-ash *in vitro* on the yam fungal pathogens. Moreover, elements such as sulphur and copper present in wood ash (Duta, 1995) are well known fungicides.

The effectiveness of lime in slowing down the rate of rot in wounded tubers in this study is encouraging. Lime is non-phytotoxic, non-toxic to consumers, safe to use, cheap and has no effect on palatability (Osagie, 1992) More studies are, however, required before the practice can be recommended for adoption.



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APPENDICES

APPENDIX 1

SURVEY ON TUBER ROT DISEASES OF WHITE YAM OBSERVED BY YAM RETAILERS AND EXPORTERS IN ACCRA.

QUESTIONNAIRE FOR YAM RETAILERS / EXPORTERS

Name.....(Retailer / Exporter)

Sex (Male / Female) Location of warehouse

Date..... Enumerator

A. VARIETIES OF EXPORTABLE YAM AND THEIR LOCAL TRANSPORTATION

1. For how long have you been retailing/exporting yam? Since 19....

2. What varieties of yam do you prefer for sale/export?

- i. Araba ii. Asana iii. Asobayere iv. Kasaati
- v. Kpajol vi. Labrakor vii. Punjo viii. Puna
- ix. Others.....

3. What are the qualities of exportable yam?

.....

.....

.....

.....

.....

.....

.....

.....

.....

4. When are these varieties available for sale/export?

- i Araba.....
- ii. Asana.....

- iii. Asobayere
- iv. Kasaati.....
- v. Kpajol.....
- vi. Labrakor.....
- vii. Punjo.....
- viii. Puna.....
- ix. Others

.....

5. Where is your source of supply?

- i. Village market
- ii. My farm
- iii. Buy at farm gate
- iv. Urban/City market
- v. Others (specify).....

6. If it is from market can you tell how long they have been stored after harvest?

- i. = Yes
- ii. = No

7. Do you consider duration of storage before buying?

- i. = Yes (condition/state of yam)
- ii. = No

8. What is the duration of storage of your selection?(Answer to Q 7 must be 1)

- i. One week
- ii. Two weeks
- iii. Three weeks
- iv. One month
- v. Two months
- vi. Three months
- vii. More than three months

9. How are they transported from the farm/market to your storehouse?

- i. Road
- ii. Rail
- iii. Air
- iv. Boat
- v. Others (specify).....

10. How are they packed for transportation?

- i. In cartons
- ii. In jute sacks
- iii. Loose stacking in lorries
- iv. Others (specify).....

11. How long does it take to transport yam from farm/market to your store?

- i. 1 day
- ii. 2-4 days
- iii. 5-7 days
- iv. 8-10 days
- v. 11-14 days
- vi. 3 weeks
- vii. One month
- viii. I don't know.

B. STORAGE

1. Where do you store your yam prior to selling/export?

- i. Roofed and open-sided storage sheds
- ii. Heaped in airy place and covered
- iii. Pit covered with soil
- iv. Air-conditioned store
- v. Enclosed room
- vi. No storage
- vii. Others (specify)

2. How long do you store your yam before retailing /export?

- i. 1-2 days ii. 3-4 days iii. 5-7 days iv. 2 weeks
- v. 3 weeks vi. One month vii. 2 months viii. No storage
- ix. Others (specify).....

3. Why do you store your yam before export?

- i. Export documents not ready
- ii. Wait for high price period
- iii. Store large quantities and export gradually
- iv. Wait for means of transport to be ready
- v. Others (Specify).....

4. Do you inspect your yam periodically when they are in store?

- i. = Yes
- ii. = No

5. Do you experience sprouting in storage?

- i. = Yes
- ii. = No

6. Which variety sprouts fastest?

- i. Araba ii. Asana iii. Asobayere iv. Kasaati
- v. Kpajol vi. Labrakor vii. Punjo viii. Puna
- ix. Others (specify).....



7. Do you experience the problem of pest in your yam store?

i. = Yes

ii. = No

8. If yes, name them

i. Yam mealy bug ii. Yam beetle iii. Scale insect iv. Rats

v. Mice vi. Coffee bean weevil vii. Yam moths

ix. Others (specify).....

C. YAM ROT

1. What is the size of a consignment?

.....

2. Have you ever experienced yam rot?

i. = Yes

ii. = No

3. When?

i. All the time

ii. During the rainy season

iii. During the dry season

iv. Others (specify).....

4. What is the average percentage of rot in your yams per annum?

(Answer to Q 2 must be 1)

i. 1-5%

ii. 6-10%

iii. 11-20%

iv. 21-30%

v. 31-40%

vi. Above 40%.

5. Are there differences in degree of rot between different yam varieties?

i. = Yes

ii. = No

6. If yes, which variety rots fastest?

i. Araba

ii. Asana

iii. Asobayere

iv. Kasaati

v. Kpajol

vi. Labrakor

vii. Punjo

viii. Puna

ix. Others (specify).....

7. Which variety rots slowest?

i. Araba

ii. Asana

iii. Asobayere

iv. Kasaati

v. Kpajol

vi. Labrakor

vii. Punjo

viii. Puna

ix. Others (specify).....

8. Is there any yam variety that does not rot?

- i. Araba ii. Asana iii. Asobayere iv. Kasaati
v. Kpajol vi. Labrakor vii. Punjo viii. Puna
ix. Others (specify).....
9. How do you prevent your yam from rot?
i. Pre-storage curing treatment by morning or evening sun.
ii. Disinfect store when new yams arrive
iii. Use of cold room/air conditioned room
iv. Apply wood-ash to bruised portions of tubers
v. Apply lime wash to bruised portions of tubers
vi. No preventive measure taken
vii. Others (specify).....
10. Can you detect yam rot in the interior of tubers with little or no external evidence?
i. = Yes ii. = No
11. If yes, how do you do it?
i. Apply pressure on tuber surface using the fingers ii. Scent
iii. Weight iv. Others (Specify).....
12. Are some of your good yams rejected by buyers/importers due to rot?
i. = Yes ii. = No
13. How do you export your yams to the final export destination?
i. Air ii. Sea iii. Road iv. Rail
14. How long does it take to reach the final export destination?
i. Few hours ii. 1-2 days iii. 3-7 days iv. 2-3 weeks
v. 3-4 weeks vi. 5-12 weeks vii. Above 12 weeks
15. In what condition are the yams kept during the export
1. Air conditioned containers
2. Containers with air holes
3. Containers without air holes
4. Others (specify).....



APPENDIX 2

Tuber weight loss (%) in Araba, Asana and Puna stored at 24-31°C and 48-83%r.h.

Storage duration (weeks)										
	4	8	12	16	20	24	28	32	36	40
Araba	1.77	4.81	6.87	11.43	16.75a	27.34	30.11a	37.78a	38.79	39.31
Asana	1.46	5.97	9.97	16.65	23.27b	33.32	39.03b	47.09b	-	-
Puna	1.99	8.22	11.05	16.12	21.00ab	29.31	34.52ab	38.00a	-	-

Means followed by the same letter in a column are not significantly different at 5% level of LSD. (All Asana and Puna tubers had rotten by the 36th week).

APPENDIX 3

Analysis of variance (ANOVA) of weight loss (%) in tubers of Araba, Asana and

Puna in storage.

20th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	44.641	22.321	6.090	0.0359
Block	3	8.285	2.752	0.753	0.5593
Error	6	21.991	3.665	-	-
Total	11	74.917			

28th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	58.104	29.052	8.659	0.0170
Block	3	1.793	0.598	179.000	0.9074
Error	6	20.130	3.355	-	-
Total	11	80.027			

32nd week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Value	Prob.
Variety	2	82.029	41.014	559.55	0.001
Block	3	0.850	0.283	3.87	0.075
Error	6	0.440	0.073		
Total	11	83.319			

APPENDIX 4

Sprouting (%) in tubers of Araba, Asana and Puna stored at 24-31°C and 48-83% r.h.

	Storage duration (weeks)			
	4	8	12	16
Araba	0.0a	40.0a	70.0a	100.0
Asana	5.0a	72.0b	96.5b	100.0
Puna	57.5b	80.0b	92.0b	100.0

Means followed by the same letter in a column are not significantly different at 5% level of LSD.

APPENDIX 5

Analysis of variance (ANOVA) of sprouting (%) in tubers of Araba, Asana and Puna in storage.

4th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	P. VALUES
Variety	2	5801.357	2900.679	37.565	0.0004
Block	3	598.919	199.640	2.585	0.1486
Error	6	463.311	77.219	-	-
Total	11	6863.587			

8th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	723.723	723.723	16.606	0.0036
Block	3	270.767	270.767	6.213	0.0286
Error	6	43.583	43.583	-	-
Total	11	2521.245			

12th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	622.432	622.432	8.685	0.0169
Block	3	345.774	345.774	4.825	0.0486
Error	6	71.667	71.667	-	-
Total	11	2712.167			

APPENDIX 6

Rotting (%) in tubers of Araba, Asana and Puna stored at 24-31°C and 48-83% r.h.

Storage duration (weeks)										
	4	8	12	16	20	24	28	32	36	40
Araba	0.0	0.0a	0.0a	0.0a	2.5a	12.5a	22.5a	25.0a	25.0a	27.5
Asana	0.0	16.5b	18.5b	27.5b	35.0b	50.0b	75.0b	95.0b	100.0b	-
Puna	0.0	0.0a	2.5a	5.0a	25.0b	50.0b	85.0b	97.0b	100.0b	-

Means followed by the same letter in a column are not significantly different at 5% level of LSD.

APPENDIX 7

Analysis of variance (ANOVA) of rotting (%) in tubers of Araba, Asana and Puna in storage.

8th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	1350.000	675.000	91.685	0.0001
Block	3	22.087	7.365	1.000	0.4547
Error	6	44.173	7.365	-	-
Total	11	1416.26			

12th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	1316.340	658.170	24.545	0.0013
Block	3	247.250	82.417	3.073	0.1123
Error	6	160.892	26.815	-	-
Total	11	1724.41			

16th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	2157.501	1078.750	23.285	0.0015
Block	3	506.250	168.750	3.642	0.0834
Error	6	277.972	46.329	-	-
Total	11	2941.723			

20th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	2179.562	1089.962	11.112	0.0096
Block	3	233.886	77.962	0.795	0.5399
Error	6	588.458	98.076	-	-
Total	11	3001.904			

24th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	1964.039	982.020	11.273	0.0093
Block	3	431.917	143.972	1.653	0.2746
Error	6	522.694	87.116	-	-
Total	11	2918.65			

28th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	3323.802	1661.901	13.533	0.006
Block	3	329.940	109.980	0.896	0.4959
Error	6	736.835	122.806	-	-
Total	11	4390.577			

32nd week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	8079.921	4039.960	63.805	0.0001
Block	3	156.074	52.025	0.822	0.5278
Error	6	379.902	63.317	-	-
Total	11	8615.897			

36th week

Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Variety	2	8990.785	4495.392	6221.996	0.0001
Block	3	2.168	0.723	1.00	0.4547
Error	6	4.335	0.723	-	-
Total	11	8997.288			

APPENDIX 8

Effect of lime and neem wood-ash on the area of rot in wounded tubers of Araba, Asana and Puna at 24-31°C and 48-83% r.h.

Mean rotten area cm² in tubers during ten weeks of storage

Araba

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10
Control	12.10a	19.83a	17.66	23.55	25.34a
Ash	10.41a	20.15a	17.50	23.33	24.52a
Lime	0.00b	6.65b	9.68	13.34	15.33b

Asana

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10
Control	31.91a	27.42a	33.21a	39.89a	45.65a
Ash	27.18a	25.10a	37.59a	41.92a	45.34a
Lime	11.98b	13.73b	14.65b	19.26b	22.00b

Puna

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10
Control	38.32a	40.32a	39.35a	47.57a	50.75a
Ash	32.65a	36.36a	44.37a	45.02a	44.42a
Lime	6.98b	10.00b	16.90b	18.27b	23.06b

Means followed by the same letter in a column are not significantly different at 5% level of LSD.



APPENDIX 9

Analysis of variance table for Arcsine transformed values of mean rotten area cm² in bruised tubers of Araba, Asana and Puna stored for ten weeks.

Araba – 2nd Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	0.835	0.418	0.4774	
Factor A	2	257.668	128.834	147.2277	0.0002
Error	4	3.500	0.875		
Total	8	262.004			

Araba – 4th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	47.506	23.753	1.0235	0.4375
Factor A	2	356.421	178.210	7.6793	0.0427
Error	4	92.827	23.207		
Total	8	496.754			

Araba – 10th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	5.117	2.558	0.4321	
Factor A	2	185.256	92.628	15.6437	0.0128
Error	4	23.684	5.921		
Total	8	214.058			

Asana – 2nd Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	40.623	20.312	0.8045	
Factor A	2	650.219	325.110	12.8772	0.0181
Error	4	100.988	25.247		
Total	8	791.831			

Araba – 4th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	141.519	70.759	3.7373	0.1215
Factor A	2	245.962	122.981	6.4955	0.0484
Error	4	72.733	18.183		
Total	8	460.214			

Araba – 6th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	23.590	11.795	2.7243	0.1792
Factor A	2	889.719	444.860	102.7506	0.0004
Error	4	17.318	4.330		
Total	8	930.628			

Asana – 8th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	0.108	0.054	0.0049	
Factor A	2	943.358	471.679	42.6645	0.0020
Error	4	44.222	11.056		
Total	8	987.688			

Asana – 10th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	14.756	7.378	0.0855	
Factor A	2	1103.861	551.930	6.3976	0.0477
Error	4	305.088	76.272		
Total	8	1423.705			

Puna – 2nd Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	217.405	108.703	0.5298	
Factor A	2	1562.093	781.047	3.8069	0.0486
Error	4	420.665	105.166		
Total	8	2200.164			

Puna – 4th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	47.305	23.653	0.2309	
Factor A	2	1630.212	815.106	7.9557	0.0404
Error	4	409.824	102.456		
Total	8	2087.340			

Puna – 6th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	40.042	20.021	0.6665	
Factor A	2	1284.253	642.126	21.3776	0.0073
Error	4	120.149	30.037		
Total	8	1444.445			

Puna – 8th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	40.606	20.303	3.4335	0.1355
Factor A	2	1580.020	790.010	133.6007	0.0002
Error	4	23.653	5.913		
Total	8	1644.279			

Puna – 10th Week					
Source	Degree of Freedom	Sum of Squares	Mean Squares	F Values	Prob.
Replication	2	74.612	37.306	0.8096	
Factor A	2	1211.082	605.541	13.1420	0.0174
Error	4	184.307	46.077		
Total	8	1470.001			

APPENDIX 10

pH of 48% neem wood-ash and 48% lime suspensions

Neem wood –ash	Lime
12.20	13.22
12.31	12.09
12.93	13.84
11.00	13.82
11.81	13.10
Mean 12.03	13.18