STUDIES ON THE INFECTION OF YAMS BY *SCUTELLONEMA BRADYS* (STEINER AND LEHEW)

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ABSTRACT

A general survey of phytoparasitic nematodes associated with yam (*Dioscorea* spp.) in the Mid-Western State of Nigeria showed that *Scutellonema bradys* and *Meloidogyne* spp. were the economically important nematodes of yam tubers. *S. bradys* was associated with the 'dry rot' of yam tubers causing storage losses estimated between 80 and 100%. Nematodes of the genus *Meloidogyne* were found associated with galling of tubers of water yam (*D. alata*).

Studies on the rate of population build-up of *S. bradys* in storage showed that *S. bradys* increased 9-fold, 8-fold and 5-fold in the tubers of *D. rotundata*, *D. cayenensis* and *D. alata* respectively during 6 months of storage. These increases in population influenced the severity of 'dry rot' disease. Results of investigations into the depth of penetration of *S. bradys* in 5 different varieties of *D. rotundata* showed that there were differences in varietal susceptibility. The bulk of the nematode population was found in the periderm to a depth of between 0 - 1.5 cm, but depth of penetration was greater in the

head portions of each of the tubers than either the middle or bottom portions.

Observations on the activities of the nematodes in tuber tissues (histopathology) suggested that the 'dry rot' was mainly due to mechanical damage to the cells and the host reaction to intracellular feeding by S. bradys. Studies on changes in the carbohydrate constituents of the yam tuber infected by S. bradys showed an increase in the percentages of monosaccharides and disaccharides like sucrose, glucose and galactose, fructose with a concomitant decrease in starch, amylose and amylopectin when compared with healthy yam.

Qualitative and quantitative determination of amino acid constituents of nematode-infected tubers of white yam (D. rotundata), yellow yam (D. cayenensis) and water yam (D. alata) showed that the relative numbers of free amino acids were not materially changed following infection by S. bradys, but a reduction occurred in the number of 'essential' amino acids in the infected tubers. Eighteen ninhydrin positive amino acids were detected in the protein hydrolysate. Except in the case of white yam and in a few other cases, increases in protein amino acids were recorded

in the infected tubers of yellow and water yam. The percentage protein was also increased by infection in all species except white yam (D. rotundata).

Observations on the rate of weight loss (cumulative percentage weight and mean percentage weight loss) in 3 different species of Dioscorea stored in a yam barn showed that there was a significant difference in the rate of weight loss between nematode-infected and nematode-free tubers of D. rotundata and D. cayenensis, but no significant difference was recorded between the infected and healthy yam tubers of D. alata. Estimation of the edible portions in nematode-infected and nematode-free tubers of D. rotundata, D. cayenensis and D. alata showed a significant difference in the percentage peeling losses between the infected and healthy tubers.

Chromatographic analysis of the incubation solution of S. bradys showed that 5 amino acids - aspartic acid, phenylalanine, hydroxylnol acetic acid, leucine and isoleucine were discharged by this nematode. The absence of the steroid group of compounds in the nematode-infected yam tubers revealed by spectrometric analysis might be disease-related as evidenced by its appearance in the

healthy tubers. Polygalacturonase and amylase activities were detected in homogenates of S. bradys.

Studies on fungi associated with the dry rot disease of yam tubers showed Aspergillus niger, Penicillium sclerotigenum, Trichoderma viride, Rhizopus nigricans and Fusarium oxysporum, Botryodiplodia theobromae and Fusarium moniliforme as the main species.

Studies on the possible interrelationshipsbetween S. bradys and 3 fungi A. niger, P. sclerotigenum and F. oxysporum showed that the presence of the nematode seemed to increase the degree of pathogenicity of Fusarium and Penicillium species on yams. But the presence of S. bradys did not increase the degree of pathogenicity of Aspergillus niger. In greenhouse experiments, the interaction between S. bradys and A. niger was found to be disadvantageous to the nematode. The presence of the fungus seemed to have some effect on the number of nematodes that invaded the roots and tubers and subsequently on nematode development. This was thought to be due to an anti-biotic action of A. niger on S. bradys.

A host range study of 30 crop plants and weeds revealed that beniseed (Sesamum indicum L.), cowpea (Vigna unguiculata (L.) Walp.), were good alternative hosts of

S. bradys. Small populations of the nematode also survived endoparasitically in the roots of Eupatorium, Synedrella, roselle (Hibiscus sabdariffa L.), kenaf (Hibiscus cannabinus L.), melon (Cucurbita pepo L.); jute (Corchorus olitorius L.); yam bean (Sphenostylis stenocarpa) (Hochst ex A Rich) Harms., soko (Celosia argentia L.) and pigeon pea (Cajanus cajan (L.) Druce). Non-hosts included maize and tobacco.

Dipping nematode-infected tubers of D. alata and D. cayenensis in hot water at temperatures ranging between 50 and 60°C for 40 minutes completely eliminated the nematode. However, at temperatures above 55°C for an exposure time of 40 minutes, the tubers so treated suffered a physiological damage and rotted very rapidly. Temperatures between 50 and 55°C had no adverse effect on percentage emergence, growth, yield and palatability of tubers of D. alata.

Field trials on chemical and cultural control of S. bradys on D. alata showed that the yield of yam was increased and the nematode population suppressed by the application of organic manure at the rate of 1.5 kg/heap

or 1,886.3 kg/ha. Although the application of nemagon at the rate of 35.2 kg/ha. considerably suppressed nematode population, the yield of yam was significantly reduced. The results showed that there is a good deal of potential for experimentation with various cultural methods of nematode control.

Studies on the effect of gamma irradiation on S. bradys showed that dosages between 5 and 15 Krad did not eliminate the nematodes completely, but suppressed sprouting and signs of deterioration in tubers. Dosages between 20 - 30 Krad eliminated about 70 - 80% of the nematode population.

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CERTIFICATION

We certify that this work was carried out by Mr. S.O. Adesiyan in the Department of Agricultural Biology, University of Ibadan, Ibadan.

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CHAPTER I

INTRODUCTION

The fundamental problem which faces most of the tropical regions of the world today is the feeding of their increasing population. Despite the vagaries of climate mentioned by Stamp (1960) in his fascinating book 'Our Developing World', it is believed that the tropical regions of the world have tremendous potentials for possible all-year-round production of a variety of food crops. Nevertheless, the practice of agriculture in the tropics is fraught with many problems. Lack of technological skill, pests and plant diseases are at the moment preventing the maximum exploitation and utilization of the existing land resources. These and other similar problems must therefore be resolved before the tropical regions of the world can be at par in production levels with the technologically developed countries of the world.

The warm climate of the tropics which is by and large regarded as a blessing is not without its disadvantages.

Many pests are able to reproduce for a longer period and more rapidly in the warmer climate. For this reason, nematode populations are believed to reach higher levels in the warmer than in the cold climates. Unfortunately, most of the nematodes that parasitise tropical crops have not received any significant attention in the past. For example, except in a few cases like the research work which revealed the devastating effects of Radopholus similis on banana, a study of the parasitic nematodes associated with the food crops of the tropics has only just begun. It is recognised, however, that a study of some aspects of parasitism on yams by nematodes has been progressing and interesting questions are being raised. Yam cultivation is economically important in Nigeria. Yam is eaten both by the rich and the poor, and the demand for yam far exceeds supply. Unfortunately, not much is known about the biology of the yam nematode. The knowledge of its behaviour, control, and interrelationships with other plant disease organisms is also inadequate. Nematodes are now recognised as pests of global concern hence the need to research into nematode problems in the hope that the problems can be identified and effectively

solved in the near future.

1.1. The objectives of the research.

The aim of this study is to establish the role of the yam nematode Scutellonema bradys in the formation of 'dry rot' disease of yams, to study the conditions favouring the occurrence of the pathogen in yams and the possible ways of control. Details of the objectives are as follows:-

(i) To evaluate existing methods of surface sterilization or decontamination of nematodes nematodes with a view to recommending the best method for in vitro studies.

(ii) To study the behaviour of Scutellonema bradys as regards survival, rate of reproduction and rot development in relation to two principal environmental factors, i.e. temperature and humidity.

(iii) To study the depth of penetration of Scutellonema bradys in yam tubers.

(iv) To study the histopathology of the nematode-infected yam tissues.

(v) To estimate the economic losses due to Scutellonema bradys by

(a) comparing the weight loss in nematode-free and nematode-infected tubers.

(b) comparing the food contents of nematode-free and nematode-infected tubers.

(c) determining the edible portion of the nematode-infected tubers.

(vi) To inoculate surface-sterilized nematodes into yams with a view to establishing the role of Scutellonema bradys in 'dry rot' formation.

(vii) To isolate the fungi associated with 'dry rot' disease of yams.

(viii) To study the relationship between Scutellonema bradys and other microorganisms attacking yams in the disease complex.

(ix) To identify substances discharged by the nematode which may be responsible for 'dry rot' formation.

(x) To conduct greenhouse experiments on host-range studies of Scutellonema bradys.

(xi) To study the distribution, levels of infestation and seasonal incidence of yam nematodes in a major yam growing part of the country.

(xii) To attempt control of Scutellonema bradys by the use of cultural and other methods such as the use of hot water treatment, nematicides, and gamma irradiation.

CHAPTER 2

REVIEW OF LITERATURE

PART I

2.1 Evidence for the occurrence of nematodes in yam tuber.

The occurrence of some parasitic nematodes in yam tuber has been long established by various workers all over the world. As early as 1880, de Man observed that yams from various tropical regions of the world were attacked by the meadow nematode which he called Anguillulina pratensis. Steiner (1931) published a brief note on the occurrence of disease in a yam tuber from Jamaica, with which there were associated large numbers of a species of nematode which he placed in the genus Hoplolaimus.

Steiner and LeHew (1933) published a description of the nematode obtained from this yam and gave it the name Hoplolaimus bradys. In 1934, Steiner and Buhrer recorded the same parasite in a yam from Puerto Rico. T. Goodey (1935), using specimens from J. West mentioned the species Dioscorea alata Linn., D. cayenensis Lam., and D. rotundata Poir, as susceptible hosts in Nigeria.

In Guatemala, Schieber (1961) found that in several experimental plantations, parasitic nematodes were found attacking three different species of Dioscorea - D. floribunda Mart. et Gal., D. composita Hemsl., D. spiculiflora Hemsl. The nematodes involved were Meloidogyne arenaria (Neal) Chitwood; M. incognita (Kofoid and White) Chitwood and Heterodera sp.

Caveness (1967) recorded seven species of nematodes infecting yams. Four species Scutellonema bradys (Steiner and LeHew), S. clathricaudatum (Whitehead), Pratylenchus brachyurus (Godfrey) Filipjev, Meloidogyne incognita (Kofoid and White), are endoparasitic in yam roots. Four species, S. bradys, P. brachyurus, M. incognita and Rotylenchulus reniformis Linford and Oliveira are endoparasitic in freshly harvested tubers. Of these species, only S. bradys persists in the tuber after long periods of storage.

Several species of these nematodes occur abundantly in tropical soils where they cause visible damage not only to yams but other field crops. The yams are affected in various ways. Firstly, the market value of yams is decreased, the carbohydrate starchy tissue is destroyed

and in some cases, the whole plant is killed. Nematode attack also results in storage losses and there are instances where tubers reserved for 'seed' may be so weakened resulting in loss of viability or giving rise to unhealthy plants.

2.2 Brief taxonomy of the plant parasitic nematodes of economic importance.

The plant parasitic nematodes comprise several hundred microscopic species of the NEMATODA - a large, size-variable PHYLUM of morphologically complex and distinct invertebrate animals. This phylum is divided into two classes; the PHASMIDIA and the APHASMIDIA. The class PHASMIDIA is further sub-divided into two ORDERS, five SUB-ORDERS, thirteen SUPERFAMILIES and forty-seven FAMILIES of which two SUPERFAMILIES and four FAMILIES feature plant parasitic nematodes of economic importance.

Some of the soil inhabiting species, all but a few of the plant parasites are grouped in the class PHASMIDIA. The class APHASMIDIA includes only a few plant parasitic nematodes.

The vast majority of the plant parasitic nematodes are to be found in the order TYLENCHIDA, and also in

the superfamilies DORYLAIMOIDEA and TYLENCHOIDEA. Two families in the superfamily TYLENCHOIDEA:- the HOPLOLAIMIDAE and the HETERODERIDAE include typically vagrant parasites of plants.

2.3 Description of Hoplolaimidae attacking yams.

(a) Scutellonema bradys (Steiner and LeHew).

S. bradys belongs to the subfamily Hoplolaiminae. This subfamily name was derived from the genus Hoplolaimus described earlier by Steiner and LeHew (1933). Goodey (1935) named it Anguillulina and Rotylenchus (Filipjev, 1936). Andrassy (1958) gave the name Scutellonema to the genus which includes several other tropical species, namely; S. clathricaudatum (Whitehead, 1959) and S. cavenessi (Sher, 1963). The latter two species are common in Nigerian soils (Bridge, Private Communication, 1972).

S. bradys is stout. The body tapers slightly in the oesophageal region towards the head which is distinctly offset by a constriction. There is little tapering of the body posteriorly and the female tail is broadly conical in shape. The male tail tapers steeply behind the cloacal opening to a cloacal terminus which is completely surrounded by the rather voluminous bursa. Prominent glands

are present at the vulval opening. Body is normally straight or slightly curved when relaxed. S. clathricaudatum and S. cavenessi are characterised by the absence of vulval glands.

The disease caused by Scutellonema bradys has been termed DRY ROT of yams by West (1934). Apparently, the parasite confines its attack to the tubers, since up to the present, no recognisable symptoms of attack has been reported in the aerial portions of the host plants. This is somewhat similar to that of potatoes attacked by Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936 in which the main seat of attack is also in the tubers. The diseased condition is revealed on peeling or lifting the skin of yam when small diserete areas are found which are at first yellowish but later become brown or black in colour. In these areas, the parasites are found and are particularly numerous in older darker lesions just beneath the skin. The primary lesions caused by the parasite do not penetrate very deep (West 1934) but are confined to the sub-dermal layers. They afford entry for various fungi, mites, and other saprophytic organisms which gradually bring about the destruction of the entire substance of the tuber.

(b) Pratylenchus (Filipjev) spp.

Members of this genus belong to the family HOPLOLAIMIDAE and subfamily PRATYLENCHINAE. About twenty-four species are described so far even though only a small proportion of these attack yams. It was originally called the meadow nematode, but is now referred to as the lesion nematode because of the lesions it forms on the host. While the species of Pratylenchus are typically root parasites, they may also attack other underground stems and tubers. They live endoparasitically and produce large numbers in roots, rhizomes and tubers where they cause dark necrotic lesions on the infested parts. Bridge (1972) found P. brachyurus in 9% of the tubers he examined in the Western State of Nigeria. Other members of the Hoplolaimidae found in yam soils are Rotylenchulus reniformis Linford and Oliveira, Helicotylenchus pseudorobustus (Steiner) Golden, Helicotylenchus dihystera (Cobb), and Hoplolaimus proporicus (Goodey).

Symptoms of P. brachyurus attack include splitting of the skin of the tuber which gives it a corky appearance and a dark brown rot extending into the starchy tissue.

2.4 Description of Heteroderidae attacking yams

Genus: Meloidogyne (Goeldi) spp.

This nematode belongs to the subfamily HETERODERINAE. In the older literature, the type of the genus was either Heterodera marionii (Cornu and Buhrer, 1938) or Heterodera radicicola (Mueller). About twenty species have been described. This is a universal pathogen with a tremendously wide host range and found attacking almost every crop. Root-knot nematodes were first reported on yams by Queva (1895) who noted that the nematodes were concerned with tissue modification in some species of Dioscorea. Young (1923) working in Florida made a report of their attack on D. alata. Species of Meloidogyne have also been observed in Nigeria (Udeaja, 1961) while Caveness (1961) found M. arenaria in D. rotundata. M. incognita var. acrita was found infesting yams in the Ivory Coast (Luc and de Guiran, 1961); Smit (1966), Schieber (1961), Jenkins and Bird (1962) also found root knot nematode associated with some wild Dioscorea species in Guatemala.

Characteristically, species of Meloidogyne form galls on tubers, but in some cases tubers may carry large

numbers of females without showing knots or galls. Feeding of the females occurs in plant tissue surrounding the head of the nematode; such tissue produces giant cells. Proliferation and hypertrophy of the cortical cells result in the formation of swellings or galls. The overall effects of root-knot nematodes on yams are similar to those of S. bradys. Yellow to brown patches are found in the cortical cells of the tuber.

2.5 Control of S. bradys in yams.

The purpose of nematode control is to improve crop yields and quality. Nematode control aims at maximising the efficient and effective use of arable lands to meet the increasing need for food and fibre throughout the world. As part of this effort, the development and use of an economical and effective method of controlling the yam nematode would go a long way in solving some of the major problems associated with the yam industry in the tropics. Unfortunately, such economical and effective methods are not easy to come by.

2.5a Chemical control.

There is little information about chemical control of S. bradys. Bridge (1972) did not achieve effective

control of the nematode population by spot application of D-D into yam hills. He, therefore, recommended that a non-phytotoxic compound should be found that can be applied as a post-plant treatment at the stage nematodes are migrating from old infected yams into the soil before invading the young developing tubers. Ayala and Acosta (1971) found D-D to be a very effective soil fumigant for nematode control in a field trial with Dioscorea alata. Ayala and Acosta (1971) found Dasanit (Bayer 25,141) at 1,250 ppm for 15 - 30 minutes, Nemafos (Ne 18,133) 625 ppm for 60 minutes and Nemagon 625 ppm for 30 minutes, effective in bath treatments of diseased propagation material without affecting its germination. Roth and Richardson (1965) reported that fumigation with methyl bromide was generally effective in the control of pest infestation on yams which showed a good tolerance to fumigation, but no data were specifically provided on nematode control. Thompson et al. (1972) in Jamaica tested the effects of methyl bromide, hydrogen phosphide and ethylene dibromide as fumigants in controlling the yam nematode in stored yams. Their results showed that

fumigation with both methyl bromide and hydrogen phosphide initially reduced the number of nematodes, but after 35 - 39 days in storage there was a steady increase in the nematode population in the tubers treated with hydrogen phosphide so that there were no significant differences from the control. Thompson et al, (1972) also concluded from their investigation that fumigation appeared to have little practical application because of the damage that was caused to the tubers.

The use of nematicides in the tropics is beset with many fundamental problems. Nematicides are expensive and, therefore, cannot be afforded by the majority of farmers whose traditional farming operations are characterised by low productivity and very little profit margin. Besides, very little is known about the effectiveness, persistence and residue effects of some of the nematicides in the tropics.

(b) Hot water treatment

It would appear that the first reported work on hot water treatment of tubers as a measure of nematode control was by Burk and Tennyson (1941). They successfully disinfested root-knot infected sweet potatoes that were

to be used for propagation by treating them for 65 minutes at 116°F. The treated roots produced good plants that were free from root-knot. This method has since been used in other parts of the world to control yam nematodes. Control of nematodes in seed yam tubers (i.e. planting material) by immersion in water at 46°C for 60 minutes, 52°C for 7 - 17 minutes or 50°C for 15 minutes was described by Ayala and Acosta (1971) and at 51°C for 30 minutes by Hawley (1956). Bridge (1972) also successfully achieved about 98% kill of the yam nematodes using hot water treatment. His exposure time was 40 minutes at water bath temperatures ranging between 50 and 55°C.

(c) Crop rotation.

Although nothing is yet reported in literature about the use of cultural methods for controlling yam nematodes, the use of crop rotation to reduce nematode population has been suggested as one of the most effective and most widely used land management practices. To be an effective control practice, crops that are unsuitable as hosts for the nematode must be included in the rotation and growth of resistant crops for two to four years must be encouraged. Planting two resistant crops between susceptible crops may

give some measure of control, but three to four years and with some nematodes seven to eight years, are sometimes necessary for effective control. The limitations are that resistant crops grown in the rotation may be of low value and consequently contribute little to the farm income. Cultural control of S. bradys, however, by rotation on non-susceptible crops is, not always possible because of its dispersal by infected tubers (Bridge, 1972).

(d) Biological control.

Although some investigators are optimistic about the potential of biological control of nematodes, very little is reported in the literature about the use of such methods involving predaceous fungi, toxic substances resulting from crop residues or other biological agents. Other methods such as irradiation, electrical treatment or plasmolysis are known to be harmful or lethal to nematodes, but their full potential for effective nematode control is yet to be investigated.

PART II: THE HOST: YAM

2.2.1 Evolution of Dioscoreaceae: its botanical features.

Yams belong to the genus Dioscorea named by Linnaeus in 1937. They are angiosperms, and the genus contains about 600 species of which only a small proportion have been studied in great detail. The genus Dioscorea may be divided into a number of sections which are taxonomically recognised. The main food yams belong to the section Enantiophyllum comprising D. rotundata Poir., D. cayenensis Lam., D. alata L., D. opposita Thunb. and D. japonica Thunb.

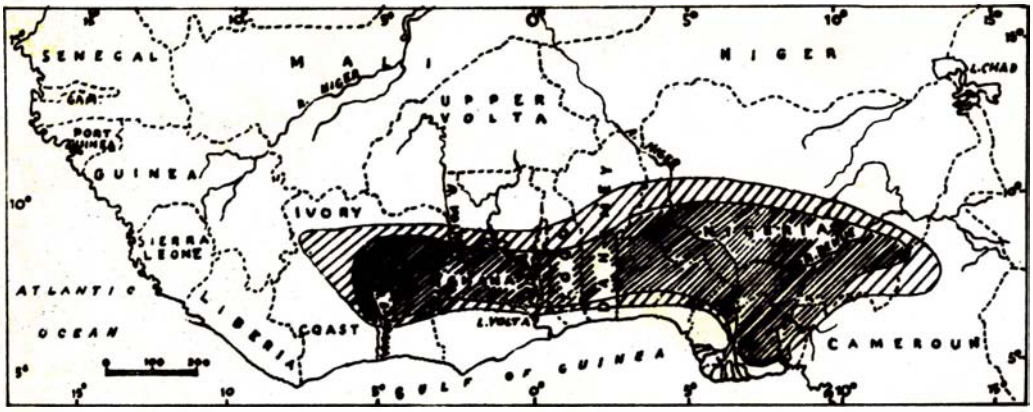
Yams first occurred in the Jurassic when Proto-Dioscoreaceae evolved in Asia. They were originally hermaphroditic and rhizomatous. But it was in the Cretaceous that the tuber began to evolve. The spread and diversification of Dioscorea with increasing tuber development, and separation of African from Asiatic species of Dioscorea probably occurred in the Miocene (Burkill, 1960).

The species in this genus are typically monocotyledonous though some may have secondary cotyledons. They are

seasonally vegetative, producing annual shoots which are twining except in a few dwarf species. They persist either underground or at the surface of the ground by means of storage organs developed in a variety of forms. These storage organs are the tubers one or a few descending into the soil, usually deeply, arising as a swelling or as swellings upon the base of the current year's stem. In most species, the base of the stem becomes indurated and lasts in the soil beyond the end of its year. The soft annually replaced tubers are protected by the depth of the soil over them. Annual stems are renewed from the hard woody knot. Although more than one tuber may be present, not uncommonly one stem alone is produced which drains the stored food from all the tubers. Storage of food occurs in the parenchyma. Leaves are never compound and are always entire. All the Dioscorea are dioecious, although occasionally both male and female flowers are borne on the same plant. Some cultivars flower only rarely.

2.2.2 Global yam production.

Asia and Africa are the continents in which yams are



THE 'YAM ZONE' OF WEST AFRICA.

FIG. 1. (Reproduced from 'Yams' by D.G. Coursey, 1967) Longmans Green & Co. Ltd.

produced to the greatest extent. In Europe and North America, yam production is insignificant. Edible yams are grown to a very limited extent probably as curiosities.

The greatest yam growing areas in the world are found in the eastern part of West Africa between the Central Ivory Coast and the Cameroun mountain chain, these areas produce more than two thirds of all yams grown in the world. Nigeria alone accounts for almost half of the global figure. The next in rank is South-East Asia. The most widely grown species here is water yam (Dioscorea alata). This species is grown mainly in Burma, Laos, Thailand, Cambodia, Vietnam and Malaysia. In India, China and Indonesia, D. alata is also most widely grown (Coursey, 1967).

Yams are also grown in significant quantities in North, Central and South America, where the total production of yams is estimated at about 714,000 metric tons per annum (F.A.O., 1966).

2.2.3 Yam production in Nigeria

(a) Acreage planted to yam and yield per hectare.

Yams are grown all over the country, but production is markedly concentrated in the Southern States. Yam is

predominantly produced in the East Central State. It is largely grown as a mixed crop in the Northern and Mid-West States, but in the Western State, yams are mainly grown as sole crops.

The total area reported planted to yam in 1965/66 was approximately 1.7 million hectares. Excluding the East Central State, 0.7 million hectares were planted in 1966/67 and 0.8 million hectares in 1967/68. Assuming the area planted in the East Central State in 1965/66 remained about the same in the following two years, the total area planted would stand at 1.3 million hectares and 1.4 million hectares in 1966/67 and 1967/68 respectively.

The average yields reported for the three years have been normal ranging between 3,633.8 and 4,105 kg per hectare. The yields recorded for Western State are, however, much higher than for the other states and ranged between 4,235.8 to 4,695.3 kg per hectare.

In 1968/69, the total area planted to yam was 1.1 million hectares. The yield in 1968/69 was 3,125.3 kg per hectare. In 1969/70, the number of acreage planted to yam was 1 million and the yield was 3,797 kg per hectare.

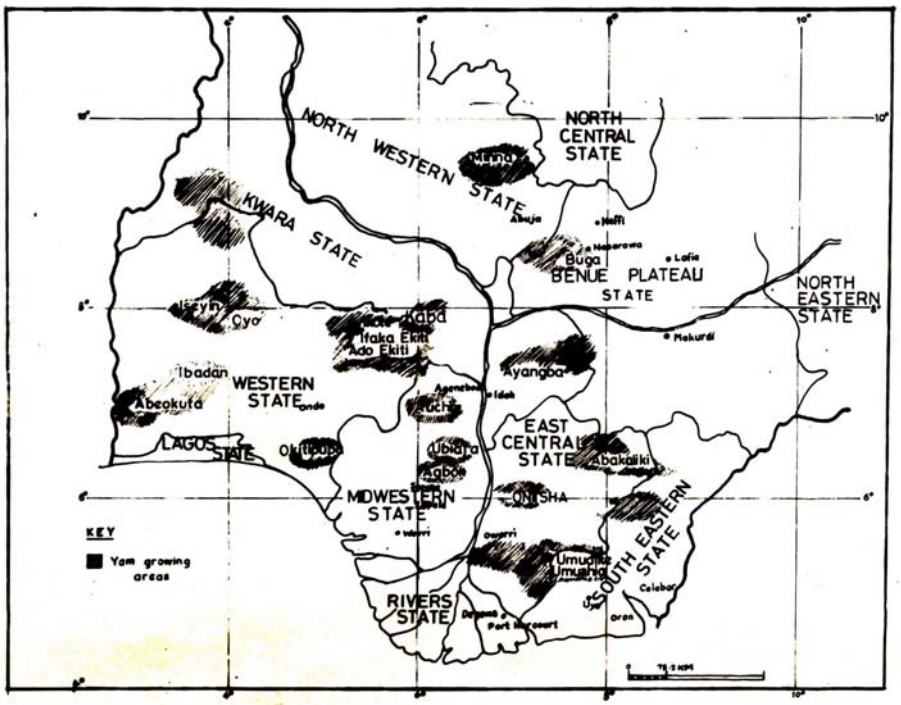


FIG.2. Major yam growing areas in Nigeria.

In 1970/71, the total area planted to yam was approximately 1 million hectares with an average yield of 4,027 kg per hectare. The total area in 1971/72 was estimated at 1.6 million hectares and the average yield of 3,223.9 kg per hectare.

Yield figures from state to state are not given here, but generally there is enormous variation in yield between the states. This is not surprising in view of the differences in climatic conditions, cultivars, the nature of cultural practices, fertilizer use, time of planting and harvesting and other management methods. All these factors and practices are known to affect the yields obtained to a very great degree.

2.2.4 Consumption of yam per head in Nigeria.

A survey of the weekly consumption of yams per head conducted by the Federal Office of Statistics in the country in 1963/64 and 1965/66 shows that yams constitute the main diet of the people in most of the states. The Benue-Plateau State tops the list with a weekly consumption unit of 13.39 kg. The figures for South Eastern and East Central States are 6.05 and 3.98 kg per caput respectively. Kwara and Mid-West States figures stand

at 4.47 kg and 2.90 kg respectively. Western State figure stands at 2.38 kg per caput.

Although the last consumption survey was conducted in 1965-1966, it is generally assumed that consumption pattern in the rural areas has hardly changed over the years in the absence of a radical change in dietary habits.

2.2.5 Economics of yam production.

Probably because yam is a non-export crop, the knowledge of the costs, efficiency and profits of yam production is fragmentary. The methods in use for yam cultivation by most farmers are in the main traditional and require only simple home-made tools. These methods require great labour inputs. The construction of the large mounds which is very laborious occurs before planting and deep digging and careful handling are required at harvesting time.

Relatively, few studies of the economics of yam production under peasant farming conditions have been carried out. At Ejura, Northern Ashanti, Ghana, Hunter et al, (1931) and Bray (1958). estimated the labour requirement to be about 109 man-days per hectare per year, and the yield of saleable yams produced between

3,763 and 6,272 kg per hectare per year. Other survey reports show that the labour requirement can vary from a little as 36 to as much as 117 man-days per hectare per year. Later studies have also shown that the labour expended accounted for approximately two thirds of the total costs of production.

A combination of all these investigations carried out under different conditions and with widely varying yields per hectare indicates that the labour required to produce 2,500 kg of actual dry food material in the form of yam is around 150 - 200 man-days per kg. These figures given in cash terms are meagre and indicate very low return for labour. In terms of actual productivity, the figures indicate that the labour of one man expended on yam farming can provide food only for his immediate family.

2.2.6 Important species grown.

The most important yam species grown are the Asiatic yam, D. alata and the West African D. rotundata and D. cayenensis, the first named in particular being cultivated almost throughout the tropics. Wild species

of D. rotundata are unknown but it is believed that D. cayenensis was selected from wild species. All these belong to the section Enantiophyllum of the genus Dioscorea, and normally produce a single large tuber annually. The other Asiatic yam, D. esculenta (Lour.) Burk. (Section Combilium) which produces large numbers of small tubers, in a manner similar to the potato, is also cultivated widely. This is not a particularly popular species in West Africa even though it occurs to some extent in other yam growing areas of the world. Cultivation of D. bulbifera is comparatively limited. It forms small aerial tubers or bulbils in the leaf exils. This is an advantage because the aerial tubers can be harvested with little efforts.

In many parts of Asia, D. hispida Dennst (Section Lasiophyton) is grown to some extent, as is the very similar D. dumetorum (Kunth.) Pax in Africa. D. trifida L. (Section Macrogynodium) is confined largely to its native Central America. This species also forms a number of small tubers. Dioscorea opposita Thunb. and Dioscorea japonica Thunb. (Section Enantiophyllum) are grown in sub-tropical areas of China and Japan.

2.2.7 Yam cultivation.

Yams require well-drained heavy loam soil and about 100 - 135 cm of rainfall, Early planting is done in November while soil is still moist and late planting in February to April. In the riverine areas of the country planting is done as soon as possible after the floods have receded. For seed yam production, close planting in May is the usual practice.

It is extremely important to select only healthy tubers especially for the November planting. Poor selection may mean losses of up to 100 per cent before the rains start. Whole tubers are the best planting material but large tubers may be cut into 'setts' for economy. Tops of yams with buds are better than bottoms or middles. Setts are usually cut one or two days before planting and cut surfaces are left to dry. Yam setts may be planted on the flat without making any mounds but are usually planted in mounds or ridges. Covering the hidden sett with a cap of grass or leaves (mulching) is essential for yams planted at the beginning or during the dry season and is desirable for all yams. The mulch is usually weighted down with a hoeful of soil and helps in

reducing soil temperature in the region of the sett. Germination starts 20 - 60 days depending on the rainfall regime, but may take longer for November yams or for bottoms and middles. Staking of the yam is essential for good yields. Live bush sticks are often used as stakes, with three or four stakes each carrying a different yam plant being tied together near the top to form a pyramidal structure. This gives greater rigidity and provides a greater support for vine growth thus withstanding violent storms.

2.2.8 Storage practices.

Like most other food crops, not all the yams harvested can be consumed immediately. Some have to be stored for fairly long periods. However, the type of storage practices varies from place to place, and within a single area, more than one technique may be used. The type of technique is often determined by the availability of capital resources. The peasant farmers of the tropics, unfortunately, have only limited resources and their storage techniques are, therefore, very simple.

Under these conditions, yamsare stored in the yam barn, which are constructed in a variety of ways but

generally avoiding excessively high temperatures and providing adequate ventilation.

Traditionally, peasant farmers who cannot afford the manpower and the cost of putting up yam barns usually leave the tubers where they were grown until they are required for sale or food. This type of storage technique is beset with set backs. The tubers are exposed to attack by agricultural pests and pilferage. In the event of heavy rains, yams left in the ground may rot. On the other hand, if the soil is very dry as it is in the dry season, harvesting of these yams when required may be difficult.

Stacking yams in heaps is another form of yam storage. Usually, each stack contains a few dozen yams. Yams stored under this condition, however, suffer from lack of adequate ventilation, especially for the tubers in the interior. Such yams are also susceptible to termite or rodent attack.

Thatched shelters, shed or store rooms with adequate ventilation are sometimes used to store yams. These prevent as much as possible the spread of rotting organisms.

The yam barn is the structure commonly used for yam

storage in many areas. This consists of a vertical framework to which the yam tubers are fastened individually by means of string, or more commonly by means of raffia. In other places, however, a yam barn may consist of a horizontal wooden platform where tubers are arranged in such a way that these yams are out of contact with each other.

2.2.9 Magnitude of storage losses.

Because yams are stored mainly during the dry season and for a long period, substantial weight losses occur during storage. Earliest observations made by Williams (1925) on D. alata grown in Trinidad showed that the variety used lost about 14.5% of its weight during about four months storage. Weight loss in stored yams was ascribed to sprouting (Campbell et al., 1962) and microorganisms (Coursey, 1961). Waitt (1961) suggested that the slightest cut or bruise may result in the rotting of yam tubers with substantial weight losses. In Puerto Rico species of D. rotundata, visible rotting of yams was associated with greatly enhanced losses in weight during storage (Anon, 1937). The storage losses are valued at about ₦20 million annually (Anon, 1959).

Gooding (1960) showed that there is, however, much variation between cultivars. For instance, he recorded a weight loss of 7.3% in 'bottle neck', a variety of D. alata, and 32.2% in D. alata variety 'monn-shine'. Despite these weight losses, however, it has been suggested that yams normally store well unless rotting organisms invade the yams during storage.

2.2.10 Nutritional value of yams.

Yams constitute the main diet of the people in the tropics not necessarily because of its simple source of calories but because it is a source of carbohydrate. Yams are also highly significant sources of iron, thiamine and especially of vitamin C, and minor, but still useful sources of protein, calcium, riboflavin and nicotinic acid. It is as a source of vitamin C (ascorbic acid) that yams are perhaps more important nutritionally. No other vitamins are known to be present in yams in any significant quantities.

Coursey et al, (1966) made a detailed study of the ascorbic acid content of forty different local varieties of D. rotundata, D. cayenensis and D. alata. From the results obtained, little variation was found between samples

of the same variety. Within individual tubers, it was found that the highest concentrations of ascorbic acid occurred near the bulbous end and also just beneath the skin, but the variation was not marked.

2.2.11 Uses other than as food.

Besides using yam as a source of food, the recent developments in the pharmaceutical industry employ various Dioscorea as sources of biologically active compounds. Some species contain diosgenin which is a valuable starting material for steroid drugs such as cortisone, sex hormones and contraceptives.

Alkaloidal or steroidal toxins are present in a number of species like D. dumetorum, D. hispida and D. drageana. The Zulus of South Africa are said to use D. dumetorum and D. drageana mixed with bait for catching monkeys. D. hispida is similarly used as a tiger poison in the Himalaya and as a fish and fowl poison in Indonesia. Many members of the Dioscorea are used in traditional medicine in Africa, among the Chinese and other Asiatic peoples.

CHAPTER 3

EXPERIMENTAL WORK AND RESULTS

3.1 Distribution of nematode parasites of yam tubers in Mid-West State, Nigeria.

In this study, the objectives of the survey of the major yam-storing and growing areas of the Mid-Western State were:-

(i) To determine the areas in the Mid-West State where plant parasitic nematodes are associated with Dioscorea species.

(ii) To identify the more common nematodes of the yam rhizosphere and the tubers.

(iii) To estimate storage losses.

(iv) To assess the effect of the present farming systems and other cultural practices on the increase and spread of nematodes.

(v) To suggest realistic nematode control measures compatible with existing agricultural practices.

During the survey, about 2 kg of soil and 9 yam tubers

of the three main species grown (D. rotundata, D. cayenensis and D. alata) were sampled per sampling site in not less than 50 major yam-growing areas, yam barns and yam markets in the Mid-Western State. The soil was placed in polythene bags and nematodes were later extracted from yam and soil samples using the tray modification of the Baermann funnel method (Whitehead and Hemming, 1965).

The areas covered during the survey are shown in Fig.3 . The results of the survey of species of nematodes associated with yams in the Mid-Western State are summarised in Table 1. A total of 11 species were recorded, 2 in the yam tubers only, 7 in the rhizosphere only and 2 (P. brachyurus and S. bradys) in both rhizosphere and tubers. The dry- and wet-rot diseases of yams were observed in all the yam barns and markets visited. It was found that about 100 hectares at Ilele and Agbadu farms had been abandoned due to S. bradys and root-knot infestation. Symptoms of nematode damage to tubers observed are similar to those reported by Goodey (1935), West (1934) and Bridge (1972). Cracking and flaking of the epidermal layers were generally noticeable as shown in Plate 1. Storage losses estimated between 80 - 100%

were observed in most of the yam barns.

Galled tubers caused by root-knot nematode species were also found to be common especially in D. alata (water yam) (Plate 2). Sections through such galled tubers showed that yellow to brown patches are found in the cortex as shown in Plate 3. Nematode activities also resulted in cell proliferation (hyperplasia) and formation of giant cells (hypertrophy). These are shown in Plate 4. Many root knot females were found embedded in the yam tissue (Plate 5).

The yam barn technique of yam storage was found commonly all over the Mid-Western State is shown in Plate 6. The yam barn consists of covered vertical framework and to this framework, the tubers are fastened individually by means of local cordage material. Examination of fifty yam barns, each yam barn usually containing 400 - 500 yams, revealed that this method of storage has one important setback. Because the tubers are in close contact with each other, the spread of decay-inducing microorganisms was facilitated; such infections occur easily through 'wounds'. This probably accounts for the high incidence of rot observed. A less important method of yam storage

found in a few places is the traditional method of burying the yams where they were grown until required for food or sale. Yams stored in this way showed a high incidence of both nematode and termite attack.

Yam propagation is by tuber cuttings (setts) and the use of small tubers known as 'seeds'. The 'seed yam' is obtained by harvesting the immature tuber early from the growing plant and leaving the bulbous head and to continue growing. In due course, this forms a new small tuber which may be used as 'seed' for the following season. The use of yam seed was found to be advantageous in that the yam seeds examined and extracted were found to be comparatively nematode free.

Localised infestation by yam beetle was also observed (Plate 7) but yam losses resulting from their attack could not be compared with losses due to nematode infestation which was found everywhere.

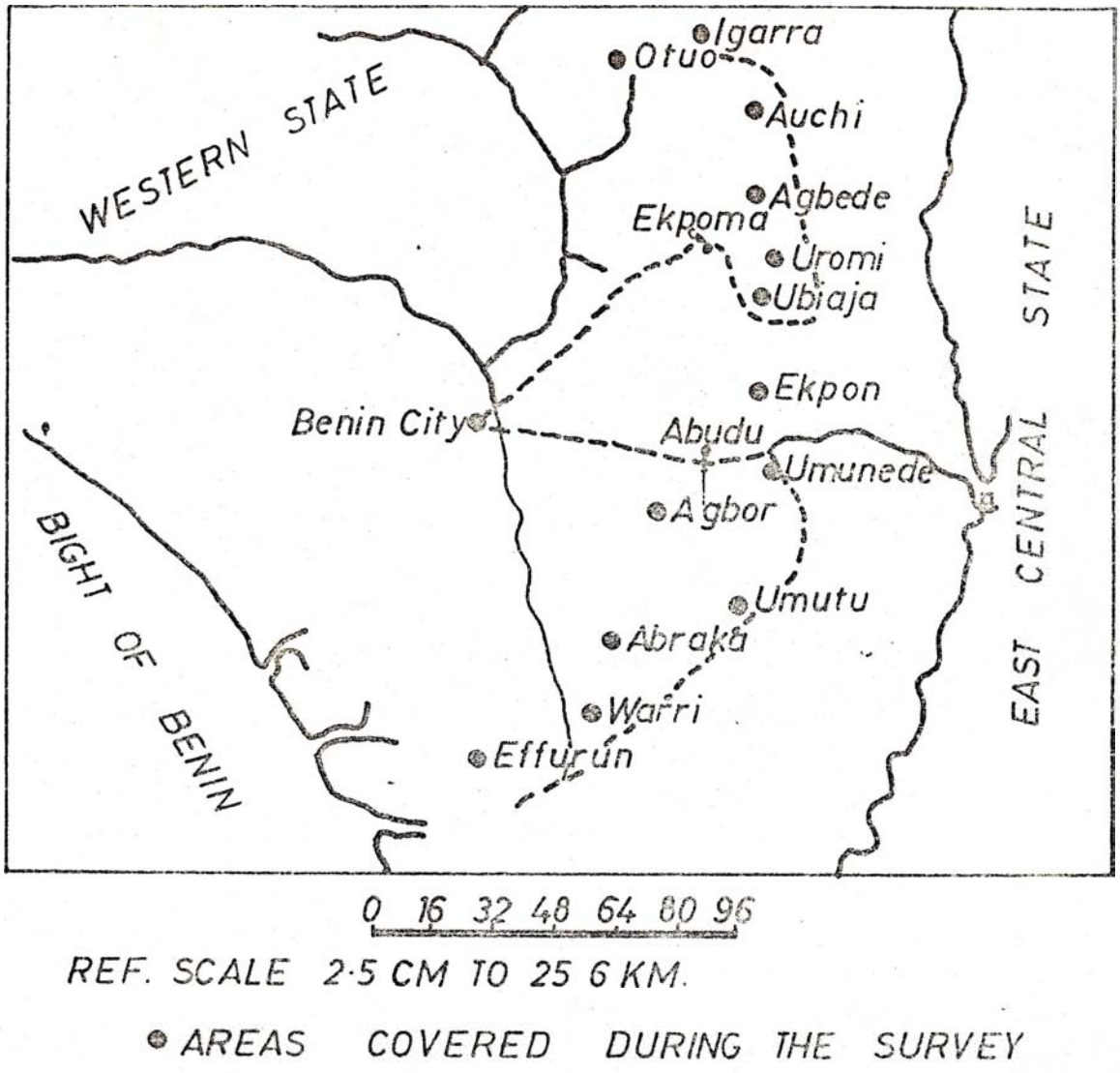


FIG. 3: MAP OF MID-WESTERN STATE, NIGERIA SHOWING THE MAJOR AREAS OF YAM CULTIVATION

TABLE 1

ECOLOGICAL SURVEY

PLANT PARASITIC NEMATODES FOUND ASSOCIATED WITH YAM TUBERS AND YAM RHIZOSPHERE IN THE MID-WESTERN STATE OF NIGERIA.

(1974)

Nematode species	Soil	Tubers
Criconemoides spp.	x	
Helicotylenchus spp.	x	
Meloidogyne spp.		x
Meloidogyne incognita		x
Pratylenchus brachyurus	x	x
Pratylenchus spp.	x	
Scutellonema bradys	x	x
Scutellonema aberrans	x	
Scutellonema clathricaudatum	x	
Scutellonema spp.	x	
Xiphinema spp.	x	

a b c

PLATE 1: (a) Yam tuber with no symptoms of nematode infection.
(b) Yam tuber with symptoms of nematode infection.
(c) Yam tuber with heavy symptoms (cracks on the skin) of nematode infection.

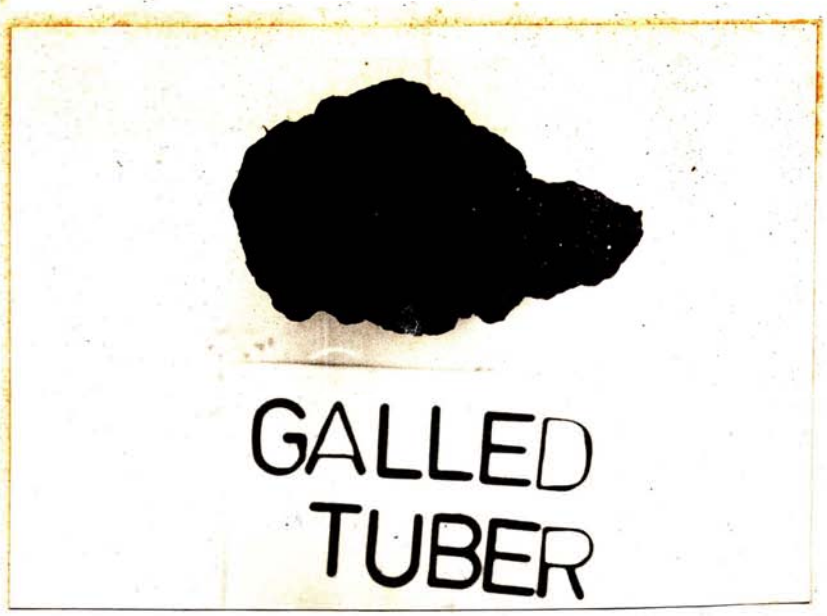


PLATE 2: A root-knot infected water yam (D. alata) from the Mid-West State. Nigeria

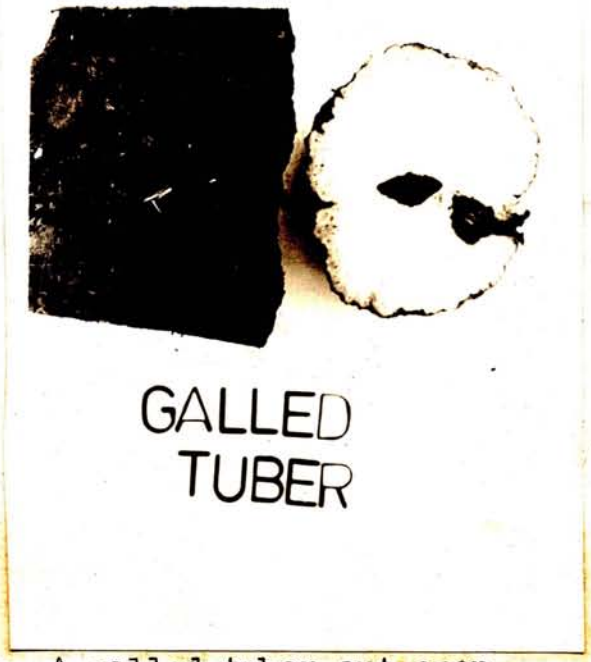


PLATE 3: A galled tuber cut open.

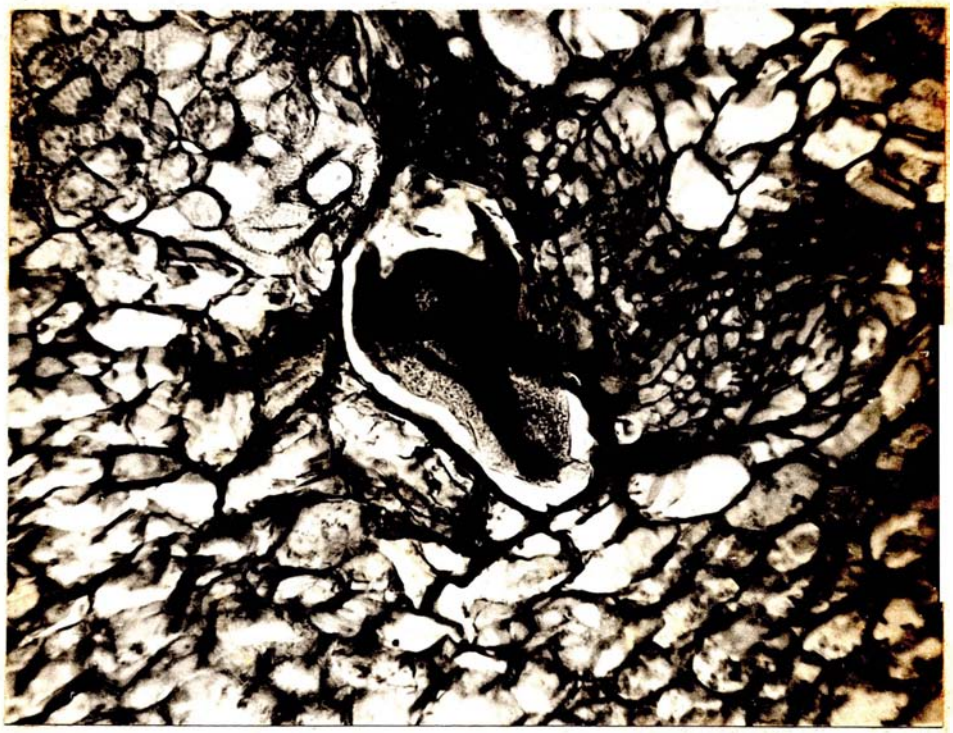


PLATE 4: A root-knot female embedded in the yam tissue. Note formation of giant cells and proliferation of cells.

PLATE 5: Root-knot females embedded in the yam tissue.

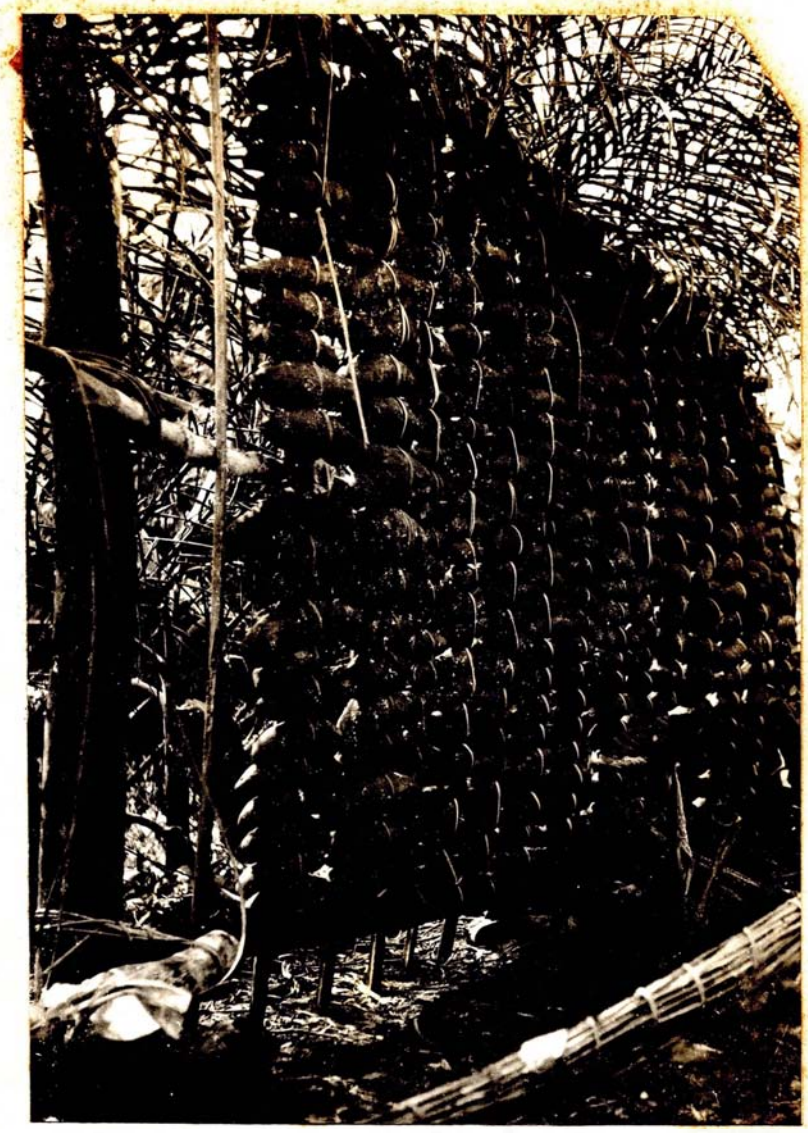


PLATE 6: A traditional yam barn used for yam storage in Mid-Western State, Nigeria.

PLATE 7: Yam showing infestation by beetle.

3.2 SURFACE STERILIZATION OF NEMATODES.

It is essential that nematodes be cleaned or be made fully axenic before they can be used for *in vitro* studies. In order to obtain a good method of surface sterilization of nematodes, four methods of surface sterilization were tested as follows:-

(i) Use of 0.1% streptomycin sulphate.

Two hundred live nematodes were first washed in sterile water and left for 30 minutes. The nematodes were then transferred into 0.1% streptomycin sulphate solution for 60 minutes and then immediately washed twice in sterile water (15 minutes each). The two hundred nematodes were then plated in agar (20 nematodes per agar plate). The plates were incubated at room temperature and observed for a period of two weeks for the number of nematode alive and the percentage of the agar plates contaminated.

(ii) Use of 0.1% streptomycin sulphate and 20 ppm of malachite green solutions.

As above, two hundred live nematodes were washed in sterile water and left for 30 minutes. The nematodes were then transferred to a known volume of 0.1% streptomycin sulphate for 30 minutes; washed in sterile water for 30 minutes and then transferred to the same volume of 20 ppm malachite green solution for 30 minutes. This was quickly followed by two washings in sterile water.

The nematodes were plated in agar, incubated at room temperature and observations carried out for a period of 2 weeks as above.

(iii) Use of 20 ppm malachite green solution only.

The same procedure as in the first method was followed but malachite green was substituted for streptomycin sulphate.

(iv) Use of 0.1% mercuric chloride.

As in (i) but mercuric chloride was substituted for streptomycin sulphate.

The nematodes used for this and subsequent experiments were extracted from yams using the tray modification of the Baermann funnel method as in (3.1). 50 g of yam, peel were cut into small pieces and spread on 'Kleenex' tissues supported on gauze in a plastic coated wire basket standing in a plastic photographic developing dish. Water was added to the collecting dish and the pieces of yam were kept constantly moist. After 48 hours, most of the nematodes would have moved into the water in the photographic dish, ready to be used. The nematodes were then withdrawn into special dropping funnels containing each of the sterilants. The nematodes were kept in the

sterilants for 30 minutes to facilitate sufficient cleaning. The nematodes having settled at the bottom of the funnel were carefully withdrawn into agar plates.

Potato dextrose agar (PDA) was found to be a suitable medium for culturing nematodes. It was prepared by mixing 39 g of the PDA powder with a litre of distilled water. This mixture was autoclaved until a clear and homogeneous solution was obtained. This was then poured into sterile petri dishes. All other handling tools used for this experiment were sterilised by exposure to U.V. light before use.

The results obtained are shown in Table 2. An attempt has been made in this investigation to determine qualitatively and quantitatively the efficiency of four methods of surface sterilization of S. bradys. The criteria for efficiency of each of the methods are (a) ability of the nematodes to remain alive after various treatments in the sterilant and (b) the ability of each sterilant to keep away contaminants during the period of observation. The significance of (a) and (b) is to facilitate correct interpretation of results obtained from sterile inoculation. From this investigation, it

appears that the method involving the use of 0.1% streptomycin sulphate gave very reliable results and can best be recommended. Nematodes were observed to be generally more active after treatment in this sterilant than in other methods. Malachite green did not appear to be a good sterilant. The recommended strength was found to be too concentrated and the nematodes had to be rinsed several times with distilled water. Even at lower concentrations, nematode survival was poor. It was only in its combination with streptomycin sulphate that 13.5% kill of the nematodes was obtained as opposed to 20% kill when only Malachite green was used at 20 ppm. With Malachite green alone sixty per cent of the plates were contaminated. The use of 0.01% mercuric chloride gave similar results with method (ii), but with only 40% of the plates contaminated.

TABLE 2

COMPARING THE EFFICIENCY OF FOUR METHODS OF SURFACE STERILIZATION OF NEMATODES (S. BRADYS).

A Sterilants	B	C	D
Method of surface sterilization	% nematodes killed or immobile*	% plates contaminated	Rating
i. 0.1% streptomycin sulphate solution	3.5	30	Good
ii. 1 : 1 ratio of a mixture of 0.1% streptomycin sulphate and 20 ppm malachite green	13.5	50	Fair
iii. 20 ppm malachite green	20	60	Poor
iv. 0.01% mercuric chloride	13	40	Fair

N.B. *Results show means of ten replicates.

A: Recommended strengths of the sterilants.

B and C: Period of observation was two weeks.

3.3 STUDIES ON POPULATION BUILD-UP OF S. BRADYS IN STORAGE.

(i) Population build-up of S. bradys.

The population build-up of S. bradys in storage was investigated using 3 species of Dioscorea (D. rotundata, D. cavenensis and D. alata). Five nematode-free tubers of each species were surface cleaned by using sterile distilled water and alcohol as described by Bridge (1973). About 400 - 500 surface sterilised nematodes were inoculated. Inoculation was done using sterile pipettes through one cylindrical core made by a cork borer in the middle of each yam tuber. The site of inoculation was then sealed with wax and wrapped in adhesive tape. (Plate 8) The tubers were stored in a yam barn for six months (October - March) and the ambient temperature and humidity were recorded using a weekly Cassela-type thermohygrograph. Control was provided by inoculating nematode-free tubers with sterile water.

After six months, the yam tubers were cut open through the site of inoculation and 50 g from each of the surrounding areas were extracted. Estimation of the nematode population was done using Peter's counting dish

under a stereomicroscope.

(ii) The effect of temperature and humidity on nematode population densities in stored tubers.

This experiment was undertaken to determine the effect of temperature and humidity on nematode population densities in stored tubers. About 200 tubers of white (D. rotundata) and water yam (D. alata) showing symptoms of nematode infection were classified into batches as follows:

50 tubers of white yam (D. rotundata)	BATCH A
50 tubers of water yam (D. alata)	
50 tubers of whate yam (D. rotundata)	BATCH B
50 tubers of water yam (D. alata)	

The tubers in BATCH A were stored under ambient conditions (23 - 32°C; 40 - 85% R.H.) for 5 months and those in BATCH B were kept in the cold store with temperatures ranging between 16 - 18°C and 80 - 85% R.H. Ten tubers from each treatment, i.e. five from white yam and five from water yam were selected at random and extracted for nematodes every month for five consecutive months. About 50 g of yam tissue from each tuber were extracted and

nematodes were counted as described before.

The results of studies on the build-up of S. bradys population in stored yams are presented in Table 3. There were substantial increases in nematode populations in all the three yam species. There were 9-fold, 8-fold and 5-fold increases in the tubers of D. rotundata, D. cayenensis and D. alata respectively. The dry rot symptoms previously described by West (1934) were observed in 12 out of the 15 tubers inoculated and the severity of the dry rot disease was related to the number of nematodes extracted. No dry rot symptom was observed in the 5 tubers inoculated with sterile water. These findings confirm that the dry rot was caused only by nematodes.

Figure 4 shows the results obtained from nematode-infected tubers stored over a 5 month period under ambient conditions and at low temperatures ranging between 16 - 18°C. The results obtained are similar to those from experiment (i). With storage at ambient temperatures (23 - 32°C) nematode populations in tubers rose to exceedingly high levels, but the low temperatures (16 - 18°C) had a slight depressing effect on nematode populations even though the initial nematode count of the

PLATE 8: Yams inoculated in the middle.

tubers stored at low temperatures was lower than those stored under ambient conditions. The lower temperatures also completely inhibited sprouting.

TABLE 3

POPULATION BUILD-UP OF *S. BRADYS* IN STORED YAMS.

Yam species	Inoculum level	Final count after 6 months/ 50 g tuber** S.E.	Population increase
D. rotundata	400-500 nematodes (Male & Female)	4,600±67.8	9x app
D. cayenensis	400-500 nematodes (Male & Female)	3,980±63	8x app
D. alata	400-500 nematodes (Male & Female)	2,765±52.6	5x app

** Means of 5 replicates.

S.E. Standard error.

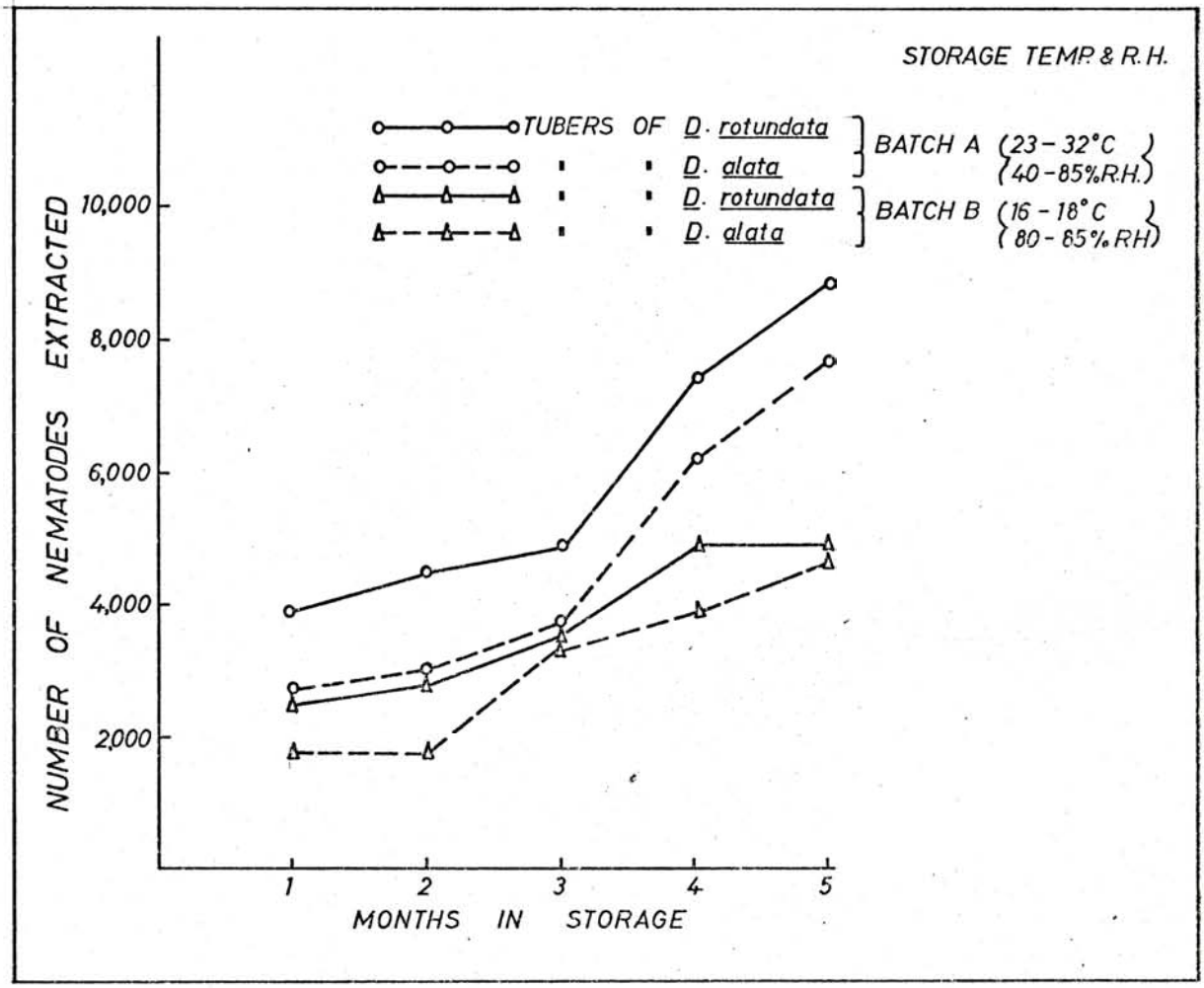


FIG. 4. FLUCTUATIONS IN NEMATODE POPULATION DENSITIES IN STORED TUBERS AS AFFECTED BY TEMPERATURE AND HUMIDITY

3.4 DEPTH OF PENETRATION.

The depth to which nematodes penetrate yam tissues gives a good indication of the extent of damage caused by nematodes. Experiments were therefore designed to find out:

(i) Whether the depth of penetration varies with different cultivars of D. rotundata.

(ii) The actual concentration of nematodes within the periderm, and

(iii) Whether there is any variation in depth of penetration of nematodes among the three portions of yam, i.e. apical (tops), middle (middles) and distal (bottoms). These investigations, it was hoped would provide information for the selection of "nematode-free planting materials" and aid in yam nematode control.

The yam tubers used for this and subsequent analyses were harvested from a plot in the Crop Collection Garden of the Department of Agricultural Biology, University of Ibadan, Ibadan, which was previously artificially inoculated with S. bradys. The tubers were washed, dried and stored in a yam barn for 3 months after which only those tubers showing distinctly recognisable symptoms of

infection were used for analyses. Clean tubers showing no apparent symptoms of nematode infection and stored for the same period were also analysed and used as control.

(i) Varietal susceptibility:

The investigation was conducted using 5 different cultivars of D. rotundata, namely; akosu, omifunfun, esinmirin, boninbonin and efon. These cultivars were harvested from an artificially inoculated plot in the University Crop Collection Garden. Using 2.5 cm diameter cork borer, 10 samples were taken from each cultivar. Each piece, usually about 3.5 cm in length was cut into 0.5 cm pieces from the peridermal layer. Each half centimetre piece was cut into smaller pieces, macerated, and extracted into small petri dishes using sieves of nylon gauze embedded in rigid polyethylene supporting rings. Most of the nematodes were recovered after 48 hours.

(ii) Distribution of nematodes within the periderm.

Using one of the susceptible cultivars - efon, a detailed study of the actual distribution of nematodes within the periderm was made. This was done by taking 10 samples from the infected portion of the tuber using a cork borer. Each sample was cut into 2 millimetre

pieces. Each piece was macerated and extracted as above and nematode counts of these were made.

(iii) Depth of penetration in the top, middle and bottom portions of the yam tuber.

Nematode infected white yams (D. rotundata) were divided into 3 portions: top, middle and bottom ends and from each of these portions, 10 g samples of yam tissues were taken at the following depths: 0-2; 2-4; 4-6; 6-8; 8-10; 10-12; and 12-14 mm. Each sample was taken starting from the periderm to the central cylinder of the yam tuber. Each piece was cut into smaller pieces, macerated and extracted into small petri dishes using sieves of nylon gauze embedded in rigid polyethylene supporting rings. Nematodes were counted after 48 hours under a stereomicroscope. Each determination was replicated ten times.

The results of studies on the varietal susceptibility are shown in Fig. 5. From the results obtained, it appears there is some degree of variation in the depth of penetration of nematodes between cultivars of the same species. The variety efon was found to be particularly susceptible to nematode penetration. Larger number of

nematodes were extracted between 0 - 0.5 cm depth and 0.5 - 1.0 cm depth than any of the other cultivars and in 8 of the ten samples, nematodes penetrated to a depth of 1.5 cm. The variety omifunfun was found to be least penetrated by nematodes. The varieties esinmirin and akosu were found to be only moderately penetrated by S. bradys. Perhaps the differences in the thickness of peridermal layer might be a factor responsible for the differences in the degree of penetration of these cultivars by the nematode.

Figure 6 shows the results of the detailed analysis of the numbers of S. bradys to a depth of 10 mm of the periderm. The majority of nematodes appear to be concentrated in the first 6 mm and that the bulk of this are to be found within 2 - 4 mm.

The results of investigations of the different portions of the yam tuber are shown in Fig. 7. The results showed that nematodes were concentrated in the top (apical) portion than either the middle or the bottom (distal) portions. Higher nematode populations were found in the 2 - 4 mm zone. Only few nematodes in zone 6 - 8 mm and only in top and middle; none in bottom. No nematodes were found beyond a depth of 12 mm.

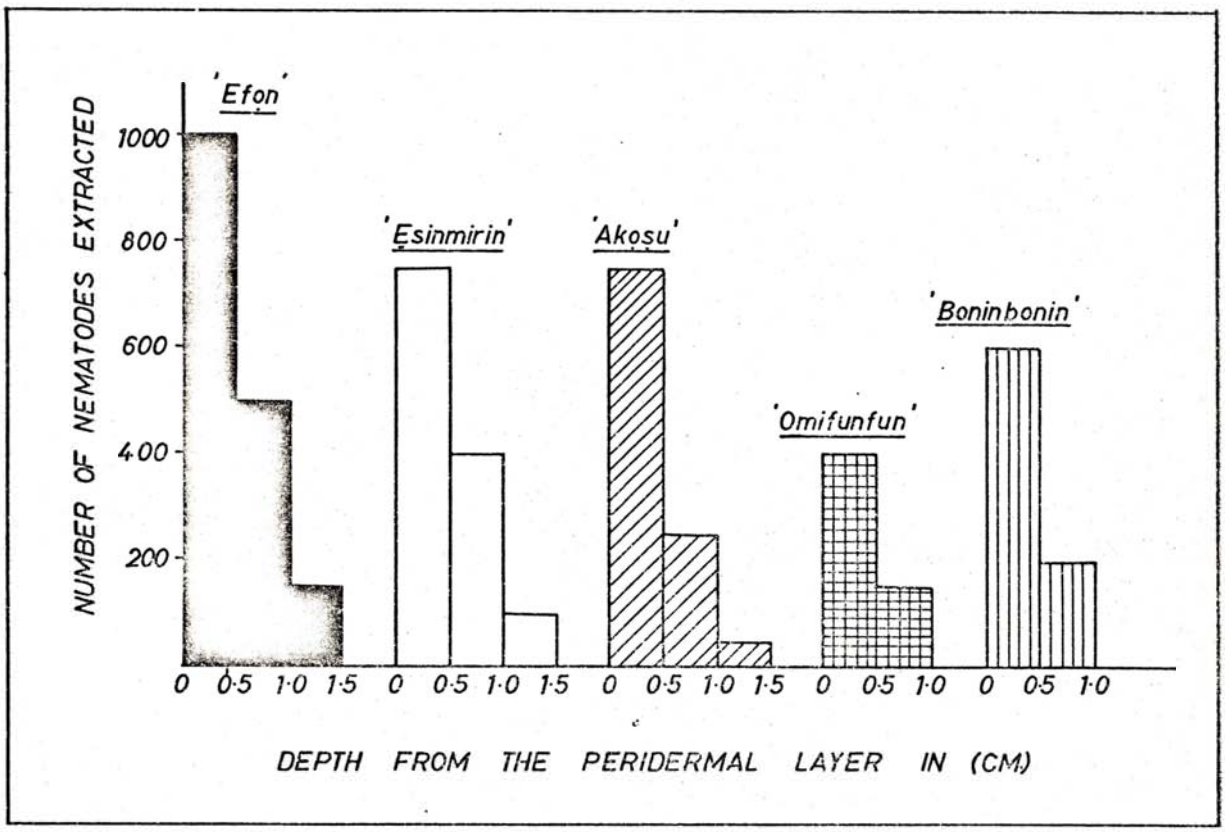


FIG. 5. DEPTH OF PENETRATION OF *Scutellonema bradys* IN FIVE VARIETIES OF *D. rotundata* Poir.

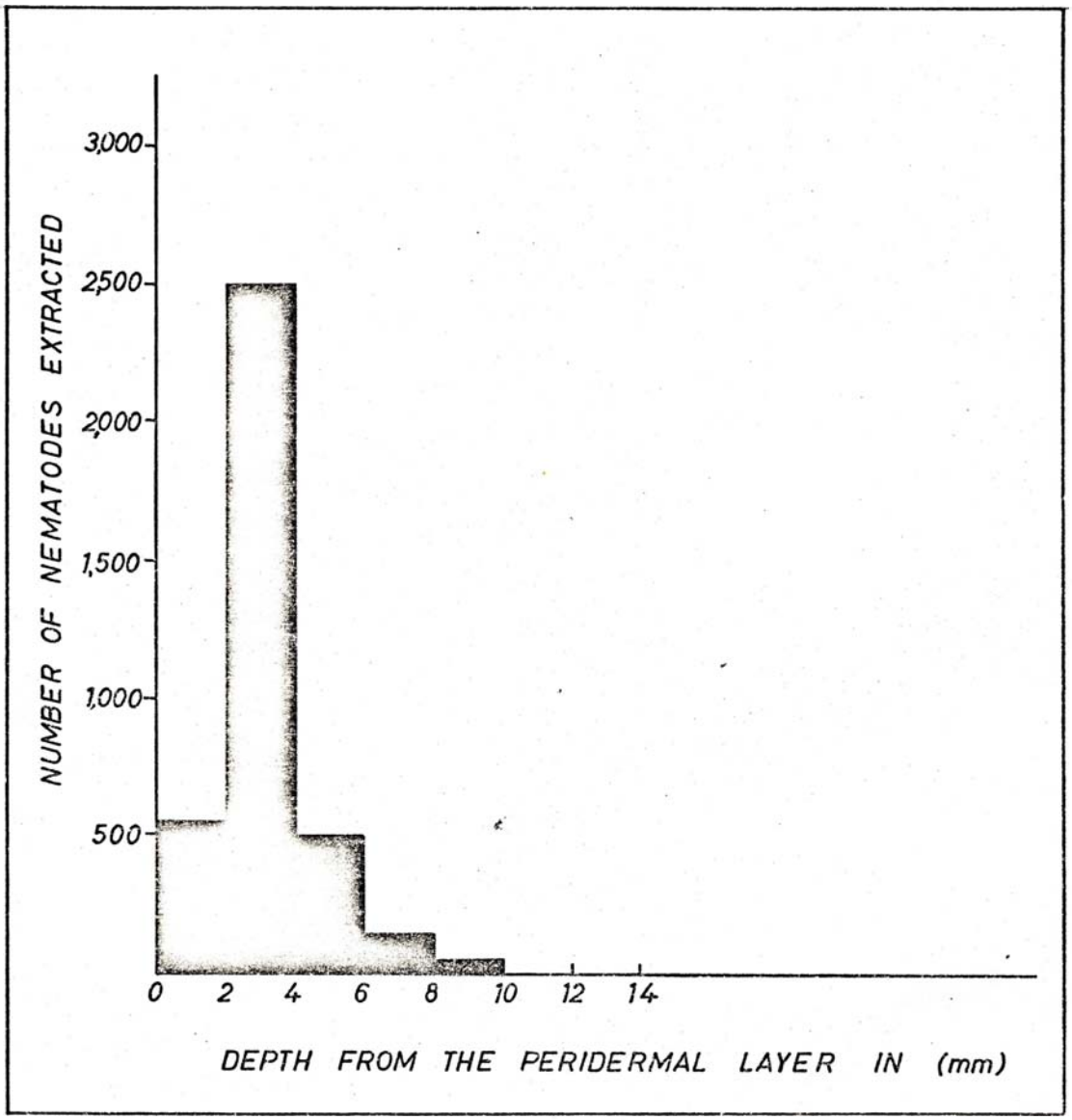


FIG. 6. DISTRIBUTION OF S. bradys within 12 mm OF THE PERIDERMAL LAYER OF THE YAM TUBER (D. rotundata var efon)

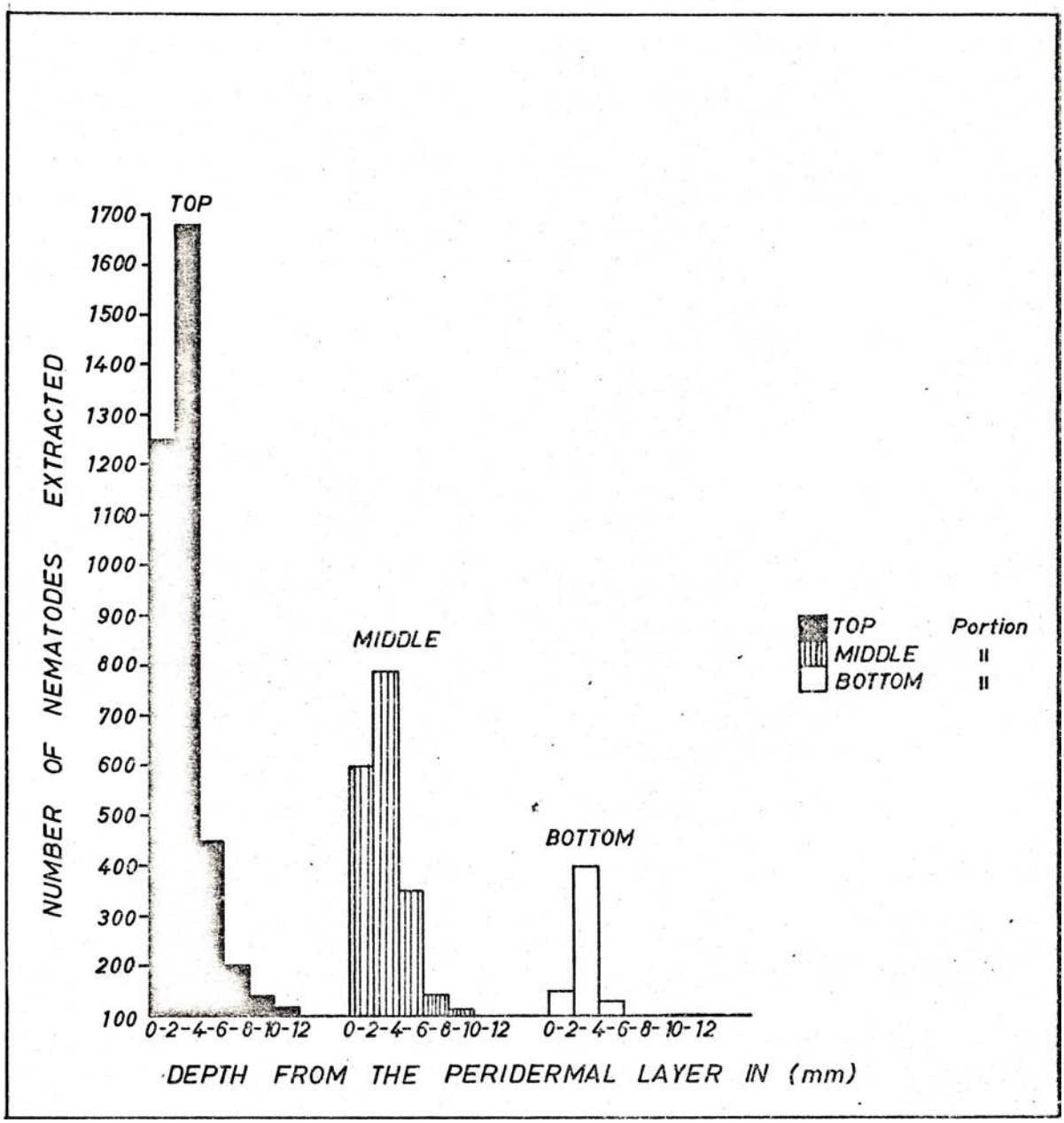


FIG. 7. DEPTH OF PENETRATION OF S. bradys IN THE TOP, MIDDLE, AND BOTTOM PORTIONS OF NEMATODE - INFECTED TUBERS OF D. rotundata Poir.

3.5 HISTOPATHOLOGY STUDIES OF THE WHITE YAM (DIOSCOREA ROTUNDATA POIR.) INFECTED WITH SCUTELLONEMA BRADYS.

In this investigation, a histological study of nematode relationships within the tissue and cells of the yam tuber was carried out. Observations were made on the overall effects of this nematode on the tissues and its probable role in aiding subsequent invasion by secondary microorganisms like fungi and bacteria was also studied.

Carefully selected portions of healthy and nematode-infected yam tubers with early symptoms of dry rot and those with early and late symptoms of wet rot were cut into pieces and sectioned at 17μ on a sliding microtome. The transverse and longitudinal sections obtained were dehydrated in Butyl alcohol series and stained either in Eosin and Haematoxylin or in Safranin and Fast green. The sections were mounted in Canada balsam, observed under high and low powers of the microscope and the information studied and interpreted in relation to the condition of the yams sectioned. Photomicrographs of these sections were taken.

The results of the observations are presented in

photographs on Plates 9 - 28.

(i) Sections across dry rot area.

Microscopic examination of both transverse and longitudinal sections of yam tuber parasitised by Scutellonema bradys reveals extensive disintegration of the epidermis (Plate 13). These nematodes seem to confine their activities to the tissues lying within the periderm layer and those immediately beneath the periderm (Plate 13). Darkened and thickened cells were noted only in areas where nematodes had fed (Plate 15). Cavities which may serve as infection sites for other invading microorganisms were present both in the epidermis and the cortex (Plate 13).

Nematode activities disrupted the yam tissue by emptying the cell contents and breaking the cell walls. Rupture of cell walls seems to follow every forward movement of the nematode. Wherever a nematode was found in the section, broken cell walls were seen around it, but farther away, all cells were intact (Plate 15). Intercellular spaces are usually very small or non-existent in yam tissue. Where they exist, they are smaller than the width of adult S. bradys. This is

probably one of the reasons why nematode migration within the tissue results in cell wall breakdown. Cavities are the result of rupture of several cells in the same locality (Plate 16). Several adults and larvae were found in such cavities (Plate 17). Even in stained sections, it was easy to differentiate the larvae and the adults, especially when they exist side by side in the same cavity (Plate 17).

No eggs were recognised in the stained sections. They may have dropped out during processing because eggs and all the larval stages were found with male and female adults whenever a yellowish portion of the yam tuber was teased out under the stereomicroscope (Plates 19, 20, 21 and 22). Observations in nearly all the sections made revealed that nematode movement is intracellular, not intercellular (Plates 15, 17 and 18). No fungi were found in early dry rot sections (Plates 15, 16, 17 and 18) indicating that fungi are clearly not associated with this stage of disease condition.

(ii) Section across wet rot area.

When early wet-rotted pieces of yam were teased under the stereomicroscope, both nematodes and fungi were

observed, but this stage of yam decay seems to be very short and transitional and rarely encountered. Advanced stage of wet rot seems to follow this 'early wet rot' condition very fast, probably within a matter of hours, not days. In 'early wet rot' the yam tissue turns yellowish-brown to brown and both nematodes and fungi were present. In 'late wet rot', dark-brown to black are the prevailing symptomatic colours and no nematodes were found. When portions of tuber with 'late wet rot' symptoms were teased out and examined under the compound microscope, fungi, bacteria and mites were found. The cause of the complete disappearance of S. bradys in 'late wet rot' is still under investigation but it is thought that some of the fungi associated with 'wet rot' e.g. Aspergillus niger probably produce substances antagonistic or toxic to the nematodes.

Another conspicuous difference observed between sections of 'early wet rot' and 'late wet rot' was that the former had many cells intact with mycelia around their cell walls as well as in the lumen of the cells. Haustoria were visible on these mycelia (Plate 24). Apparently, not every invasion of the fungus is associated with cell

rupture. On the other hand, individual cells are hard to recognise in 'late wet rot' stage. Instead, what is visible in the stained sections of 'late wet rot' is a completely disintegrated mass of watery tissue (Plate 14). The cells were dead and have shrivelled together and have brown to black deposits on their walls. Numerous strands of mycelia were visible in the cavities formed in the yam tissues. Chlamydospores of Fusarium, probably F. oxysporium were found in 'late wet rot' areas (Plates 23, 24).

No starch grains were found. Secretions from the fungi probably diffuse scores of cells ahead of the growing mycelia because their discoloration and disintegration effects on cells and starch grains respectively were evident far away from mycelia, more so than the effects of nematode secretions (Plate 27).

(iii) Longitudinal sections through yam roots (Plate 28).

Longitudinal sections through yam roots show that the root cortex is the favourite feeding site. Nematodes are embedded within a cavity in cortical cells. This cavity is a result of the destruction of parenchymatous cells by the nematode. From close examination of these sections, it appears that nematode entered the roots

PLATE 9: A healthy yam tuber cut open.

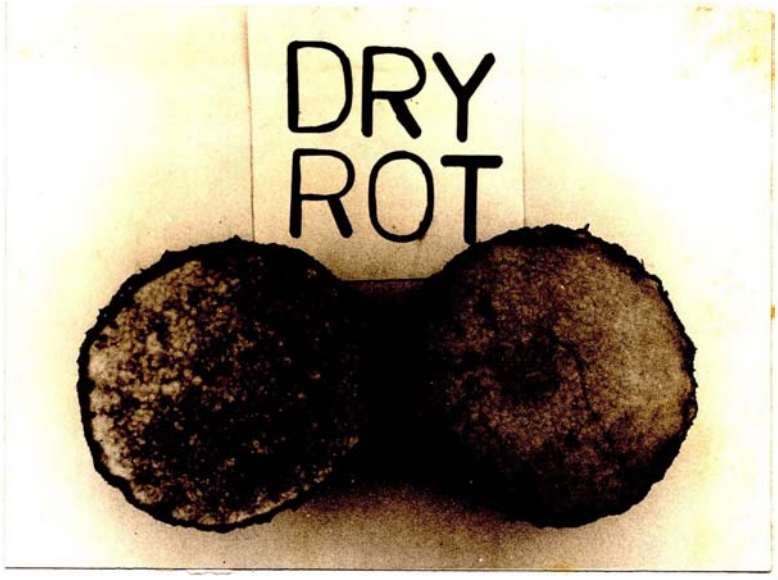


PLATE 10: Yam with symptoms of 'dry-rot' disease.

PLATE 11: Yam with symptoms of 'wet-rot' disease.

PLATE 12: Transverse section of a healthy yam.

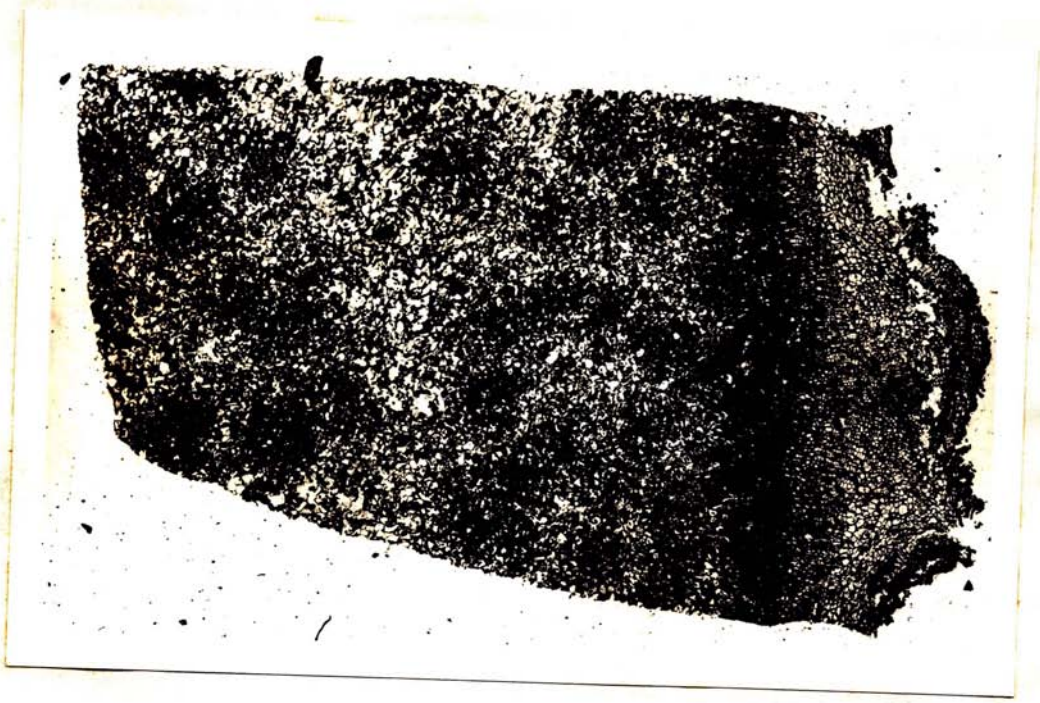


PLATE 13: Transverse section of a dry-rotted yam.

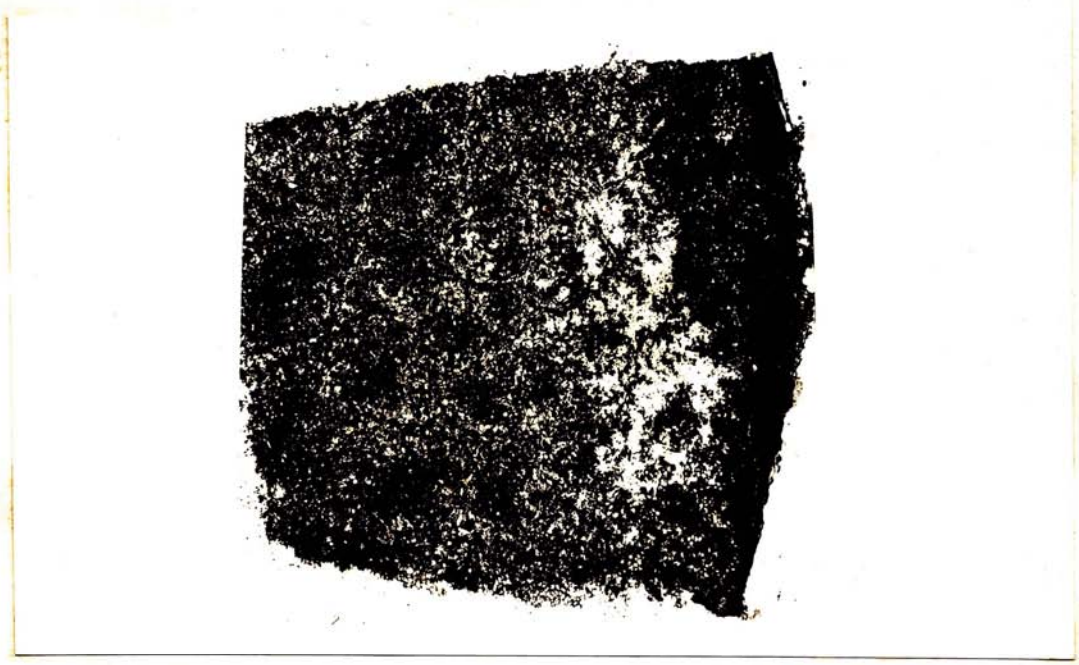


PLATE 14: Transverse section of a wet-rotted yam.

PLATE 15: Intracellular movement of S. bradys results in cell rupture and necrosis.

PLATE 16: Nematodes in cavity formed inside the yam tissue.

PLATE 17: Section showing infectivity of larvae and adults.

PLATE 18: Robust appearance of adult nematode inside yam tissue.

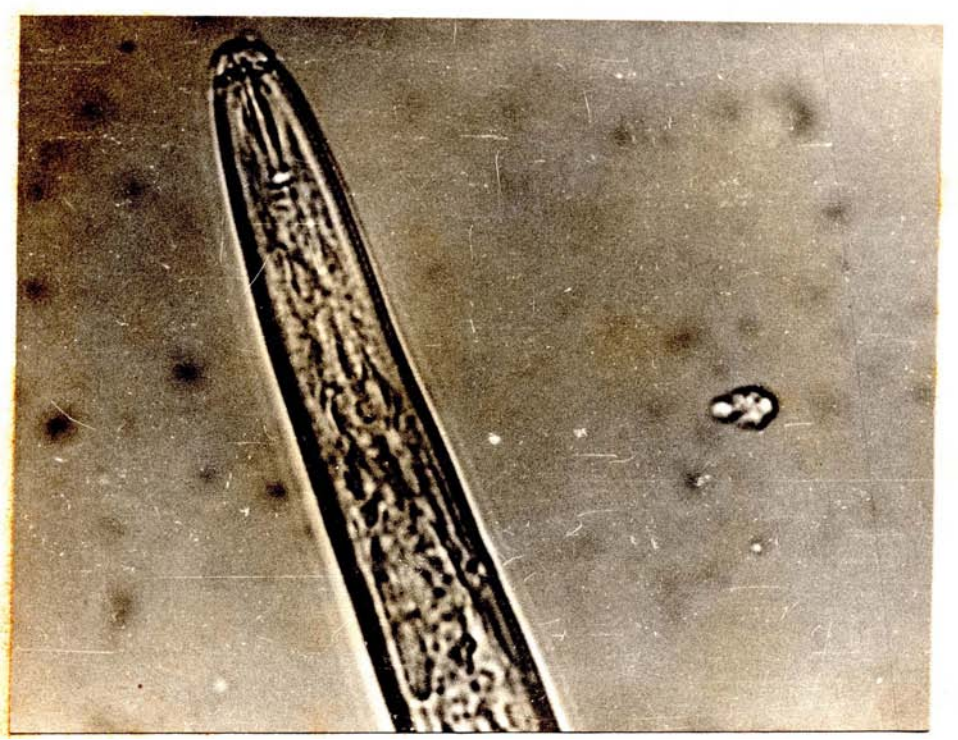


PLATE 19: Head end (male) of *S. bradys*.

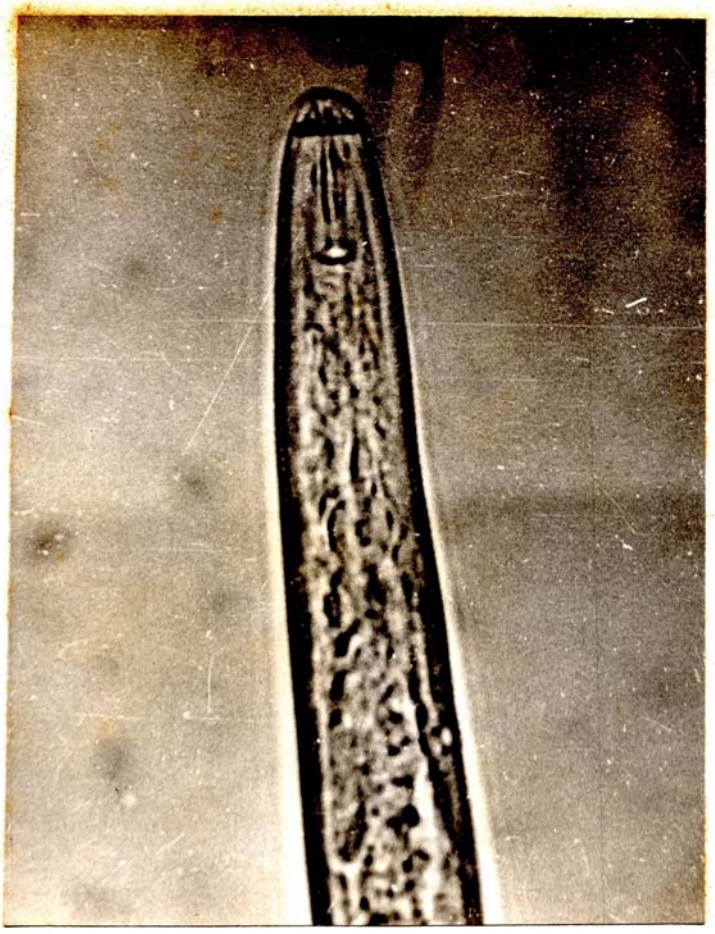


PLATE 20: Head end (female) of S. bradys.

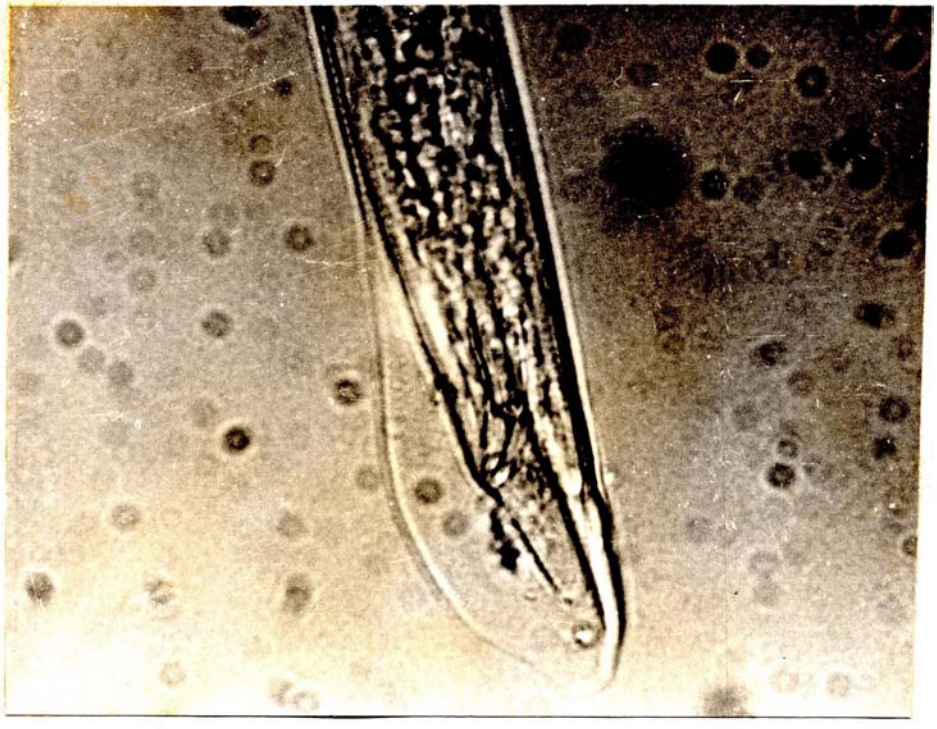


PLATE 21: Tail end (male) of S. bradys.

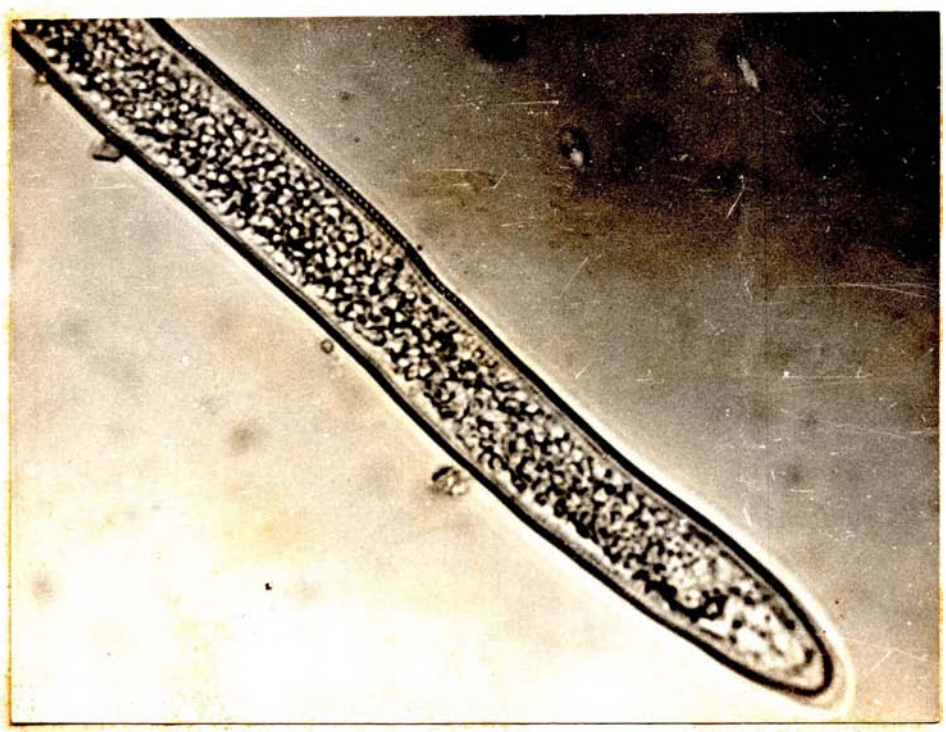


PLATE 22: Tail end (female) of S. bradys.

PLATE 23: Invasion of yam by fungal mycelium at the wet rot stage. Note gradual distortion of starch grains inside yam cells not yet reached by fungal mycelia, but already affected by their secretions.

PLATE 24: Invasion of yam cells by fungal mycelium at the wet rot stage. Note complete disappearance of starch grains and presence of chlamydospores of Fusarium sp.

PLATE 25: Starch grains inside the cells of a healthy yam tuber.

PLATE 26: Starch grains (partially digested) in the cells of a dry-rotted yam.

PLATE 27: Starch grains (completely digested) in a wet-rotted yam.

PLATE 28: Longitudinal section of a yam root showing infectivity by nematodes. Note that the cortex is the favourite feeding site.

through lenticels. Since photosynthates are stored in the parenchyma cells, such tissues would provide excellent environment for the development, growth and reproduction of Scutellonema bradys.

3.6 CHANGES IN CARBOHYDRATE CONSTITUENTS INDUCED IN DIOSCOREA ROTUNDATA VAR. EFON BY SCUTELLONEMA BRADYS.

Although a considerable number of analyses for proximate composition of yam tuber have been carried out in different parts of the world, comparatively little information is available on the changes in carbohydrate constituents of yam tubers resulting from nematode attack. It is necessary to study the action of nematodes on the starch and sugar content, since the food value of yams is determined primarily by their carbohydrate content. In this investigation, the carbohydrate constituents of the yam tuber at the dry and wet rot stages are compared with those of apparently healthy yam tuber from the same source, and using the widely used white yam (Dioscorea rotundata var. efon).

(i) Preparation of samples.

About 50 g samples were removed from 5 mm depth below the suberised epidermis of uninfected tubers and those

showing symptoms of dry and wet rot. These tubers were separately cut into smaller pieces and oven-dried for 24 hours in an electric oven pre-set at 80°C to reduce moisture content sufficiently to inhibit further changes and facilitate grinding. Each of the samples was later ground to powder using a 'Moulinex' blender.

(ii) Quantitative estimation of starch.

An aliquot sample, weighing 0.2 g from each of the ground yam samples was put into separate 50 ml centrifuge tubes. Twenty-five ml of hot 80% ethanol was added, stirred thoroughly and centrifuged after five minutes. The alcoholic solution in each tube was decanted and discarded. Thirty ml of freshly heated 80% ethanol was again added to each of the tubes, stirred and centrifuged as before. The alcoholic solution (containing soluble sugars) was again discarded. This washing treatment was repeated many times until a test with anthrone was negative. The residue was used for the estimation of starch using the method of McCready et al. (1950). By this method, starch is estimated and extracted by the Glucose - Anthrone Sulphuric Acid reaction.

(iii) Estimation of Amylose.

The percentage amylose in each yam sample was estimated by a modification of the autoanalyser procedure or simplified amylose procedure of Williams et al. (1958). Ten milligrams of powdered samples were weighed into a flask. Each sample was then wetted with about 10 drops of 95% ethanol and 2 ml of 1 N sodium hydroxide. The mixture was heated for five minutes in a boiling water bath to gelatinize the starch, and this was transferred quantitatively with water washings into a 100 ml volumetric flask. The flask, with its contents was cooled and the sample was brought up to volume with water. The starch solutions were transferred into sample cups of the autoanalyser for amylose determination. Calibration was done using yam samples of pre-determined amylose content.

(iv) Sugar estimation.

About 20mg of ground yam sample was measured into a beaker to which 40 ml of 80% ethanol was added and left in a water bath for one hour with occasional stirring. The sample was allowed to settle and the supernatant liquid was decanted into a 100 ml volumetric flask. Proteins were precipitated from the sample by the addition

of 1 ml of saturated lead acetate. The sample was shaken properly and left to stand for 15 minutes. About 2 g sodium bicarbonate was added to the sample and the sample was shaken and made upto 100 ml with ethanol. After centrifuging, the sample was decanted. The samples in the sugar solution were analysed using the "Dubois Phenol-Sulphuric Acid Colorimetric Method" (Dubois et al., 1956).

The results of the effect of nematode infection, the dry and wet rot phases of the disease on starch and sugar levels in infected yams are shown in Table 4.

Starch decreased from 60.8% to 39.5% in dry rotted tubers and to 22.2% in wet rotted tubers. Amylose also decreased from 27% to 20% in the dry rotted tuber and to 2% in the wet rotted tuber. Percentages amylopectin also decreased from 33.8 in the healthy yam to 19.5 in the dry rotted tuber and to 20.2 in the wet rotted tuber. These results showed that at these two stages of infection there is a degradation of starch to low polymer sugars aided in the first instance by the presence of nematodes and later by fungi and bacteria.

Results of all the analyses showed that all the three yam samples, i.e. healthy yam, and those with symptoms of dry and wet rot disease contain sucrose, glucose, fructose, and galactose. Small, though noticeable increases in the sugars were generally observed in the infected tubers. Sucrose and glucose increased from 0.3% in the healthy yam to 0.5% in the dry rotted tubers and to 0.4% in the wet rotted tubers. Galactose increased from 0.3% to 0.4% in the dry rotted tubers but fell back to 0.3% in the wet rotted tubers. Fructose was unchanged by infection.

TABLE 4

CARBOHYDRATE DETERMINATION

I	II	III	IV	V	VI	VII	VIII
Type of yam	% Starch	% Amylose	% Amylopectin	% Sucrose	% Glucose	% Galactose	% Fructose
H	60.8	27	33.8	0.3	0.3	0.3	0.1
D	39.5	20	19.5	0.5	0.5	0.4	0.1
W	22.2	2	20.2	0.4	0.4	0.3	0.1

N.B. Results show means of three replicates.

I - H: Healthy yam

D: Dry rotted yam

W: Wet rotted yam

IV - The values of % amylopectin were obtained by the difference between % starch and % amylose.

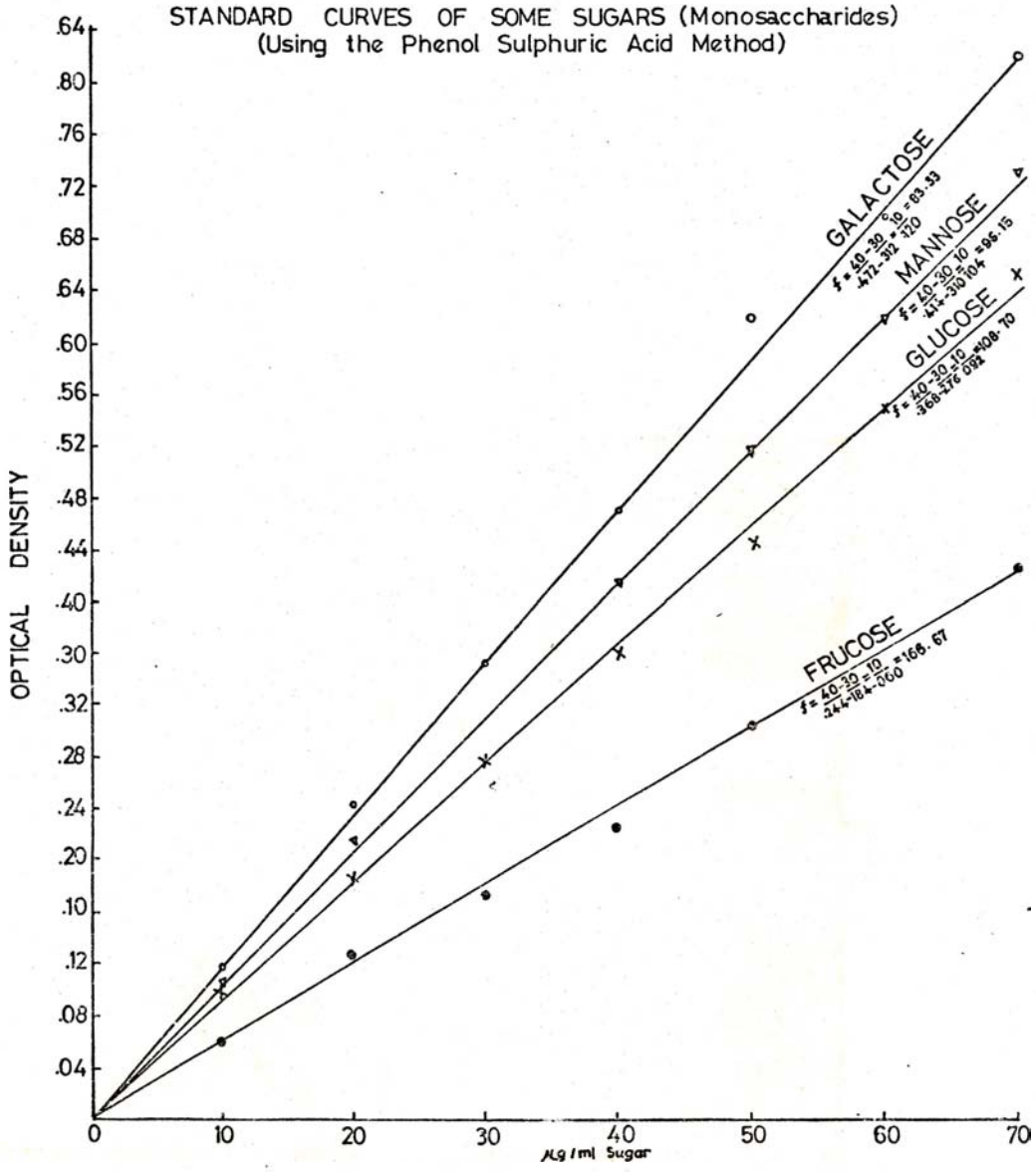

STANDARD CURVES OF SOME SUGARS (Monosaccharides)
(Using the Phenol Sulphuric Acid Method)
OPTICAL DENSITY
.64
.80
.76
.72
.68
.64
.60
.56
.52
.48
.44
.40
.30
.32
.28
.24
.20
.10
.12
.08
.04
0
10
20
30
40
50
60
70
µg / ml Sugar
GALACTOSE
MANNOSE
GLUCOSE
FRUCOSE

FIG. 8.

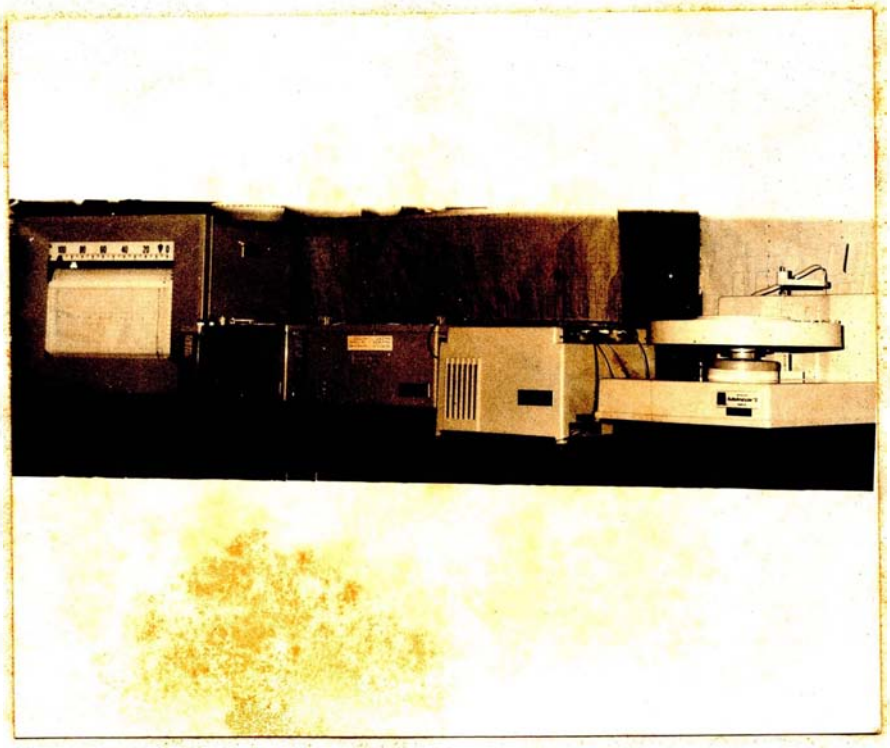


PLATE 29: Autoanalyser equipment for amylose determination.

3.7 QUALITATIVE AND QUANTITATIVE CHANGES IN THE FREE AND PROTEIN AMINO ACIDS IN THE HEALTHY AND NEMATODE-INFECTED TUBERS IN THREE SPECIES OF DIOSCOREA.

(i) Extraction of free amino acids.

Samples of healthy and nematode-infected yam species (D. rotundata, D. cayenensis and D. alata) were analysed for their amino acid constituents by the method described by Oke et al. (1973). About 100 g samples were removed from 5 mm depth below the suberised epidermis of healthy and infected tubers. These were cut into small pieces, mixed with 100 ml of distilled water and homogenised in a blender. To prevent foaming, a few drops of amyl alcohol was added. The supernatant was collected and made up roughly 70% alcohol by the addition of ethanol. The extract was desiccated in a vacuum and later vacuum dried. Identification of the various amino acids was effected by means of paper chromatography (2-dimensional ascending chromatography) using Whatman No. 1 papers. The spots were separated in n-butanol: acetic acid and water at the ratio of 12 : 3 : 5 by volume as the first dimension, and phenol : water at the ratio of 80 : 20 w/v for the

second dimension. The chromatogram was sprayed with ninhydrin to locate the acids.

(ii) Extraction of protein - amino acids by column chromatography.

The Perkin Elmer automatic amino acid analyser was used to quantitatively determine the amino acid constituents of yam samples using the method of Moore and Stein (1951). About 100 mg of the freeze-dried yam were hydrolysed with 10 ml of 6 N NCl in a pyrex test tube. The air in the test tube was quickly replaced with nitrogen to prevent oxidative decomposition of some amino acids. Hydrolysis of the yam samples was accomplished by placing the sealed test tubes containing the samples in an oven equipped with a mechanical fan and operating at 110°C for 24 hours. At the end of the heat treatment, the test tubes were cooled and the hydrolysate filtered. About 5 ml of the hydrolysate was evaporated in vacuo to get rid of the HCl. The film of amino acid in the vacuum flask was then taken up in buffer. An aliquot of this solution was placed on the column for separation of the amino acid mixture. The peaks obtained were then compared with those of standard amino acids and the

necessary calculations carried out. Tryptophan is extensively destroyed by acid hydrolysis and so could not be estimated by this method.

The results of the effect of S. bradys infection on the free amino acids, protein amino acids and protein nitrogen in three species of Dioscorea are shown in Tables 5, 6 and 7 respectively.

(a) Free amino acids.

In this investigation, 13 ninhydrin-positive amino acids were extracted from the uninfected white yam (D. rotundata). These were glycine, arginine, isoleucine, leucine, lysine, proline, serine, threonine, alanine, histidine, aspartic acid, glutamic acid and amino acid mixture. In the nematode-infected white yam, there was a similar distribution of amino acids but there were three fewer than in the healthy yam. Lysine, leucine and isoleucine were not detected by paper chromatography. In D. cayenensis, 11 amino acids were detected in the uninfected yellow yam and only 10 amino acids in the nematode-infected tuber. However, unlike in D. rotundata, leucine and isoleucine were present. In the healthy tuber of water yam (D. alata), 10 ninhydrin-positive amino acids were

detected. These include two 'essential' amino acids - lysine and tyrosine. Other essential amino acids like leucine and isoleucine were absent. In the infected tuber the number of amino acids was only two fewer than those detected in the healthy tuber. The nematode-infected water yam was deficient in almost all 'essential' amino acids except histidine.

(b) Protein amino acids.

Eighteen ninhydrin-positive amino acids were detected in the protein hydrolysate. There was no correlation between the total number of amino acids found in the free amino acid pool and the total number found in the protein hydrolysate. Methionine peaks were insufficiently resolved to be measurable in the healthy yam samples of both D. rotundata and D. cayenensis. Proline peak was also insufficiently resolved in the healthy yam sample of yellow yam (D. cayenensis). Tryptophan and cystine are extensively destroyed by acid hydrolysis and so could not be estimated by this method.

Generally, the amounts of each protein amino acid from both healthy and infected tubers varied considerably with each yam species. In the white yam (D. rotundata),

all amino acids detected except glycine, methionine, and ammonia, decreased in the infected tuber. Serine was unchanged by infection. Ammonia, glycine, and methionine, however, increased in the infected tuber. In the yellow yam (D. cayenensis), the reverse was the case, all the amino acids detected increased in the infected tuber with the exception of arginine which was unchanged by infection. In the water yam the trend was similar to that of yellow yam. All the amino acids increased as a result of infection except glutamic acid, valine, isoleucine, and phenyl alanine which were not changed by infection. Methionine, leucine, and arginine decreased in the infected tuber. In both the white yam and yellow yam, methionine was not detected in the healthy tubers, but was detected in the infected tubers. Proline was not detected in the healthy sample of yellow yam, but was substantially detected in the infected yam. Ammonia, alanine and glycine increased as a result of infection in all the three species of Dioscorea. Except in the white yam, the percentage protein increased as a result of infection. Similar trends were also observed for the protein nitrogen determined.

TABLE 5

EFFECT OF SCUTELLONEMA BRADYS INFECTION ON THE FREE AMINO ACIDS IN THREE SPECIES OF DIOSCOREA

	White yam *D. rotundata*		Yellow yam *D. cayenensis*		Water yam *D. alata*	
	Healthy	Nematode infected	Healthy	Nematode infected	Healthy	Nematode infected
Glycine	+	+	+	+	+	+
Arginine (Monohydrochloride)	+	-	+	-	+	+
DL-isoleucine	+	-	+	+	-	-
DL-leucine	+	-	+	+	-	-
DL-lysine (Monohydrochloride)	+	-	+	-	+	-
Tryptophane	-	-	-	-	-	-
L-tyrosine	-	-	-	-	+	-
DL-valine	-	+	-	-	-	-
Hydroxy inolacetic acid	-	-	-	-	-	-
Methionine	-	-	-	-	-	-
Phenylalanine	-	-	-	-	-	-
DL-proline	+	-	-	+	-	-
DL-serine	+	+	-	+	+	+
Threonine	+	+	+	+	-	-
Alanine	+	+	+	+	+	+
DL-histidine	+	+	+	+	+	+
Aspartic acid	+	+	+	+	+	+
L-cystine	-	+	-	-	-	-
L-glutamic acid	+	+	+	-	+	+
Amino acid mixture	+	+	+	+	+	+

N.B. + (Amino acid detected)

\- (Amino acid not detected).

TABLE 6

EFFECT OF SCUTELLONEMA BRADYS INFECTION ON THE PROTEIN AMINO ACIDS IN THREE SPECIES OF DIOSCOREA

Percentage	Dioscorea rotundata		Dioscorea cayenensis		Dioscorea alata	
Amino acids; moisture and protein	Healthy	Nematode infected	Healthy	Nematode infected	Healthy	Nematode infected
Aspartic acid	0.58	0.50	0.49	0.64	0.72	0.74
Threonine	0.19	0.18	0.16	0.23	0.25	0.29
Serine	0.25	0.25	0.20	0.35	0.39	0.41
Glutamic acid	0.67	0.55	0.48	0.67	0.90	0.90
Proline	0.18	0.16	*	0.22	0.27	0.28
Glycine	0.18	0.21	0.13	0.23	0.23	0.26
Alanine	0.20	0.21	0.14	0.26	0.27	0.30
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	0.21	0.19	0.12	0.26	0.31	0.31
Methionine	*	0.05	*	0.06	0.08	0.07
Iso-leucine	0.17	0.16	0.11	0.21	0.26	0.26
Leucine	0.33	0.29	0.21	0.39	0.49	0.48
Tyrosine	0.16	0.14	0.08	0.19	0.22	0.20
Phenylalanine	0.32	0.21	0.33	0.36	0.37	0.37
Histidine	0.11	0.09	0.09	0.12	0.13	0.14
Lysine	0.29	0.23	0.21	0.28	0.33	0.35
Ammonia	0.18	0.22	0.10	0.30	0.24	0.28
Arginine	0.65	0.31	0.32	0.32	0.74	0.61
Tryptophane	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Protein	6.5	4.9	5.9	6.4	7.4	7.9
Moisture	11.6	11.9	13.5	14.2	10.5	11.3

* Peaks insufficiently resolved or formed to be measurable.

N.D. Not determined.

Results are expressed as % amino acid (i.e. g/100 g of sample).

TABLE 7

EFFECT OF SCUTELLONEMA BRADYS INFECTION ON THE PERCENTAGE PROTEIN NITROGEN IN THREE SPECIES OF DIOSCOREA

	Dioscorea rotundata		Dioscorea cayenensis		Dioscorea alata	
Amino acids as % nitrogen	Healthy	Nematode infected	Healthy	Nematode infected	Healthy	Nematode infected
Aspartic acid	0.06	0.05	0.05	0.07	0.08	0.08
Threonine	0.02	0.02	0.02	0.03	0.03	0.03
Serine	0.03	0.03	0.03	0.05	0.05	0.06
Glutamic acid	0.06	0.05	0.05	0.06	0.09	0.09
Proline	0.02	0.02	*	0.03	0.03	0.03
Glycine	0.03	0.04	0.02	0.04	0.04	0.05
Alanine	0.03	0.03	0.02	0.04	0.04	0.05
Valine	0.03	0.02	0.01	0.03	0.04	0.04
Methionine	*	0.005	*	0.01	0.01	0.01
Iso-leucine	0.02	0.02	0.01	0.02	0.03	0.03
Leucine	0.03	0.03	0.02	0.04	0.05	0.05
Tyrosine	0.01	0.01	0.01	0.01	0.02	0.02
Phenylalanine	0.03	0.02	0.03	0.03	0.03	0.03
Histidine	0.02	0.02	0.02	0.03	0.03	0.03
Lysine	0.06	0.04	0.04	0.05	0.06	0.07
Ammonia	0.15	0.18	0.08	0.25	0.28	0.23
Arginine	0.21	0.10	0.10	0.10	0.24	0.19
Total nitrogen	0.81	0.68	0.51	0.90	1.07	1.09
% nitrogen	1.04	0.785	0.93	1.02	1.18	1.26
% recovery	78	87	55	88	91	87

N.B. (a) The results are expressed as % nitrogen (i.e. g/100 g of sample).
(b) The poor recovery of nitrogen from Dioscorea rotundata (healthy) and Dioscorea cayenensis (healthy) is partly due to the low levels of proline and methionine and their consequent poor resolution.
(c) Non-protein nitrogen was not determined.

3.8 THE EFFECT OF NEMATODE INFECTION ON PERCENTAGE WEIGHT LOSS AND EDIBLE PORTIONS IN THREE SPECIES OF DIOSCOREA.

(i) Weight Loss.

This investigation was carried out using 3 species of Dioscorea (D. rotundata, D. cayenensis, and D. alata). Yams with symptoms of nematode infection and non-infected yam tubers were stored for 12 weeks in a yam barn. The infected and non-infected yam tubers were divided and laid out as follows:

D. rotundata (uninfected)	- 20 tubers	BATCH A
D. cayenensis "	- 20 "	
D. alata "	- 20 "	
D. rotundata (nematode-infected)	- 20 tubers	BATCH B
D. cayenensis " "	- 20 "	
D. alata " "	- 20 "	

Weight losses were determined by weighing each of the tubers weekly and recording the weekly loss in weight. Records of temperature and humidity were taken in the yam barn with a weekly Cassela-type thermohygrograph.

(ii) Estimation of edible portions in nematode-infected tubers.

60 infected yam tubers made up of 20 of D. rotundata, 20 of D. cayenensis and 20 of D. alata were weighed individually.

The tubers were then peeled until the nematode-damaged portions had been removed, and only the edible portions remained. The tubers were again weighed. The differences in weight were recorded. 20 clean yam tubers of each variety were also weighed and peeled in the same way; the data obtained from these were used as control data.

The results of these investigations are shown in Tables 8 and 9 and Fig. 9.

It is noteworthy that there is considerable variation in weight losses between and within the three different yam species stored under the same environmental conditions. Table 8 shows the means of weight loss for both the uninfected and nematode-infected tubers as 19.1, 22.7 and 12.2 for the infected tubers of D. rotundata, D. cayenensis, and D. alata respectively even though weight loss as high as 54.2% was recorded for one of the infected tubers of

D. rotundata. In the healthy tubers, the means of total weight loss were 12.7%, 16.6% and 8.9% for D. rotundata, D. cayenensis and D. alata respectively. The differences in the mean percentage weight loss between the nematode-infected and uninfected tubers of D. rotundata and D. cayenensis were statistically significant but not in D. alata. It can also be seen that the least percentage weight loss was recorded in D. alata and highest was recorded in D. cayenensis.

The results of the cumulative percentage weight loss for the three different yam species (uninfected and nematode-infected) are shown in Fig. 9. The results show that there is considerable variation in the cumulative percentage weight loss among the three different species and between the uninfected and the nematode-infected yam tubers. Cumulative percentage weight losses of 14%, 13.9% and 8.8% were recorded for the uninfected yam tubers and 24%, 22.3% and 11.9% for the nematode-infected yam tubers of D. rotundata, D. cayenensis and D. alata respectively.

Table 9 shows the results of the estimation of percentage edible portions in nematode-infected and

"uninfected tubers" of *Dioscorea*. The results showed peeling losses as 28.1, 26.2 and 26.3 for the infected tubers of *D. rotundata*, *D. cayenensis* and *D. alata* respectively even though peeling loss as high as 57% was recorded for one of the tubers of *D. cayenensis*. In the healthy tubers, the means of peeling losses were 9%, 9% and 6.9% for *D. rotundata*, *D. cayenensis* and *D. alata* respectively.

TABLE 8

PERCENTAGE WEIGHT LOSS IN NEMATODE-INFECTED AND UNINFECTED TUBERS OF DIOSCOREA

	Dioscorea rotundata		Dioscorea cayenensis		Dioscorea alata	
	Range	Mean of total weight loss**	Range	Mean of total weight loss**	Range	Mean of total weight loss**
Nematode infected yam tubers	(2.99-54.20)	19.07±1.71	(10.40-38.90)	22.65±1.66	(3.20-26.50)	12.20±1.70
Uninfected yam tubers	(0 -33.30)	12.70±1.71	(5.70-28.60)	16.55±1.90	(3.40-14.10)	8.93±0.50

1 ** Mean of twenty tubers ± standard error

Level of significance between the healthy and nematode-infected tubers of

(i) D. rotundata = P $0.05 <$ - significant

(ii) D. cayenensis= P $0.05 <$ - significant

(iii) D. alata = P $0.05 >$ - not significant.

TABLE 9

ESTIMATION OF THE EDIBLE PORTIONS OF NEMATODE-FREE AND NEMATODE-INFECTED TUBERS OF DIOSCOREA. PERCENTAGE PEELING LOSSES DUE TO DRY ROT DISEASE ASSOCIATED WITH SCUTELLONEMA BRADYS.

	D. rotundata		D. cavenensis		D. alata	
	Range	Mean**	Range	Mean**	Range	Mean**
Nematode-infected yam tubers	(16.60-51.70)	(a) 28.05±2.12	(13.30-57.10)	(c) 26.19+2.47	(15.60-50.0)	(e) 26.30±2.25
Uninfected yam tubers	(3.40-20.00)	(b) 9.01±1.05	(3.60-19.00)	(d) 9.02±0.85	(3.2 -12.3)	(f) 6.88±0.57

** Mean of twenty tubers ± Standard Error.

Level of significance between

(a & b) = P 0.05< - Significant.

(c & d) = P 0.05< - Significant

(e & f) = P 0.05< - Significant.

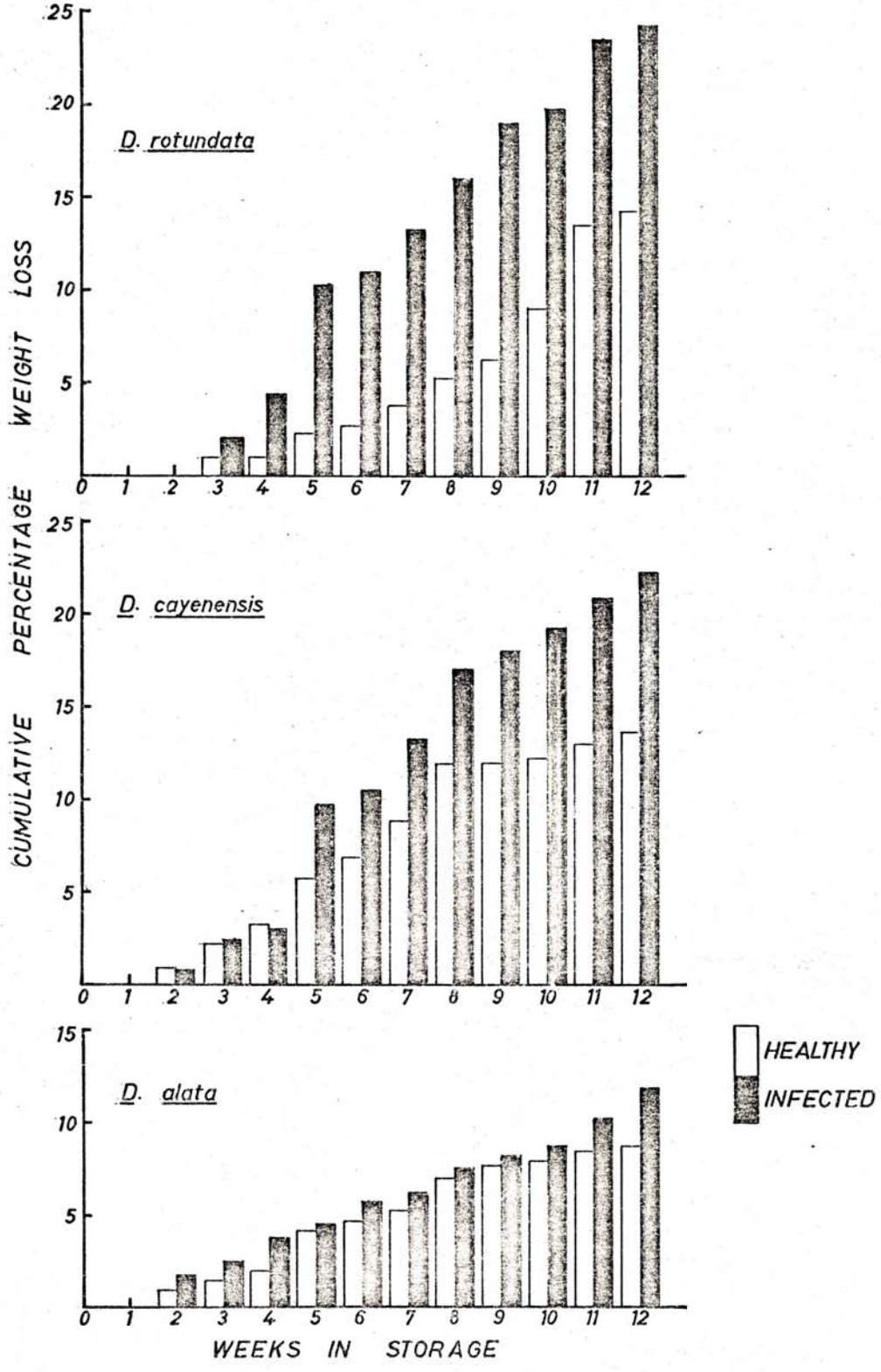


FIG. 9. WEIGHT LOSS IN HEALTHY AND NEMATODE-INFECTED YAM TUBERS

3.9 SUBSTANCES DISCHARGED BY THE YAM NEMATODE S. BRADYS

In this study, the substances discharged by the yam nematode were investigated by methods described by Krusberg and Myers (1964) and by spectrometric analysis, a previously unexplored method.

(i) Sources of the nematodes and extraction procedure.

Nematodes were extracted from infected yam tubers using the tray modification of the Baermann funnel method. Since contaminating microorganisms might metabolise substances discharged by nematodes and secrete other metabolites, the nematodes were surface-sterilized using 0.1% streptomycin sulphate. The nematodes were further irrigated with a fine mist of sterile distilled water. The final distilled water rinse was repeated five times after which the nematodes were used in experiments.

(ii) Preparation of the surface sterilized nematodes for microchemical tests.

The surface sterilized nematodes were placed in flasks containing distilled water and 1% glucose adjusted with 1 N HCl to pH 5.0 and incubated in a water bath shaker at 30°C for 48 hours. The incubation solution was checked for microbial contamination by pipetting about

1 ml of the solution into melted potato dextrose agar (PDA) in a test tube. The P.D.A. and the incubation solution were mixed and poured into petri dishes. The petri dishes were incubated at 30°C for 48 hours after which microbial colonies were counted. After 48 hours, the counts of microbial colonies were found to be negligible (less than 200) and the incubation solution was used immediately.

The solution was divided into two portions. In the first portion, the nematodes were filtered off and the second portion contained all the nematodes together with a bit of the incubation solution. A few drops of the first solution were chromatographed and the second solution containing the nematodes was used for spectrometric analysis.

(iii) Chromatographic analysis of incubation solution.

Nematodes incubation solutions were tested microchemically to determine the classes of compounds present. A few drops of the incubation solution (about 0.1 ml) were used in each test for amino acids, urea and other amides. A two dimensional ascending technique with n-butanol: acetic acid and water (12 : 3 : 5) as

the first dimension and phenol: water (80 : 20 w/v) as the second dimension. The papers were left for 24 hours and then sprayed with ninhydrin and dried at 80 - 100°C for some minutes when most of the amino acids showed up brightly.

(iv) Preparation of nematode homogenate for spectrometric analysis.

The concentrated suspensions of nematodes were pelleted by centrifugation, and the supernatant removed with a pipette. To eliminate most bacteria and soluble substances present in the suspensions, the nematodes were re-suspended and centrifuged for a few seconds at 680 G several times with distilled water. After each washing, the supernatant was discarded and the process repeated with fresh solution. These massed nematodes were ground to prepare homogenates. The nematode homogenate was evaporated on a rotary evaporator (attached to a vacuum pump) until it was reduced to a powdered form. The powdered extract was again mixed with benzene, which removed excess water leaving the extract completely dry. The resulting extract was then mixed with Niyol and run on the Infra red and U.V. equipment. The remainder of

the original extract (i.e. without Niyol) was dissolved in D6 (Deuterodimethylsulphoxide) and run on the Nuclear Magnetic Resonance (N.M.R.) equipment.

As a form of control, the solution containing the extracted nematode, i.e. dry rot extract was similarly treated as the nematode homogenate (iv). The dry rot solution was evaporated on a rotary evaporator, mixed with Niyol and run on the Infra-red and later the Nuclear Magnetic Resonance equipment as above.

(v) Enzymes of S. bradys.

About 5 ml of the massed nematodes (obtained from i) in 1% sodium chloride solution were placed and homogenized for 30 minutes in a 15 ml Ten-Broeck ground glass tissue grinder held in an ice bath. Microscopic examination revealed that all the nematodes were macerated. The homogenates were then filtered, and the clear nematode extract was assayed for pectinases, cellulases, amylases and invertases.

(vi) Assay systems for hydrolytic enzymes.

Cellulolytic and pectolytic enzymes were separately assayed by viscometric methods (Levinson et al., 1950). Solutions of 1% carboxymethylcellulose

(Cellulose gum Type CM32) and 1% pectin in 0.05 M potassium phosphate buffer, were prepared as substrates and a small amount of toluene was added to inhibit bacterial activity. The pH of the solution for the cellulose gum was 5.0 and that of pectin was adjusted to 6.0. About 10 ml of the substrate were added into a Volac No. 0507 viscosity pipette which was supported in a constant temperature water bath at 27 $\pm$ 0.5°C. The initial time flow of the substrate alone was measured. Then 3 ml of the enzyme were added and thoroughly mixed with the substrate in the viscosity pipette. Time flow measurements were thereafter made at five minute intervals. Extracts from infected yam (from which nematodes had been extracted) clean yam, and 0.1 M Nacl were tested in the same way. The clean yam extract was used as control.

(vii) Relationship between nematode homogenate in ml and percentage drop in viscosity.

The ability of the extracts of nematode homogenate to degrade pectin using various quantities of the nematode homogenate was tested viscometrically. Five different quantities of the enzyme were used 2.5, 5, 7.5, 10, and 12.5 ml. About 2.5 ml of the enzyme were

pipetted each time into the viscometer pipette containing 10 ml of the substrate mixed thoroughly with the substrate and thereafter viscosity measurements were made at five minutes interval.

(viii) Assay systems for Amylase and Invertase.

The substrates for the amylase and invertase assays consisted of 1 g of starch in 100 ml 0.02 M potassium phosphate buffer (pH 6.9), and 5 g of glucose in 100 ml 0.02 M potassium phosphate buffer (pH 5.0) respectively. To 2 ml of each of the substrates, 2 ml of the enzyme from the nematode extracts and infected yam were separately added and each mixture was incubated at between 24 - 28°C for two hours. Controls of 0.1 M Nacl solution and homogenate boiled for five minutes (B' HOM) were incubated along with others. A colorimetric technique using 3, 5 dinitro salicylic acid reagent (D.N.S.A.) which reacts with reducing sugars to give a coloured solution was employed to measure amylase (Bernfeld, 1955) and invertase activities (Sumner and Somers, 1953). Approximately, 2 ml of the reaction mixtures were combined with 2 ml of D.N.S.A. reagent, boiled for 5 minutes, made up to 24 ml with distilled water and read on a colorimeter

operating at 525 nm. The controls were treated in the same manner. The absorbance of D.N.S.A. was subtracted from all readings, and the amounts of enzymes were determined by reference to standard curves previously constructed for maltose (amylase) and glucose (invertase). N.B. The D.N.S.A. reagent used was prepared by dissolving 1 g of 3, 5, dinitrosalicylic acid in 20 ml of 2 N NacH and 50 ml of water. About 30 g of Rochelle salt (sodium-potassium tartrate) were added and the final volume was made to 100 ml with distilled water.

Table 10 lists five amino acids found to be discharged by the yam nematode S. bradys. By a series of chromatographic analyses, the amino acids detected in incubation solution were iso-leucine, leucine, aspartic acid, hydroxyinol acetic acid, and phenylalanine. Phenylalanine was found to be present in appreciable amounts. From this investigation, it appears that S. bradys discharges both essential and non-essential amino acids. The essential amino acids discharged were leucine and iso-leucine whereas the non-essential amino acids discharged were aspartic, hydroxyinol acetic acid and phenylalanine.

TABLE 10

SUBSTANCES DISCHARGED BY S. BRADYS

AMINO ACIDS DISCHARGED INTO 1% AQUEOUS GLUCOSE SOLUTION BY SURFACE-STERILIZED NEMATODES S. BRADYS INCUBATED AT 30°C FOR 48 HOURS

Amino acid	Scutellonema bradys
Histidine	-
Isoleucine	+
Leucine	+
Arginine	-
Alanine	-
Valine	-
Glycine	-
Aspartic acid	+
Tyrosine	-
Threonine	-
Methionine	-
Hydroxy proline	-
Lysine	-
Serine	-
Glutamic acid	-
Proline	-
Tryptophane	-
Hydroxyl indol acetic acid	+
Phenyl alanine	++
Urea	-

N.B. + Amino acid present
++ Amino acid present in appreciable amount
- Amino acid absent.

Because insufficient quantities of dry rot and nematode extracts contributed to the failure of the U.V. and I.R. spectra to give meaningful results, extracts from clean, nematode-infected, and wet rotted yams had to be examined for other classes of compounds using the N.M.R. equipment. The results of the various peaks obtained are shown in Figs 10 and 11. The solvent peaks are shown in Fig. 10 and the peaks obtained from the freeze-dried yam extracts are shown in Fig. 11. In the clean yam extract, there is a peak at 1.0δ which is characteristic of the steroid-like group of compounds. But in the dry and wet rotted yam extracts there is a peak shift from 1.0δ to 8.2δ . The peak at 8.2δ is characteristic of the aromatic group of compounds. It is probable that as a result of nematode infection, the steroid group of compounds are converted to the aromatic compound indicated in this analysis.

The data obtained from the viscometric tests (for Pectinase and Cellulase enzymes) were calculated by the methods reported by Krusberg (1960). The data were

analysed according to the formula:

$$\frac{A - B}{A - W} \times 100 = \% \text{ loss in viscosity}$$

where A = initial flow time

B = flow time after an interval

W = flow time of distilled water.

The results obtained for pectinase enzyme are shown in Fig. 13. Extracts of nematode homogenate and the infected yam tissue (from which nematodes had been extracted) reduced the viscosity of pectin. by 60% and 50% respectively. No viscosity reduction was observed with carboxymethyl cellulose, i.e. negative results were obtained for the cellulase.

The results of the relationship between nematode homogenate in ml and percentage drop in viscosity are shown in Fig. 14. The results show that the percentage reduction in viscosity increased with increase in nematode homogenate up to 10 ml, thereafter no further viscosity reduction was observed.

The results presented in Table 11 show some amylase activity in both the nematode homogenate and the infected yam. This enzyme appears to be present in larger amounts

in the infected yam tuber than in the nematode homogenate. Table 12 shows that negative results were obtained for the invertase enzyme.

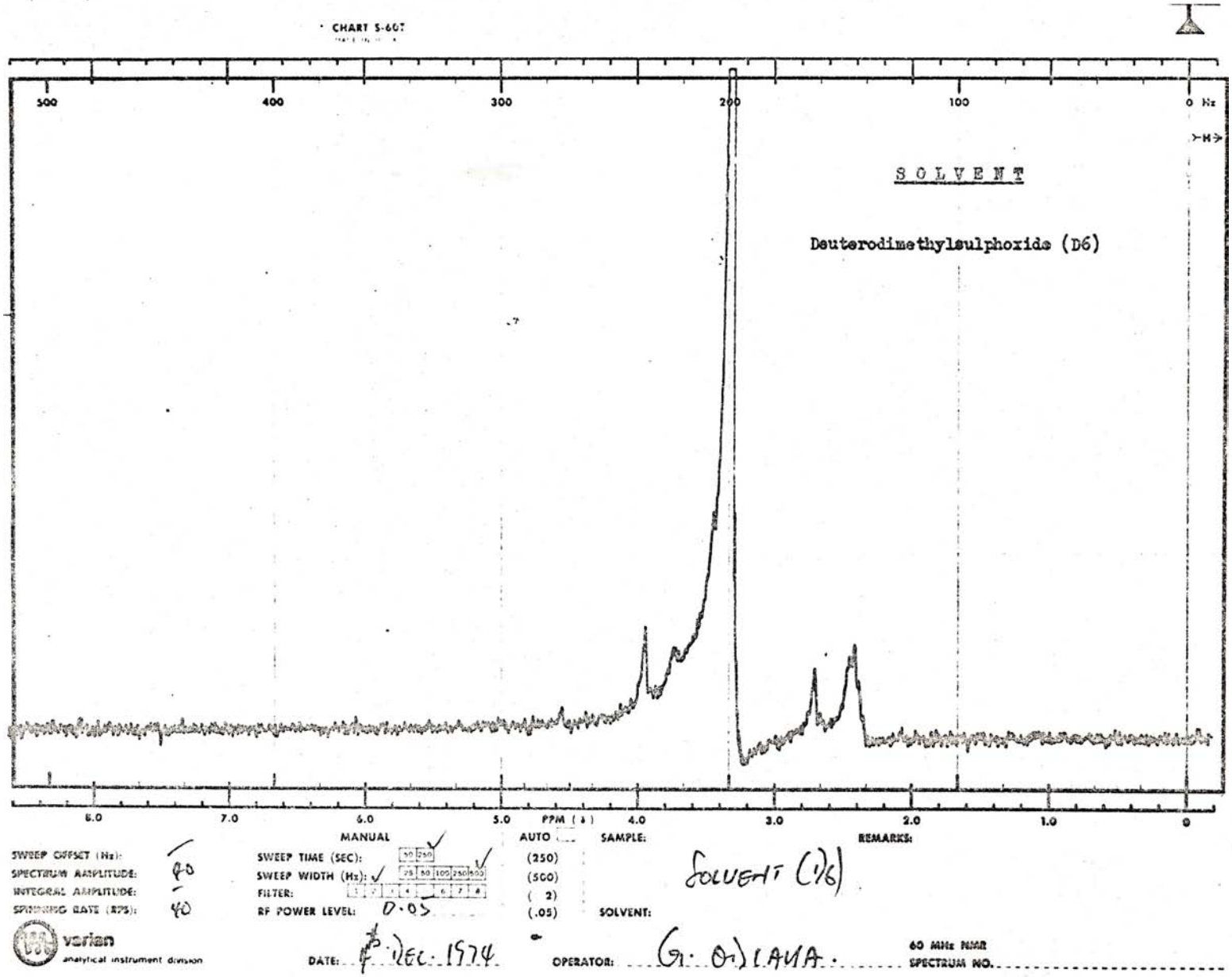


FIG. 10: N.M.R. PEAKS OF DEUTERODIMETHYLSULPHOXIDE (D_6)

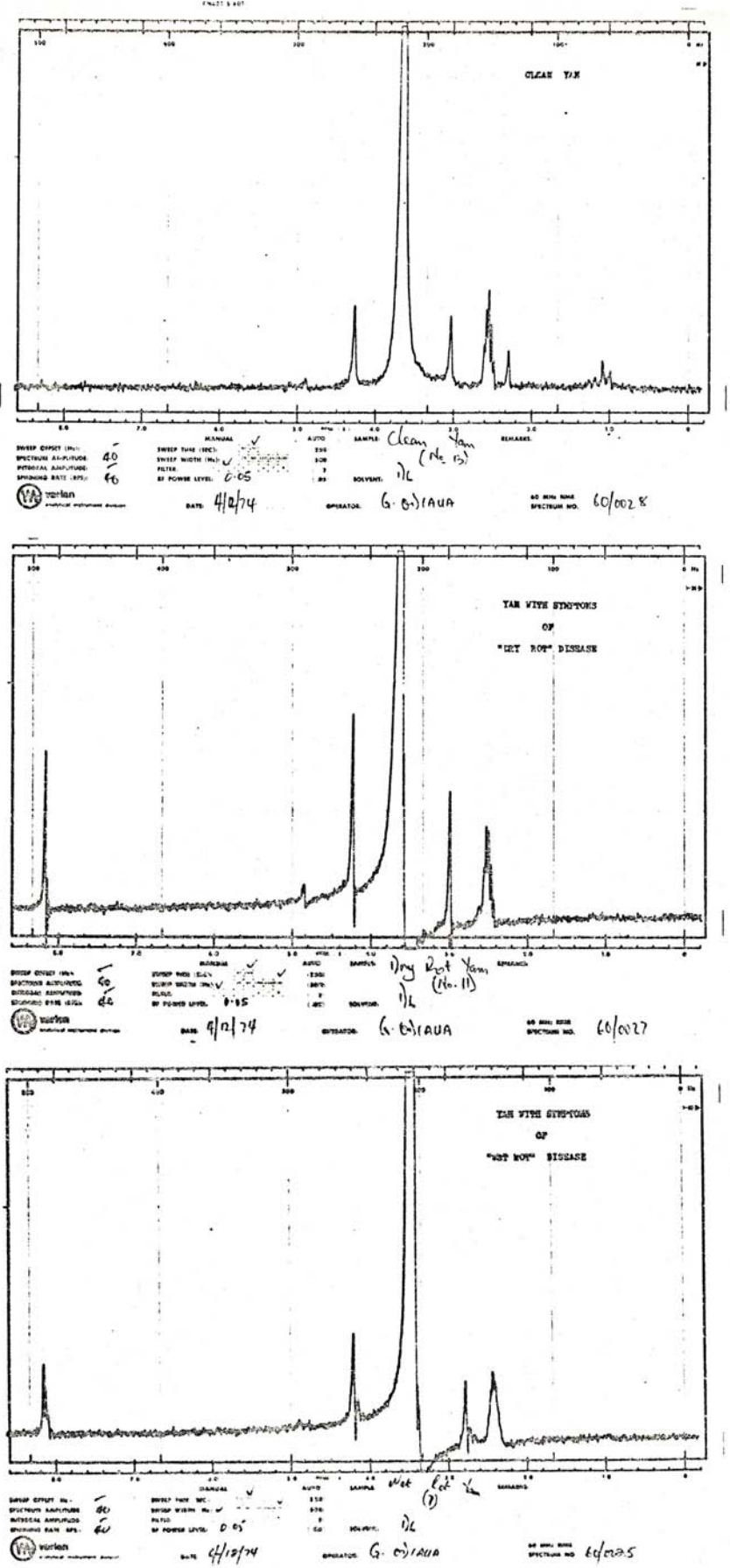


FIG. 11: N.M.R. PEAKS OF CLEAN, DRY ROT AND WET ROT YAM EXTRACTS

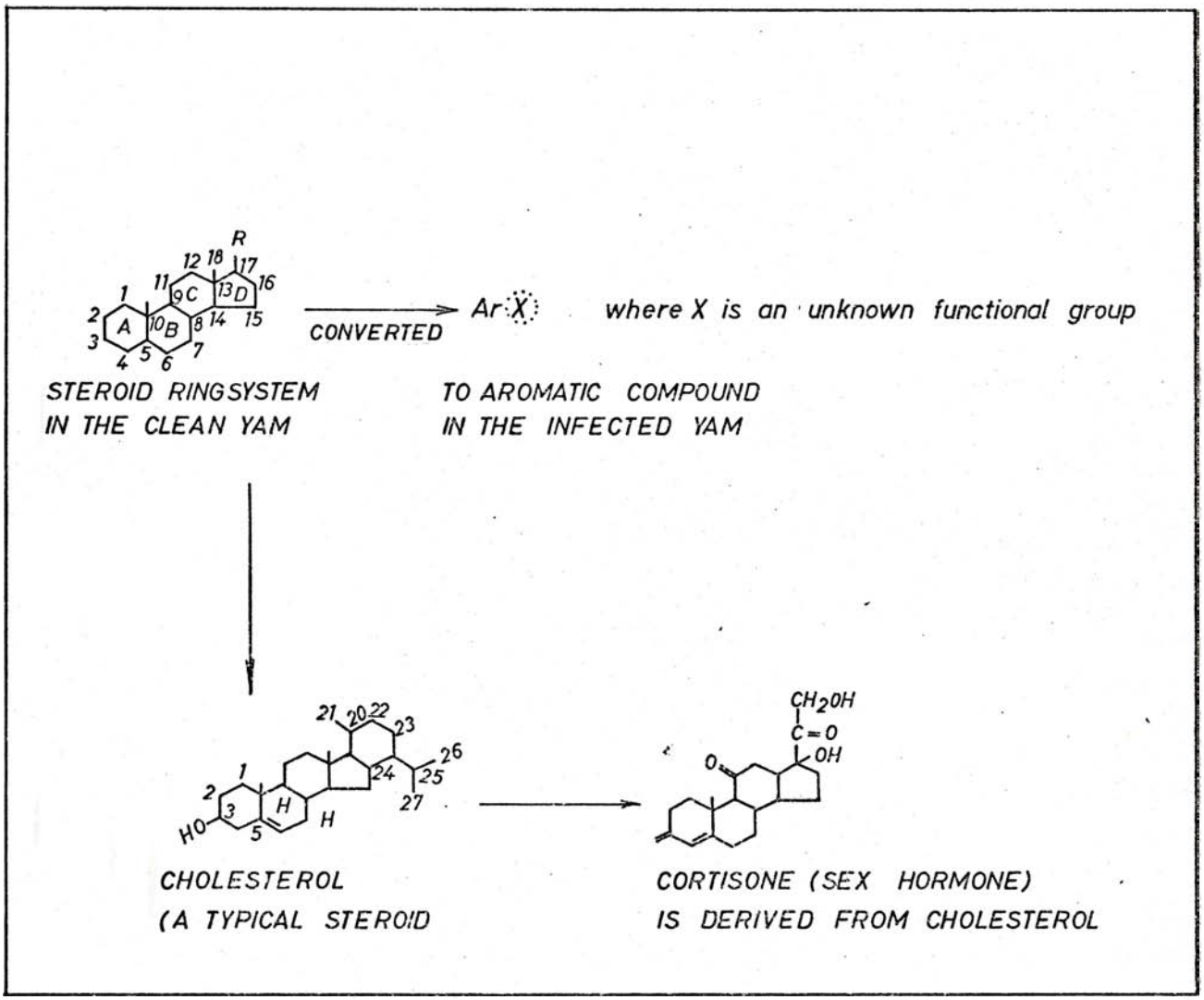


FIG. 12. SOME PHARMACOLOGICALLY ACTIVE CONSTITUENTS OF THE YAM TUBER (*DIOSCOREA* SPP.)

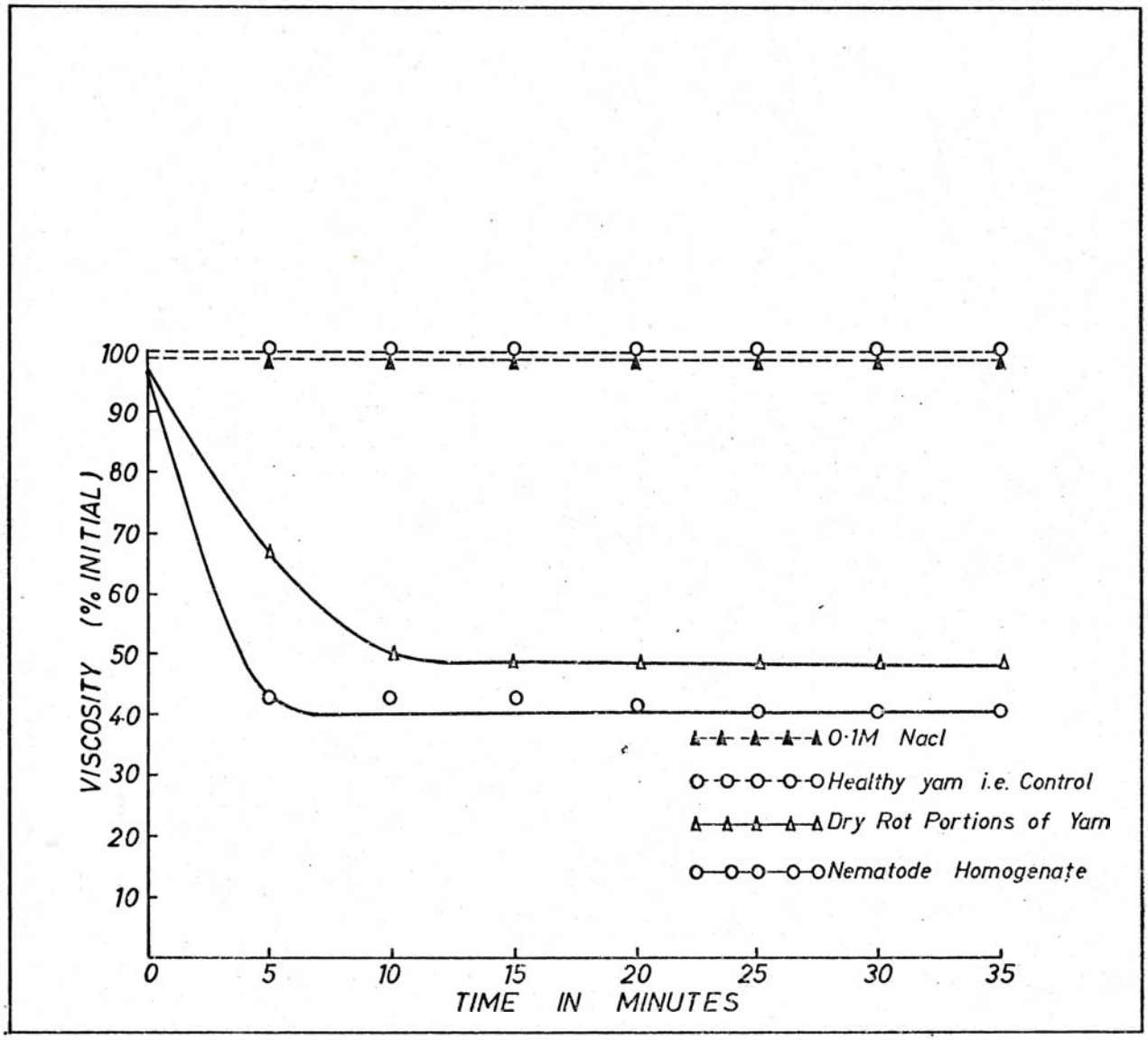


FIG. 13. HYDROLYSIS OF A 1% PECTIN SOLUTION BY HOMOGENATES OF *Scutellonema bradys*

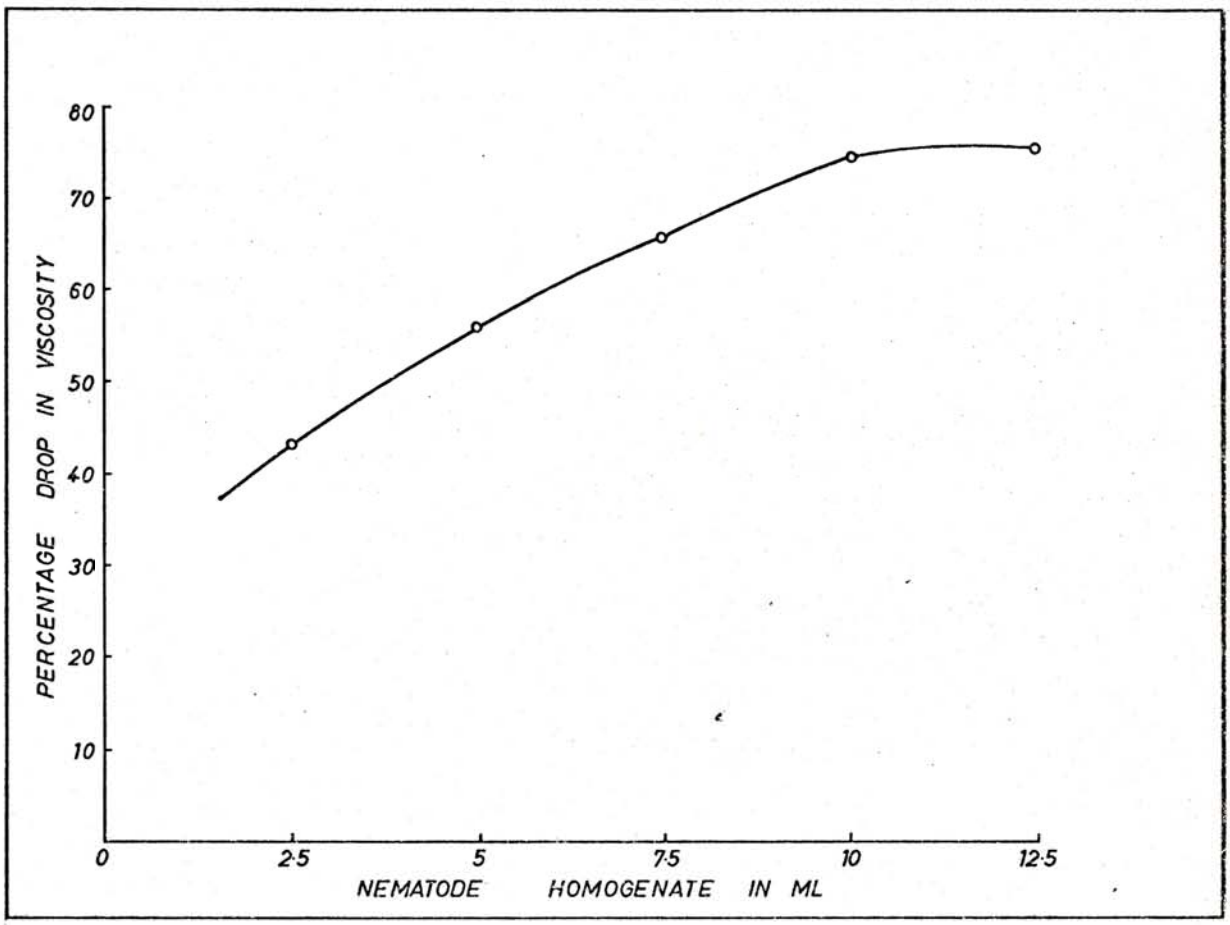


FIG. 14. RELATIONSHIP BETWEEN NEMATODE HOMOGENATE (S. bradys) IN ML AND PERCENTAGE DROP IN VISCOSITY

TABLE 11

Amylase activity of homogenates of S. bradys

Enzyme source		Distilled water (blank)	Nacl (blank)	D.N.S.A. (blank)	Homogenate (starch-substrate)	Boiled homogenate (starch-Sub)
1. Scutellonema bradys	(i)	0.00	0.01	0.076	0.40	0.41
	(ii)	0.00	0.01	0.076	0.46	0.46
2. Infected yam	(i)	0.00	0.01	0.076	1.15	1.10
	(ii)	0.00	0.01	0.076	1.25	1.20

TABLE 12

Invertase activities of homogenates of S. bradys and Infected yam

Enzyme source		Distilled water	Nacl (blank)	D.N.S.A. (blank)	Homogenate (sucrose-sub)	Boiled homogenate (sucrose-sub)
1. Scutellonema bradys	(i)	0.00	0.01	0.076	0.00125	0.00125
	(ii)	0.00	0.01	0.076	0.00125	0.00125
2. Infected yam	(i)	0.00	0.01	0.076	0.005	0.005
	(ii)	0.00	0.01	0.076	0.005	0.005

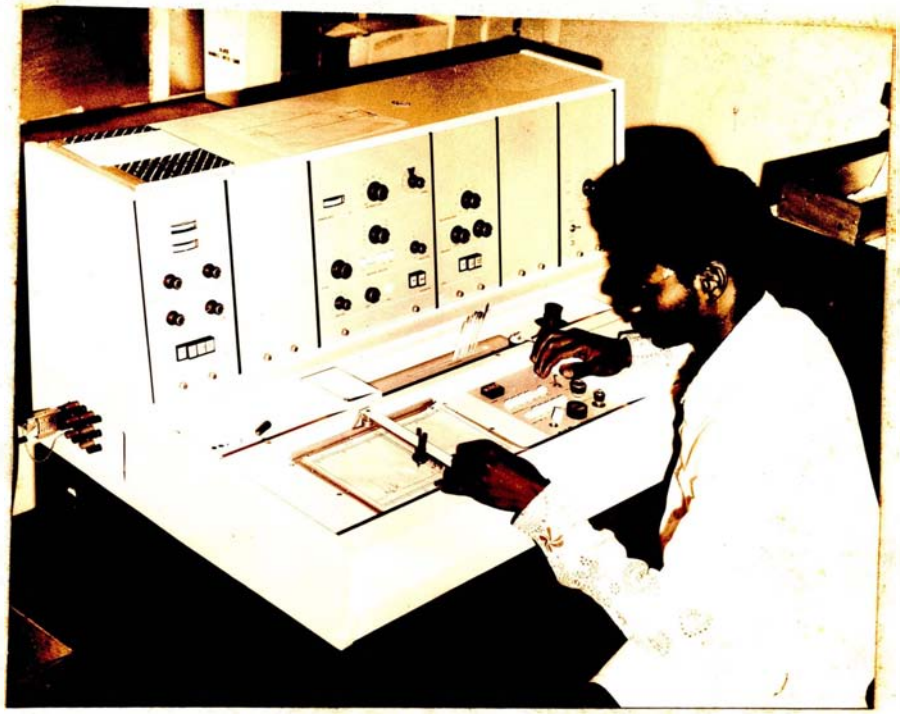


PLATE 30: A technician operating the Varian T-60 N.M.R. Spectrometer.

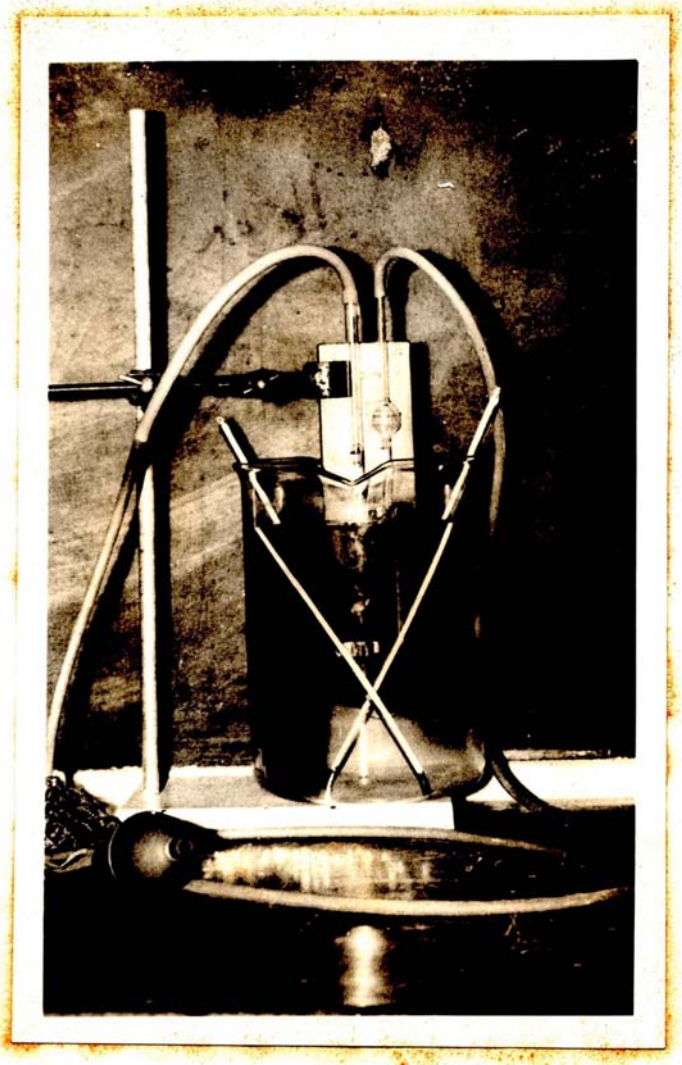


PLATE 31: Apparatus for viscometric measurement of pectinase enzyme.

3.10 FUNGI ASSOCIATED WITH THE DRY ROT DISEASE OF YAM TUBERS.

To isolate the fungi which may be associated with 'dry rot' disease of yams, tubers of *D. rotundata*, *D. alata* and *D. cayenensis* showing recognisable symptoms of dry rot disease were investigated. As a control healthy tubers were also used. The isolation of fungi was done in two ways.

(i) Small pieces of diseased and healthy tissue were removed aseptically (using a flamed scalpel), plated on potato dextrose agar (P.D.A.) in oven-dried petri dishes and incubated at 25°C for 14 days. The fungi that grew were subcultured on P.D.A. to obtain pure culture of the isolates. These fungi were later identified as far as possible.

(ii) The second method involved keeping the yam pieces in oven-dried petri dishes inside humidity chambers. During 14 days of incubation, the fungi that grew were subcultured on to agar plates and identified.

The results are shown in Tables 13 and 14.

It is very interesting to note from this experiment that the two methods used in isolating the fungi gave different results. By the direct plating of diseased portions of the dry rot tissue on agar, only two fungi were isolated even though profuse mycelial growth was observed. Fusarium oxysporum Schlecht and Rhizopus nigricans Ehrenb. were isolated by this method. From the other method involving keeping the yams in humidity chambers for 14 days before plating the diseased tissues on agar, the following fungi were isolated:- Aspergillus niger van Tiegh; Botryodiplodia theobromae Pat., Fusarium moniliforme var subolutinans Woron and Reinking., Penicillium sclerotigenum Yamamoto., Trichoderma viride Link ex Fr., and Rhizopus nigricans Ehrenb. Aspergillus, Pencillium, Rhizopus and Fusarium sp. were the most commonly isolated. Penicillium sp. were more commonly isolated from yellow yam (D. cayenensis) and the two Aspergillus sp. were not host specific. The fungus was isolated from all the three yam species.

TABLE 13

FUNGI ASSOCIATED WITH DRY ROT DISEASE OF YAMS

FUNGI ISOLATED FROM DRY ROT PORTIONS OF YAMS BY DIRECT PLATING METHOD

Fungi	D. rotundata	D. cayenensis	D. alata
Aspergillus niger	-	-	-
Aspergillus sp.	-	-	-
Botryodiplodia theobromae	-	-	-
Fusarium moniliforme	-	-	-
Fusarium oxysporum	+	+	+
Penicillium sclerotigenum	-	-	-
Penicillium sp.	-	-	-
Trichoderma viride	-	-	-
Rhizopus nigricans	+	+	+

\+ Fungi isolated

\- Not isolated

Profuse mycelial growth without spores observed on most plates.

TABLE 14

INFECTED YAM PIECES KEPT INSIDE HUMIDITY CHAMBERS FOR 14 DAYS AND PLATED ON TO AGAR

Fungi	D. rotundata	D. cayenensis	D. alata
Aspergillus niger	+	+	+
Aspergillus sp.	+	+	+
Botryodiplodia theobromae	+	-	-
Fusarium moniliforme	+	-	-
Fusarium oxysporum	+	+	+
Penicillium solerotigenum	-	+	-
Penicillium sp.	+	+	+
Trichoderma viride	+	-	+
Rhizopus nigricans	+	+	+

+ Fungi isolated.

- Fungi not isolated.

3.11 THE INTERRELATIONSHIPS OF SCUTELLONEMA BRADYS AND FUNGI ASSOCIATED WITH WET ROT OF YAMS.

Plant parasitic nematodes are often regarded solely as pathogens in their own right, capable of producing a recognizable disease condition. Such a concept may be valid in some cases but not in all cases. There is now an increasing awareness especially amongst those concerned with plant protection, that symptom expression of a particular disease in plants may not be attributable to one pathogen only but that often a group of concurrent pathogenic infections may produce disease conditions. By carefully controlled experiments, possible interaction between S. bradys and some fungi previously isolated from yam were studied with the hope that the results would elucidate the role of various organisms and such complex interactions and indicate the basis for the development of an effective disease control programme.

(i) Greenhouse Experiment.

One greenhouse experiment was conducted to study the effect of the interaction of S. bradys and Aspergillus niger on the growth of water yam (D. alata)

and the fungus were tested on water yam as follows: Nematode-free yam setts of water yam (Dioscorea alata) of approximately equal weights (50 g) previously surface sterilised for 20 minutes in 1:10 commercial bleach (Clorox), and rinsed in sterile distilled water several times, were planted in autoclave-sterilised sandy loam soil. The autoclaved pots were wrapped up in silvery paper and maintained in a disinfected greehouse. Sterile water was used for watering the pots using specially adapted sterile test tubes embedded inside the pots. The tops of the test tubes were covered with absorbent cotton wool to let in air, and to prevent or minimize the entry of contaminants. The following treatments were applied to the pots at three treatment times, i.e. at planting; two months after planting and three months after planting. Each treatment was replicated six times.

Treatment A: Pots were inoculated with approximately 500 males and 500 females of Scutellonema bradys alone.

Treatment B: Pots were inoculated with the fungus Aspergillus niger.

Treatment C: Pots were inoculated with both Scutellonema bradys and Aspergillus niger. (The fungus was inoculated three weeks after the nematodes).

Treatment K - Control: No nematode, no fungus.

For the nematode inoculation, a nematode suspension containing roughly 1000 surface sterilized male and female adults of S. bradys was pipetted into the soil through the embedded test tubes. The emerging vines of the tuber were supported with twines in place of the usual yam stakes. After six months, the yams were harvested and assessed for the following:

(a) Incidence of dry rot.

(b) Tuberization and dry rot interactions.

(c) The effect of the nematode/fungal interaction on nematode development.

(d) The effect of the nematode/fungal interaction on the establishment of the fungus.

(ii) Storage Experiment.

This experiment was conducted with 40 healthy tubers of D. rotundata. The yam tubers were previously surface sterilized by three washings in 1:10 commercial

Clorox. Three fungal species - namely, Aspergillus niger, Penicillium sclerotigenum and Fusarium oxysporum, previously isolated from rotting yam, and the yam nematode S. bradys were tested for pathogenicity by inoculation into healthy yam tubers according to the method described by Okafor (1966). One cylindrical core was removed at the middle of each yam tuber with a sterile 2 cm cork borer. Five mm discs of 7-day old fungal cultures were placed into the holes in the tubers, and the cores of yam from the tubers were replaced. Inoculation of the surface sterilised nematodes was done using small micro pipettes. The replaced cores were then waxed and further sealed with adhesive tapes. The following treatments were applied to the yam tubers:

1. Inoculation with the nematode Scutellonema bradys alone.
2. Inoculation with the nematode Scutellonema bradys and Penicillium sclerotigenum.
3. Inoculation with the nematode Scutellonema bradys and Fusarium oxysporum.
4. Inoculation with the nematode and Aspergillus niger.
5. Inoculation with the nematode S. bradys + Penicillium + Fusarium and Aspergillus.

6. Inoculation with the nematode Fusarium oxysporum alone.
7. Inoculation with the nematode Penicillium sclerotigenum alone.
8. Inoculation with the nematode Aspergillus niger alone.
9. Inoculation with dry rot extract.
10. Inoculation with sterile distilled water as control.

Each of these treatments was replicated four times. (One spot was inoculated per tuber and four tubers were used for each treatment). The yams were then stored in a yam barn for three months and afterwards an assessment of the following was made:

(a) The role of S. bradys in dry rot formation.

(b) The role of fungi in dry rot formation.

(c) The combined effects of the nematode and each of the fungus, and all the three fungi.

After 3 months of storage, the tubers were cut open from the site of inoculation and photographs of the infected areas were taken. Plates 33, 34, and 35 show the yams that have been inoculated with Fusarium oxysporum, Penicillium sclerotigenum, and Aspergillus niger and Plates 36, 37, and 38 show the yams that have been

inoculated with the nematode S. bradys and Fusarium oxysporum, nematode + Penicillium sclerotigenum and nematode + Aspergillus niger. Plate 39 shows yams that were inoculated with S. bradys and all the three fungi, and Plate 40 shows the yam that was inoculated with the dry rot extract only, Plate 41 shows the yam that was inoculated with S. bradys alone. In the first case. where each fungus was inoculated alone, penetration of fungal mycelium was restricted despite the fact that a form of 'wound' had been caused artificially using a cork borer, a general mottling of the yam was, however, observed. But in the second case, where both the nematode and fungus were inoculated in pairs simultaneously the infection spread was much greater especially where nematodes were inoculated with Penicillum and Fusarium species. Where the nematodes and all the three fungi were inoculated, the depth of penetration was also much enhanced. But with A. niger, the presence of the nematodes did not influence the depth of penetration of the fungus in any positive way. In this case where the nematodes were inoculated alone, infection was similar to that previously described as 'dry rot' recognised by the yellowish necrotic lesions restricted to the cells beneath the

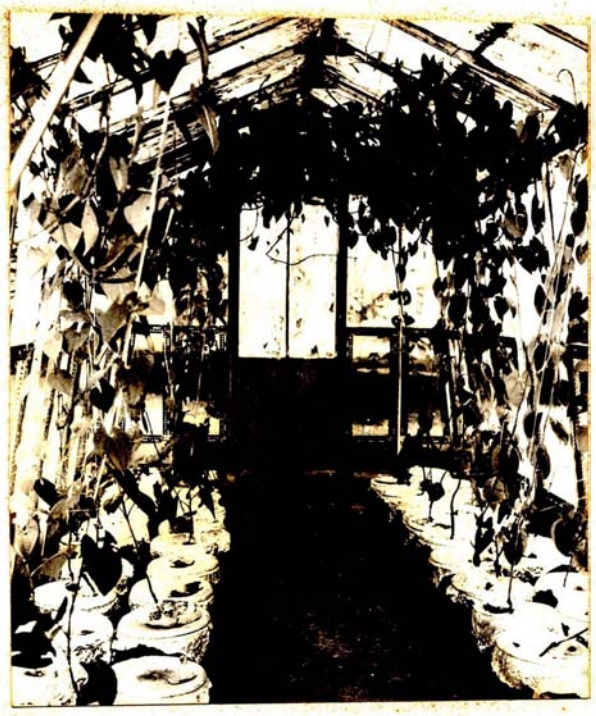


PLATE 32. A nematology greenhouse showing the experiment for the study of interrelationships between *A. niger* and *S. bradys*.

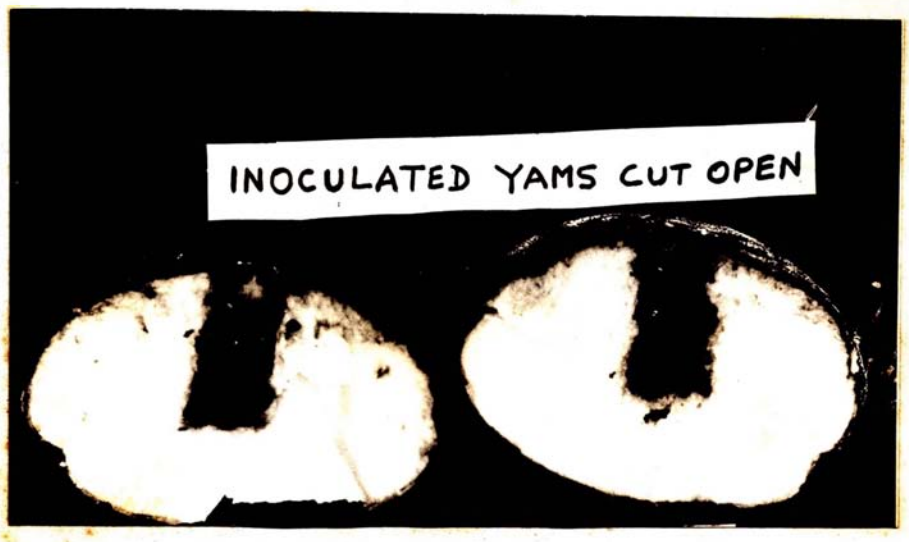


PLATE 33. Yam inoculated with Fusarium oxysporum only.

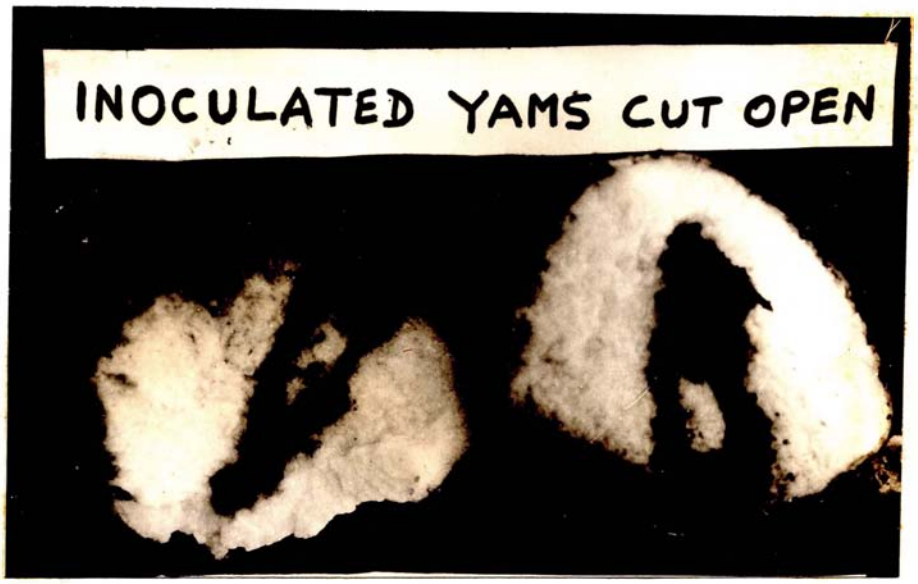


PLATE 34. Yam inoculated with Penicillium sclerotigenum only.

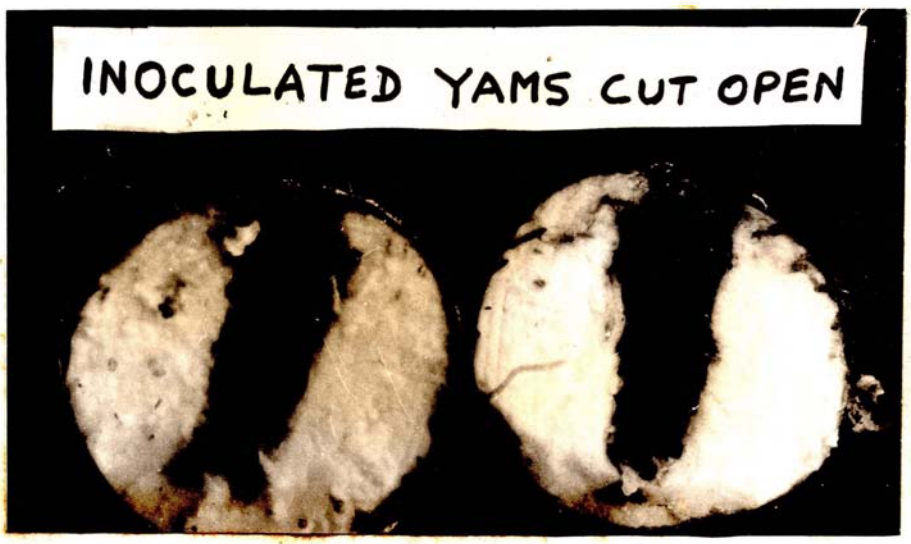


PLATE 35. Yam inoculated with Aspergillus niger only.

PLATE 36. Yam inoculated with S. bradys and F. oxysporum.

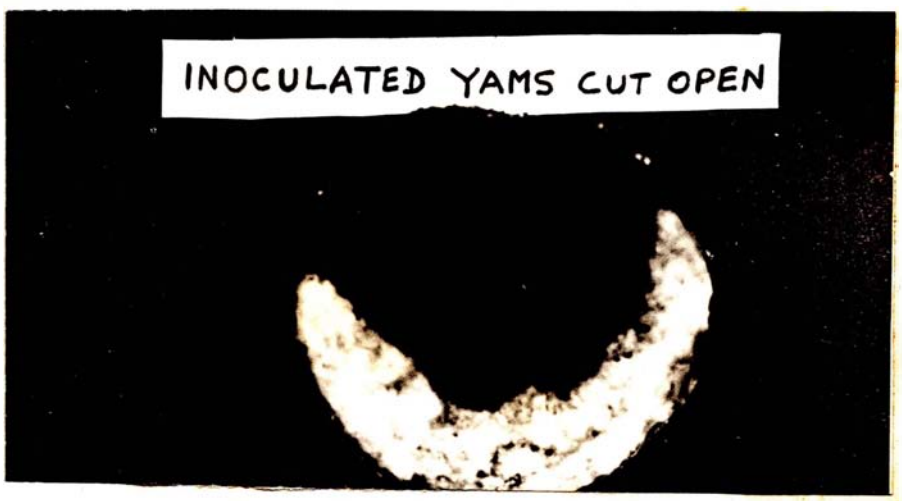


PLATE 37. Yam inoculated with S. bradys and P. sclerotigenum.

PLATE 38. Yam inoculated with S. bradys and A. niger.

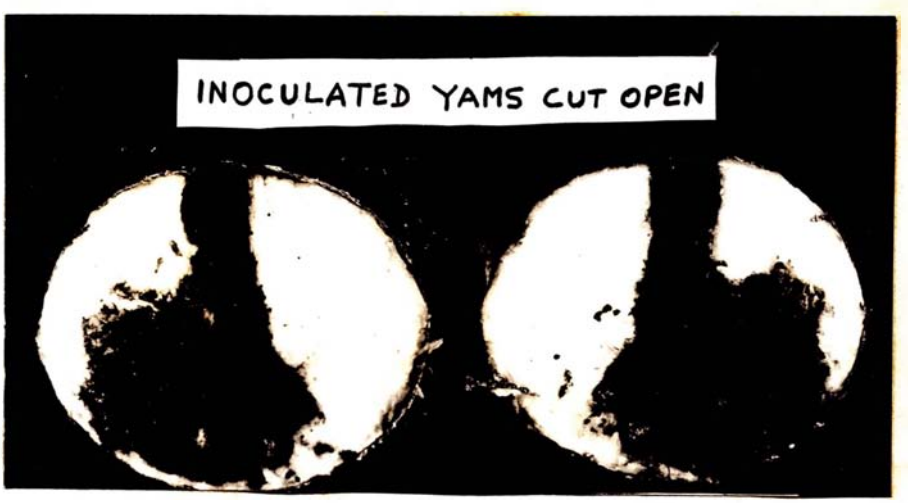


PLATE 39. Yam inoculated with S. bradys, F. oxysporum, P. sclerotigenum and A. niger.

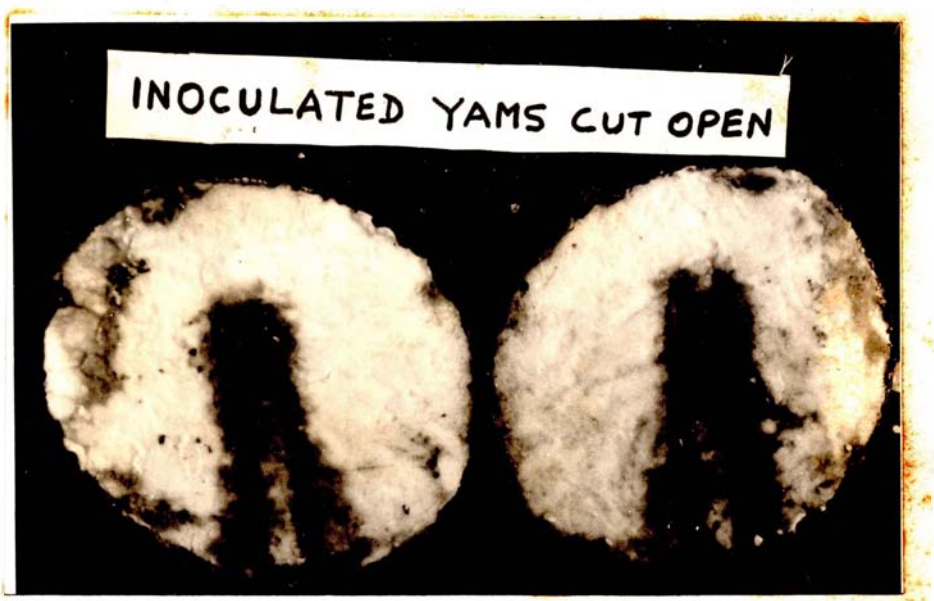


PLATE 40. Yam inoculated with dry rot extract.

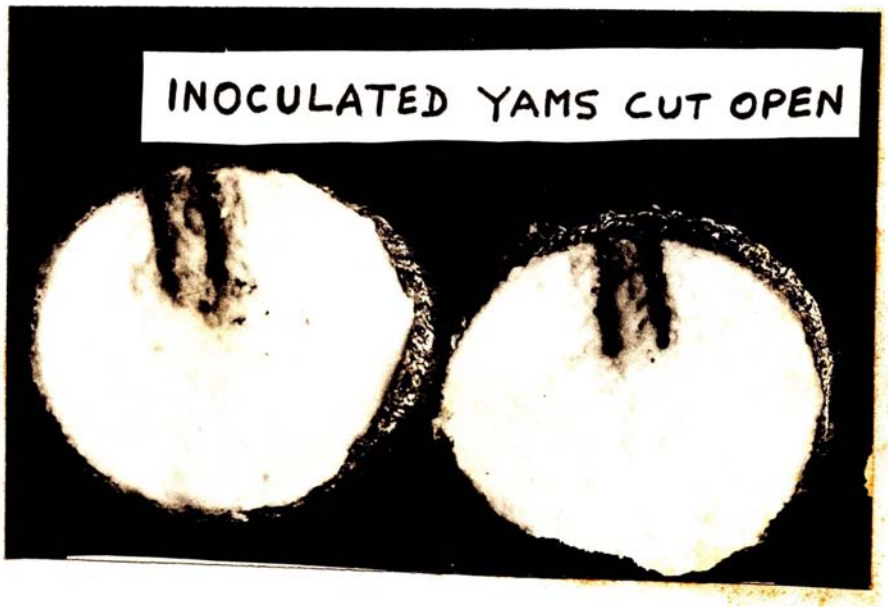


PLATE 41. Yam inoculated with S. bradys only.

periderm (Plate 41).

The mean fresh weights of tubers obtained in the greenhouse experiment are shown in Table 15. The weights of tubers from each treatment were subjected to statistical analysis (completely randomized block design) and the results show that there were no statistically significant differences in weights of tubers from each treatment.

TABLE 15

MEAN FRESH WEIGHT OF TUBERS IN GRAMMES

GREENHOUSE EXPERIMENT

Treatments	A	B	C	D	E	F	G	H	I	K
Mean fresh weight in G*	137	121	115	126	122	133	139	131	117	141

*Mean of six replicates.

No significant difference among mean fresh weights of tubers at all levels in the analysis of variance.

Treatments.

A Yam inoculated with S. bradys at planting.

B " " " A. niger at planting.

C " " " S. bradys and A. niger at planting.

D " " " S. bradys 2 months after planting.

E " " " A. niger 2 months after planting.

F " " " S. bradys and A. niger 2 months after planting.

G " " " S. bradys 3 months after planting.

H " " " A. niger 3 months after planting.

I " " " S. bradys and A. niger 3 months after planting.

K Control i.e. Untreated

Nematodes were extracted from the tubers, roots and soil after harvest. Nematodes were extracted from 50 g of tuber from each treatment using the method described earlier. Nematode extraction from the roots was done by cutting 10 g fresh roots from each treatment, into millimetre pieces and these were macerated in 100 ml of water for 3 minutes in a Waring blender. These were later extracted into small petri dishes using sieves of nylon gauze embedded in rigid polyethylene supporting rings. Most nematodes were recovered after 48 hours, and only one species of nematode (S. bradys) was recovered from yam roots extracted. The soil extraction procedure was similar to that used for the tubers, but in this case, 200 cc of soil from each treatment were extracted for 48 hours. The results of nematode population in the tuber, root and soil for each of the treatments are presented in Fig. 15.

In treatments A, D, and G, i.e. where nematodes were inoculated at planting, 2 months after planting and 3 months after planting respectively, fewer nematodes were extracted from the soil, and greater numbers of nematodes were extracted from the tubers and the roots. It was clear that

majority of the nematodes entered the roots and tubers and reproduced in them. The average population increase was of the order of 3.8x, 3x and 3x for treatments A, D, and G, respectively. But in C, F, and I, i.e. where the nematodes and A. niger were inoculated at planting, 2 months after planting and 3 months after planting, much greater numbers of nematodes were extracted from the soil. This implies that fewer numbers of nematodes entered and reproduced in the roots and tubers. No nematodes were extracted in the tubers, roots and soil from control pots.

Fungi were isolated from yam tubers and roots by plating small pieces of surface sterilized yam roots and tubers from all the treatments and control on agar (P.D.A.) and incubated at 25°C for a period of two weeks. Some of the plates contained a mixed population of fungi and were subcultured and incubated for a further period of two weeks before identification. The lists of fungi isolated are shown in Tables 16 and 17.

Fungi were isolated from soil by two methods:

(a) Soil dilution and

(b) Direct plating methods

In the soil dilution method, 10 cc of soil from each treatment were thoroughly mixed in 100 ml of water in a volumetric flask and afterwards the following soil dilution series were prepared from this, namely, 10^{-1}, 10^{-2}, 10^{-3}, ... 10^{-7}. Using graduated pipettes, 1 ml of each of these dilutions was pipetted into agar and incubated at 30°C for two weeks. In the direct plating method, about 0.5 g of soil (from each treatment) was made into a paste, sprinkled in each agar plate and incubated for two weeks. By both the soil dilution and direct plating methods, two species of fungi were found predominantly in the plates and these were Aspergillus niger and Fusarium oxysporum.

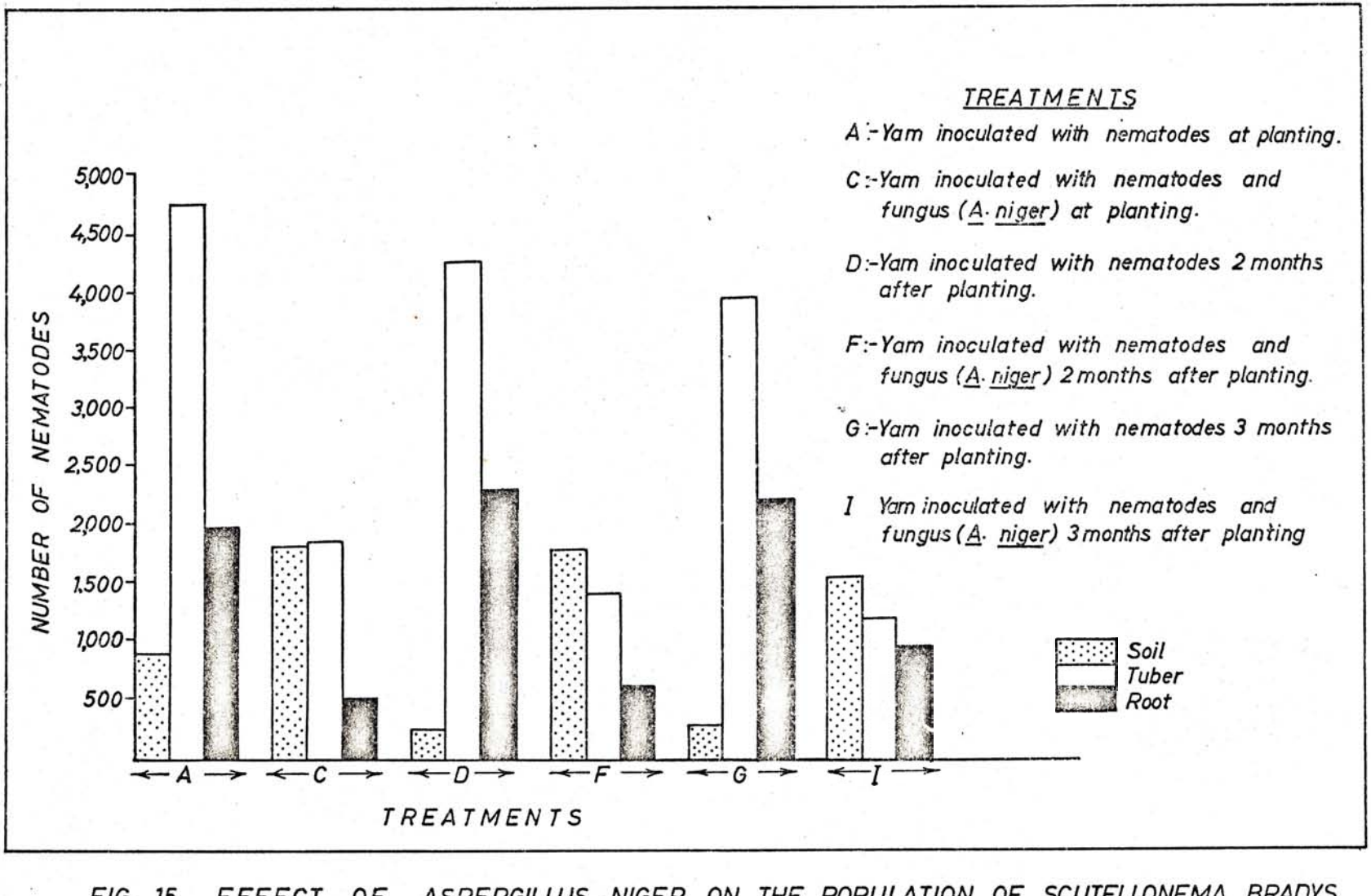


FIG. 15. EFFECT OF ASPERGILLUS NIGER ON THE POPULATION OF SCUTELLONEMA BRADYS.

TABLE 16

FUNGI ISOLATED FROM YAM TUBER

Treatments	Fungi isolated
A	*Rhizopus* spp. and *Fusarium* sp.
B	*Aspergillus niger* and *Rhizopus* sp.
C	*Aspergillus niger*, *Fusarium* sp. and *Rhizopus* sp.
D	*Rhizopus* spp. and *Fusarium* sp.
E	*Aspergillus niger* and *Rhizopus* spp.
F	*Aspergillus niger* and *Fusarium oxysporum*
G	*Rhizopus* spp.
H	*Rhizopus* spp. and *Fusarium* sp.
I	*Aspergillus niger*
J	*Rhizopus* spp. and *Fusarium* spp.

TABLE 17

FUNGI ISOLATED FROM YAM ROOTS

Treatments	Fungi isolated
A	*Fusarium* spp.
B	*Aspergillus niger*, *Rhizopus* spp. + *Fusarium* spp.
C	*Aspergillus niger*
D	*Aspergillus niger* + *Fusarium oxysporum*
E	*Aspergillus niger* + *Rhizopus* spp.
F	*Aspergillus niger*
G	*Rhizopus* spp.
H	*Aspergillus niger* + *Fusarium oxysporum*
I	*Aspergillus niger* + *Rhizopus* spp.
J	*Rhizopus* spp.

3.12 COMPARISON OF POSSIBLE NEMATICIDAL EFFECTS OF *A. NIGER*, *P. SCLEROTIGENUM* AND *F. OXYSPORUM*.

In the previous experiment involving *Aspergillus niger* and *S. bradys*, the fungus did not support the development of a large population of the nematode hence it was thought that this was due to a possible antibiotic action of *A. niger*. To investigate this further, a study was carried out to compare the anti-nematode effects of three fungi previously isolated from yams - *A. niger* (Plate 42), *P. sclerotigenum* (Plate 43), and *F. oxysporum* (Plate 44) on *S. bradys*.

The fungi were grown in Potato Dextrose Agar (P.D.A.) for 21 days and filtrates of the fungi were obtained by gently washing the spores into sterile distilled water. The spores were diluted with distilled water to obtain the following five dilutions (20,000, 15,000, 10,000, 5,000, and 1,000 spores per ml). Suspensions of *S. bradys* were added to the different spore concentrations in vials and incubated at 28°C. Three replicates of each spore concentration were prepared for each fungus and a control series of distilled water. The percentages of immobilised nematodes were determined after 12 hours. The results of

this investigation are shown in Fig. 16.

After 12 hours incubation, the nematodes were examined under a stereomicroscope and the percentage number of nematodes killed or immobilized was determined. Of the three fungi, Aspergillus was most active at spore concentrations between 15,000 and 20,000 when 32% and 55% of the nematodes were killed respectively. The maximum percentage numbers of nematodes immobilized by Penicillium and Fusarium species were 24% and 25% respectively. Immobilized nematodes did not recover when removed from Aspergillus niger spores and later died. The lethal and immobilizing effects of spores of Aspergillus on S. bradys may be due to some toxic substances being produced by the fungus.

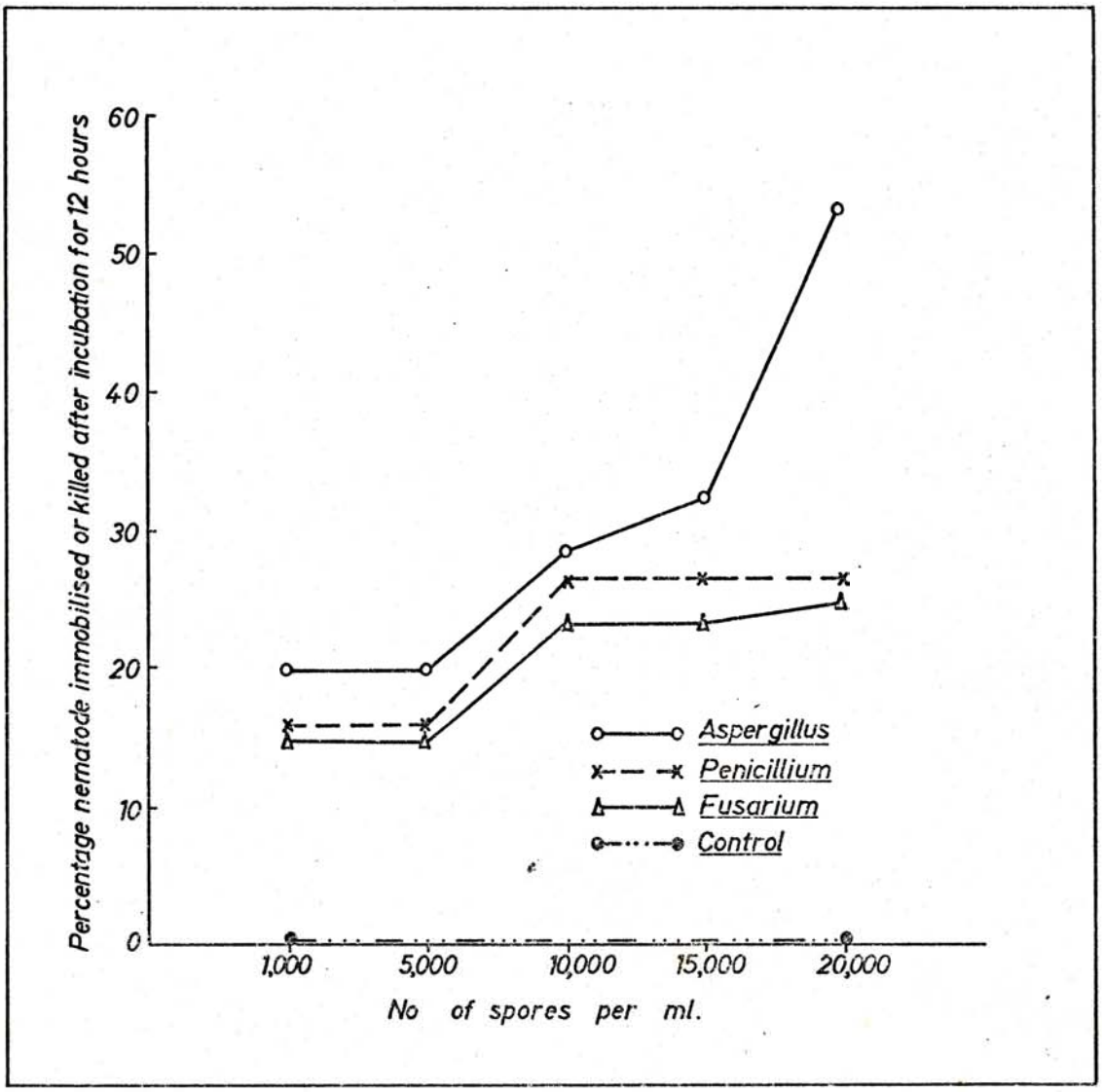


FIG. 16. A COMPARISON OF THE POSSIBLE NEMATICIDAL PROPERTIES OF A. niger, P. sclerotigenum AND F. oxysporum

PLATE 42. Culture of A. niger.

PLATE 43. Culture of P. sclerotigenum.

PLATE 44. Culture of F. oxysporum.

3.13 HOST RANGE STUDIES OF THE YAM NEMATODE - SCUTELLONEMA BRADYS.

Autoclaved sandy loam, consisting of 3 parts of loam and one part of sand were thoroughly mixed, potted and planted to 30 test plants. The seedlings of these test plants were inoculated each around the roots with a suspension of about 500 - 1000 eggs and larvae of S. bradys. All plants were maintained in plastic pots in the greenhouse. After 30 days, the roots of the plants were harvested, washed and 10 g from each test plant was communited in a Waring blender for 30 seconds. The communited roots were then extracted into small petri dishes using sieves of nylon gauze embedded in rigid polyethylene supporting rings. Estimation of nematodes was made after 48 hours.

Table 18 lists the test plants in descending order of nematode population. Host plant efficiency was rated in terms of the highest nematode population increase. Twenty out of thirty test plants were found to be suitable hosts for S. bradys even though only 3 plants supported a significant population increase. Substantial population increase were obtained with Benniseed, Cowpea (var New Era) and Cowpea (var Ife Brown). The high rate of reproduction on cowpea

is of considerable importance. Four other legumes were found to be moderate hosts to this nematode. These were Yam bean, Green gram, Pigeon pea and Pueraria. Of equal interest is that tobacco, cotton, groundnut and maize did not support the nematode.

TABLE 18

HOST RANGE STUDIES OF S. BRADYS

	Common names of plants	Scientific names	No. of nematodes per 10 g root tissue	Host plant efficiency rating
1.	Benniseed	Sesamum indicum Linn.	2,540	Good
2.	Cowpea (var 'New Era')	Vigna unguiculata (L.) Walp.	1,664	-do-
3.	Cowpea (var 'Ife Brown')	Vigna unguiculata (L.) Walp.	1,196	-do-
4.	Eriosema	Eriosema psoraleoides (Lam.) G. Don	342	Moderate
5.	Siam weed	Eupatorium odoratum Linn.	252	-do-
6.	Yam bean	Sphenostylis stenocarpa (Hochst ex A. Rich) Harms	239	-do-
7.	Synedrella (a common weed)	Synedrella nodiflora Gaertn.	208	-do-
8.	Green gram	Vigna aureus (L.)	172	-do-
9.	Mallow	Urena lobata Linn.	157	-do-
10.	Pigeon pea	Cajanus cajan (L.) Druce	152	-do-
11.	Okra	Abelmuschus esculentus Linn.	143	-do-
12.	Kenaf	Hibiscus cannabinus Linn.	140	-do-
13.	Loofa gourd	Luffa aegyptiaca Mill	135	-do-
14.	Soko	Celosia argentia Linn.	131	-do-
15.	Tomato	Solanum lycopersicum Linn.	129	-do-
16.	Melon	Cucurbita pepo L.	128	-do-
17.	Pueraria	Pueraria phaseoloides (Roxh.) Benth.	118	-do-
18.	Roselle	Hibiscus sabdariffa Linn.	116	-do-
19.	Jute	Corchorus olitorius L.	97	Poor
20.	Guinea corn	Sorghum spp.	96	-do-
21.	Maize	Zea mays	-	Non-host
22.	Tobacco	Nicotiana tabacum Linn.	-	-do-
23.	Indian spinach	Basella alba Linn.	-	-do-
24.	Cotton	Gossypium hirsutum Linn.	-	-do-
25.	Groundnut	Arachis hypogea L.	-	-do-

TABLE 18 (cont'd)

	Common names of plants	Scientific names	No. of nematodes per 10 g root tissue	Host plant efficiency rating
26.	Water leaf	Talinum triangulare (Jacq.) Wild	-	-do-
27.	Tridax	Tridax procumbens Linn.	-	-do-
28.	Hot pepper	Capsicum frutescens Linn.	-	-do-
29.	Snake gourd	Tricosanthes anguina	-	-do-
30.	Green tete	Amaranthus viridis Linn.	-	-do-

N.B. Host plant efficiency rating

Good host = a number increase of 2 - 3 times

Moderate = entry accomplished

Poor = entry just accomplished

Non-host = entry of nematode into the root not accomplished.

3.14 CONTROL OF SCUTELLONEMA BRADYS BY HOT WATER TREATMENT.

Because of the simplicity and inexpensive application of hot water treatment, this method has been proposed by a number of workers as a means of eliminating nematodes from yam tubers (Bridge, 1972). Treatment is carried out by the immersion of the tuber in hot water at a known temperature for a specified length of time using a thermostatically controlled hot plate. Besides the effects of hot water treatment on nematode mortality, it is important to determine the effects of such treatment on germination, growth, yield, palatability and storage of yam tubers.

(a) Effect of hot water treatment on nematode mortality.

The initial countsof nematode population were made using 50 g of nematode-infected tubers of water yam (D. alata) and yellow yam (D. cayenensis). Each extraction was replicated five times. Thereafter, the infected tubers were subjected to the following treatment temperatures.

(i) Heating from cold to 50°C

(ii) Hot water at 55 - 55°C for 40 minutes

(iii) Hot water at 55 - 60°C for 40 minutes.

After treatment, the tubers were allowed to cool for 24 hours, then and the resulting live-nematode count was taken. These treatment temperatures were selected from the initial laboratory screening experiments conducted on the effect of hot water dip on germination and growth of yam tubers.

(b) Effect of hot water treatment on storage.

Hot water treatment at 50 - 55°C for 40 minutes gave consistently good results of nematode kill without apparent danger to tuber growth, hence it was selected for this investigation. Eighty yam tubers of D. rotundata were subjected to hot water treatment at 50 - 55°C between October 1972 and March 1973. Twenty yams each were treated on four different occasions as follows:

(i) Immediately after harvest in October.

(ii) Immediately before the November planting (Early planting).

(iii) In January, i.e. after 4 months of storage and before February planting (Late planting).

(iv) After dormancy has broken - March i.e. after 6 months of storage.

Twenty untreated yams were stored under the same conditions as control. The treated and untreated yams were

stored in the yam barn and observations on rotting and other effects were carried out weekly. Ambient temperature and humidity in the yam barn were recorded using the Cassela-type thermohygrograph.

(c) Effect of different hot water treatments on germination, growth and yield of water yam (D. alata).

The three treatment temperatures in (a) were employed in this experiment. After treating the tubers of water yam at these temperatures, the tubers were cut into setts weighing approximately 150 g. These yam setts were then planted on a 99 m^2 plot in the Teaching and Research Farm. The layout was a completely randomised block design.

(d) Palatability and acceptability tests.

After harvest, the tubers from (c) were assessed using a panel of ten tasters and assessors. The following qualities were assessed - (i) external appearance of whole tubers (ii) internal appearance when cut, (iii) taste and appearance of yams when boiled with salt, (iv) pounding quality, and (v) peeling losses.

The results of the effect of different hot water treatments on nematode mortality are shown in Table 19. Hot water

treatment of nematode-infected tubers of D. alata and D. cayenensis at temperatures between 50 and 60 for 40 minutes completely eliminated the nematode population. Heating yam tubers from cold to 50°C also significantly reduced nematode population, but did not completely disinfest the tubers.

TABLE 19

EFFECT OF DIFFERENT HOT WATER TREATMENTS ON NEMATODE MORTALITY

Treatment	Mean nos of nematodes per 50 g tuber** (± Standard Error)		Percentage kill		Efficiency rating
	D. alata	D. cayenensis	D. alata	D. cayenensis	
(A) Heating to 50°C from cold	200±14.1	50±7	99.98	99.99	Good
(B) 50 - 55°C for 40 mins	Nil	Nil	100	100	V. Good
(C) 55 - 60°C	Nil	Nil	100	100	V. Good
(D) Control	10,000±100	4,500±67	-	-	-

**Means of five replicates.

Table 20 shows the effect of the timing of hot water treatment (50 - 55°C for 40 mins) on the deterioration of tubers. All the yam tubers which were treated with hot water immediately after harvest, i.e. in October rotted completely before the end of the storage period. Those tubers treated after 2 and 6 months of storage showed very little signs of deterioration, and hot water did not appear to affect their sprouting potential.

TABLE 20

EFFECT OF THE TIMING OF HOT WATER TREATMENT (50 - 55°C FOR 40 MINS) ON THE DETERIORATION OF TUBERS DURING SUBSEQUENT STORAGE

Different treatment times	Percentage no. of rotted yams at the end of storage period	Percentage sprouting
(I) October (immediately after harvest)	100	100
(II) i.e. Late November before early planting i.e. after 2 months of storage	20	100
(III) January i.e. after 4 months of storage	20	100
(IV) March (after dormancy has broken i.e. after 6 months of storage	5	100
(V) Control i.e. untreated	15	100

The results of the effect of different hot water treatments on germination, growth and yield of water yam are shown in Table 21. A comparison of the treatment means using Duncan's Multiple Range Test is shown in Table 22, and the table of the analysis of variance is shown in Appendix 1. All the treatment temperatures except those between 55 and 60°C did not have any adverse effect on percentage emergence, growth and yield of tubers of water yam (D. alata). Treatment with hot water at 55 - 60°C completely inhibited emergence and no yields were obtained from this treatment.

TABLE 21

EFFECT OF DIFFERENT HOT WATER TREATMENTS ON GERMINATION, GROWTH, AND YIELD OF WATER YAM (D. ALATA)

Treatments	Percentage emergence	Mean yield in (kg)**	Mean nos of nematodes per 50 g tuber ± S.E.	Rating
(A) Heating to 50°C from cold	75	16.5	280±16.7	Good
(B) 50-55°C	90	25.1	Nil	V. Good
(C) 55-60°C	0	0	-	Poor
(D) Control i.e. Untreated	95	35.9	3,440±58.7	Poor

*Means of five replicates.

**Means of twenty tubers.

TABLE 22

COMPARISON OF TREATMENT MEANS USING DUNCAN'S MULTIPLE RANGE TEST

D = 9.0a

B = 6.3b

A = 4.1b

Means followed by the same letter are not significantly different at 5% level.

Table 23 shows that yams treated at temperatures between 50 and 55°C for 40 minutes were accepted by majority of tasters. Those tubers heated from cold to 50°C were rated next. The untreated control were the least acceptable and were rejected by most people because of the unattractive appearance, taste and high percentage of inedible portions.

TABLE 23

PALATABILITY AND ACCEPTABILITY TESTS

Grading	Heat from cold to 50°C	50 - 55°C for 40 minutes	Control
A	86	92	-
B	14	8	-
C	-	-	17
D	-	-	83

Results are expressed as percentage of tasters

A - Very Good

B - Good

C - Fair

D - Poor

3.15 CULTURAL AND CHEMICAL METHODS OF CONTROL OF THE YAM NEMATODE SCUTELLONEMA BRADYS.

This trial was set up in the Crop Collection Garden of the Department of Agricultural Biology, University of Ibadan. The plot used was approximately 851 m^2 containing 120 yam heaps. The heaps were set approximately 1 m apart. Each of these heaps had earlier been artificially inoculated with peels of nematode-infected yam tubers. The heaps were watered daily to facilitate the establishment of the nematodes. After a week, various treatments for nematode control were applied. There were six treatments each of which was replicated twenty times in a completely randomized design. The following treatments were applied to the heaps.

1. D-D Mixture (1, 2-dichloropropane and 1, 3-dichloropropene in the liquid form was applied at a depth of 15 cm at the rate of 25.3 litres a.i. per hectare using the conventional chisel-type applicator).

2. Organic manure (Cow dung): 1.5 kg of organic manure was thoroughly mixed with each heap (1886.3 kg/ha.).

3. Wood Ash: This was used to coat yam setts before planting.

4. Nemagon (D.B.C.P.): Mixture of 1, 2-dibromo-3-chloro propane in the granular form, was applied at the rate of 114 g per heap (35.2 kg a.i./ ha.). This was also thoroughly mixed with the soil.

5. NPK fertilizer: Nitrogen (in form of $NH_4(SO_4)_2$) was applied at the rate of 100.8 kg/ha. Phosphate (in form of P_2O_5) was applied at the rate of 67.2 kg/ha and Potassium (in form of KNO_3) was applied at the rate of 33.6 kg/ha.

6. Control.

The six treatments except (3) were applied in advance of planting to give time for the toxic materials to diffuse out of the soil and for the organic matter to decompose. Yam setts were planted two weeks after applying these treatments.

The yield of water yam (D. alata) and nematode population as influenced by chemical and cultural methods of control are presented in Table 24 and a comparison of

the treatment means using Duncan's Multiple Range Test is shown in Table 25. Treatment with organic manure gave the highest yield, followed by wood ash. The control was ranked third and D-D, N.P.K. and Nemagon came fourth, fifth and sixth, respectively. In this investigation, the yield of water yam was increased, and the nematode population depressed by the use of organic manure. D-D was ineffective both in terms of yield and reduction of nematode population. Wood ash was effective in terms of yield but only slightly effective in reducing nematode populations. Application of N.P.K. considerably reduced nematode population but surprisingly did not increase yield. Although nemagon was very effective in reducing the nematode populations it also caused significant depression of yields. This suggests that nemagon may be phyto-toxic to yams at the levels used.

TABLE 24

YIELD OF WATER YAM (DIOSCOREA ALATA) AND NEMATODE POPULATION AS INFLUENCED BY CHEMICAL AND CULTURAL METHODS OF NEMATODE CONTROL.

Treatments	Rate of application	Yield in kg/hectare	Mean nos of nematodes ± S.E./(50 g tuber)***
(1) D-D. Mixture of 1, 2-dichloropane and 1, 3-dichloropropene	25.3 litres/ha. (liquid form)	2326.4	4,870±64.79
(2) Organic manure	1.5 kg/heap (1886.3 kg/ha)	3357.5	1,410+37.55
(3) Wood ash	To coat yam 'setts' before planting	2628.2	3,350±57.8
(4) Nemagon Mixture of 1, 2-dibromo-3-chloropane	35.22 kg/ha (granular form)	1169.5	260±16.12
(5) N.P.K. (Mixed as fertilizer)	N=100.8 kg/ha P= 67.2 kg/ha K= 33.6 kg/ha	2100	810+28.46
(6) Control	Untreated	2464.7	14,480±120.3

***Means of five replicates.

TABLE 25

COMPARISON OF TREATMENT MEANS USING DUNCAN'S MULTIPLE RANGE TEST

Treatments	Mean yield (kg/ha)*
2	3,357.5 a
3	2,628.2 ab
6	2,464.7 ab
1	2,326.4 b
5	2,100.0 b
4	1,169.5

*Means followed by the same letter are not significantly different at the 5% level.

PLATE 45. A nematode control trial on water yam (*D. alata*) in the Crop Collection Garden, Department of Agricultural Biology, University of Ibadan.

3.16 PRELIMINARY STUDIES ON THE EFFECT OF GAMMA RADIATION (FROM A COBALT 60 SOURCE) ON STORAGE LIFE OF NEMATODE-INFECTED WHITE YAM (D. ROTUNDATA VAR EFON).

Recent investigations have shown that ionizing radiation treatment can be used to increase the storage life of potatoes (Sparrow et al., 1954; Brownell et al., 1954). Other workers like Sawyer et al. (1955) have also shown that ionizing radiation can increase the storage life of potatoes without adversely affecting their taste or appearance. Very little is reported in the literature on the effect of gamma radiation on nematodes infesting yam tubers. This investigation was initiated to study the effect of different dosage levels of irradiation on

(a) the storage life of nematode-infected tubers of D. rotundata, and

(b) nematode mortality.

(i) Effect of gamma radiation on storage.

The variety of D. rotundata used was specially selected because of its high degree of susceptibility to nematode infestation. The radiation source was a Gamma 3,500 irradiation unit (Noratom-Norcontrol As, Vinderen, Oslo 3, Norway). Because the sample chamber has a small volume of 3.5 litres, only small tubers were used and ten tubers

were selected for each treatment. Each group of ten tubers was irradiated at 5, 7.5, 10, 12.5 and 15 krads. Ten tubers similarly selected were used as control. After irradiation, the yams were placed on platforms in a well ventilated yam barn for observations on nematode populations and sprouting.

(ii) Effect of gamma radiation on nematode mortality.

About 60 actively motile larvae and adults (mixed population) of S. bradys were picked into each of the glass blocks. Each glass block has an approximate surface area of 24 cm^2. These 21 glass blocks were divided into 3 groups. Each group containing seven blocks was irradiated at 0, 5, 10, 15, 20, 25 and 30 krads (i.e. each treatment was replicated three times). Each glass block was examined for the number of dead nematodes immediately after irradiation and twenty-four hours after irradiation. From the means of dead nematodes recorded for each treatment, the mortality percentages were calculated.

(iii) Effect of gamma radiation on nematode-infected yam peels of D. rotundata.

Yam peels with obvious symptoms of nematode infection were carefully removed from six tubers of D. rotundata.

They were sliced into small pieces and mixed thoroughly. About 25 g of the yam peel were weighed into each petri dish. There were 14 petri dishes in all, and these were divided into two groups of seven. Two each were irradiated at 0, 5, 10, 15, 20, 25, and 30 krads, giving two replications for each treatment. These yam peels were extracted immediately after irradiation, and nematodes numbers were estimated after 48 hours.

Table 26 shows the effect of gamma radiation on the numbers of nematodes and the storage life of nematode-infected tubers of white yam (D. rotundata var. efon). The nematode counts at 0, 4, and 12 weeks after irradiation are given. Although nematode counts in the control were consistently higher than in all other treatments, there were no marked differences in the counts for all treatments except in the case of 15 krad when less than 1,000 nematodes were extracted. Sprouting and incidence of rotting were, however, completely inhibited at all levels of irradiation.

TABLE 26

PRELIMINARY INVESTIGATIONS INTO THE EFFECT OF GAMMA RADIATION ON THE STORAGE LIFE OF NEMATODE-INFECTED TUBERS OF WHITE YAM (DIOSCOREA ROTUNDATA VAR. EFON).

Dosage in Krads	Mean nos of nematodes extracted weeks after irradiation ± standard error*			Incidence of sprouting	Incidence of rotting
	TIME IN WEEKS				
	0	4	12		
0	2,000 ± 44.7	2,560 ± 50.6	4,110 ± 64	100%	60%
5	960 ± 31	1,200 ± 34.6	1,500 ± 38.7	0	0
7.5	1,500 ± 38.7	500 ± 22.3	2,100 ± 45.8	0	0
10	Eggs	300 ± 17.3	1,000 ± 31.6	0	0
12.5	Eggs	1,600 ± 40	1,420 ± 37.7	0	0
15	Eggs	Nil	500 ± 22.4	0	0

*Means of five replicates.

The results of the effect of gamma radiation on Scutellonema bradys, immediately after irradiation and 24 hours after irradiation are shown in Fig. 17. These results show that there is a positive correlation between percentage kill and dosage. Mortality increased progressively with increase in dosage and followed a more or less sigmoid curve. Only 80 - 82% kill was, however, recorded.

Figure 18 shows that larval stages are more susceptible to irradiation than adults.

Figure 19 shows the effect of gamma radiation on the nematodes present in infected yam peels.

Because nematodes were not completely eliminated in the yam tuber at 15 Krads in experiment (i) after 3 months, it was necessary to increase the dosage to determine the levels at which nematodes would be killed. Figure 19 shows that there was little difference between the untreated yam peels and those irradiated at 5 Krad. Considerable decline in nematode numbers was observed as from 10 Krads. This number dropped to low levels as from 20 Krads. Not all the nematodes were, however, eliminated even at the highest dosage of 30 Krads.

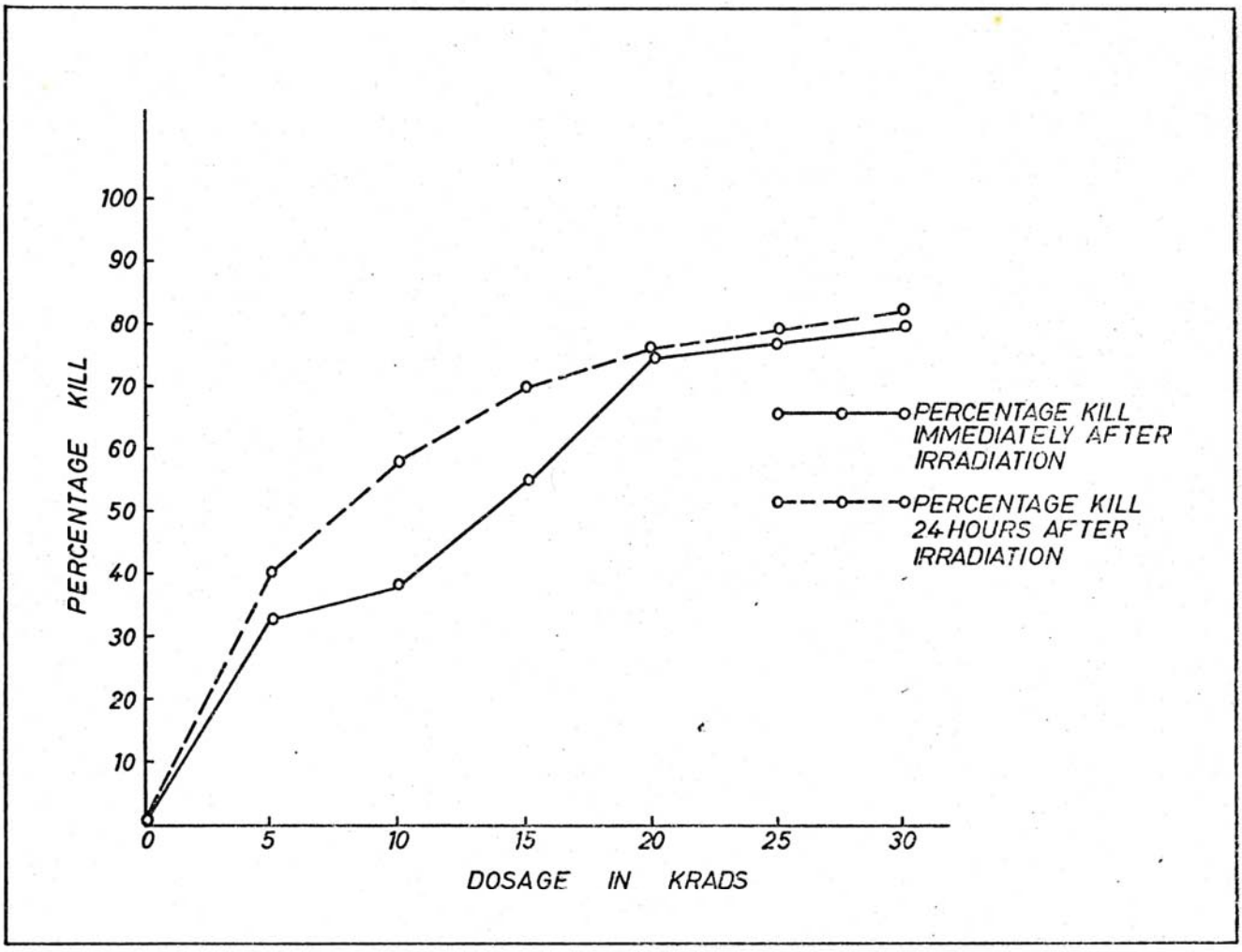


FIG. 17. EFFECT OF DIRECT IRRADIATION ON THE MORTALITY OF S. bradys

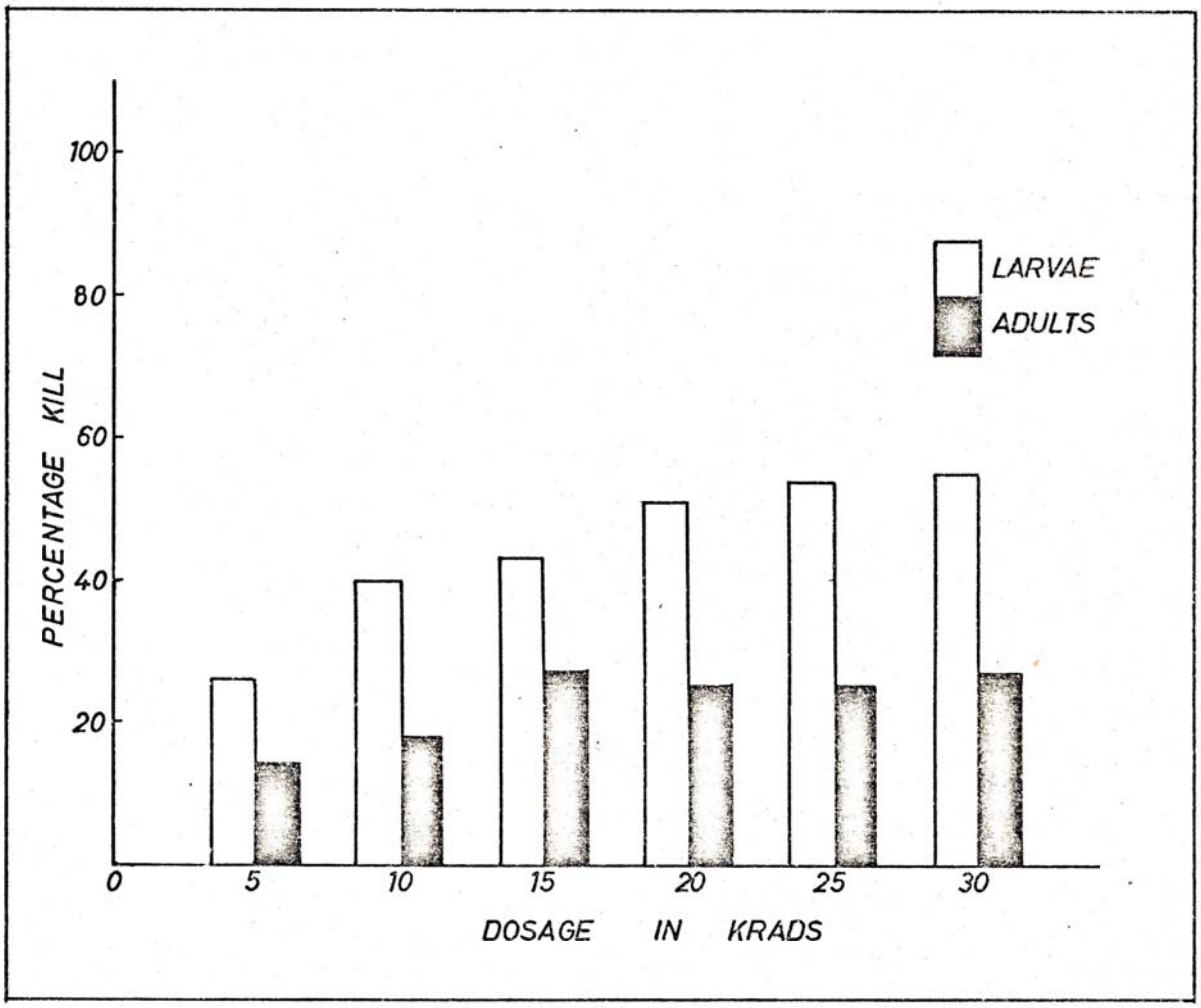


FIG. 18. EFFECT OF DIRECT IRRADIATION ON THE LARVAE & ADULTS OF S. bradys

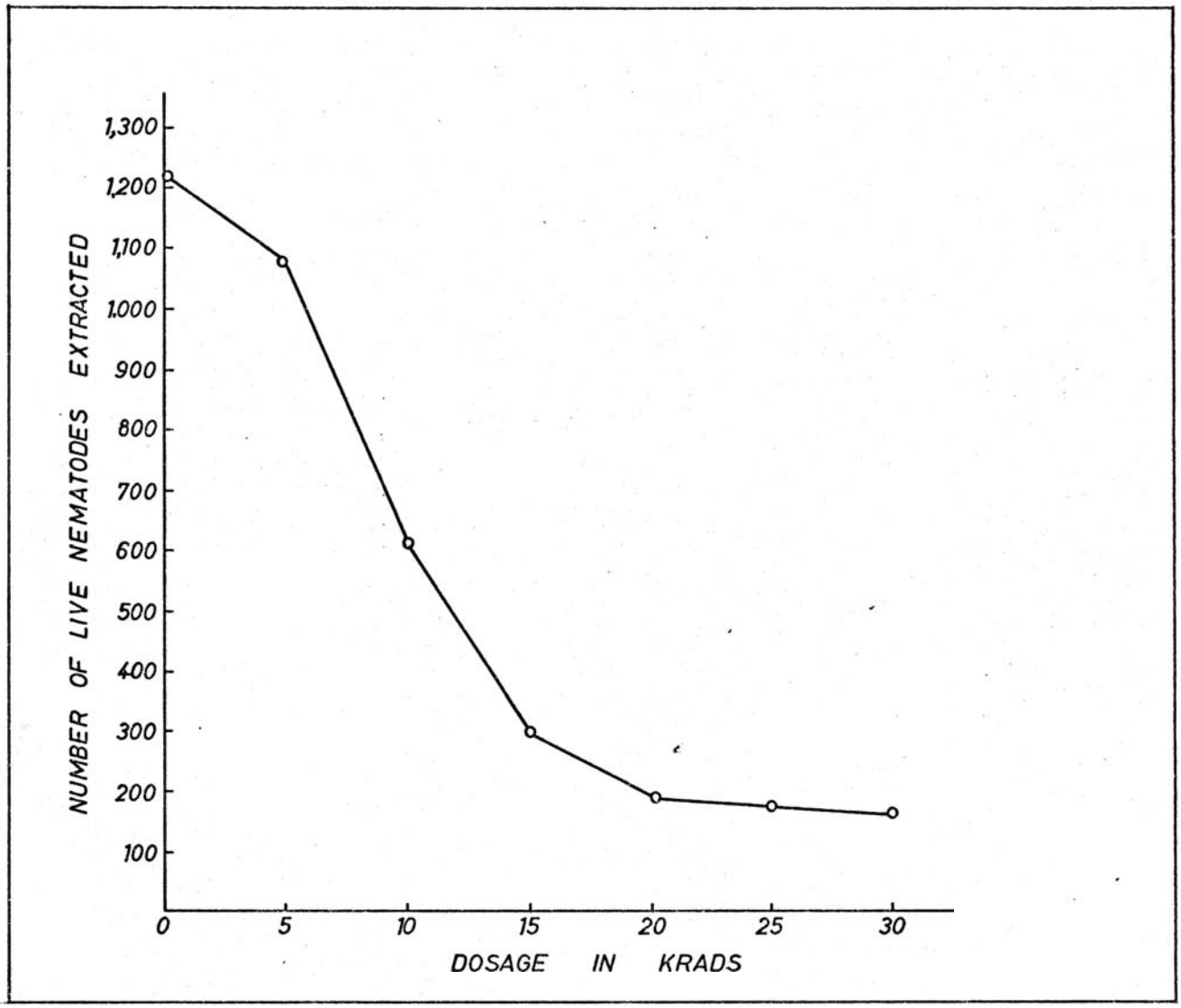


FIG. 19. EFFECT OF GAMMA RADIATION ON S. bradys IN YAM PEELS

CHAPTER 4

DISCUSSION

Distribution of nematode parasites of yam in the Mid-Western State of Nigeria.

Although S. bradys has always been referred to in the older literature as the yam nematode, it is getting clear in recent surveys carried out in various parts of the world that the nematode genus or species associated with dry rot of yam tubers varies from one geographical location to the other. West (1934), Steiner and Buhrer (1934) associated S. bradys with yam rot in Nigeria and West Indies respectively. Schieber and Lassmann (1961) found that the root-knot nematode was the principal cause of damage to yam tubers in Guatemala. Unny and Jerath (1965) found S. bradys, M. incognita, and M. javanica as the most important endoparasitic nematodes of yams in Eastern Nigeria. Bridge (1973) found S. bradys as the only nematode of economic importance in Western Nigeria. On the other hand, Thompson et al. (1973) reported P. coffeae as the principal nematode associated with yam

tuber decay in the West Indies. The survey results from this study reveal that both S. bradys and M. incognita are important in yam tuber decay in the Mid-Western State of Nigeria and that this degree of importance varies with locality and yam species or cultivar. Reference to S. bradys as the 'yam nematode' is therefore likely to assume only local significance or become obsolete as further surveys may reveal other nematode genera which are equally important in yam decay.

The Mid-Western State probably has the potential to produce more yams but pest and disease problems are limiting yam production. Despite the fact that the proceeds from the yam farms form the bulk of the farmers' annual income (Consolidated Results of Crop Estimation Surveys, 1968-1971) this problem is not being given serious consideration. Storage losses are serious and could inhibit the development of the yam industry if not prevented or controlled. The costs of nematicides are prohibitive and as has been shown in this study nematicides may not necessarily be effective and cannot be recommended to the peasant farmer. The use of nematode-free yam 'seeds' should be encouraged. Mixed cropping with other

susceptible crops like cowpea and beniseed should be avoided as they are good alternative hosts to the yam nematode; successive cropping of yam should be avoided. Fallow or planting of non-host plants like maize and tobacco for one growing season will starve the remaining yam nematodes. It should be brought home to the farmers that yam barns with better ventilation and storage facilities are likely to enhance not only the storage of, but also the economic returns from their yam production.

Methods of surface sterilization.

Many workers have attempted using one method or the other to obtain axenic cultures of nematodes, but it appears that very few have attempted to estimate the efficiency of their treatments qualitatively and quantitatively. Mountain (1955) placed single females of Pratylenchus penetrans in successive drops of sterile water and then later transferred the nematodes into 0.1% streptomycin sulphate for about fifteen minutes, followed by further washing in sterile water. He claimed that he obtained axenic cultures. Zuckerman and Brzeski (1966) obtained axenic nematodes by using 0.5% Hibitane diacetate followed by rinsing with sterile water. Dolliver et al. (1962) and

Myers (1967) independently axenised Aphelenchoides ritzemabosi (Schwartz) Steiner. and Aphelenchoides sacchari Hooper. respectively using 0.01% aqueous mercuric chloride. Peacock (1959) also claimed to have obtained axenic larvae from Meloidogyne egg masses by washing the larvae for five minutes in 0.1% letavlon (cetyltrimethyl ammonium bromide), followed by careful rinsing in sterile water, immersion in 0.5% Hibitane diacetate for fifteen minutes and finally washed in distilled water.

Sterile inoculation of nematodes is one of the scientific methods for determining the role of plant nematodes as causative agents of diseases. There is convincing evidence that by the application of Koch's postulates, the complex host-parasite relationship involving nematodes can be better understood. Obtaining axenic cultures of nematodes is also useful in morphological and morphometric measurements which are diagnostic tools employed in taxonomic studies.

Build up of population.

From these investigations and similar investigations by Bridge (1973), there is overwhelming evidence that

Scutellonema bradys has the ability to reproduce fast in yams stored under ambient conditions. In Bridge's investigations, increases in nematode population were recorded in 6 out of 8 tubers of D. rotundata inoculated with S. bradys. In two of the tubers, a 14-fold increase was recorded after 5 months of storage.

In the second experiment, results obtained for the infected tubers were similar to those obtained in the inoculated ones. Increases in nematode numbers were recorded throughout the five-month period despite the fact that the infected tubers were selected for nematode count at random. Cool conditions i.e. 16 - 18°C did not have any depressing effect on nematode population for the first 3 months when compared with the tubers stored under ambient conditions. But after the first 3 months, whereas the nematode number of tubers stored under ambient conditions was on the increase, the number in tubers stored under cool conditions began to show noticeable decreases. The effect of the cool conditions began to show after the first 3 months. Of equal interest is the effect of the cool conditions in suppressing sprouting whereas those tubers stored at ambient conditions sprouted luxuriantly. The suppressing effect of the cool conditions on nematode

number after the third month, and on sprouting appears to be an advantage in that tubers under such cool conditions will keep for considerably longer periods. Wallace (1963) divided into five arbitary phases the influence of temperature on plant nematodes namely

(1) non-lethal low temperatures at which activity is inhibited.

(2) optimum temperatures.

(3) non-lethal high temperatures at which activity is inhibited.

(4) lethal low temperatures, and

(5) lethal high temperatures.

Theoretically, it is believed that every species should have a temperature range corresponding to each of these categories, but complete data on any one species are lacking. The temperature limits at which a plant parasitic species reproduces or is killed are variable and depend to a large extent on the host plant and time of exposure. Results of studies on temperature relations of nematode reproduction in plants are sometimes difficult to interprete because the host plant itself may often be affected by temperature.

The ability of S. bradys to survive and reproduce at

tropical ambient conditions in yam tubers confers some degree of success on this nematode. The effect of the feeding and reproduction of S. bradys on stored tubers is the major problem in yam storage. With such high numbers of nematodes produced at the end of storage period, the quality of such infected-tubers drops resulting in tubers which are virtually inedible and also unsuitable as planting materials.

Depth of penetration.

In all the 5 cultivars of D. rotundata examined, the bulk of the nematode population was found mainly under the skin i.e. to a depth of between 0 to 0.5 cm. This is in general agreement with observations made by West (1934), Goodey (1935) and Bridge (1972). Penetration may extend beyond the 1 cm depth as was found in three varieties namely 'akosu', 'esinmirin', and efon. In these varieties, nematodes were extracted beyond the 1 cm depth in most of the samples. In thus confining their destruction to the surface layer of the yam tissues the nature of attack of S. bradys resembles that of A. dipsaci when parasitizing potato tubers. This parasite also does not penetrate very deeply below the skin of potato.

Within the tubers, the population of S. bradys builds up considerably with time. This is shown by the fact that from each five milimetre piece of infected portions of the yam tissue, several hundreds of S. bradys were extracted. These nematodes were found to occur mainly between 0-4 mm of the peridermal layer of yam where they cause extensive cell damage. As a result of this infection, cracks are developed on the skin and after some time, it peels off easily.

The apparent preference of S. bradys for the oldest part of the tuber i.e. top end seems to be simply because it was the first to be formed during the process of tuberization and remains longer under the soil surface and probably in constant contact with the nematodes. It is only in the later growth of the yam tuber that other portions are infected. At harvest, the top end must have been severely attacked by nematodes whereas at the middle or bottom portions, the nematode populations remain low.

Information obtained from these experiments are very useful in many respects. Since it is now established that S. bradys is capable of penetrating only to a depth of about 1.5 cm, eradication of the nematodes in the yam tuber may be achieved by chemical or hot water dip. Knowing

that the nematodes prefer the oldest portion of the yam also aids in the selection of planting material. The top end may not be used for propagation if infected even though it germinates fastest. The middle and bottom ends can be used for propagation, and although they tend to germinate slowly. These two portions i.e. middle and bottom are more likely to produce better quality yam tubers than the top end.

Histopathology studies.

Most of the findings in this work are in perfect agreement with the observations and conclusions made by Goodey (1935). In Western Nigeria, as many as 100,000 nematodes are found in an average dry rotted tuber. Early nematode infections of yam, especially by S. bradys appear symptomless to the naked eye, even though the nematodes are there in hundreds at that stage. By the time the symptoms become obvious (which is usually after some months in storage), the nematodes are there in thousands or hundreds of thousands per tuber. This is in agreement with the recent observations by Bridge (1972) who recorded a maximum nematode population of 62,000 per 10 g of tuber.

Observations of diseased pieces of yam tuber under

the microscope and the histological studies indicated that yam decay is a disease process which can be separated conveniently into 2 main types 'dry rot' and 'wet rot' each having an early and an advanced stage. Fortunately, the infection of S. bradys on white yam (D. rotundata) can be distinguished by the characteristic colour it imparts on different stages of yam deterioration. 'Early dry rot' is associated with cream and light yellow colour while 'late dry rot' ranges from dark yellow to light brown. 'Early wet rot' usually has brown to dark brown colour, but 'late wet rot' ranges from dark brown to black. Throughout the course of these investigations, only nematodes were found in stained sections of dry rotted tubers and fungi in wet rot sections. In the pathogenicity tests carried out by Bridge (1973), the dry rot symptoms have been reproduced experimentally by inoculating surface sterilized Scutellonema bradys into clean yam tubers. This symptom was not produced when fungi were similarly inoculated into clean yam. The fact that Adeniji (1970); Ogundana (1970) found that the fungal isolates used could not establish on yams in the absence of wounds also lends support to the argument that

yam decay is initiated by nematodes. The nematodes are good modifiers of the yam substrate which is starch. This is reduced to simple sugars and in the presence of these sugars the fungi grow actively. The 'dry rot' stage is a slow process compared with the 'wet rot' stage. Once the 'wet rot' stage starts, yam decay becomes very fast and nematodes seem to disappear. The periods when nematodes and fungi can be found side by side, in yam tissue - 'late dry rot' or 'early wet rot' are transitional and very short.

Whereas the nematodes are confined to the outer 1.5 cm of tuber, fungi are found in the deepest region of the infected tuber. Besides, fungal secretions are found several cms ahead of their advancing mycelia. Oxygen requirement may account for the more superficial penetration of the nematodes.

From the observations and explanations above, it is obvious that yam decay is a disease syndrome involving microorganisms of both plant and animal origin. It is also obvious that it involves a process that can be separated into 4 main stages 'early dry rot', 'late dry rot', 'early wet rot' and 'late wet rot'. Each of these stages can be recognised by the naked eye and is

associated with definite colouration and certain microorganisms. Disease interactions in which nematodes are followed by fungi to produce an aggravated disease condition are already well documented in other plant tissues (Powell, 1963).

Carbohydrates.

Studies on determination of carbohydrate constituents of the yam tuber have been reported earlier. A number of analyses reported by Oyenuga (1955-1959), and recent observations made in Nigeria by Ketiku and Oyenuga (1970) have indicated that in the case of D. rotundata, there are two principal sugars present, sucrose and glucose and that sucrose in present in greater amounts than glucose. The results of this investigation agree with these observations. A few analytical results so far available also confirm that during storage of yams, there is metabolic breakdown of the principal component, starch, hence sweetish taste is developed, suggestive of the accumulation of sugars in the tissues. But in none of these studies was the accummulation of sugars associated with the presence of decay organisms namely, nematodes, fungi and bacteria which normally attack yams in storage. The present study goes further to show that the

interconversions of starch to soluble sugars may be influenced by the presence of nematodes and secondary invaders like fungi and bacteria which attack yams in storage. The subsequent decrease in sugar levels in the wet rotted yam when compared with the dry rot phase indicates the possible conversion and utilisation of some sugars by nematodes.

The conversion of starch to simple sugars induced by the presence of nematodes has far reaching consequences on the yam tuber. The sugar increases recorded in the nematode-infected yam tuber explains one of the ways by which nematode may pre-dispose yam to infection by secondary invaders like fungi and bacteria. This pre-disposition is done by the provision of food in form of simple sugars (monosaccharides) for the fungi which may be associated with the dry rot of yam. These simple sugars have been shown to support the growth of fungal isolates (Ogundana et al, 1970). In the presence of these sugars, the fungal spores grow actively and later invade the yam tissue. Their activities result in progressive deterioration of the entire substance of the yam tubers. The invasion by fungi and bacteria may subsequently speed up other physiological processes like respiration and

water loss. These processes can affect the storage life of the tubers adversely.

There are other ways in which the nematode-induced hydrolysis of high polymer carbohydrates to low polymer sugars in yam tuber could lead to economic losses. The low polymer sugarslike sucrose, glucose and galactose are water soluble, and therefore can be leached out of the yam tubers which are exposed to rain in the open barn. Secondly, these sugars would be lost easily during the usual processing steps like washing and boiling in water before eating, and one is finally left with more fibrous and less nourishing pieces of yam.

Qualitative and quantitative amino acids.

The free amino acids in plant may be regarded as a pool which is of great importance in nitrogen metabolism. This component constitutes usually about 70 - 80% of the non-protein nitrogen, and in some cases, these amino acids act as a reservoir from which protein may be formed. The results obtained show that, although the relative number of amino acids was not materially reduced following infection by Scutellonema bradys, qualitative differences may exist in the number of "essential" amino acids. In

this study, fewer number of "essential" amino acids was consistently detected in the infected tuber than in the uninfected. Perhaps, in the nematode-infected tubers the "essential" amino acids are utilized more by the nematode than the "non essential" ones.

From the results obtained in the protein amino acids, increases in amino acids were generally observed in the infected tubers except in the case of white yam (D. rotundata). If one believes previous calculations based upon known concentration of amino acids in larvae of M. incognita (Mcclure et al., 1973) which showed that the direct contribution of nematodes to the amino acid composition to the root was not significant (less than 0.1%), then the greater quantity of protein amino acids in the infected than in the uninfected tissues indicates a plant response to nematode infection. However, Howell et al., (1966) and Saxena (1972) have remarked that interpretation of biochemical changes in host-parasite complexes is difficult especially where obligate endoparasites are involved. Increased levels of amino acids upon infection by root-knot nematodes have been noted by Owens et al. (1966). They found that free amino acids increase very sharply in the galls of tomato.

Increased levels of amino acids have also been found in the galls of alfalfa caused by D. dipsaci (Howell et al., 1966) and in galls initiated by Longidorus africanus on grape vine roots (Epstein et al, 1971). An increase in proteins with a concomitant decrease in free amino acids has also been observed for black rot of sweet potato (Ipomea batatas L.) caused by Ceratocystis fimbriata Ellis and Halst (Uritani, 1961).

The small increase recorded in protein, and protein amino acids in the infected tubers of yellow and water yam could conceivably arise directly from a stimulation in nitrogen metabolism of the host, or from oxidation of certain phenols by the host to form quinones that combine with and thereby inactivate some proteins. Howell et al., (1966) noted that increase in levels of free amino acids in galled when compared with healthy tissues of alfalfa and pea could be due to (a) increased translocation into the areas of infection (b) increased rates of synthesis, and (c) decreased rates of translocation out of the gall and/or decreased rates of breakdown.

The results of these investigations show that the amounts of protein, protein amino acids and free amino acids may alter as a result of nematode infection, but how

this is brought about is still largely a matter of speculation. In view of the substantial contribution which may be made to protein nutrition and to pharmaceutical industries generally by amino acids in yams, it is highly desirable that much more extensive investigation on the amino acid composition of yam proteins should be undertaken.

Weight losses.

Many workers have reported that substantial weight losses in yam tubers do occur during storage. The earliest observations were made by Williams (1925) who found that the species of D. alata he used lost weight to the extent of 14.5% during about four months of storage. Gooding (1960) in Trinidad showed that variations in water loss do occur among different species and cultivars of the same species. Observations in the Caribbean area in Puerto Rico showed that visible rotting was associated with greatly enhanced losses in weight during storage (Anon, 1937). Weight losses of 7.8%, 11.0%, and 28% were recorded for healthy, slightly infected, and badly infected tubers respectively.

The results of this study are in agreement with the above findings that there is a considerable variation in

the weight loss amongst different species and between the healthy and infected tubers.

Because yams are living storage organs, basic metabolic processes like respiration and carbohydrate metabolism are going on all the time in the tuber. The process of respiration converts the carbohydrates in the yam to carbon dioxide and water, the latter being lost by evaporation. It is, therefore, reasonable to assume that there will be a correlation between the respiratory rate and weight loss during storage. It is also reasonable to assume that the presence of microorganisms like nematodes will make a definite contribution to a higher respiratory activity in the nematode-infected yam tuber and hence a higher rate of weight loss than in the healthy yam. This fact is well documented by Coursey et al; (1969) in decayed tubers infected with Botryodiplodia theobromae Pat. and Penicillium cyclopium Westling.

From the data obtained for both the means of total weight loss and the 'cumulative percentage weight loss' for the "uninfected yam tubers", it appears that by using yam barns with better ventilation and avoiding the rottening organisms like nematodes and fungi, yams can be stored for longer periods without any major loss of quality. The higher

degree of weight loss resulting in the greater loss of quality in the nematode infected tubers than in the uninfected is an indication of the relative economic importance of the yam nematode - Scutellonema bradys in yam storage in Nigeria.

Estimation of the edible portion.

The primary importance of nematodes as pests of yams is in the reduction in market value and edible portion which result from their infestation (Smit, 1967). This investigation showed that more than a quarter of the fresh weight of the nematode-infected yam tuber can be lost or rendered inedible. Figures recorded for the mean of 20 tubers were 28.1%, 26.2%, and 26.3% for infected D. rotundata, D. cayenensis and D. alata respectively. At the later stage of dry rot disease, more severe losses of the edible portion can occur. The highest peeling loss recorded for one of the species at an advanced stage was 57%.

In the "nematode free tubers", figures recorded for the mean of 20 tubers were 9%, 9% and 6.9% for D. rotundata, D. cayenensis and D. alata respectively. The differences in peeling losses between the nematode-infected and nematode free yams were found to be statistically significant

at the 5% level.

Although control measures involving the use of hot water treatment, chemical and cultural methods have been used to suppress the population of this nematode in yam tubers, further investigation on how to reduce such peeling losses are essential to assist the small scale farmers who derive their main income from yams. The cumulative amount of losses due to S. bradys represents a staggering total which the teeming masses of our under-nourished people cannot afford.

Substances discharged by S. bradys.

By a series of chromatographic analyses, five ninhydrin-positive amino acids were discharged by a mixed population (adults and larvae) of S. bradys. These were iso-leucine, leucine, aspartic acid, hydroxy inol acetic acid and phenyl alanine. Phenyl alanine appeared to be actively secreted or excreted by this nematode because it gave a rather deep purple colour with ninhydrin. Discharged substances probably represent secretory and excretory products, and those lost due to inefficiency of body function.

The synthesis and discharge of amino acids by plant-parasitic nematodes are particularly interesting because

of their possible function in the nematode and host tissues. Myers and Krusberg (1965) studied the amino acids discharged by three plant-parasitic nematodes, D. dipsaci, P. penetrans and Meloidogyne incognita. Aspartic acid, glutamic acid, serine, glycine, ornithine, threonine, alanine, methionine sulphoxide, asparagine, lysine, arginine and iso-leucine/leucine were discharged by D. dipsaci and M. incognita. P. penetrans discharged all except asparagine, lysine, arginine, and iso-leucine/ leucine. Anders (1961) suggested that tryptophan, lysine and histidine in aphid saliva were responsible for plant galls caused by these insects. Myuge (1957) suggested that ammonia discharged by D. destructor Thorne. into infected plant tissues was the major cause of tissue damage. Tryptophan - C^{14} was produced when root-knot nematodes were incubated in acetate C^{14}. The discharge of phenyl alanine in appreciable amounts by S. bradys is interesting in that this amino acid may possibly contribute to phenol metabolism in host tissues. It is, therefore, likely that the amino acids discharged by S. bradys will contribute to the physiological and biochemical changes and deterioration induced in infected

yam tissues. Identification of substances discharged by plant parasitic nematodes is therefore important in many ways. The substances released by nematodes into parasitized plant tissues will very likely contribute to symptoms of disease. Interactions between plant nematodes and other plant pathogens may also be better understood in relation to substances discharged by them.

Enzymes of S. bradys.

Nematode homogenates and the nematode infected yam tissues were tested for the activities of four enzymes, two of which were detected. Pectolytic activity was detected in the nematode homogenate and the infected yam but was not detected in the healthy yam. No cellulase activity was detected in all the extracts.

The presence of pectic enzymes in nematode homogenate and in the infected yam tissue elucidates the role of S. bradys in cell wall separation. In the past, the mechanisms by which nematodes injure host plants have been largely a matter of speculation. Myuge (1957), claimed that a protopectinase excreted by Ditylenchus allii (Beijerinck) Fil. and Sch. Stek. dissolved intercellular protopectinaceous layers and caused tissue maceration.

Supportive histological and histochemical evidence was, however, not presented. Mountain (1960) hypothesised that since pectic compounds are important structural components of the middle lamella, pectolytic enzymes might be agents of their dissolution. But there is little published evidence that pectinases are effective in destruction of the middle lamella.

One of the mechanisms of action of S. bradys as demonstrated by this study is that the nematode secretes into the yam tissues pectic enzymes responsible for breaking down the cohesiveness between cells. The pectinase detected in vitro must have been produced by the feeding activities of the nematode since healthy yam did not cause any decrease in viscosity of the pectin. The absence of cellulase activity further lends support to the argument that intracellular movement of S. bradys leads to a mechanical cell wall separation and not to a dissolution of the cellulose cell wall.

Amylase activity was demonstrated in both the nematode homogenate and in the nematode infected yam tissue. From this investigation, greater amylase activity was detected in the infected yam than in the nematode. Perhaps the nematode interferes with plant metabolism by causing a

further release of the enzyme by the plant. The detection of amylase agrees with earlier findings (Adesiyan et al., 1975) that in the nematode-infected yams some hydrolysis of starch to glucose by this enzyme takes place. Incomplete hydrolysis of starch gives maltose.

Although the presence of invertase enzyme in some plant parasitic nematodes has been demonstrated by various workers (Zinojev, 1957), its presence was not detected in this study. It is possible that this enzyme was present in the nematode but that its activity was interfered with at the pH used, or probably, activity was lost during the preparation of the extracts. There are many unknown factors in extracts that can interfere with the activity of some enzymes. However, this study illustrates the importance of pectinases and amylases in the pathogenicity of S. bradys.

Yam tissue extracts examined for other compounds.

The N.M.R. absorption at 1.0 δ is thought to be due to the aliphatic or steroidal group of compounds. This peak is shifted to about 8.2 δ in both the dry rot and wet rot yam extracts. Absorption at this peak is due to the aromatic group of compounds. The biochemical alteration

of the steroidal to aromatic compounds in the infected yams is very interesting because of the effects this might have on the use of yams in the pharmaceutical industry. There has been considerable interest in recent years on yams (Dioscorea sp.) by pharmaceutical industry overseas. Yams have been found to contain the steroid diosgenin which is easily converted to the cortisone group of drugs. Thus we propose that the absence of steroid group of compounds in the infected tubers appears to be disease-related as evidenced by its appearance in the healthy tubers. The appearance of the aromatic group of compounds in the infected tubers may probably help to establish a basis for further investigations as these groups of compounds may be involved in modifying host susceptibility.

Fungi associated with the dry rot of yams.

This preliminary study on the isolation of fungi associated with the 'dry rot' of yam gave basic information and results on which further investigations were based. Only two fungi were isolated from the dry rot portion by the 'direct plating' method and these were identified as Fusarium oxysporum and Rhizopus nigricans. Fusarium sp.

and Rhizopus nigricans caused no decay of yam under experimental conditions (Adeniji, 1970). The failure of other fungi to germinate may be due to other factors like pH of the medium, effect of toxic secretions by other organisms, or spore dormancy. When these yams were, however, transferred into humidity chambers, many fungi grew out. Aspergillus niger, Penicillium sp., Trichoderma viride were most commonly isolated. It appears from this investigation that keeping the yam pieces first in humidity chambers before plating them in agar created favourable conditions for germination.

Investigations carried out by Adeniji (1970) and Ogundana et al. (1970) showed that fungi were easily isolated from decayed portions of yams by plating in agar without necessarily keeping them first in humidity chamber. Perhaps the stage of the yam decay was important in determining the type of fungi that would be isolated.

Interrelationship.

In experiments involving interactions of two organisms of proven pathogenicity, many factors need to be considered critically, e.g.

(a) The effect of the nematode on the fungus.

(b) The effect of the fungus on the nematode.

(c) The additive effects of the two pathogenic components on their host.

Effect of nematodes on the fungus.

In these experiments, the presence of nematodes seemed to have brought about greater depth of penetration of mycelia of Penicillium sclerotigenum and Fusarium oxysporum in yam tissues than the absence of nematodes. Adeniji (1970) was able to test the pathogenicity of fungi like Aspergillus niger and Botryodiplodia theobromae on yams only after 'holes' had been made on the tubers. Ogundana (1970) also found that no fungal penetration of tubers occurred in the absence of wounds. In this study, despite the 'wounds' initially caused on the tubers before inoculating the fungi, penetration by mycelium was restricted when fungi were inoculated alone. The presence of nematodes seemed to have increased the degree of pathogenicity or infection in these two cases. But the presence of nematodes did not seem to have helped the penetration of A. niger.

All plant parasitic nematodes wound in some degree either by a simple micro-puncture or by rupturing or separating cells. These micro-punctures aid the penetration

of pathogens incapable of self establishment. Besides creating micro-punctures, the nematodes can aid in the digestion of host tissues which in this case is starch. The presence of nematodes has been shown to aid the digestion of starch to sugars. These simple sugars form a food base for the fungi; the food base reinforces the invasive potential of fungi and bacteria. Such is thought to be the way in which the yam nematode Scutellonema bradys aids in the infection of the yam tuber by secondary pathogens like Penicillium and Fusarium species.

Effect of the fungus on the nematode.

The effect of the fungus on the nematode in the storage and greenhouse experiments can be viewed from two angles:

(a) Effect on survival of the nematode and

(b) Its effect on reproduction.

Because majority of plant parasitic nematodes are obligate parasites and because they are primarily the initiator or incitant of such a complex, they are especially vulnerable to competition. The balance may shift with time, the nematode eventually becoming worse off when the competitive organisms become dominant. The nematodes

begin to disappear due to lack of food and because the environment has been made unsuitable by toxic secretions by the invading secondary organisms.

In the greenhouse experiment, the interaction between S. bradys and A. niger was disadvantageous to the nematode. The presence of the fungus seemed to have some effect on the number of nematodes that invaded the roots and tubers and subsequently on nematode development. This may be due to a possible antibiotic action of A. niger on S. bradys. The antibiotic action may have had a lethal immobilizing effect on S. bradys. Such antibiotic action of A. niger was previously reported by Mankau (1969) and Murad (1966). This incompatibility between plant parasitic nematodes and many decay promoting organisms has been reported by many workers. Davis (1962) and Davis and Jenkins (1963) noted that the presence of fungal hyphae prevented maturation of female Meloidogyne spp. James (1968) found that fungi lowered the hatch of Heterodera rostochiensis, decreased the number of cysts produced by the nematode and larval invasion of tomato roots. In some cases, where the nematode is not an obligate plant parasite, it may benefit by feeding on the mycelium (Mankau and Mankau 1963; Baker et al., 1954).

The additive effects of two pathogens on the host.

It was clear from visual observation of the tubers that association of A. niger and the nematode did not produce an aggravated condition. The difference between the weights of tubers from the inoculated pots and the aseptically grown and uninoculated ones was comparatively small, indicating that the fungus and nematodes added to the soil were not capable of independently causing a significant growth reduction.

From these investigations, it is evident that S. bradys obviously has a profound influence on the development of dry rot disease of yam. It is the primary pathogen in this complex. It is, therefore, unnecessary to use fungicidal pesticides for economic control of the disease. Although fungi may be associated with 'dry rot' of yam they are not a party to its formation. There is also some evidence from these investigations that modification of host's tissues is an integral basic component of interaction and that this may even be more important than mechanical injuries.

The role of fungus in nematode diseases has been little studied. But in this experiment, it appears fungi are secondary invading organisms which aggravate the nematode-

induced rot. They are, therefore, secondary pathogens in yam decay. Although the interaction between S. bradys and A. niger on yams is disadvantageous to S. bradys it is not sufficient for useful economic suppression of nematode in yams.

Comparative nematicidal activities of A. niger, P. sclerotigenum and F. oxysporum.

Of all the three fungi, only Aspergillus niger was found to be nematicidal at high spore concentration - 20,000 spores per ml. At this high concentration, only about 53% of the nematode population was immobilized. Fusarium and Penicillium species were not as nematicidal, even though about 25% of the nematode population was immobilized at the same spore concentration.

Many fungi are known to support the development of a large population of nematodes, but some fungus isolates sustain very limited reproduction, and others allow little or no increase in the numbers of the nematode. The possibility that such fungi produced antibiotics has been demonstrated by some workers. Mankau (1969) found that Aspergillus niger culture filtrate was nematicidal to Aphelenchus avenae Bastian at as low a concentration as

10%, whereas Fusarium sp. was not nematicidal at all concentrations. Similar studies were carried out by Murad (1966) who showed that black Aspergillus species had an antibiotic action on a nematode Polodera chitwoodi (Bassen) Dougherty and that the 'toxin' produced by this fungus had similar effects on rhabditiform nematodes. Mankau (1969) in his experiment, detected that toxic amounts of oxalic acid were produced in soil under conditions favourable for rapid or extensive growth of Aspergillus niger, and concluded that the lethal immobilizing of A. avenae by culture filtrates of A. niger may be due to toxic concentrations of oxalic acid produced by the fungus.

Host range studies.

A few workers in the past three years have investigated the survival of Scutellonema bradys in crops other than yam. Bridge (1973) reported that yam is the only good host to S. bradys and that the nematode survived endoparasitically in the roots of melon, sorghum, okra, tomato, kenaf and pigeon pea. He listed cassava, maize, hot-pepper, pineapple, pawpaw, cocoyam, oil palm, groundnut, yam bean, roselle, and rice as non-hosts. Odihirin and Ososami (unpublished data, 1974) found cowpea, and

groundnut to be "very good" hosts. Tomato, yam bean, and Eupatorium were rated as "fairly good" hosts. Celosia, roselle, maize and Tridax were among the plants listed as non-hosts.

The results of this investigation showed that beniseed, cowpea ('New Era' and 'Ife Brown' varieties) are good hosts to Scutellonema bradys because about 2-3 fold population increase was recorded in these hosts in 30 days. Small populations of S. bradys just survived endoparasitically in the roots of 17 plants including kenaf, roselle, tomato, melon, Synedrella and Eupatorium. Ten plants were rated non-hosts and these included maize, tobacco, Tridax and water leaf (Talinum triangulare).

The results obtained in some respect agree with the results obtained by Bridge (1973) and Odihirin and Ososami (1974). Roselle and yam bean were found to be fairly susceptible. However, groundnut was not found to be a host. For the first time, beniseed Sesamum indicum is being reported as a good host and tobacco as a non-host.

Because of the economic importance of S. bradys on yams,

the need to search for other crops that harbour this nematode cannot be over-emphasised. Such alternative hosts can be excluded in rotation sequence. Rotation of non-hosts like maize, tobacco or cotton with susceptible hosts may give an effective control of nematode population in the soil. Such non-hosts will be a suitable alternative to nematicides which cannot be afforded by majority of the farmers in the tropics. Even where farmers can afford nematicides, an effective crop rotation involving the use of non-hosts is also necessary so that the nematodes which escape from the nematicide are deprived of any host and thereby starved to death. However, such non-hosts must be high value crops to compensate the farmers for income derived from the sale of yams.

The high rate of reproduction of S. bradys on legumes is particularly disturbing. Equally, disturbing is the fact that some weeds like Eupatorium odoratum and Synedrella and some vegetables like jute (Corchorus olitorius L.) and soko (Celosia argentia) commonly used in mixed cropping all support populations of S. bradys. These weeds and vegetables growing in rows with highly susceptible crops like yams, cowpea and beniseed with their roots intermingled, would encourage the build-up of large nematode populations

and perhaps lead to greater damage and loss. It is, however, interesting to note that crops like tobacco, cotton, and maize did not support the reproduction of the nematode. These crops may be of value in rotations. The results reported here show that S. bradys is able to reproduce on a considerable range of plants. These crops should not therefore be used in rotation without supplementary chemical control where this nematode is a problem. Weed control and exclusion of legumes in yam plots are very important in preventing population increase and the use of non-hosts may also aid in population reduction.

Control of S. bradys by hot water treatment.

It is obvious from the results obtained in this investigation and other investigations conducted by Hawley (1956), Ayala et al; (1971) and Bridge (1973) that there is a great deal of potential in the use of hot water treatment as a means of eliminating nematodes from yam tubers. Dipping nematode-infected tubers in hot water maintained at temperatures between 50 and 60°C for 40 minutes completely suppressed nematode population. Although heating yam tubers from cold to a temperature of

50°C also significantly suppressed the nematodes, the nematodes were not entirely eliminated. Temperatures between 50 - 55°C did not adversely affect germination and palatability of water yam (D. alata), but yield was significantly reduced at 5% when compared with the control. However, nematode population in the treated was completely suppressed and this has a long term advantage over the control.

Despite the literature on the usefulness of hot water treatment in suppressing nematode populations, very little work has been done on the effect of hot water treatment on yams at different stages of storage. From this investigation, however, it was observed that of all the treatment times, only yams treated immediately after harvest rotted completely. Only a small proportion of the yam tubers treated between November and March rotted and hot water did not affect their sprouting potential. The high percentage of rotted yams recorded for those treated in October is to be expected in that the new yams are immature, the skin being soft and fragile, hot water probably penetrated deep into the tissues causing physiological damage.

Because of its ability to suppress nematode populations

in infected tubers, hot water treatment was considered useful in prolonging the storage life of nematode-infected tubers. This method is particularly successful on nematodes because they do not penetrate deep into the tubers. Hot water dip also attempts to 'clean' yam setts before planting, but the method cannot be expected to be effective if treated yam setts are planted in nematode-infected soils. It can, however, be used in combination with other control measures. It appears that from the results obtained from these investigations, hot water treatment as a means of nematode control in tubers has a great deal of potential for the future.

Chemical and cultural methods for control of S. bradys.

There had been reports on the use of organic amendments in the control of plant diseases (Walker, 1969; Morgan and Collins, 1964; Krusberg, 1961; Johnson et al., 1967). These reports indicate that there is stimulation of microbial activity or an increase in the antagonism of microorganisms in soil by the addition of organic amendments. Laan (1956) observed that organic manuring suppressed the rate of infestation and reproduction of Heterodera rostochiensis. Singh (1964) postulated that

reduction in nematode populations by organic matter added to the soil may be due to toxicity to the nematodes, interference with its respiration and alteration of the oxygen, nitrogen and pH status of the soil. Organic amendment is also thought to influence the increase of nematophagous fungi like Arthrobotrys oligospora (Fres) and Dactylaria candida (Nees) Sacc. and the mononchid types of nematodes generally.

Factors affecting the decline of plant parasitic nematodes in soil during degradation of organic material are not well understood. But it is thought that simple nitrogenous compounds which are intermediate breakdown products might be responsible for reducing nematode populations. On the other hand, with the addition of organic manure the plants are kept growing vigorously. Nutrients are released fast into the soil by the organic manure and these probably contribute to rapid tuberization. From the results obtained in this study, it appears that organic manure plays a double role of increasing yield of the tuber and suppressing the nematode.

Theoretically, the addition of fertilizers should promote healthy, well-nourished plants which are able to

withstand some kind of diseases. But in this case fertilizers as applied in this work did not seem to have any remarkable improvement on yield of water yam, but the nematode population was suppressed considerably. Heald and Burton (1968) reported that nitrogen fertilizer components are detrimental to nematodes. Results obtained from this experiment suggest that one benefit of nitrogen, phosphate and potassium fertilization might be reduction of populations of S. bradys in soil.

The use of wood ash to coat yam 'setts' before planting is a traditional practice amongst yam growers in the tropics, but there are at present no published data on its beneficial effects on yams. From this study, it was observed that tuberization was enhanced with wood ash. The reason for this might be due to supply of nutrients to the soil as was the case with organic manure. Wood ash also caused a slight suppression of nematode population.

Although application of nematicides has made it possible to reduce nematode population in soil to low levels, success in usage depends to a large extent on several factors like, dose, method of application and the type of crop. Nemagon was especially effective against S. bradys

in this experiment, but yam appears sensitive and probably susceptible to phytoxicity and did not do well with nemagon. Earlier work in the Netherlands has, however, shown that tobacco and potato are also sensitive to nemagon.

Although some degree of nematode control was achieved by the use of these treatments when compared with the control, no single treatment seemed to have achieved complete suppression of the nematode and increase in yield simultaneously. Some treatments are, however, better than others. Even though nematicides can be very effective in reducing nematode population to low levels in soil, the costs are often prohibitive and cannot be afforded by majority of yam growers in the tropics. The residue effects of nematicides in soil and food and resistance of species or biotypes to nematicides have been little studied in the tropics; whereas cultural control is inexpensive and often a valuable adjunct to the chemical control of plant pests. The results of this experiment demonstrate that there is a good deal of potential for trials with various cultural, biological, and chemical methods of control.

The effect of gamma radiation on the yam nematode.

The results of the irradiation experiments show that to achieve complete suppression of nematode population in infected tubers, a dose of 30 Krads and above which was found to have an adverse effect on the internal tissue of yams was necessary (Adesuyi and MacKenzie, 1973). These preliminary investigations also confirm their finding that sprouting of dormant tubers was suppressed at dosages between 5 and 15 Krads whereas the untreated yams sprouted luxuriantly. There was also no incidence of rot recorded in all the tubers irradiated after 3 months of storage. About 60% of the tubers in the control group rotted completely.

Earlier reports by Kahan and Gorodeiski (1966) showed that sprouting was prevented in stored potatoes between 10 and 14 Krads. In this investigation, lower doses were required than those reported for potatoes. Sparrow and Christensen (1954), Sawyer and Dallyn (1955) found that the lowest dose found to inhibit the development of embryonated eggs is the same as the dose found to inhibit completely the sprouting of potatoes. But in this investigation, this was not so. At 5 Krads when sprouting

of yams was suppressed, the nematode numbers were not reduced. Although nematode numbers were considerably reduced at high doses, this condition would be disadvantageous to the yam tubers. Irradiation at high dosages is known to decrease natural resistance of potatoes to phytopathogenic organisms (Metlitskii, 1968). So far, no dose has been found to inhibit sprouting and to suppress nematode population completely. However, doses between 10 and 15 Krads showed some promise.

CHAPTER 5

SUMMARY

1. Distribution of nematode parasites of yam tubers in the Mid-West, Nigeria.

A survey of the yam growing areas of Mid-Western Nigeria, showed that root-knot nematodes, especially Meloidogyne incognita, were as important as Scutellonema bradys in causing yam losses in the field and in storage. The root-knot species were found associated with only tubers of water yam (D. alata) in the Mid-Western State.

Reports from other countries give growing evidence of a wider geographical distribution of nematode genera associated with yam tuber decay. Scutellonema bradys is therefore not the only nematode capable of initiating decay in yam tubers. Other species like Pratylenchus coffeae (West Indies) and root-knot nematodes (Guatemala and Nigeria) may be equally important in some countries or in certain localities of the same country. Reference to S. bradys as the 'yam nematode' in older literature is therefore likely to assume only local significance in future.

Yam decay is obviously a serious problem in Mid-Western

Nigeria. Infestation of tubers ranged from 80 - 100% in yam barns and markets. All available evidence shows that nematodes have spread from farm to farm by vegetative propagation through the use of infected yam setts or seeds and also from tuber to tuber in the yam barn because of the method of storage which involves close contact of tubers.

Yam 'seeds' were found to be freer of nematode infection than yam 'setts' cut from regular tubers. They are therefore recommended for yam propagation in preference to yam 'setts'.

2. Surface sterilization of nematodes.

Four known methods of surface sterilization of nematodes were tested, namely, 0.1% streptomycin sulphate; 1 : 1 ratio of 0.1% streptomycin sulphate and 20 ppm malachite green; 20 ppm of malachite green; and 0.01% mercuric chloride. The method involving the use of 0.1% streptomycin sulphate alone appears most efficient from this investigation.

3. Studies on population build-up of S. bradys.

Studies were undertaken on the population dynamics of S. bradys at ambient temperatures and at cool temperatures ranging between (16 - 18°C). Results showed that there

were increases in nematode populations during storage at ambient temperatures. These increases occurred throughout the five month period of storage whereas at lower temperatures nematode populations only increased for the first 3 months, but thereafter the populations remained low. Sprouting was also completely inhibited at low temperatures, whereas yams stored at ambient conditions sprouted luxuriantly.

4. Depth of penetration.

The results obtained demonstrated that there are differences in the depth of penetration amongst the five cultivars of D. rotundata.

The nematodes (S. bradys) produce severe damage in the white yam, colonising mostly the first 4 mm of the periderm, even though the nematode population could be found in the periderm to a depth of between 0 - 1.5 cm.

The oldest portion, adjacent to the stems (top) contained the highest population, while fewer nematodes were recovered from the central (middle) and posterior (bottom) portions of the tuber.

5. Histopathology studies.

The histopathological studies indicate that:

The early stage of yam decay is the dry rot stage

caused by the yam nematode Scutellonema bradys in the Western State of Nigeria.

So far, there has been no anatomical evidence of fungi in dry rot sections. The nematodes are primarily responsible for providing infection sites for fungi and bacteria.

The 'wet rot' stage is essentially associated with fungal and bacterial activities. Whereas nematodes employ a slow kill at the dry rot stage, the disintegration of cells at the wet rot stage is fast. Fungi are found in the wet rot sections only, especially the chlamydospores and mycelia of Fusarium species.

Yam decay involves fungi and nematodes each playing a vital role at one stage or another in the process.

Longitudinal sections through nematode infected roots of yam tubers revealed the cortex as the favourite feeding site.

6. Carbohydrates

Rapid and reliable methods for the determination of carbohydrates are described.

The results of this study agreed with earlier observations that there is conversion of starch to soluble

simple sugars during storage, and these interconversions were influenced by the presence of decay organisms like nematodes and fungi.

The conversion of starch to simple sugars aided by the presence of nematodes helps to explain how nematodes may predispose yam to infection by other secondary organisms, e.g. fungi and bacteria.

7. Amino acids.

Detection of free amino acids in 3 species of healthy and nematode-infected tubers of Dioscorea was carried out by means of paper chromatography.

About 13 ninhydrin-positive amino acids were extracted from the uninfected white yam (D. rotundata) wereas 10 were detected in the infected. Eleven free amino acids were detected in the uninfected yellow yam (D. cayenensis) whereas only 9 were identified in the infected yam. Only 10 and 8 free amino acids were extracted in the healthy and nematode-infected tubers of D. alata respectively.

In the nematode infected yam tubers fewer essential amino acids were detected than in the healthy tubers.

Eighteen ninhydrin-positive materials were detected in the protein hydrolysate. The infected tubers of .

D. cayenensis and D. alata for the most part had more protein amino acids than did the corresponding healthy tubers.

There was no correlation between the absolute number of free amino acids in the yam tubers and the protein amino acids.

The absolute amount of each protein amino acids from both the healthy and infected tubers varied considerably within each of the yam species. However, the relative amounts varied within rather narrow limits between the healthy and the infected tubers of each species.

Except in the case of white yam (D. rotundata), percentage protein also increased in the nematode-infected tubers.

8. Weight loss in healthy and nematode-infected tubers

Considerable variation in water loss was found to occur between the infected and "uninfected yam tubers" of yellow yam (D. cayenensis) and white yam (D. rotundata). The nematode-infected yam tubers of both species were found to lose more weight than the uninfected. This loss was found to be statistically significant at 5%.

9. Measurement of edible portion of the nematode-infected tubers.

Percentage peeling losses due to dry rot recorded were 28.1; 26.2; and 26.3 for species of D. rotundata, D. cayenensis and D. alata respectively. The highest peeling loss recorded for the infected tuber was 57%. The difference in peeling losses between the healthy tubers and nematode infected tubers was statistically significant.

10. Substances discharged by Scutellonema bradys.

Amino acids emitted and extracted from surface-sterilized larvae and adults of S. bradys were identified by paper chromatography. Five amino acids identified were aspartic acid, phenylalanine, isoleucine, leucine and hydroxyinol acetic acid.

In addition to the amino acids discharged by S. bradys, the presence of pectolytic and amylase enzymes was demonstrated in aqueous extracts of a population of mono-axenic cultures of S. bradys isolalated from nematode-infected yam. In viscometric tests, pectin was degraded at pH 6, and amylase was detected spectrophotometrically at pH 6.9. The pectolytic enzymes are also produced in vitro by the nematode, and amylase activities

were not detected in healthy yam extracts and sodium chloride. However, the difficulty in obtaining sufficient quantity of nematodes for enzyme experiments can severely limit the type and number of enzymes detected.

Yams showing symptoms of dry rot and wet rot were examined with N.M.R. Spectrometer for other classes of compounds. The conversion of steroid compounds in the healthy yam to aromatic compounds (i.e. substances having fragrant odours) in both the dry and wet rot yams was revealed. This conversion is probably disease related.

11. Host range studies.

From green house experiment involving 30 test plants, 20 were found to be 'hosts' to Scutellonema bradys. Beniseed and cowpea were regarded as good hosts. Eupatorium, Synedrella, yam bean (Sphenostylis stenocorpa) are among the hosts. Tobacco, maize and cotton are non-hosts.

12. Fungi associated with the dry-rot of yam tubers.

The direct plating methods of diseased portions of yam tissues in agar (P.D.A.) supported the growth of two fungi, namely, Fusarium oxysporum and Rhizopus nigricans, even though plenty of mycelial growth was observed on most plates. The keeping of yams in humidity chambers before plating them on agar showed the growth of more

fungi like Aspergillus niger, Aspergillus sp., Penicillum sclerotigenum, Trichoderma viride, and Botryodiplodia theobromae.

13. Interrelationships.

Results from this investigation showed that S. bradys obviously has a profound influence on the development of 'dry rot' disease of yams. It is, in fact, the primary pathogen in this complex.

Although fungi may be associated with 'dry rot' disease, they are not responsible for its formation.

Association of Aspergillus niger and nematode (S. bradys) did not produce any aggravated condition in the disease complex because the fungus inhibited nematode reproduction.

The presence of the fungus A. niger seemed to have some anti-nematode effect and to have some adverse effects on the numbers of nematodes that invaded the roots and tubers and subsequently on nematode development.

14. Control of S. bradys by hot water treatment.

Observations on the use of hot water treatment as a means of controlling yam nematodes revealed that dipping nematode-infected tubers of water yam D. alata and

D. cayenensis in hot water at 50 - 55°C for 40 minutes completely suppressed the nematode.

Germination, growth, storage life, yield, and palatability of tubers of D. alata so treated were not adversely affected. The best time to use hot water starts as from two months after harvest, that is during the post-dormancy period.

15. Some cultural and chemical methods of control of S. bradys.

Five different methods of nematode control were employed. Treatment involving organic manuring gave the highest yield of yam tubers, and considerably reduced nematode population. Application of nemagon was effective in controlling S. bradys but it reduced the yield of water yam probably due to some degree of phytoxicity. Application of D-D was ineffective as it neither increased yield nor reduced nematode population. Application of wood ash increased the yield of water yam while NPK fertilizer reduced nematode population.

16. Control of S. bradys by gamma irradiation.

About 70 - 80% reduction of nematode population was achieved at 15 Krads and above. In order to achieve

complete suppression of nematode population in infected tubers, a dose of 30 Krads and above was required, but this led to rotting of tubers. Complete suppression of sprouting was achieved as from 5 Krads.

REFERENCES

Acosta, N. (1974). Depth of penetration of phytoparasitic nematodes in yam tubers. Nematropica 4(1) p.7 - 11.

Adeniji, M.O. (1970). Fungi associated with storage decay of yam in Nigeria. Phytopathology 60(4) p.590 - 592.

Adesiyan, S.O., R.A. Odihirin and M.O. Adeniji (1974). Histopathology studies of the yam tuber (Dioscorea rotundata) infected by Scutellonema bradys Steiner and LeHew. Int. Biodetn. Bull. (In press).

_____ (1975). Economic losses caused by the yam nematode Scutellonema bradys in Nigeria. Pl. Dis. Reptr. 59(6) p.477 - 480.

_____ (1975). Changes in carbohydrate constituents induced in the yam tuber (Dioscorea rotundata Poir) by a plant parasitic nematode, Scutellonema bradys. Int. Biodetn. Bull. (In press).

_____ (1975). Distribution of nematode parasites of yam tubers in Mid-West State, Nigeria. Niger. Pl. Protect. J. (In press).

Adesuyi, S.A. and J.A. MacKenzie (1973). The inhibition of sprouting in stored yams (Dioscorea rotundata) by gamma radiation and chemicals. I.A.E.A. S.M.-166/35 p.127 - 136.

Aist, S. and R.D. Riggs (1969). Amino acids from Heterodera glycines. J. Nematol. 1(3) p.254 - 259.

Allard, H.A. (1945). Some behaviour of the yams (Dioscorea) of the family Dioscoreaceae. Castanea 10(1) p.8 - 13.

Anders, F. (1961). Untersuchungen uber das cacidogen, Princip der Reblaus (Vurus vitefolii Shimer). III. Biochemische Untersuchungen uber das galleninduzierende. Agens. Biol. Zentralb. 80: 199 - 233.

Andrassy, I. (1958). Hoplolaimus tylenchiformis. Daday 1905 (Syn. H. coronatus Cobb 1923) und die Gattungen der Unterfamilie Hoplolaminae Filipjev 1931. Nematologica 3(1), p. 44 - 56.

Anon (1937). U.S.D.A. Annual report for 1936 of the Puerto Rico Experiment. Station. p. 40 - 42.

____ (1938). U.S.D.A. Annual report for 1937 of the Puerto Rico Experiment. Station p. 42 - 49.

____ (1952). Dioscorea. Wealth of India. Raw Mater. 3 p. 67 - 76. C.S.I.R. New Delhi.

Anon (1959). Compte Economique Togo - Etudes economiques Quest Africaines, No. 3 Bangue centrals des Etats de l'Afrique de l'Ouest.

____ (1961). Note in A. Rep. Minist. Agric. Eastern Nigeria 1960 - 61.

____ (1962). Annual report for 1961 - 62 of the Research Division, Ministry of Agriculture and Natural Resources, Western Nigeria.

____ (1964). Note in A. Rep. Minist. Agric., Nigeria 1962-63.

Atkinson, C.F. (1892). Some diseases of cotton. Ala. Polytech. Inst. Agric. Expt. Sta. Bull. 41 p.61-65.

Ayala, A. and N. Acosta (1971). Observations on yam (Dioscorea alata) nematodes (Abstr.). Nematropica 1: 39 - 40.

Baker, A.D., G.L. Brown and A.B. James (1954). Relationship of fungi, mites and the potato rot nematode. Science 119: 92 - 93.

Baudin, R.H. (1956). Maladies parasitaires designames en Cote d'Ivoire. Revue Mycol. 21: 87 - 111.

Bernfeld, P. (1955). Amylases, and . In S.P. Colowick and N.O. Kaplan, ed. Methods in enzymology. Vol. 1. Academic Press, New York. p. 149 - 158.

Bray, F. (1958). A study of yam farming in North Mampong, Ashanti. Mimographed report of the Faculty of Agriculture. University of Ghana.

Bridge, J. (1972). Nematode problems with yams Dioscorea spp. in Nigeria. PANS 18: 89 - 91.

_____ (1973). U.K. O.D.A. sponsored plant Nematological Research in Nigeria. March 1971 - November 1972.

Brown, D.H. (1931). The cultivation of yams. Trop. Agric., Trin. 8(8) 201-6; 8(9) 231 -6.

Brownell, L.E., J.V. Nehemias, and J.J. Bulmer (1954). Designs for potato irradiation facilities. Univ. of Michigan, Engineering Research Institute Report No. 1943: 7 - 23 - p.37p.

Bruhn, C. and K. Wolfgang (1962). Control of root-knot nematodes in Dioscorea tubers (Abstr.). Phytopathology 53: 24.

Burk, E.F., and G. Tennyson (1941). Hot water treatment for control of nematodes in sweet potato seed roots. Proc. Am. Soc. hort. Sci. p.299 - 302.

Burkill, I.H. (1951). The rise and decline of the greater yam in service of man. Advmt. Sci., Lond. 7(28), p.443 - 8.

Burkill, I.H. (1960). The organography and evolution of Dioscoreaceae the family of the yam. J. Linn. Soc. 56: 319 - 412.

Campbell, J.S., V.O. Chukwueke, F.A. Teriba, and H.U.S. Ho-A-Shu (1962). Some physiological experiments with the white Lisbon yam (Dioscorea alata L.). The breakage of the rest period in tubers by chemical means. Emp. J. exp. Agric. 30(118), 108 - 14.

Caveness, F.E. (1961). Report on nematology project, U.S.A.I.D. Project 61-13-050. Ministry of Agriculture and Natural Resources, Ibadan, Nigeria.

_____ (1965). Report on nematology project, U.S.A.I.D. Project 620-11-110-050. Ministry of Agriculture and Natural Resources, Ibadan, Nigeria.

_____ (1965). Controlling nematodes in the Nigerian garden - In Nematology Studies 1960-65. End of Progress Report on the nematology project. Project 620-11-110-050.

_____ (1965). Shadehouse host ranges of some Nigerian nematodes. Pl. Dis. Reptr. 51 No. 1. pp.33-37

Chitwood, B.G. and M.B. Chitwood (1950). An introduction to nematology (revised edition) Monumental Printing Company, Baltimore Md. 213pp.

Christie, J.R. (1959). Plant nematodes. Their Bionomics and Control. Department of Entomology Agricultural Experiment Station, University of Florida, U.S.A.

Clemente, (1918). A study of Dioscorea with starch determinations and cooking tests. Philipp. Agric. 6(8): 230 - 246.

Correll, D.S., B.G. Schubert, H.S. Gentry, and W.O. Hawley (1955). The search for plant precursors of cortisone. Econ. Bot. 9: 305 - 375.

Coursey, D.G. (1961). The magnitude and origins of storage losses in Nigerian yams. S. Sc. Food. Agric. 12(8) 574 - 580.

_____ (1963). Storage losses in yams. Niger. Grower and Producer 2(3): 43 -5.

_____, L.E. Fellows, and C.B. Coulsen (1966). Respiration in yam tuber tissue. Nature, London. 210 (5042), 1292-3.

_____ (1967). Yams. Longmans, Green and Co. Ltd.

_____ and J.D. Russull (1969). A note on endogenous and biodeteriorative factors in the respiration of dormant yam tubers. Int. Biodetn. Bull. 5(1) 27 - 30.

Dade, H.A. and J. Wright (1931). Note in Bulletin No. 23 (Year book 1930). Dep. Agric. Gold Coast p.248 - 251.

Dallimore, C.E. (1955). Methods of increasing the efficacy of soil fumigation in experimental fields. Pl. Dis. Reptr. 39(6): 511 - 514.

Davis, R.A. (1962). Interactions of nematodes and pea (Pisum sativum L.) diseases. Ph.D. Thesis, Rutgers University. (Abstract in Dissertation abstracts, 24 p. 2646).

Davis, R.A., .and W.R. Jenkins (1963). Effects of Meloidogyne spp. and Tylenchorynchus claytoni on pea wilt incited by Fusarium oxysporum f. pisi race 1 (Abstract) Phytopathology 53: 745.

Dharkar, S.D. (1966). Radiation preservation of some fruits and vegetable. Application of Food Irradiation in Developing Countries. Technical Reports Series No. 54 I.A.E.A. p. 115 - 22.

Dixon, W.B. (1961). Nematological Investigations 1958 - 1961. Minist. Agric. Lands Jamaica Bull. 59 (N.S.).

Dixon, M. and E.C. Webb (1964). Enzymes. Academic Press, New York. 950 p.

Dolliver, J.S., A.C. Hildebrandt and Riker (1962). Studies of reproduction of Aphelenchoides ritzemabosi (Schwartz) on plant tissues in culture. Nematologica, 7: 294 - 300.

Dropkin, V.H. (1955). The relations between nematodes and plants. Expl. Parasit. 4: 282 - 322.

DuBois, M., K. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith (1956). Colorimetric method for the determination of sugars and related substances. Analyt. Chem. 28: 350-356.

Epstein, E. and E. Cohn (1971). Biochemical changes in terminal root galls caused by an ectoparasitic nematode, Longidorus africanus Amino acids. J. Nematol. 3: 334-340.

Esau, K. (1953). Plant Anatomy. John Wiley and Sons, New York.

F.A.O. (1966). World Crop Statistics, 1948 - 64.

Fassuliotis, G. and A.H. Sparrow (1955). Preliminary report of X-ray studies on the golden nematode. Pl. Dis. Reptr. 39(7) p.572.

Federal Office of Statistics, Lagos, Nigeria (1973). Rural Economic Survey of Nigeria.

(i) Rural Consumption Enquiry of Food Items 1963- 1966.

(ii) Consolidated Results of Crop Estimation Surveys 1965 - 1971.

Filipjev, I.N. (1936). 'On the classification of the Tylenchinae' Proc. helminth. Soc., Wash., 3(1), 80-2.

Goodey, T. (1935). 'Observations on a nematode disease of yams'. J. Helminth. 13(3), 173 - 190.

_____ (1963). Soil and Freshwater Nematodes. Methuen and Co. Ltd., London. John Wiley and Sons Inc., New York.

Gooding, H.J. (1960). West Indian Dioscorea alata cultivars. Trop. Agric., Trin., 37: 11 - 30.

Hawley, W.O. (1956). Hotwater treatment for the control of root knot nematodes on Dioscorea floribunda. Pl. Dis. Reptr. 40: 1045 - 1046.

Hayward, L.A.W. and H.M. Walker (1962). Yam storage in Nigeria. CCTA/FAO Symp. Stored Food Freetown.

Heald, C.M. and G.W. Burton (1968). Effect of organic and inorganic nitrogen on nematode populations of turf. Pl. Dis. Reptr. 52: 46 - 48.

Hollis, J.P. (1963). Action of plant parasitic nematodes on their hosts. Nematologica 9 p.475 - 494.

Howell, P.K. and L.R. Krusberg (1966). Changes in concentrations of nitrogen and free and bound amino acids in alfalfa and pea infected by Ditylenchus dipsaci. Phytopathology 56: 1170 - 1177.

Hunter, T. and T.V. Danso (1931). Food farming in the Ejura district. Bulletin No. 23 (Year book 1930). Gold Coast Depart. Agric.

James, G.L. (1968). The interrelationships of the causal fungus of brown root rot of tomatoes and potato root eelworm. Heterodera rostochiensis Woll. Ann. Appl. Biol. 61: 503 - 510.

Jenkins, W.R. and G.W. Bird (1962). Nematodes associated with wild yam Dioscorea spp., with special reference to the pathogenicity of Meloidogyne incognita. Pl. Dis. Reptr., 46(12): 858 - 860.

Johnson, L.F., A.Y. Chambers and H.E. Reed (1967). Reduction of root knot of tomatoes with crop residue amendments in field experiments. Pl. Dis. Reptr. 51: 219 - 222.

Kahan, R.S. and N. Temkin-Gorodeiski (1968). Storage tests and sprouting control on up-to-date variety of potatoes and on an experimental onion, variety (Beit Alpha) Preservation of fruit and vegetable by radiation (Proc. Panel Vienna, 1966). IAEA Vienna, p.29 - 37.

Ketiku, A.O. and V.A. Oyenuga (1960). Preliminary report on the carbohydrate constituents of cassava root and yam tuber. Nigerian J. Sc. 4(1) p.25 - 30.

Krusberg, L.R. and L.W. Nielsen (1958). Pathogenesis of root knot nematodes to the Puerto Rico variety of sweet potato. Phytopathology 48: 30 - 39.

_____ (1960). Hydrolytic and respiratory enzymes of the species of Ditylenchus and Pratylenchus. Phytopathology 50: 9 - 22

Krusberg, L.R. (1961). Studies on the culturing and parasitism of plant parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissues. Nematologica 6: 181 - 200.

_____ (1967). Pectinases in Ditylenchus dipsaci. Nematologica 13: 443 - 451.

Laan, P.A. van der (1956). The influence of organic manuring on the development of potato root eelworm, Heterodera rostochiensis. Nematologica 2: 112 - 125.

Lawton, J.R.S. and L.R. Lawton (1967). The morphology of the dormant embryo and young seedlings of five species of Dioscorea from Nigeria. Proc. Linn. Soc. Lond. 178(2): 153 - 159.

Levinson, H.S. and E.T. Reese (1950). Enzymatic hydrolysis of soluble cellulose derivative as measured by changes in viscosity. Expl. Parasit. 6: 245 - 260.

Linford, M.B., F. Yap and J.M. Oliveira (1938). Reduction in soil populations of the root-knot nematodes during decomposition of organic matter. Soil Sci. 45: 127-140.

Linnaeus, C. (1737). Genera Plantarum, Wisshoss, Leyden.

Lordello, L.G.E. (1959). A nematosis of yams in Pernambuco, Brazil caused by a new species of the genus Scutellonema. Rev. bras. Biol. 19: 35 - 41.

Luc, M. and G. de Guiran (1961). Les nematodes associes aux plantes de l'Ouest Africain. Liste preliminaire. Agron. trop., 15: 434 - 449.

Mai, W.F. and B.F. Lownsbery (1948). Studies on the host range of the golden nematode of potato Heterodera rostochiensis Woll. Am. Potato J. 25: 290 - 294.

de Man, J.G. (1880). Die Einheimischen, Frei in der reinen und im Sussen Wasser lebenden Namatoden. Vorlaufiger Bericht und descriptivsystematischer Theil. Tijdschr. Ned. Dierk. Vereen., 5 p.1 - 104.

Mankau, R. and S.K. Mankau (1963). The relationships of Aphelenchus avenae to phytopathogenic soil fungi. In Doeksen, J. and Drift. J. van der (Editors) Soil organisms. North Holland Publishing Co., p.271 - 280.

_____ (1969). Nematicidal activity of Aspergillus niger. Phytopathology 59(8): p. 1170.

Martin, F.W. and S. Ortiz (1963). Origin and anatomy of tubers of Dioscorea floribunda and D. spiculiflora. Bot. Gaz. 124: 416.

Martin, F.W. and M.H. Gaskins (1968). Cultivation of the sapogenin bearing Dioscorea species. Prod. Res. Rep. U.S. Dep. Agric. No. 23.

Mcclure, M.A. and J. Robertson (1973). Infection of cotton seedlings by Meloidogyne incognita and a method of producing uniformly infected root segments. Nematologica 19: 428 - 434.

McCready, R.N., J. Guggiz, V. Silviera and H.S. Owens (1950). Determination of starch and amylase in vegetables. Analyt. Chem. 22: 1156 - 1158.

Metlitskii, L.V. (1968). Irradiation of potatoes and other vegetables to prevent sprouting. Status of the food irradiation program. U.S. Govt. Printing Office, Washington 591 - 595.

Moore, S. and W.H. Stein (1951). In Textbook of Biochemistry (Edit. West/Todd/Mason/Van Bruggen). 4th Edition 1968. The MacMillan Company - New York. London.

Morgan, G.T. and W.B. Collins (1964). The effect of organic treatments and crop rotation on soil populations of Pratylenchus penetrans in strawberry culture. Can. J. Pl. Sci. 44: 272 - 275.

Mountain, W.B. (1955). A method for culturing plant parasitic nematodes under sterile conditions. Proc. helminth. Soc. Wash., 22: 49 - 52.

Murad, J.L. (1966). Possible antibiotic action of Aspergillus sp against a nematode Pelodera chitwoodi. Tex. J. Sci. 18: 89 - 90.

Murdock, G.P. (1959). Africa. Its peoples and their cultural history. McGraw-Hill, New York.

Myers, R.F. and L.R. Krusberg (1965). Organic substances discharged by plant parasitic nematodes. Phytopathology 55: 429 - 437.

_____ (1965). Amylase, cellulase, invertase and pectinase in several free living, mycophagus and plant parasitic nematodes. Nematclogica 11: 441 - 448.

_____ (1967). Axenic cultivation of Aphelenchoides sacchari Hooper. Proc. helminth. Soc. Wash., 34: 251 - 255.

Myuge, S.G. (1956). The physiology of the nutrition of gall nematodes. C.R. Akad. Sci. U.R.S.S. 108: 164 - 165.

_____ (1957). Contributions to the trophic character of the potato stem nematode. (In Russian). Akad. Nauk. SSSR Isv., Spr. Biol. 3: 357 - 59.

National Research Council (1968). Principles of plant and animal pest control. Vol. 4. National Academy of Sciences. Washington, D.C.

Njoku, E. (1963). The propagation of yams (Dioscorea spp.) by vine cuttings. Jl. W. Afr. Sci. Ass., 8(1): 29-32.

Odihirin, R.A. (1964). Host parasite relationships of garden balsam (Impatiens balsamina L.) and certain nematodes. M.S. Thesis, Rutgers State University, New Brunswick, New Jersey.

_____ and O. Ososami (1974). Grain legumes as alternative hosts of the yam nematode Scutellonema bradys. Nigerian Agric. J. (In press).

Ogundana, S.K., S.H.Z. Naqvi and J.A. Ekundayo (1970). Fungi associated with soft rot of yams (Dioscorea sp.) in storage in Nigeria. Trans. Bri. mycol. Soc. 54(3): 445 - 451.

_____ (1971). Studies on soft rot of yams in storage. Trans. Br. mycol. Soc. 56(1): 73 - 80.

Okafor, N. (1966). Microbial rotting of stored yams (Dioscorea spp.) in Nigeria. Expl. Agric. 2: 179 - 182.

Oke, O.L. (1973). Changes in soluble amino acids of some tropical starchy roots during chilling. Paper presented at the 3rd International Symposium on Tropical Root Crops. I.I.T.A., Ibadan, Nigeria.

Okiy, G.E.O. (1960). Indigenous Nigerian food plants. Jl. W. Afr. Sci. Ass., 6(2): 117 - 121.

Owens, R.O. and H.N. Specht (1966). Biochemical alterations induced in host tissues by root-knot nematodes. Contr. Boyce Thomson Inst. 23: 181 - 198.

Oyenuga, V.A. (1968). Nigerian food and feeding stuffs; their chemistry and nutritive value. 3rd ed. Ibadan University Press.

Paterson, D.D. (1942). Cultivation costs and labour requirements for crops grown on the College farm. Trop. Agric., Trin., 19(6): 104 - 115.

Peachey, J.E. (Edit.) (1969). Nematodes of Tropical Crops. Tech. Commun. Commonw. Bur. Helminth. No. 40.

Peacock, F.C. (1959). The development of a technique for studying the host parasite relationship of the root-knot nematode Meloidogyne incognita under controlled conditions. Nematologica 4: 43 - 55.

Phillips, T.A. (1964). An agricultural notebook with special reference to Nigeria. Longmans.

Pitcher, R.S. (1963). Role of plant parasitic nematodes in bacterial diseases. Phytopathology 53: 35 - 39.

_____ (1965). Interrelationships of nematodes and other pathogens of plants. Helminth. Abstr. 34: Part 1 p. 1 - 17.

Powell, N.T. (1963). The role of plant parasitic nematodes in fungus diseases. Phytopathology 53: 28 - 35.

Queva, C. (1894). Recherches sur l'anatomie de l'appareil vegetatif des Taccacees et des Dioscories. Mem. Soc. Sci. Agric., Lille, Ser. 4, 20(1): 1 - 457.

Roth, H. and H. Richardson (1965). J. Econ. Ent. 58: 1086.

Sasser, J.N. and W.R. Jenkins (Edit.) (1960). Nematology: Fundamentals and Recent Advances with emphasis on Plant Parasitic and Soil Forms. Univ. of North Carolina Press, Chapel Hill, N. Carolina, U.S.A.

Sawyer, R.L. and S.L. Dallyn (1955). The effect of gamma irradiation on storage life of potatoes. Am. Potato J. 32: 141 - 143.

Saxena, A.P. (1972). Studies on free amino acids content in Meloidogyne javanica Treub and in root-knots of jute plant. Z. Parasitenkd 40: 101 - 105.

Schieber, E. (1961). Parasitic nematodes on Dioscorea in Guatemala. Pl. Dis. Reptr., 45: 425.

_____ and D.K. Lassmann (1961). Root-knot nematode on Dioscorea in Guatemala. Pl. Dis. Reptr., 45: 981.

Sher, S.A. (1963). Revision of the Hoplolaiminae (Nematoda). III. Scutellonema Andrassy, 1958. Nematologica 9: 421 - 443.

Singh, R.S. (1964). Organic matter and biological control of plant parasitic nematodes. Uttar Pradesh Agricultural University Magazine Year 1963-64 p.58 - 64.

Smit, J.J. (1967). In Yams (Ed. Coursey, D.G.), London: Longmans. p. 115 - 20.

Smith, L. and C. Ellenby (1967). Some biochemical changes in the contents of the cyst of Heterodera rostochiensis during its maturation. Nematologica, 13: 395 - 405.

Sobulo, R.A. (1972). Studies on white yam (Dioscorea rotundata). Expl. Agric. 8: 99 - 106.

Southey, J.F. (Edit.) (1965). Plant Nematology. Tech. Bull. Minist. Agric., Lond., No. 7.

_____ (1970). Laboratory methods for work with plant and soil nematodes. Tech. Bull. Minist. Agric., Fish. Fd., Harpenden. No. 2.

Sparrow, A.H. and E. Christensen (1954). Improved storage quality of potato tubers after exposure to C^{60} gammas. Nucleonics 12(8): 16 - 17.

Stamp, L.D. (1960). Our Developing World. Faber and Faber.

Steiner, G. (1931a). Tylenchus pratensis parasitising yams (Dioscorea sp.) from West Africa. Pl. Dis. Reptr., 15: 21.

_____ (1931b). A nematosis of yams caused by a new species of Hoplolaimus. Pl. Dis. Reptr. 15: 121.

_____ and R.R. LeHew (1933). Hoplolaimus bradys, n.sp. (Tylenchidae, Nematoda), the cause of a disease of yam (Dioscorea sp.). Zool. Anz. 101(9/10): 260 - 264.

_____ and E.M. Buhrer (1934a). Observations of interest on nematode diseases of plants. Pl. Dis. Reptr., 18(7), 100.

_____ (1934b). Disease symptoms produced by Anguillulina pratensis in yams. Phytopathology 24: 164 - 5.

_____ (1937a). Observations on the yam nematode (Rotylenchus bradys) (Steiner and LeHew, 1933). Filipjer, (1936). Phytopathology 27: 865 - 7.

Sumner, J.B. and F. Somers (1953). Chemistry and methods of enzymes. 3rd Edition. N.Y. Academic Press (revised and enlarged).

Taylor, A.L. (1952). Infection of tomato by Heterodera from tobacco soil. Pl. Dis. Reptr. 36: 54.

Taylor, T.A. (1964a). Studies on the Nigerian yam beetles. Part 1. Jl. W. Afr. Sci. Ass., 8(2): 180 - 9.

_____ (1972a). The concept of Agricultural Biology in Tropical Agriculture. Seminar paper, International Rice Research Institute, Los Banos, Phillipines, Sept. 1972.

Thompson, A.K., B.O. Been, and C. Perkins (1973). Nematodes in stored yams. Expl. Agric. 9: 281 - 286.

Thorne, G. (1961). Principles of Nematology. McGraw-Hill Book Co., New York, Toronto and London.

Tisdale, W.B. (1931). Development of strains of cigar wrapper tobacco resistant to black shank (Phytophthora nicotianae Breda de Haan). Univ. Fla Agric. Expt. Sta. Bull. 226. 45.

Tracey, M.V. (1958). Cellulases and chitinase in plant nematodes Nematologica 3: 179 - 183.

Triffitt, M.J. (1929). On the occurrence and significance of H. schachtii infecting certain weeds. J. Helminth. 7: 215 - 222.

Udeaja, P.E. (1961). Note in Ann. Rep., Federal Dept. of Agricultural Research, Nigeria, for 1959-60.

Unny, K.L. and M.L. Jerath (1965). Parasitic nematodes on Dioscorea spp. in Eastern Nigeria. Pl. Dis. Reptr. 49: 875 - 876.

Uritani, I. (1961). The role of plant phenolics in disease resistance and immunity. p. 98 - 124. In Symposium on Biochemistry of plant Phenolic Substances. Colo. State Univ., Fort Collins. 169p.

Waitt, A.W. (1961). Note in Annual Report for 1959-60 of the Federal Department of Agricultural Research, Nigeria.

Walker, J.T. (1969). Pratylenchus penetrans (Cobb) population as influenced by microorganisms and soil amendments. J. Nematol. 1(3) p. 260 - 264.

Wallace, H.R. (1963). The Biology of Plant Parasitic nematodes. Edward Arnold Ltd., London.

West, J. (1934). Dry rot of yams. Bull. imp. Inst., Lond. 32: 449 - 450.

Whitehead, A.G. and J.R. Hemming (1963). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55: 25 - 38.

Williams, R.D. (1925). West Indian Yams. Bull. Dep. Agric. Trin. and Tobago. 21(1): 1 - 26.

Williams, V.R., W.T. Wu., H.Y. Tsai, and H.G. Bates (1958). J. Agr. Fd. Chem. 6: 47 - 48.

Yamamoto, K., Yoshitani and M. Maeda (1955). Studies on the Penicillium and Fusarium rots of Chinese Yam and their control. Scient. Rep. Hyogo Univ. Agric. 2: 69 - 79.

Young, R.A. (1923). Cultivation of the true yams in the Gulf Region U.S.D.A. Bull No. 1167.

Zinorger, V.G. (1957). Enzymatic activity of the nematodes parasitizing plants. (In Russian). Zool. Zh. 36: 617 - 620. English summary.

Zuckerman, B.M. and M.W. Brzeski (1966). Methods for the study of plant parasitic nematodes in gnotobiotic root culture. Nematologica 11: 453 - 466.

APPENDIX AI

STATISTICAL ANALYSES OF DATA

(1) Effect of hot water treatment on the yield of water yam (D. alata)

(COMPLETE RANDOMIZED BLOCK DESIGN)

B L O C K S

Treatments	I	II	III	IV	Treatment totals	Treatment means
A	2.4	3.3	6.85	3.95	16.5	4.1
B	8.0	3.675	7.45	5.95	25.075	6.3
D	9.1	10.35	9.3	7.2	35.95	9.0
Block totals	19.5	17.325	23.6	17.1	77.525	-

N.B. Wts. in kilo grams.

ANALYSIS OF VARIANCE

Sources of variation	d.f.	s.s.	m.s.	F.
Between treatments	2	47.51	23.76	7.71*
Between blocks	3	9.03	3.01	0.98
Erro	6	18.46	3.08	
Total	11	75		

From tables	0.05	0.01
Between treatments	5.14	10.92
Between blocks	4.76	9.78

COMPARISON OF TREATMENT MEANS USING DUNCAN'S MULTIPLE RANGE TEST

Treatments	Mean yield/tuber (kg)
D. i.e. Control	9.0 a
B i.e. 50 - 55°C for 40 minutes	6.3 b
A i.e. heat to 50°C from cold	4.1 b

Means followed by the same letter are not significantly different at the 5% level.

APPENDIX 2

YIELD OF WATER YAM (D. ALATA) AS AFFECTED BY CULTURAL AND CHEMICAL METHODS OF NEMATODE CONTROL

COMPLETE RANDOMIZED DESIGN

	REPLICATES WTS. IN KG																				Treatment totals	Treatment means
Treatments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	( )	( )
1	2.75	0.8	1.60	2.80	2.75	1.50	1.75	3.1	0.75	0.60	1.20	0.75	1.60	1.75	2.25	1.60	1.80	2.20	1.75	3.60	36.90	1.85
2	3.75	0.8	1.50	1.50	0	1.20	2.0	3.75	4.75	2.75	2.80	3.50	3.25	2.60	3.10	3.70	3.25	2.20	3.40	3.60	53.40	2.67
3	2.5	2.25	3.60	1.0	1.50	1.40	4.25	2.0	4.6	0.75	4.0	4.5	3.40	3.0	0.60	1.25	0.60	1.0	2.0	0	41.70	2.09
4	0.75	0.50	0	0.40	0.75	1.0	1.40	2.0	0.1	1.5	0.25	0.75	3.5	0.25	1.40	1.20	1.25	1.40	0.20	0	18.60	0.93
5	0.80	0	0.75	4.75	1.20	0	0.25	2.75	1.50	1.50	1.60	2.80	3.75	2.20	0	1.5	2.75	1.50	1.80	1.50	33.30	1.67
6	3.25	0.75	0.75	0.50	2.40	2.30	1.50	2.20	2.20	1.45	3.25	1.20	1.50	3.0	3.0	3.75	1.75	2.0	0.60	1.75	39.10	1.96
Totals																					223.00	-

ANALYSIS OF VARIANCE

COMPLETE RANDOMIZED DESIGN

Source of variation	d.f.	s.s.	m.s.	F	Required F.		
					0.05	0.01	0.001
Treatment	5	32.37	6.67	5.17***	2.29	3.17	4.42
Error	114	147.37	1.29				
Total	119	179.74					

COMPARISON OF TREATMENT MEANS USING DUNCAN'S MULTIPLE RANGE TEST

Treatments	Mean yield per tuber* (kg)
2	2.67 a
3	2.09 ab
6	1.96 ab
1	1.85 b
5	1.67 b
4	0.93

*Means followed by the same letter are not significantly different at the 5% level.

APPENDIX B

PUBLICATIONS

1. Adesiyan, S.O., R.A. Odihirin and M.O. Adeniji (1974). Histopathology studies of the yam tuber (Dioscorea rotundata) infected by Scutellonema bradys Steiner & LeHew. Int. Biodetn. Bull. (In press).

2. ———————————————— (1975). Economic losses caused by the yam nematode Scutellonema bradys in Nigeria. Pl. Dis. Reptr. 59(6) p.477 - 480.

3. ———————————————— (1975). Changes in carbohydrate constituents induced in the yam tuber (Dioscorea rotundata Poir) by a plant parasitic nematode, Scutellonema bradys. Int. Biodetn. Bull. (In press).

4. ———————————————— (1975). Distribution of nematode parasities of yam tubers in Mid-West State, Nigeria. Niger. Pl. Protect. J. (In press).