STUDIES ON THE FALSE SMUT OF RICE, *ORYZA SATIVA* L., INDUCED BY *USTILAGINOIDEA VIRENS* (CKE.) TAK.

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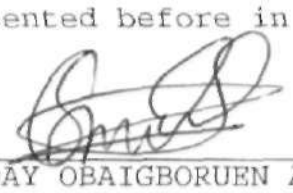
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DECLARATION

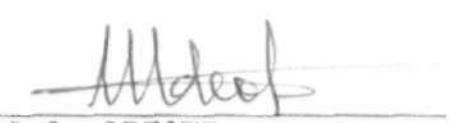
I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any application for higher degree.



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CERTIFICATION

This thesis entitled "STUDIES ON THE FALSE SMUT OF RICE, ORYZA SATIVA L. INDUCED BY USTILAGINOIDEA VIRENS (CKE.) TAK." by Monday Obaigboruen Ahonsi meets the regulations governing the award of the degree of MASTER OF SCIENCE, Crop Protection, of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is affectionately dedicated to my parents, Napoleon and Margaret, my senior brother, Godfrey and my younger brother and sisters Okhaide, Marian, Oigha, Bose and Peace in recognition of the sacrifice they made throughout the period of my education.

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ABSTRACT

The incidence and severity of false smut of rice incited by (Ustilaainoidea virens (ke.) Tak.) in the major rice producing areas of Edo State was investigated in 1992. Although the disease was present in all the areas surveyed, it was most prevalent and severe in Ovbiowun-Emai, and Uokha, both in Owan East of Edo State. The mean incidence and severity of false smut in the area between mid-August and mid-September were 28.5% and 13.9% respectively compared to 8.9% and 3.4% for late-September to mid-October.

A pathogenicity trial conducted at the University of Benin, Benin City in 1993 revealed that false smut was reproduced only when rice panicles were sprayed with either chlamydospores or conidia suspension at 'heading' stage. Inoculation at 'booting' or 'milk-grain' stage respectively did not cause any infection.

Field trials were conducted at Ovbiomun-Emai, in 1993 and 1994 to determine the effect of sowing date, plant density, varietal resistance, and fungicidal sprays on the incidence and severity of the disease.

Rice sown with the early rains, between early-April and early-May, were virtually free from false smut infection. On the other hand, mid-season sowing, between mid-May and early-June, recorded the highest incidence and severity of false smut.

There were no significant differences (P = 0.05) among the severities of false smut at three spacing levels (0.2 x 0.2m, 0.4 x 0.4m and 0.4 x 0.8m) tried.

Of the seven rice varieties screened against false smut under

field conditions, at two sites, ITA 316, Ex-china and IRAT 170 were the most resistant. The other four varieties were susceptible.

Delsene M, Benlate, Tecto, and Cupravit, at the three concentrations tried in-vitro, completely inhibited mycelial growth of U. virens; while Dithane M-45 and Rovral reduced mycelial growth by 62.2% and 47.8% respectively. Four sprays each of the six fungicides at an interval of 7-days, starting at the sighting of the first 'boot', were found to reduce incidence and severity of the disease in the field. However, the best result was obtained by spraying with cupravit. This resulted in 75.9% reduction in disease incidence and significantly increased grain yield by 43.2% when compared with the control. Dithane M-45 sprays also resulted in 47.0% disease reduction, but did not increase grain yield significantly. Delsene M sprays apparently gave a 100% disease control, but was highly phytotoxic to the rice plants at the recommended rate.

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CHAPTER I

1. INTRODUCTION

Rice, Oryza belongs to the family Gramineae. This family is made up of about 10,000 species (Gill, 1988). The genus Oryza, made up of about 25 species is widely cultivated nearly under all ecological conditions of the world (Grist, 1975). Among all these species, Oryza sativa L. and Oryza glaberrima Steud. are the most widely cultivated. They are said to have been derived from wild rice cultivars (Grist, 1975).

O. sativa is of Asian origin and is more widely cultivated throughout the world, while O. glaberrima originated from Africa and its cultivation is restricted to the continent (Grist, 1975; Gupta and O'toole, 1986). The earliest cultivation of O. sativa in Nigeria was about 1890 (Grist, 1975), when upland varieties were introduced to the high rain forest zone in western Nigeria. This rapidly replaced O. glaberrima, then in extensive cultivation. Grist (1975) also noted that O. glaberrima is now mostly confined to the far north of Nigeria and to Sierra Leone.

Rice is the most important staple food for more than half of the world's population. This includes millions of Asians who depend almost entirely on it (Grist, 1975; Thurston, 1984; Gupta and O'toole, 1986). Rice is now a very vital source of carbohydrate in the food basket of many countries including Nigeria. Husked rice grain contains approximately 9.7% water, 7.7% protein, 78.1% carbohydrate, 1.1% fibre and 1.6% ash (Purseglove, 1976).

Rice is used in brewing and baking industries for making

wine, beer and baking of cake, biscuits and bread. Rice is also used in textile industries as laundry starch source. Rice bran is high in protein, hence it is used in livestock feed as roughage and protein source. Rice straws can be fed to animals, though it is of inferior quality compared to that of other cereals. It can be used in mat, hat, bag and thatch making. Rice husk can be used for fuel and for making light weight concretes.

Rice is primarily cultivated in the tropics and subtropics but it is also important in temperate countries such as Italy, Japan, and Korea (Thurston, 1984). About 91% of the world rice crop is grown in Asia; 5.8% in the two Americas; 2.8% in Africa, and 0.4% in Europe (Konokhova, 1985). Among the rice producing countries in Africa are Malagasy Republic, Egypt, Sierra Leone, Guinea and Nigeria.

The rice plant will flourish under widely different climatic conditions. Although the highest yields are obtained in countries enjoying a subtropical or warm temperate climate, the crop thrives well over a wide range of conditions within about 45° north and 40° latitude south of the equator (Grist, 1975).

Rice is adapted to regions of high temperatures and prolonged sunshine. The average temperature required throughout the life of the plant ranges from 20 to 37.7°C. The total temperature required (sum of daily mean temperature during the growing period) is between 1,130 and 1,500°C (Grist, 1975).

In most tropical countries, rice cultivation is entirely dependent on seasonal rainfall. The water supply for about 80% of the world's area planted to paddy comes from rainfall. The

high variability of tropical rains renders the success of paddy cultivation uncertain in areas other than the great deltas and basins of large rivers. An annual rainfall of 750-1000mm is adequate for rice, provided the rainfall is regular, or where irrigation facilities are adequate, the cultivation of rice can be done successfully.

Rice is grown in all types of soils from sandy loam to heavy clays, but best adapted to heavy textured soils. A heavy, not completely impervious, soil is the optimum requirement. Rice can tolerate a wide range of soil pH (pH 4.5 - 8.7) but it thrives better in neutral to highly acid soils than in alkaline soils (Grist, 1975).

The three major systems used in rice cultivation are upland, floating and lowland or paddy. Upland (rainfed) rice is cultivated much like other small-grain cereals without flooding. Much of the rice in tropical America and Africa is upland, but it constitutes less than 10% of that grown in Asia, in areas subjected to deep flooding as found in Thailand, Bangladesh, and Vietnam. The plant elongates rapidly in the rising water which may reach a depth of 5 meters. Harvesting is carried out using boats or after flooding has subsided.

The most important system for growing rice is the paddy or lowland rice culture system. There are variations of this system. The two major sub-systems are paddy rice with or without artificial water control. Generally, the first operation after flooding by natural rainfall or irrigation is to plough the field so as to burry the weeds stubbles and rice straws. The wet paddies are harrowed or puddled a few times to obtain a soft

level and weed free planting bed. Most of the rice grown in Asia in paddy culture is transplanted from small seed beds sown in advance. After transplanting, irrigated rice paddies are continuously flooded until shortly before harvest. On rainfed farms, flooding may be intermittent.

Crop protection problems associated with rice production include insect pests, rodents, birds, nematodes, weeds and diseases. The occurrence and intensity of any one of these problems varies from region to region, and from one cultural system to the other.

More protection problems are encounterred in the warm, humid environment of the tropics than the cooler, drier environment of the temperate regions. Further more, weed, pest and disease problems are probably more on upland rice than lowland rice.

Weeds are very important constraint to tropical agriculture. The paddy system of submerging weeds gives rice an early advantage over later-germinating weeds. One of the most important advantages of flooding is that it is a very effective weed control system for the tropics (Thurston, 1984).

Thurston (1984) noted that flooding also reduces the number of fungal propagules, insects, and nematodes in the soil and, by controlling weeds, which harbour rice pathogen and insects, reduces disease and insect damages.

Diseases are serious problems in rice production throughout the world. Padwick (1950) listed 231 different fungi recorded on rice; however, not all of them could cause disease. In addition, there are bacterial and viral diseases, as well as nematodes and non-parasitic diseases.

The global importance of rice diseases is difficult to estimate. Crammer (1967) calculated world wide losses due to rice disease as 8.9%.

Tungro virus, sheath blight, and bacterial leaf blight are the most important rice disease problems of south east Asia (Thurston, 1984).

The most serious bacterial disease of rice, bacterial leaf blight (Xanthomonas campestris pv. oryzae) and bacterial leaf streak (X. campestris pv. oryzicola), are important only in Asia; however, X. campestris pv. oryzae has been reported from Africa and tropical America (Thurston, 1984). Thurston (1984) reported that bacterial leaf streak is not found in temperate rice-growing areas, but in the Asian tropics where under favourable weather conditions can cause serious losses.

There are now over 14 known rice virus or virus-like diseases, but only three occur outside Asia (Thurston, 1984; Ou, 1985; Gupta and O'toole, 1986). Tungro is the most important and widespread virus disease of rice in south east Asia. The main vector of the virus is the leafhopper, Nephotettis virescens (Thurston, 1984). Tungro has been reported (Thurston, 1984) to cause severe epidemics in Indonesia, where 30,000 to 50,000 hectares of rice were affected in the 1930's. In 1966, over 300,000 hectares were severely damaged (Ou, 1985). Ou (1985) also reported that hundreds of thousands of hectares were affected in the Philippines in 1971. Fortunately however, resistant varieties have been found.

In Nigeria, just like in any other tropical country, diseases are a major constraint to rice production. Most of the

reported rice diseases in Nigeria are caused by fungal pathogens (Awoderu, 1974; 1984).

Awoderu (1974) reported that the two major fungal diseases of rice in Nigeria are rice blast (Pyricularia oryzae Cav.) and brown spot (Cochliobolus miyabeanus (Ito & Kuribayash) Dreschler ex Dastur). The loss in grain yield attributable to these diseases ranges from 11.5 - 39.6% and 12-43% respectiely. Mancozeb, blasticidin-S, fentin hydroxide, benomyl and edifenphos as foliar sprays effectively control rice blast in the laboratory and field (Awoderu, 1974). Brown spot is controlled by spraying with thiram, mancozeb or blasticidin-s (Awoderu, 1974). A more useful approach to the control of these diseases adopted in Nigeria as reported by Awoderu (1974) is the search for horizontally resistant varieties. In the same report he listed other fungal diseases of rice in Nigeria in order of importance as, false smut (Ustilaginoidea virens (Cke.) Tak.), basal sheath rot (Rhizoctonia solani Kuhn), Leaf scald (Rhynchosporium oryzae Itash. & Yok.), narrow brown leaf spot (Cercospora oryzae Miyake) and bakanae disease, a foot rot caused by Fusariuun moniliforme Sheldon. He mentioned that two suspected cases of virus diseases that has been reported are; grassy stunt disease (transmitted by Nilaparvata lugens Stal.) and orange leaf disease (transmitted by Inazuma dorsalis Mots.) and that no bacterial disease of rice has been found in Nigeria. However, in 1981, the rice yellow mottle virus (RYMV) disease was reported in Badeggi (Rossel, et.al., 1982). The new disease was first found to occur in August 1981, infecting a few stands of some lines of rice ADT 32, ITA 212 and ARC 11775) in an advanced yield nursery plot of the

National Cereal Research Institute (NCRI) Badeggi. In September 1981, a similar disease was reported on FARO 15, Americana, and other traditional varieties in farmers rainfed lowland rice fields near Doko, Bida. Apparently, the RYMV is indigenous to Africa (Rossel, et al., 1982). Erinle (1981) has also reported two bacterial disease of rice, bacterial leaf blight (Xanthomonas campestris pv oryzae) and bacterial leaf streak (X. campestris pv oryzicola) from Northern Nigeria. Bacterial leaf blight was found only in Samaru, Kaduna State, while the only record of bacterial leaf streak has been from Gusau in Sokoto State (Erinle, 1981).

Observations on farmers' fields in some rice producing areas of Edo State of Nigeria by the present author showed that false smut of rice (Ustilaginoidea virens) is a serious disease of rice grain. In each of the years 1989, 1990 and 1991, many farms in Owan East Local Government area of the State were found to be affected by smut.

Though the disease is generally regarded as minor globally, and not warranting any special method of control (PANS, 1974; Raymundo, 1980; Notteghem and Baudin, 1981; Awoderu, 1984; Ou, 1985; Gupta and O'toole, 1986), there have been several reports of severe incidence, or even epidemics in Africa and Asia (Reinking, 1918; Seth, 1945; Awoderu, 1969; 1974; Awoderu and Onuorah, 1974; and Adeoti, 1987).

Very little or no attention has been paid to this disease by pathoLogists globally. In Nigeria, the very few records on the disease are mere reports of severe incidence.

It must be noted, however, that the status of a disease is

usually not static. This may be due to factors such as changes in varieties planted and production system; environmental changes; and development of virulent strains of the pathogen. For instance, Thurston (1984) reported that sheath blight of rice was a minor disease in tropical Asia before the introduction of the high yielding International Rice Research Institute (IRRI) cultivars, which changed the status to probably the most serious rice disease especially in the lowland tropics of Asia today.

The present observation on the false smut of rice has shown that it is a destructive disease of rice grains in the fields of the humid region of Edo State of Nigeria.

It is in the light of this, and in order to maximise the farmers' output, that the present research was directed at the following objectives:

1 Surveying the major rice producing areas of Edo State, with a view to assessing the extent of damage due to the disease.

2 Isolating and identifying the pathogen associated with the disease and establishing its pathogenicity.

3 Screening some upland rice cultivars for resistance to the disease.

4 Studying the effect of sowing date on the incidence and severity of the disease.

5 Studying the effect of plant density on the incidence and severity of the disease.

6. Evaluating some fungicides for their efficacies in controlling the disease.

CHAPTER 2

2. LITERATURE REVIEW

2.1 History and Nomenclature

Cooke (1878) was the first to describe the fungus causing false smut of rice, otherwise known as green smut. He named the fungus Ustilago virens, basing his description upon a specimen, from India. However, Patouillard (1887) named the material sent to him from Japan Tilletia oryzae. Brefeld (1895) changed the latter to Ustilaginoidea oryzae (Pat.) Brefeld. Takahashi (1896) studied the fungus and called it Ustilaginoidea virens ((Ke.) Tak. The fungus was still considered as a true smut by Omori (1896) who named it Sphacelotheca virens. Ou (1985) reported the discovery of the teleomorphic state of the fungus in 1934 by Sakurai, who wrongly recombined the anamorphic epithet virens with the teleomorphic genus Claviceps as Claviceps virens (Cke.) Sakurai.

Since this name is not nomenclaturally acceptable, Hashioka (1971) provided the name Claviceps oryzae - sativae for the teleomorph. The nomenclature of this fungus is still controversial. Ou (1985) noted that the anamorph can, however, be correctly referred to as Ustilaginoidea virens (Cke.) Tak. and this is the name that is in general use presently.

The fungus was also described at a very early date in Chinese literature, but without a scientific name. Ou (1985) reported that, the presence of the disease was believed to be an indication of a year of good crop. This is because the conditions favourable for the growth of the fungus, particularly high moisture or rainfall, are similar to those resulting in good

growth of the rice crop.

2.2 Distribution

False smut of rice has been reported to be present in most of the major rice-growing areas of the world (Ou, 1985). Such areas include, Burma, Ceylon, China, India, Indonesia, Japan, Malaysia, Pakistan, Philippines, Taiwan, Thailand and Vietnam in Asia, Bolivia, Brazil, Colombia, Guyana, Mexico, Panama, Peru, Surinam, Trinidad, USA, and Venezuala in America; Congo, Cote d'Ivoire, Ghana, Guinea, Liberia, Madagascar, Mozambique, Nigeria, Rhodesia, Sierra Leone, Sudan, Tanzania and Zambia in Africa; and Fiji, Italy and Papua - New Guinea.

2.3 Symptoms

The fungus transforms individual grains of the panicle into greenish spore balls of a velvety appearance (Copeland, 1924; Abeygunawardena, 1969; Grist, 1975; Ou, 1985). The spore balls are small at first and are visible in between the glumes, growing gradually to reach 1cm or more in diameter, and enclosing the floral parts. The green colour is superficial, being the colour of the outer membrane of the balls; the inner part of the balls is orange-yellow near the surface and white in the centre (Grist, 1975; Ou, 1985). The membrane burst as a result of further growth and the colour of the ball becomes orange and later yellowish-green or greenish-black (Ou, 1985). At this stage the surface of the ball cracks. When cut open, it is white in the centre, and consists of tightly woven mycelium together with the glumes and other tissues of the host.

Usually, only few grains in a panicle are infected, but several may sometimes occur (Ou, 1985).

2.4 Description of the Fungus

Ustilaginoidea virens (Cke.) Tak. is the name generally accepted for the fungus causing the false or green smut of rice. Ou (1985) reported that chlamydospores formed on the spore balls are borne laterally on minute sterigmata on radial hyphae, and are spherical to eliptical, warty, olivaceous, measuring 3 - 5 x 4 - 6μm. Younger spores are small; paler, almost smooth. Chlamydospores germinate in culture by germ tubes which become septate and form conidiophores bearing condia at the tapering apex. These condia are ovoid and very minute.

Some of the green spore balls develop one to four sclerotia in the centre. These sclerotia overwinter in the field and produce stalked stromata the following summer or autumn. The stromata which form swellings at the tip of the stalk, are more or less globose, and contain perithecia around the periphery. Each flask-shaped perithecium contains about 300 asci. The asci are cylindrical with a hemispherical apical appendage, 180 - 220 x 4μ, and 8-spored. Ascospores are hyaline, filiform, unicellular, 120-180 x 0.5-1 μ, (50-80 x 0.5 - 1.5 μ). Ikegami (1963) traced the development of the sclerotia and noticed that they are usually very numerous in the larger, greenish-black smut balls. Hashioka, et. al. (1966) compared the fine structure of the chlamydospores of the fungus with that of the true cereal smut spores, and found distinct differences in spore wall ornamentation.

Hashioka et al. (1951) reported that chlamydospores germinated in water to produce fine germ tubes bearing 1-3 conidia. They observed more growth of germ tubes and a greater number of conidia when the chlamydospores were grown in sugar solution or potato decoction than in water. Chlamydospores germination and mycelial growth were best at 28°C and slight at 12°C; no growth occured at 36°C (Ou, 1985). Seth (1945) obtained the best growth at 26°C. Ou (1985) also described the growth to be best at pH 6.02 - 6.72. There was little or no growth at pH 2.8 and only slight growth occurred at pH 9.1. Thus, growth of the fungus is best favoured by slightly acidic to neutral pH.

Hashioka and his co-workers also produced perithecia and mature stromata from sclerotia kept for 4-5 weeks in moist sand at 24-30°C. In culture, chlamydospores are produced in 20-40 days on Saito's soy agar, rice decoction agar, and steamed rice (Ou, 1985). Lepori (1951) found that growth was better on malt agar and peptone glucose broth than on potato agar.

Seth (1945) studied the germination of the spores, and found that after 24 hours, about 10% of the incubated spores germinated, each producing a short unbranched germ tube. After another 48 hours, the germ tubes were found profusely branched, septate and bearing clusters of small pear shaped hyaline conidia, which were formed both terminally and laterally at the hyphal tips. After a few days, on Oat meal agar, the fungus produced a white fluffy felt-like mycelium. This mycelium continued its growth and after about three weeks produced many white compact, almost round, sclerotial bodies. The sclerotia later on turned orange-yellow and finally olive-green in colour

and became slightly powdery in appearance. On examination, they were found to correspond closely to those obtained from the host plant, in having a central white portion of compact hyphae finally surrounded by powdery Olive-green layer consisting of mature spores. The spores obtained from these bodies germinated in the usual way and produced sclerotia.

The longevity of the spores on sclerotial bodies of the fungus was studied by Seth (1945). Sclerotial bodies were kept in glass tubes at laboratory temperature. The spores from these were periodically examined by growing them in plain agar medium to find out how long they would remain viable. He noted that the spores remained viable up to a period of about 8 months from the time of their formation.

Seth (1945) also studied the germination of sclerotia and found that growth commenced on Oat meal agar in the 3rd day producing many typical sclerotial bodies after three weeks. However, no ascus-bearing structures appeared though the cultures were kept in the laboratory for a period of over three months.

Sharma (1977) found that propagules in liquid media grew into mycelium when transferred to solid medium. He suggested that the fungus may survive in nature as propagules in pond water.

Yamashita (1965) detected alkaloids in infected plant parts and considered that the sclerotia of U. virens (Cke.) Tak. resembled the ergots formed by certain other fungi.

2.5 Economic Importance

Copeland (1924) reported that false or green smut

(Ustilaginoidea virens (Cke.) Tak.) is perhaps as widespread as Pyricularia oryzae but less destructive. He reported that U. virens is always present in Philippine rice fields and sometimes results in heavy yield losses. The disease is known in Japan, but not considered very disastrous. In Louisiana (USA) the loss in yield due to the disease is usually less than 1%. In Sumatra, the farmers had learnt to live with it.

Grist (1975) also regarded the disease as though widely distributed, rarely causes much damage. Ou (1985) reported that the disease usually causes little damage except in restricted areas under special conditions. However, Reinking (1918) reported a severe infection in the Philippines during damp weather. Seth (1945) made reference to an epidemic in Burma in 1935.

Awoderu and Onuorah (1974) and Awoderu (1974) regarded the disease as important and a major grain disease of rice wherever it occurs in Africa. Awoderu (1974) cited a case of severe incidence of this disease at Massagha swamp, Calabar Province; at Itu on the Inyoung Creek and Adairi in Onitsha Province of Nigeria in 1951. Awoderu (1969) also referred to a severe incidence of the disease at Ado-Ekiti (Western State of Nigeria) on a 2 hectare plot of Ofada rice variety imported from Zaire. Adeoti (1987) also reported a severe occurrence of the disease on rice cultivars GEB 24/37 and TOS 103 (lowland cultivars) in Badeggi, Nigeria, during the month of September. In Madagascar major damage, and virulent forms of the pathogen causing crop losses of about 10% in Fiji have been reported (Awoderu and Onuorah, 1974).

2.6 Disease Cycle

Ou (1985) reported that the fungus survives the winter by means of sclerotia as well as chlamydospores in the temperate regions. The chlamydospore is said to play an important role in secondary infection, which is a major part of the disease cycle. Chlamydospores are air-borne but because of the presence of a sticky material onthe smut balls they are not so free.

Hisada (1936) from field observation, suspected that infections occur when plants were at the booting stage. As a result of histological studies, Raychaudhuri (1946) described two types of infection; one type takes place at a very early stage of flowering, when the ovary is destroyed, but the style, stigma and anther lobes remain intact and are ultimately buried in the spore mass. The second type takes place when the grain is mature, absorb moisture, swell and force the lemma and palea apart. The fungus finally contact the endosperm and the growth is greatly accelerated. Ultimately, the whole grain is replaced and enveloped by the fungus.

2.7 Reproduction of the Disease

Reproducing the disease artificially (i.e by inoculation) has not been very easy. Seth (1945) inoculated panicles just at the time of opening and at the milk-grain stage. The method he used consisted of inserting the spores within the glumes by means of sterilized water. Some of the panicles after treatment were enclosed in tubes and some left exposed. In this work which he did for two consecutive years, he found out that majority of the inoculated panicles mature quite normally and all were free from

infection. Seth (1945) also inoculated seeds before planting, to determine if the disease might be systemic but, he found that all plants matured normally and remained free from infection.

However, Yoshino and Yamamoto (1952); and Ikegami (1960) successfully inoculated rice plants with chlamydospores and ascospores (Ikegami) by injecting a spore suspension into the leaf sheath enclosing the young panicle. They inferred that infection occurs at the rather short period just before heading. As a support to this inference, Hashioka, et al. (1951) observed that 99.6% of the smut balls contained intact anthers, indicating that most infections take place just before heading.

Ikegami (1962; 1963) inoculated coleoptiles of germinating seeds with spore suspensions and found that the fungus infected the host tissues. Later hyphae were found in the growing points of the tillers until the late tillering stage, but the fungus was not isolated from the reproductive parts. Kulkarni & Moniz, (1975) reported successful inoculation by applying chlamydospore suspensions with a camel hair brush both to fertilized and unfertilized ovaries, but infection of seeds by smearing them with chlamydospores was not successful. They suggested that chlamydospore balls in soil may germinate to produce secondary conidia that can be dispersed by wind and rain and initiate infection.

2.8 Factors Enhancing Disease Development

Ou (1985) reported that the development of false smut is generally believed to be favoured by high moisture. Ikegami (1960) reached the conclusion that plants grown under conditions

of high fertility, favourable for the vegetative growth of rice, were more susceptible to the disease. Rao (1964) and Singh (1974) also reported that weather and nutritional conditions affect the incidence of the disease. Rao (1964) found that varieties with medium duration (130-145 days) registered maximum infection while those with shorter and longer durations were comparatively less affected. This was attributable to higher percentage relative humidity and lower maximum temperature during the period of flowering of the medium duration varieties. He also observed a correlation between the incidence and the number of cloudy days. It appears that the actual precipitation has less effect on incidence of the disease than the number of cloudy days and low maximum temperature. However, cloudiness and lowering of temperature are phenomena associated with precipitation.

Rao (1964) also reported that application of nitrogen fertilizers increases the incidence of false smut. He found a positive correlation between fertilizer levels and incidence of the disease. He also found that time of fertilizer application influences the incidence. There was a gradual increase of infection with later applications, the highest infection being noticed when the fertilizer was applied at the time of flowering. Awoderu (1974) attributed a high incidence of the disease in a particular location in Nigeria to poor management. According to him, the farm was weedy and surrounded by thick forests.

2.9 Host-Range and Varietal Resistance

Haskel and Diehl (1929) reported that a species of

Ustilaginoidea on maize tassels from Louisiana and Panama was similar morphologically to U. virens (Cke.) Tak. on rice, but that it is not known if the fungus from maize could attack rice. Rao & Reddy (1955) found U. virens (Cke.) Tak. on Oryza officinalis Wall. in India. Veeraghaven (1962) also reported the finding of the fungus on wild species of Oryza.

Little information is available on varietal resistance, but certain varieties have been noted to be more frequently attacked than others. For instance, Rao (1964) observed in India that 186 varieties remained free from infection among 297 examined in a single area, despite the occurrence of environmental conditions favouring disease development. In Nigeria, Adeoti (1987) found severe infection on only two cultivars - GEB 24/37 and TOS 103 out of 13 cultivars screened for rice diseases at Badeggi in Niger State.

2.10 Control

A satisfactory control measure is not known. However, Awoderu and Onuorah (1974) have recommended picking and subsequent destruction of smutted grains as well as the use of hot water treatments. They also recommended the importation of grains from areas known to have virulent strains of the fungus to be in conformity with strict plant quarantine measures. This recommendation is possibly made due to the fact that grains harvested from infected field whether smutted or not are likely to carry some spore balls. And it is common to find infected seeds in seed lots harvested from infected field. Through sowing of such seeds, chlamydospores and sclerotia of the fungus are

likely to be introduced into the field. These will probably germinate in soil at the time of flowering producing secondary conidia that could be dispersed by wind and water and initiate infection.

CHAPTER 3

3. PRELIMINARY STUDIES

3.1 Materials and Method

3.1.1 Symptomatology

Studies of the development of symptoms of false smut of rice, was carried out in 1992 on farmers' field. Visits were made to infected fields and observations taken on symptoms of the disease at different stages of its development. Infected panicles were then collected in paper envelopes and taken to the laboratory for isolation of the pathogen.

3.1.2 Isolation and morphological studies of the Pathogen

3.1.2.1 Preparation of Potato Dextrose Agar (PDA) Media

The PDAS media used for this study were prepared following the procedure given below.

Peeled irish potatoes	200g
Dextrose	20g
Agar	20g
Water	1000ml

The peeled potatoes were cut into small pieces, boiled for one hour in 1000ml of water. Thereafter, the material was filtered through muslin cloth. To this was added 20g each of dextrose and agar. The volume was made up to 1000ml and reboiled to dissolve the agar. Five hundred millilitres (500ml) each was poured into 1000ml Erlenmeyer flask (Pyrex), plugged with cotton wool and autoclaved for 15 minutes at 1.2Kg/Cm^2.

Earlier, Petri dishes were put in steel canisters, covered and sterilized in the oven for 6 hours at 120°C to ensure

adequate sterilization.

Just before pouring the molten Potato dextrose agar (PDA) into the petri dishes, 5ml of 0.5% streptomycin sulphate solution was pipetted into 500ml of the molten PDA to prevent bacterial growth.

Pouring was done when the molten PDA had cooled to about 35-40°C and under sterile conditions. Approximately 15-20ml of Potato Dextrose Agar-Streptomycin (PDAS) medium was poured per petri dish and the plates were left for 24 hours to set before use.

PDA slants were prepared in McCartney bottles. Appropriate volumes of the PDA were poured into the bottles after reboiling the PDA to dissolve the agar. These were then autoclaved for 15 minutes at 1.2Kg/Cm^2 after which they were allowed to set in slanting position.

3.1.2.2 Isolation Methods

(1) Sclerotial Plating after Sterilization

(a) Smut balls were teased into pieces using a small knife. To these pieces in a McCartney bottle, 1% sodium hypochloride, NaOCl (Milton) was added. The bottle was corked, shaken and allowed to stay for 5 minutes. The milton was thereafter drained off, and the pieces washed in two exchanges of sterile distilled water. Inoculation on PDAS plates was done by plating the pieces directly on the PDAS medium using forceps and needle that were disinfected in ethanol and flamed. Five plates were inoculated. The whole process was carried out under sterile conditions in a sterilized chamber. The plates were then incubated at 27$\pm$2°C for

72 hours.

(b) Smut balls were ground with a pestle and mortar into small granules. The granules were then introduced into milton solution in a McCartney bottle and shaken continously for 5 minutes. The milton was then drained off and the smut residues (pieces of sclerotia) rinsed with sterile distilled water through muslin cloth. The pieces of sclerotia were plated on PDAS in a Petri dish directly, using a flamed inoculation needle, and five plates were thus inoculated. The whole process was carriedout in sterilized chamber. The plates were then incubated at 27±2°C for 72 hours.

(2) Chlamydospore Plating using Serial dilution technique:

The smut balls were first shaken in 1% sodium hypochloride (Milton) for 10 minutes and then allowed to dry between double layers of filter paper for 24 hours. Thereafter, they were ground into small granules in a mortar with a pestle.

Serial dilutions of the smut granules were made as follows: Approximately 0.1g of the ground smut balls was weighed out into 10ml of Milton solution in a sterilized McCartney bottle and shaken gently and continously for 5 minutes to form the stock suspension. One millilitre of the suspension was transferred into 9ml of sterilized distilled water, in another sterilized McCartney bottle with the use of a sterile pipette. From this dilution, 1ml was in the same way transferred into 9ml of sterilized distilled water in another sterilized McCartney bottle. The serial dilutions were continued in this manner until a $1:10^7$ dilution was obtained.

Two methods of inoculation on PDAS plates were used.

(a) Use of pipette: from each dilution, five PDAS plates were inoculated respectively by adding 1ml of chlamydospore suspension onto the surface of agar plate with the use of a sterilized pipette. The agar plate was gently rotated so as to allow the suspension to cover the agar surface uniformly. The whole process was carried out in a sterilized chamber. The plates were thereafter incubated at 27±2°C for 72 hours.

(b) Use of inoculation loop: from each dilution, five PDAS plates were inoculated respectively, with the use of a heat sterilized 0.3cm diamater inoculation loop. Each plate was inoculated by dipping the inoculation loop into the appropriate suspension (dilution) and using this to touch the agar surface. A minimum of 4 spots on an agar plate were inoculated. The whole process was carried out in a sterilized chamber. The plates were thereafter incubated at 27±2°C for 72 hours.

3.1.2.3 Purification and Examination of Culture

A pure culture of the fungus was obtained through the sub-culturing technique. Colony characteristics of the hyphae growing from the agar plates inoculated with false smut fungus were examined after 72 hours of incubation. Hyphae were removed with a flamed inoculation needle onto fresh PDAS plates and incubated for another 48 hours at 27±°C. Thereafter, fungal hyphae growing out of the colonies transferred onto the PDAS plates were again transferred onto fresh PDAS plates and incubated for 7 days at 27±°C before detailed microscopic examination was carried out. Colony characteristics of the fungus were observed daily, during the 7 days growth period. The

culture was left on the laboratory bench for three weeks.

The pure culture was maintained on PDA slants in McCartney bottles for further work.

3.1.3 Reproduction of disease symptoms by artificial inoculation

This experiment was carried out in the screen house, at the back of Botany Department, University of Benin, Benin City. A local upland rice cultivar, 'Agbede', known to be susceptible was used as the test plant. The seedlings were raised on heat sterilized sandy loam soil in plastic pots (26cm diameter and 19cm deep).

NPK 15:15:15 fertilizer was mixed with the heat sterilized soil using 1g of fertilizer per pot. The mixing was done prior to sowing and two days before the soil was packed into the pots. Five seeds were sown per pot. The seedlings were later thinned down to two healthy ones at 3 weeks after sowing.

The seven treatments included inoculation with chlamydospore suspension at 'booting' stage, 'heading' stage, and at 'milk-grain' stage respectively; inoculation with conidia suspension at the same 3 different stages as above; plus the control treatment which was not inoculated. The pots were arranged in a completely randomized block design. There were 10 replicates per treatment, with each pot of plant serving as a replicate.

3.1.3.1 Inoculation with chlamydospore suspension

Chlamydospore suspension was prepared from false smut infected rice panicles, earlier collected from an infected field. The suspension was obtained by grinding the smut balls into small

granules which were introduced into sterile distilled water and then filtered. The filtrate (spore suspension) was then adjusted to aproximately $1x10^8$ spore/ml using a haemocytometer and a compound microscope.

Inoculation at 'booting' stage was done using the method described by Yoshino and Yamamoto (1952) and Ikegami (1960). Five ml of chlamydospore suspension was injected into the leaf-sheath enclosing the young panicle, using a hypodermic syringe and a needle.

Inoculation at 'heading' stage and 'milk-grain' stage were carried out by spraying to run-off the young panicle using a handsprayer.

In each of the cases, the test plants were covered for 12 hours with moistened transparent 'polyethylene' bags (70cm long and 30cm wide) immediately after inoculation. Thereafter, they were uncovered and sprinkled with water, and covered again for another 12 hours. The cycle continued for 72 hours before the plants were finally uncovered. Symptoms of the disease were observed as they developed.

3.1.2.2 Inoculation with conidia suspension

Conidia suspension was prepared from pure culture of the pathogen on PDAS plates. The suspension was obtained by scraping nine-day old mycelial culture of Ustilaginoidea virens on PDAS into sterilized distilled water in 1000ml conical flask, using a scapel. This was shaken vigoriously and then filtered. The filtrate (conidia suspension) was then adjusted to approximately $1x10^8$ spore/ml using a haemocytometer and a compound microscope.

Inoculation of rice plants at the 3 different stages 'booting', 'heading', and 'milk-grain' respectively, followed the same procedures as described for that of inoculation with chlamydospore suspension. Symptoms of the disease were observed for each treatment, as they developed.

3.2 Results and Discussion

3.2.1 Symptomatology

The first visible symptom appeared between 1 to 2 weeks after flowering as a small, whitish-yellow ball of mycelium between the glumes (lemma and palea). It later grew into a large greenish spore ball of a velvety appearance (plate 1). The enlargement of the spore ball forced the palea and lemma apart (Plate 2), the greenish spore ball remaining between them in some cases. In most other cases, however, the greenish spore ball overgrew the glumes and covered them up.

A fully grown spore ball was examined in detail by cutting it accross. It was found to consist of three layers; a greenish powdery but not completely loosed outer layer giving the ball its greenish appearance; followed by a yellow layer, and then a whitish core, both of which were sclerotic in nature. Thus, some few balls were yellow, while others were greenish (Plate 3) at maturity.

A view of an infected field simply reveals the disease as greenish to black balls quite more visible and larger than the healthy brown grains (Plate 4).

3.2.2 Isolation and morphological studies of the Pathogen

The pathogen, Ustilaginoidea virens (Cke.) Tak., could not be separated from surface contaminants (Rhizopus sp. and Aspergillus sp.) when sclerotial plating after sterilization method, was adopted. However, there was a high reduction in growth of the surface contaminants when dilution technique was used. Growths on plates inoculated with 1ml chlamydospore suspension per plate were not as good as those on plates inoculated by the loop method because of bacterial contamination. Using the loop method, the best result was obtained at $1:10^4$ dilution. The pathogen, U. virens (Cke.) Tak. was successfully isolated on PDAS medium.

Chlamydospores from diseased material inoculated on PDAS plates germinated within 48 hours of inoculation, produced white fluffy cottony mycelium in culture. At 72 hours after inoculation, the mycelium developed an orange-yellow colour with a dark brown back-ground at the centre (Plate 5). At 2 weeks after inoculation the orange-yellow colouration at the centre became mixed up with a deep brown colour which covered the fluffy growth (with a rough topography), leaving the outer narrow ring white (Plate 6). At this time the dark brown background had also expanded from the centre outwards (Plate 7).

Four days after inoculation, conidia were found in the culture. Hyphae were profusely branched and septate, bearing bunches of minute pear-shaped conidia at their tips. In group, on hyphal tips the conidia appeared like short bunches of grape fruits. Detailed examination revealed the individual conidia as been small, pear-or egg-shaped or ovoid., hyaline with some

inclusions. The ovoid shaped conidia were seen attatched (with their tapering end) to the hyphal tip.

The above observations agreed with that reported by Seth (1945), and Ou (1985). Seth (1945) described the culture as a white fluffy felt-like mycelia which after two weeks produced white roundish sclerotial bodies that later turned orange-yellow and then greenish yellow. Both authors, described the spores (conidia) as been very minute, pear-shaped or ovoid, hyaline, and bore on the tips of profusely branched, septate hyphae.



Plate 1: Developmental stages of false smut of Rice

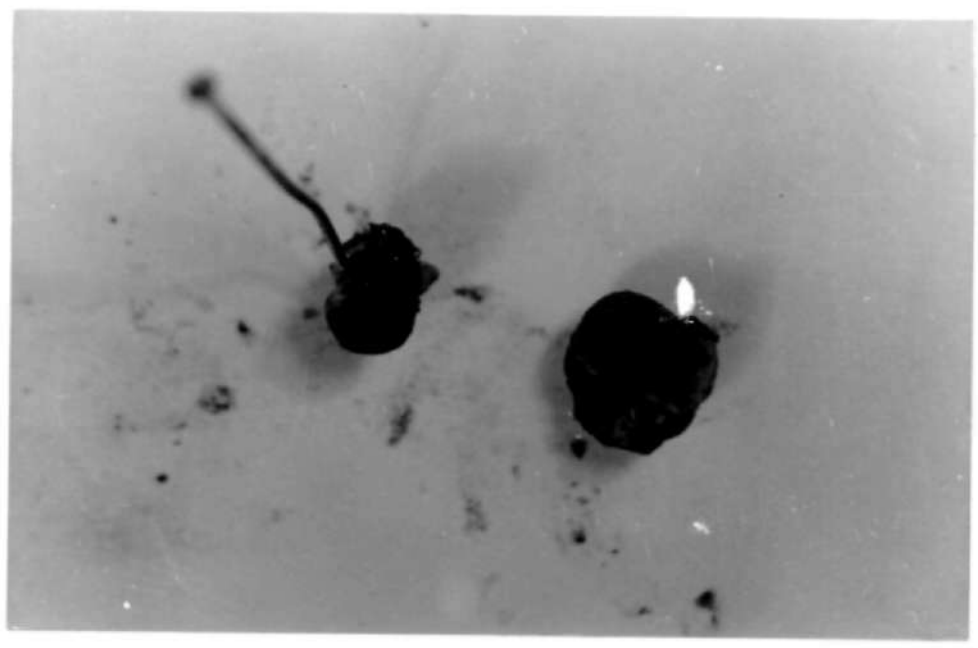


Plate 2: Two fully grown false smut balls

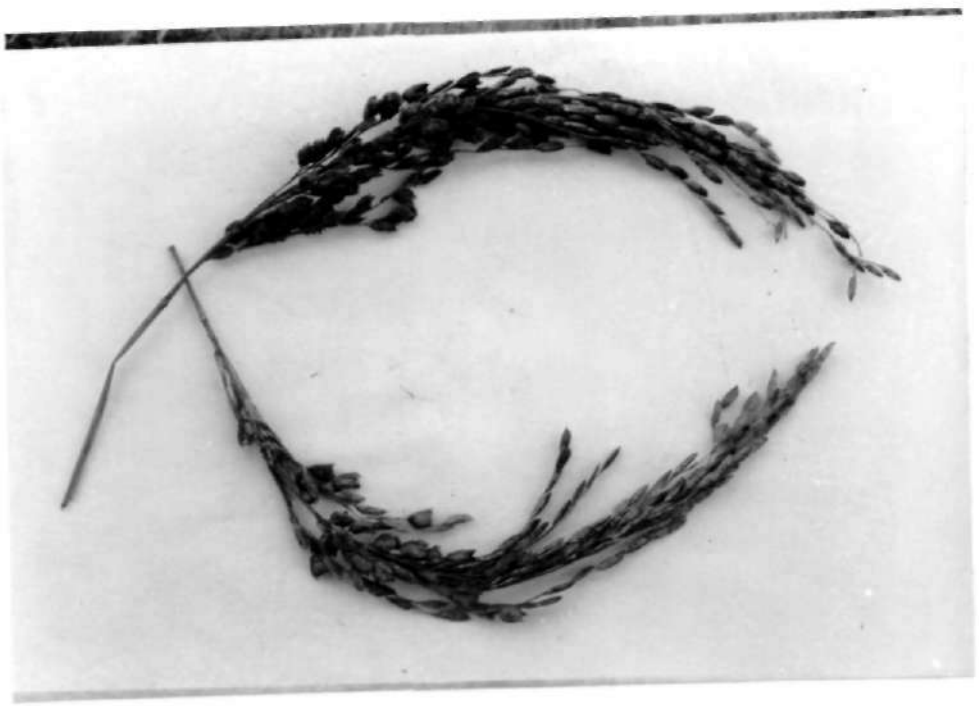


Plate 3: Smuttted panicles showing the superficial greenish outer layer (top) and the yellow inner layer of the smut balls (bottom).



Plate 4: A view of a smut infected rice field

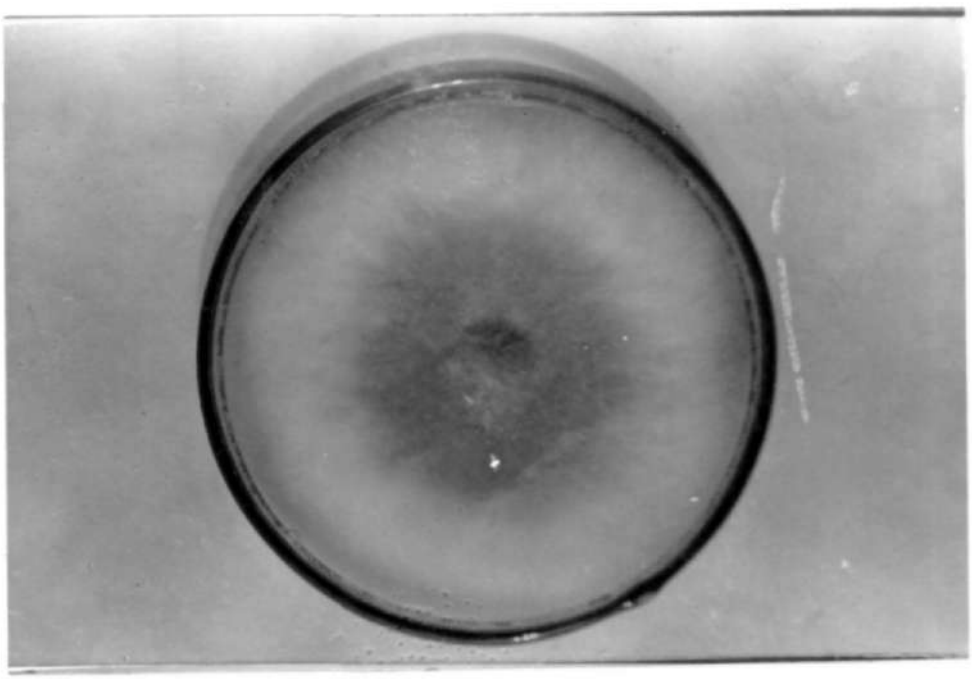


Plate 5: Cultural characteristics of Ustilaginoidea virens on PDAS at 7 days after inoculation

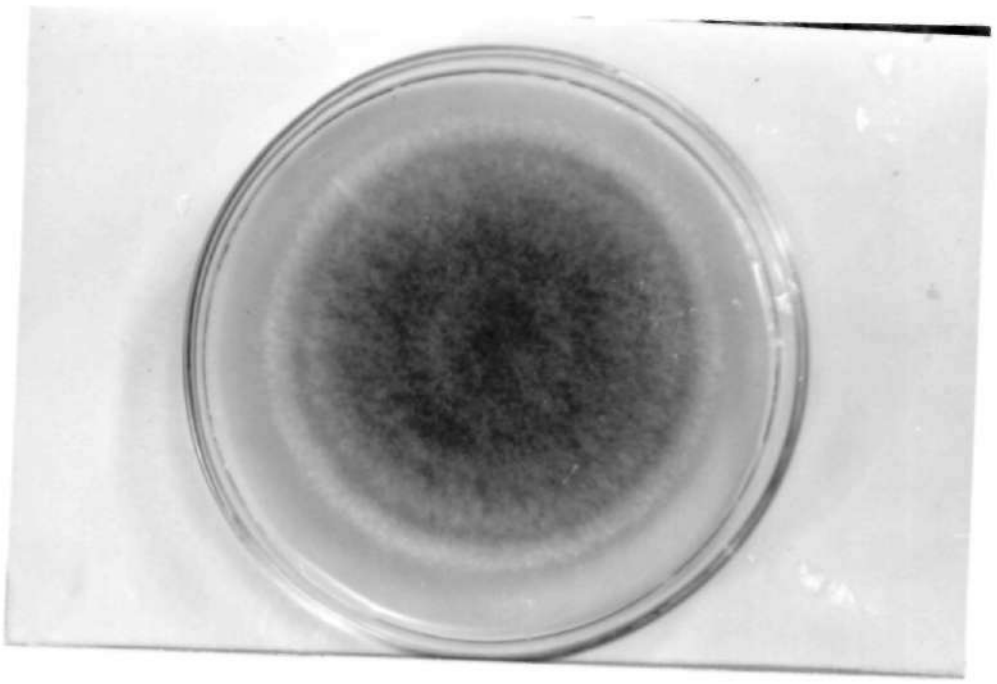


Plate 6: Cultural characteristics of Ustilaginoidea virens on PDAS at 14 days after inoculation (Front View)

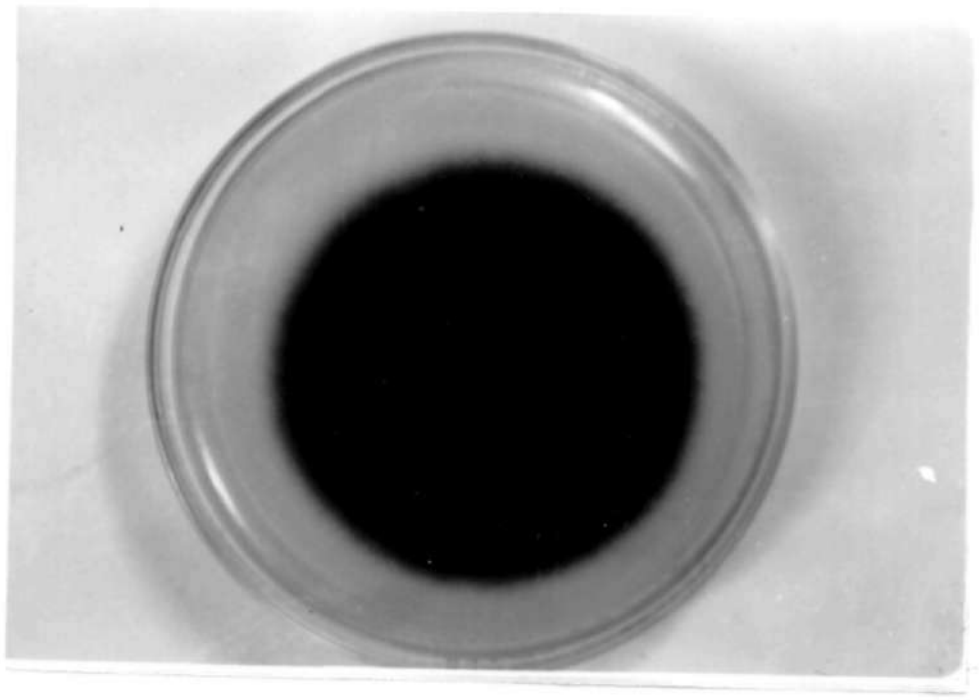


Plate 7: Cultural characteristics of Ustilaginoidea virens on PDAS at 14 days after inoculation (Back View)

3.2.3 Reproduction of disease symptoms by artificial inoculation

The results (Table 1) showed that, typical symptoms of false smut of rice were reproduced only when the rice plants were inoculated with either chlamydospores or conidia at 'heading' (or 'flowering') stage.

Inoculation with chlamydospores at 'booting' stage using a hypodermic syringe, resulted in 78% empty panicles. Typical false smut symptoms were not observed. However inoculating young panicles with chlamydospores at 'heading' stage using a hand sprayer, resulted in 37.5% incidence of panicles showing typical symptoms of rice false smut, as well as 40% incidence of empty panicles. When plants were inoculated with chlamydospores at 'milk-grain' stage using a handsprayer, the panicles matured normally, and without any smut symptoms, nor empty panicles.

Reactions of rice plants to inoculation with conidia, at these three stages, respectively, were very much the same as that described for inoculation with chlamydospores, above. The incidence of empty panicles was 64.8% when plants were inoculated with conidia at 'booting' stage. No typical false smut symptoms was observed. At 'heading' stage, inoculation with conidia, resulted in 30.2% incidence of panicles showing typical false smut symptoms. Here also, 38.5% incidence of empty panicles was observed. No symptoms of false smut, nor empty panicles was observed when inoculation with conidia was done at milk-grain stage.

Table 1: Reproduction of False smut symptoms by artificial inoculation

Treatment	Ave. % incidence of typical false smut symptoms	Ave. % incidence of empty panicles
Inoculation with chlamydospore at 'booting' stage	0.00	78.00
Inoculation with chlamydospore at 'heading' stage	37.50	40.00
Inoculation with chlamydospore at 'milk-grain' stage	0.00	0.00
Inoculation with conidia at 'booting' stage	0.00	64.80
Inoculation with conidia at 'heading' stage	30.20	18.45
Inocuation with conidia at 'milk-grain' stage	0.00	0.00
control (without inoculation)	2.00	0.00

Observation of empty panicles especially when plants were inoculated at 'booting' stage might be due to a disturbance in the physiological processes of the rice plant as a result of the inoculation process. This is likely so, because, the reproductive phase is very sensitive and fragile.

Various methods had been used by previous workers to reproduce false smut symptoms. However, it appeared that a very effective, easily reproduciable and standardised method of inoculation is yet to be fully established.

For instance while inoculation of flowers by Seth (1945) was unsuccessful, Kulkarni and Moniz (1975) successfully inoculated flowers with chlamydospores. Also Yoshino and Yamamoto (1952) and Ikegami (1960) reported successful inoculation with chlamydospores, and ascospores respectively at 'booting' stage. On the other hand, Kulkarni and Moniz (1975) inoculated plants at 'booting' stage without causing any infection.

The present findings agreed with those of Kulkarni and Moniz (1975). However, while Kulkarni and Moniz (1975) reported that plants inoculated at 'booting' stage developed normal panicles, most of them were observed in the present study to be empty.

The inference from the present observations is that successful infection is likely to occur only after the opening of flowers, probably after fertilization, as proposed by Kulkarni & Moniz (1975).

More importantly, production of the typical symptoms of the disease by inoculation of rice flowers with conidia from culture, confirms that, the isolated organism, U. virens is the pathogen of false smut of rice.

CHAPTER 4

4 SURVEY FOR INCIDENCE AND SEVERITY OF FALSE SMUT OF RICE IN THE MAJOR RICE PRODUCING AREAS OF EDO STATE

4.1 Introduction

In each of the years, 1989, 1990 and 1991, many false smut infected rice fields were observed in some parts of Edo State. To assess the severity of the disease, a survey was carried out twice (coinciding with when majority of early and late planted rice matures respectively) in 1992 in the major rice producing areas of Edo State. The first survey was done in the month of September, while the second was in October, 1992.

4.2 Materials and Methods

4.2.1 Area surveyed

The survey for incidence and severity of false smut of rice was carried out in parts of the rain forest belt of Edo State, Nigeria. The towns and villages on which outskirts, samples were taken, included Ilushi, Ukhun, and Idoa in Esan; Agenebode, Agbede in Etsako; and Ilievbe, Ovbiowun-Emai, Uokha in Onwan. These areas are within longitude 5-6°E and latitude 6-7°N.

4.2.2 Survey method

In each of the surveys, visits were made to rice farms and three farms on the outskirt of each town were selected at random for sampling. The sampled quadrats in each field were randomly selected to cover the field. The size of each quadrat was 2m x 2m, but the number of quadrats selected in various farms varied; depending on the estimated size of the farm. Ten quadrats were selected per hectare, while five were selected from any farm less

than a hectare.

4.2.3 Disease assessment

The incidence and severity of false smut were visually assessed in each of the rice farms visited. The disease incidence was assessed in each sampled quadrat by counting the number of infected rice stands within the quadrat and expressing this as a percentage of the total number of rice stands in the quadrat. Severity of disease in a sample quadrat was assessed by adapting the method described by Williams et. al. (1976) for pear millet disease. The procedure is as follow: first, each panicle in an infected stand was given a numerical disease rating as provided by the key below:

Key

Numerical disease rating	Description of severity of infection
1	no disease
3	< $^1/_4$ of the panicle infected
5	≥ $^1/_4$ but < $^1/_2$ of the panicle infected
7	≥ $^1/_2$ but < $^3/_4$ of the panicle infected
9	$^3/_4$ and above of the panicle infected

The average panicle ratings for a stand was then approximated to the nearest numerical rating in the scale as the severity score for that stand. Thereafter, the percentage severity of the disease in a sampled quadrat was estimated, using the formula given below:

$$\text{Percentage severity (or \%)} = \frac{Y_1(1-1) + Y_3(3-1) + Y_5(5-1) + Y_7(7-1) + Y_9(9-1)}{N \times 8} \times 100$$

infection)

Where Y = number of plants (stands) in each infection category (severity rating) and N is the total number of plants. Data obtained were then subjected to statistical analysis based on a randomized complete block design.

4.3 Results and Discussion

The survey carried out in September 1992 (Table 2) showed that the mean percentage smut incidence ranged between 17.8 and 53.8; while the mean percentage smut severity was between 7.7 and 29.0, in the area surveyed. Except for the significantly higher (P = 0.05) incidence and severity recorded in Ovbiowun-Emai, there were no significant differences (P = 0.05) in the disease incidence and severity among the locations. Furthermore, the disease incidence and severity recorded in Ovbiowun-Emai, was not significantly (P = 0.05) different from that observed in Uokha (a close neighouring town) which was also significantly (P = 0.05) equal to observed incidence and severity in other locations.

In the survey carried out in October, 1992 (Table 3), the highest mean percentage disease incidence (18.6) was also recorded in Ovbiowun, and this was significantly (P = 0.05) higher than those of Ukhun, Idoa, Agbede, Agenebode and Ihievbe. It was however, not significantly (P = 0.05) different from the disease incidence in Ilushi and Uokha which were at par with the disease incidence in other locations.

A comparison of the results obtained in the various locations from the two surveys (Table 4) showed that, the

incidence and severity of false smut of rice was significantly (P = 0.01) higher during the first survey carried out in September, than in October. This suggests that there was a higher incidence of the disease in mid-August to mid-September than in late September to mid-October.

The survey revealed false smut to be an important grain disease of rice in some areas of Edo State. Contrary to the general belief that only very few grains are infected in a panicle or stand where an infection occurs (Ou, 1985), a whole stand could be smutted as revealed from the survey. It was however observed that although the disease was prevalent in the area, not all farmers' fields were infected. In most of the infected fields, the disease was, however, severe.

The disease was observed to be most prevalent in Ovbiowun-Emai and Uokha, both in Owan East of Edo State. The high prevalence might be due to the variety grown. It was found that most farmers grow a local variety ("Agbede") while very few grow

Table 2: Incidence and severity of false smut of rice in some major rice producing areas of Edo State, between September 2nd -16th, 1992

Location	Disease Incidence/ Site (%)	Mean Disease Incidence (%)	Disease Severity/ Site (%)	Mean Disease Severity (%)
Ukhun	16.25 30.00 7.22	17.82[b]	6.25 15.00 1.80	7.68[b]
Idoa	25.86 39.78 10.34	25.33[b]	9.48 18.28 4.02	10.59[b]
Ilushi	14.29 19.67 32.65	22.20[b]	9.92 8.61 17.35	11.96[b]
Agbede	30.68 14.94 25.00	23.54[b]	14.49 7.47 13.28	11.75[b]
Agenebode	18.64 11.34 35.59	21.86[b]	5.51 3.87 17.37	8.92[b]
Ihievbe	20.21 18.87 35.87	24.72[b]	10.90 8.49 17.54	12.31[b]
Uokha	30.21 53.06 31.76	38.34[ab]	14.06 26.53 15.88	18.82[ab]
Ovbiowun-Emai	66.10 42.55 52.73	53.79[a]	40.25 18.88 27.73	28.95[a]
SED±		9.94		5.92
LSD (0.05)		21.32		12.70

Means within the same column followed by the same letter do not differ significantly at P = 0.05

Table 3: Incidence and severity of false smut of rice in some major rice producing areas of Edo State, between October 3rd -13th, 1992

Location	Disease Incidence/ Site (%)	Mean Disease Incidence (%)	Disease Severity/ Site (%)	Mean Disease Severity (%)
Ukhun	3.92 2.08 6.67	4.22^{c}	0.98 0.52 2.00	1.17^{b}
Idoa	3.53 5.66 7.41	5.53^{bc}	0.88 1.89 1.85	1.54^{b}
Ilushi	18.63 8.93 12.31	13.29^{ab}	7.35 2.23 4.23	4.60^{ab}
Agbede	6.90 1.85 5.26	4.67^{bc}	2.16 0.46 1.32	1.31^{b}
Agenebode	3.33 3.51 8.16	5.00^{bc}	0.83 0.89 3.57	2.02^{b}
Ihievbe	9.43 5.49 12.24	9.05^{bc}	2.83 1.65 3.57	2.68^{b}
Uokha	9.64 5.68 16.33	10.55^{abc}	3.92 1.99 6.63	4.18^{b}
Ovbiowun-Emai	5.43 18.52 31.92	18.62^{a}	2.45 8.33 18.62	9.80^{a}
SED±		4.14		2.508
LSD (0.05)		8.88		5.38

Means within the same column followed by the same letter do not differ significantly at P = 0.05

ITA 150. The cultivation of these two varieties both of which are susceptible (Chapter 7) to the disease might have led to a build up of the pathogen's inoculumn in the area.

The survey further revealed that panicles scoring up to 5 and sometimes less in the severity scale, are not harvested by the farmers, but left on the field. This practice increases grain yield loss, and at the same time enhances the build up of inoculumn on the field.

False smut of rice is not restricted to Edo State. The disease is wide spread and has been reported from most of the major rice-growing areas of the world (Ou, 1985). However, its occurrence appears to be favoured only by the high humidity and low maximum temperature respectively, of the tropics during the mid-growing season. The present observation might be as a result of this favourable conditions in the zone surveyed.

Gupta and O'toole (1986), has reported the disease as being prevalent in the humid tropic, uncommon in the Guinea savanna, and totally absent in the sudanian savanna. In Nigeria, the disease has been previously reported to be prevalent in the southern part (Awoderu, 1969; 1974; Awoderu and Onuorah, 1974) where humidity is usually very high during the mid-growing season. It has also been reported from Niger State (middle-belt of Nigeria) by Adeoti (1987) in the month of September. In the northern part of the country, the disease has not been previously reported. There was however an occurrence of the disease in

Table 4: A comparison of the observations of the incidence and severity of false smut of rice in some major rice producing areas of Edo State (between September, 2nd to 16th and October 3r to 13th, 1992)

Location	Mean Disease Incidence (%)		Mean Disease Severity (%)	
	Sept. 2nd-16th	Oct. 3rd-13th	Sept. 2nd-16th	Oct. 3rd-13th
Ukhun	17.82	4.22	7.68	1.17
Idoa	25.33	5.53	10.59	1.54
Ilushi	22.20	13.29	11.96	4.60
Agbede	23.54	4.67	11.75	1.31
Agenebode	21.86	5.00	8.92	2.02
Ihievbe	24.72	9.05	12.31	2.68
Uokha	38.34	10.55	18.82	4.18
Ovbiowun	53.79	18.62	28.95	9.80
Mean	28.45	8.87	13.87	3.41
T Value	6.66**		6.79**	

** = Highly significant (i.e, significant at P = 0.01)

Samaru, in the northern Guinea zone of Nigeria, in 1994. This might also be associated with the high and extended rainfall experienced during the month of September and October, 1994.

CHAPTER 5

5 EFFECT OF SOWING DATE ON THE INCIDENCE AND SEVERITY OF FALSE SMUT OF RICE

5.1 Introduction

Observations made during the 1992 surveys suggested flowering period and therefore, the sowing date of a cultivar to have some influence on the incidence of false smut of rice. This same opinion was held by most of the farmers whose farms were sampled for the disease.

To determine the effect of sowing date on the incidence and severity of false smut of rice, field trial was conducted at two sites on the outskirt of Ovbiowun-Emai, in the 1993 wet season. The aim was to evaluate sowing date as a cultural method of controlling the disease in the rainforest zone of Nigeria.

5.2 Materials and methods

A local rice cultivar 'Agbede' earlier known to be susceptible to false smut was used. The experiment was carried out at two sites on the outskirt of Ovbiowun, using the randomized complete block design. Starting from April 2nd, six sets of planting each separated from the other by an interval of two weeks, were carried out separately at both sites. There were three replicates per treatment (sowing date) and the size of each plot was 4m x 5m.

Each plot was hoe weeded three times before crop maturity. NPK 25:10:10 compound fertilizer was applied in two splits, by hill application, at the rate of 20kg N/ha: 8kg P_2O_5/ha: 8kg

k/ha. The first application was done three weeks after sowing and the second, eight weeks after sowing.

Disease incidence and severity was assessed for each of the sowing dates at 120 days after sowing when the rice had reached full maturity. Disease assessment was done using the same procedure described in section 4.2.3.

5.3 Results and Discussion

The results (Table 5) showed that, the highest mean percentage incidence (51.65%) and severity (31.51%) of false smut was recorded on rice sown in mid-May. This was significantly (P = 0.01) higher than the disease incidence and severity recorded on those sown in early-April, mid-April, early-May and Mid-June respectively; but similar to disease incidence and severity on early-June sown crop. There were no significant differences (P = 0.05) among the disease incidence and severity observed on rice sown in early-April, mid-April, and early-May respectively. These were however, significantly (P = 0.01) lower than the disease incidence and severity in the mid-June sowing. Rice sown in early-April were completely free from false smut infection.

The above results showed that early planting of rice during the cropping season, might effectively help in escaping severe damage due to false smut infection. Since such rice will head and mature before the peak of the rains.

Table 5: Effect of sowing date on the incidence and severity of false smut of rice in Ovbiowun-Emai, during the 1993 cropping season

Sowing Date		Site	Disease Incidence (%)	Mean Disease Incidence (%)	Disease Severity (%)	Mean Disease Severity (%)
I	April, 2nd (July, 30th)	1	0.00		0.00	
	April, 3rd (July, 31st)	2	0.00	0.00[c]	0.00	0.00[c]
II	April, 16th (Aug. 13th)	1	3.85		1.28	
	April, 17th (Aug. 14th)	2	2.38	3.12[c]	0.60	0.94[c]
III	May, 2nd (Aug. 29th)	1	2.47		0.62	
	May, 3rd (Aug. 30th	2	0.00	1.24[c]	0.00	0.31[c]
IV	May, 16th (Sept. 14th)	1	52.6		27.37	
	May, 17th (Sept. 15th)	2	50.84	51.65[a]	35.64	31.51[a]
V	June, 2nd (Sept. 29th)	1	39.09		25.54	
	June, 3rd (Sept. 30th)	2	57.99	48.54[a]	36.96	31.25[a]
VI	June, 16th (Oct. 13th)	1	29.01		14.78	
	June, 17th (Oct. 14th)	2	30.26	29.64[b]	15.49	15.14[b]
S.E.D ±				5.63		3.63
LSD (0.05)				11.74		7.57

Figures in parenthesis = Date of disease assessment

Figures folowed by the same letter within the same column are not significantly different at (P = 0.05)

Rice sown between mid-May and early-June, flowered between mid-August and early-September when the weather was characterised by high cloud cover, frequent rainfall and high relative humidity. By late-September, when the mid-June sown rice flowered, the weather had become less cloudy; hence the corresponding decrease in the incidence and severity of the disease. Rice sown between early-April and early-May flowered between late-June and early-August when the weather was almost dry, especially during the day.

The present findings are in agreement with those of Rao (1964) who found that varieties of medium duration (130-145 days) registered maximum infection by false smut, while those of shorter and longer durations were comparatively less affected. He attributed this to a higher percentage relative humidity and lower maximum temperature during the period of flowering of the medium duration varieties. He also found a correlation between the incidence and the number of cloudy days.

Thus, timing of sowing to avoid flowering during damp weather will be a good cultural method of controlling the disease.

CHAPTER 6

6 EFFECT OF PLANT DENSITY ON THE INCIDENCE AND SEVERITY OF FALSE SMUT OF RICE

6.1 Introduction

A field trial to determine the possible effect of plant density on the incidence and severity of false smut of rice was conducted during the 1994 cropping season. The trial was located on the outskirts of Ovbiowun-Emai, in Edo State.

6.2 Materials and Methods

A local rice cultivar 'Agbede' was used for this experiment. The experiment was in a randomized complete block design with three treatments composing of three spacing levels, 0.20 x 0.20m, 0.4 x 0.4m, and 0.4 x 0.8m, each replicated three times. The size of each plot was 4m x 5m.

For each of the three spacing levels, twelve seeds were sown per hole. The seedlings were however, thinned down to eight per stand, two weeks after germination.

The plots were hoe weeded three times before the plants reached maturity. NPK 25:10:10 compound fertilizer was applied in two splits by hill application, at the rate of 20kg N/ha: 8kg P_2O_5/ha: 8Kg K/ha. The first application was done three weeks after sowing and the second, eight weeks after sowing.

At 120 days after sowing when the rice was fully matured, disease incidence and severity were assessed for each of the treatments. Disease assessment was done following the procedures described in section 4.2.3.

6.3 Results and Discussion

Results obtained (Table 6) showed that the lowest percentage incidence (35.2%) of false smut was recorded on rice sown at 0.4 x 0.8m spacing level (lowest plant density). This was significantly different (P = 0.05) from the percentage incidences (64.8%, and 64.7%) recorded at 0.2 x 0.2m, and 0.4 x 0.4m, respectively, which were not significantly different from each other. There were however, no significant differences (P = 0.05) among the percentage disease severities observed at the three spacing levels.

The findings did not indicate any clear effect of plant density on the severity of false smut of rice, at the three spacing levels tried. An increase in the density of rice plant was expected to have increased the % relative humidity of the micro-environment, and hence an increase in both the incidence and severity of the disease.

From the results, it appeared that an increase in plant density increased the average number of rice plants infected by false smut in the field without increasing the severity of the disease on a rice plant.

The results (Table 6) also revealed that grain yield increased with increase in plant density. In a plant density experiment carriedout at Ibadan and Ikene, Nigeria, by IITA (1982) at spacing levels ranging from 10 x 10cm to 50 x 50cm, it was also found that the grain yield of all the varieties tried increased with plant density within the range investigated.

Table 6: Effect of plant density on incidence and severity of false smut of rice in Ovbiowun-Emai during the 1994 cropping Season

Spacing (M)	Replicate	Disease Incidence (%)	Mean Disease Incidence (%)	Disease Severity (%)	Mean Disease Severity (%)	Yield (t/ha)	Mean Yield (t/ha)
0.2	1	55.46		25.84		1.634	
x	2	63.59	64.80[a]	31.72	32.87	1.550	1.446[a]
0.2	3	75.35		41.04		1.155	
0.4	1	72.73		46.97		0.860	
x	2	46.47	64.65[a]	27.02	39.56	1.015	0.888[b]
0.4	3	74.75		44.70		0.789	
0.4	1	32.73		20.45		0.761	
x	2	23.64	35.15[b]	18.18	23.79	0.874	0.752[b]
0.8	3	49.09		32.73		0.620	
SED ±			7.17				0.0895
LSD (0.05)			19.90				0.025

Figures followed by same letter in the same column are not significantly different at P = 0.05

The inference is that, wide spacing of rice, or the use of low seedrate, to reduce the incidence of false smut might be unnecessary.

CHAPTER 7

7 SCREENING OF RICE CULTIVARS FOR RESISTANCE TO FALSE SMUT

7.1 Introduction

The use of resistant cultivars in disease control remains the most feasible, most economic, easiest, safest and most effective means (Agrios, 1978). This is so because it is the method which does not involve the farmers in extra input of paying for pesticides with the attending risks in their use. In developing resistant cultivars, the first step is to look for source(s) of resistance.

This experiment was therefore designed to identify upland rice cultivar(s) that could serve as source(s) of resitance to false smut, under field conditions.

7.2 Materials and Methods

Seven upland rice cultivars, 'Agbede', ITA 335, ITA 315, ITA 316, ITA 150, IRAT 170, and Ex-China, were screened for resistance to false smut of rice under field conditions, using 'Agbede' (local cultivar) as the check. The trial was carried out in 1993 cropping season at two different sites, on the outskirts of Ovbiowun-Emai.

At each of the two sites, the experiment was set up in a randomized complete block design, with each cultivar serving as a treatment. There were three replicates per treatment, each plot measuring 4m x 5m.

Each of the cultivars screened, except Ex-china and 'Agbede' were collected from Edo State Agricultural Development Project Unit, Irua. The Ex-China cultivar was collected from the National Seed Service, Zaria while the local cultivar, 'Agbede'

was collected from a farmer in Ovbiowun-Emai. The cultivars were sown at different dates depending on the maturation period of each cultivar as inquired from the source with the target of all, flowering during the damp weather of mid-August to early-September. To further enhance the development of the disease at heading, NPK 25:10:10 compound fertilizer was applied in two splits at the rate of 20Kg N/ha: 8Kg P_2O_5/ha: 8Kg K/ha. The first application was done three weeks after sowing, and the second at 'booting' stage, by hill application. Hoe weeding was done three times on each plot before crop maturity.

At 'flowering', development of the disease was further encouraged by broadcasting pieces of smut balls obtained from previously infected fields uniformly on the field.

At maturity, the cultivars were assessed for percentage disease severity as described in section 4.2.3.

The grading of cultivars for their reaction to attack by false smut was adapted from Orji (1985). The grading was as follow:

Percentage Disease severity		Reaction group
0	=	Highly resistant (HR)
<1	=	Resistant (R)
1-5	=	Moderately resistant (MR)
5.1-10	=	Moderately susceptible (MS)
10.1-20	=	Susceptible (S)
and > 20	=	Highly susceptible (HS)

7.3 Results and Discussion

The results (Table 7) indicated that the seven rice cultivars reacted differently to false smut infection under the same field conditions.

From the combined analysis of data obtained from the two sites, it was found that, ITA 315, ITA 335, and the check 'Agbede', were highly susceptible; while ITA 150 was susceptible. ITA 316, Ex-China and IRAT 170 were moderately resistant, resistant, and highly resistant respectively.

It should, however, be noted that, IRAT 170 though appeared to be highly resistant to false smut attack at both sites, was severely infected by blast especially in one of the sites.

Little information is available on varietal resistance to false smut of rice (Ou, 1985). Certain varieties have however, been noted to be more frequently attacked than others. In India for example, Rao (1964) observed that 186 varieties remained free from infection among the 297 screened under the same environmental conditions which were favourable for the disease development. In Nigeria, Adeoti (1987) found severe infection only on two cultivars, GEB 24/37 and TOS 103, out of the thirteen cultivars screened for disease under the lowland rainfed conditions.

The forgoing results have shown that a wide range of reaction of rice varieties to false smut, exists. The present findings have indicated that ITA 316, Ex-china, and IRAT 170, are the most promising genotypes for resistance to false smut of rice, among the seven varieties screened.

Table 7: Reaction of seven upland rice cultivars to false smut under field conditions at two sites in Ovbiowun-Emai during the 1993 cropping season

Variety	Site	Disease Incidence (%)	Mean Disease Incidence (%)	Disease Severity (%)	Mean Disease Severity (%)	Reaction group/ site	Reaction group/ var.
	1	28.16		13.73		S	
ITA 315	2	52.84	40.50[a]	35.61	24.67[a]	HS	HS
	1	32.67		18.13		S	
ITA 335	2	53.83	42.92[a]	33.01	25.57[a]	HS	HS
	1	7.07		1.77		MR	
ITA 316	2	10.05	8.56[b]	3.00	2.39[c]	MR	MR
	1	28.87		12.47		S	
ITA 150	2	43.38	36.13[a]	18.01	15.24[b]	S	S
	1	0.00		0.00		HR	
IRA 170	2	0.00	0.00[b]	0.00	0.00[c]	HR	HR
	1	2.78		0.69		R	
Ex-China	2	3.94	3.36[b]	0.52	0.61[c]	R	R
	1	32.56		20.88		HS	
'Agbede' (Check)	2	48.60	40.56[a]	29.60	25.24[a]	HS	HS
SED ±			7.85		4.46		
LSD (0.05)			16.20		9.21		

Figures followed by same letter in the same column are not signficantly different at P = 0.05

CHAPTER 8

8. EVALUATION OF SOME FUNGICIDES FOR THEIR EFFECACIES IN CONTROLLING FALSE SMUT ON RICE

8.1 Introduction

Fungicides have continued to serve as a quick and effective means of controlling fungal disease of plants, despite their associated hazards. They are of two major types, the non-systemics, and the systemics. The non-systemic (contact) fungicides do not enter the plant tissue, but remain on the outside of the plant where they kill the growing fungal hyphae but do not damage the plant (Hill and Waller, 1990). On the other hand, the systemic fungicides must enter plant tissue to be effective.

Not much is known about the chemical control of false smut. This could probably be due to its rating as a minor disease (Ou, 1985). Not withstanding while there have been a few reports from Asia on the chemical control of false smut disease of rice (Ou, 1985), there have been no such reports from Africa, however. A trial was therefore conducted in 1994, to evaluate the efficacies of six fungicides for the control of false smut of rice.

The six fungicides tested were carefully selected from both systemic and non-systemic fungicides. They included, Delsene M, Dithane M-45, Cupravit, Benlate, Rovral, and Tecto. The formulation used, trade and common names of the fungicides as well as their chemical compositions are given in Table 8.

Table 8: Formulation, chemical nature, common and Trade names of fungicides evaluated in the laboratory and field for their efficacies against U. virens

Trade name	Common name of fungicide (a.i)	Chemical nature of fungicide (a.i)	Formulation of fungicide used
Benlate	Benomyl	Methyl-1-(butylcarbamoyl) benzimidazole 2-yl carbamate	50WP
Delsene M	Carbendazim + Maneb	Carbendazim = (methyl benzimidazole 2-yl carbamate)-10% Maneb = (manganesse ethylene bisdithio-carbamate) - 64%	80WP
Cupravit	Copper oxychloride	50% copper, as copper oxychloride	50WP
Rovral	Iprodione	3-(3,5-dichlorphenyl)-1-isopropyl carbamoyl *hydantion*	50WP
Tecto	Thiabendazole (TBZ)	2-(thiazol-4-yl) benzimidazole	Fl
Dithane M-45	Mancoceb	2.5%cn ion + 20%Mn as in maneb	80WP

8.2 Materials and Methods

8.2.1 In-vitro assessment of the effect of six fungicides on growth of U. virens

The six fungicides were evaluated for their efficacies in controlling the growth of U. virens in the laboratory. Each of the fungicides was evaluated at three concentrations, low, medium, and high as shown in the table below:

Table 9: Concentration of fungicides evaluated in the laboratory for their efficacies against U. virens

Fungicide	Concentration levels:- Low (0.5x)	Medium (x)*	High (2x)
Benlate	0.75g/lit.	1.5g/lit.	3g/lit.
Delsene M	1.5g/lit.	3g/lit.	6g/lit.
Dithane M-45	3.13g/lit.	6.25g/lit.	12.5g/lit.
Cupravit	5g/lit.	10g/lit.	20g/lit.
Rovral	3.2g/lit.	6.4g/lit.	12.8g/lit.
Tecto	0.3ml/lit.	0.6ml/lit.	1.2ml/lit.

*represents the concentration used in the field (Adeoti, personal communication).

To assess the fungicides, appropriate concentrations of the above formulations were incorporated into 200ml of Potato Dextrose Agar (PDA) respectively.

To prepare PDA-fungicide mixture, the following method was adopted. The appropriate quantity of the fungicide were added separately into 500ml conical flask. To each of the wettable powders in conical flask, little volume of sterile distilled water was added enough to dissolve the chemical. Appropriate volume of motten PDA at about 45°C was then added, to make the volume up to 200ml. The flask was carefully shaken to ensure proper mixing of the chemical and the PDA. Appropriate volume of streptomycin solution (2ml, from 1g/200ml solution) was also incorporated into the PDA-fungicide mixture before pouring to prevent the growth of bacteria.

To ensure that equal quantity of the medium was poured into each plate, the following method was adopted. McCartney bottles were sterilized along with the media in the autoclave. Using one

20ml McCartney bottle for each concentration (filling the bottle up to a marked point on the neck), 20ml of the medium was poured into each petri dish. The whole process was carried out in a closed, U-V light illuminated, inoculation room; sterilized with IZAL. Seven petri dishes were poured from each concentration. The plates were left on the bench in the sterilized inoculation room, for 24 hours, to set before use.

A 0.6cm (diameter) cork borer was used to transfer the fungus from 7-day old culture, onto the centre of each fungicide-agar plate. 'Check' treatments consisting of PDAS plates alone were similarly inoculated for each fungicide concentration in the experiment.

The plates were arranged in completely randomized design, in an incubator and incubated at 27±2°C.

Fungitoxicity was assessed by measuring the radial (diameter of) growth of the fungus after 7-days.

8.2.2 Evaluation of effects of six fungicides on the control of false smut of rice under field conditions

A field trial to evaluate the efficacies of six fungicides on the control of false smut on rice was carried out during the 1994 wet season. The trial was located on the outskirts of Ovbiowun-Emai. Delsene M, Dithane M-45, Cupravit and Benlate were evaluated in the field at their respective recommended rates of 1.2, 2.5, 4.0, 0.6Kg/ha; while Rovral, and Tecto were evaluated at the rates of 2.6Kg/ha and 240ml/ha respectively (Adeoti, personal communication) using the local cultivar 'Agbede'.

The experimental layout was a randomized complete block

design with three replicates, each plot measuring 4m x 5m. A 1m border separated one plot from the other.

The plots were hoe weeded three times. NPK (25:10:10) fertilizer was applied in two splits (first at 3 weeks after sowing, and the second at 'booting' stage) at the rate of 20kg N/ha: 8Kg P_2O_5/ha: 8kg K/ha.

Spraying started immediately the first 'boot' was sighted. Spraying was carriedout four times at 7 day intervals. For each fungicidal spray, 400 litres of water was used per hectare using CP3 knapsack sprayer.

Percentage disease severity and grain yield were assessed at harvest. From the data obtained percentage Disease control (PDC) and Yield Deviation (PYD) were estimated for the fungicides, respectively, applying the formulae given by Pathak (1972). The formulae are as follow:-

$$PDC = \frac{\text{Disease severity in control} - \text{Disease severity in treatment}}{\text{Disease severity in control}} \times 100$$

$$PYD = \frac{\text{Yield in treatment} - \text{yield in control}}{\text{yield in control}} \times 100$$

8.3 Results and Discussion

8.3.1 In-vitro assessment of the effect of six fungicides on growth of U. virens

The results (Table 10) showed that Delsene M (Carbendazim + Maneb), Benlate (benomyl), Cupravit (copper oxychloride), and Tecto (Thiabendazole, TBZ) completely inhibited mycelial growth

Table 10: Effect of three concentrations of six fungicides on the growth of U. virens on Agar medium

Fugicide	Concentration of fungicide in agar (g/lit. or Ml/lit.	Growth of U. virens 7-days after inoculation	
		Mean radial diameter of colony (cm)	Percentage growth reduction (PGR)
	3.13	4.2	53.33
	6.25	3.4	62.22
Dithane M-45	12.50	2.7	70.00
	5.00	0.0	100.00
Cupravit	10.00	0.0	100.00
	20.00	0.0	100.00
	0.30	0.0	100.00
Tecto	0.60	0.0	100.00
	1.20	0.0	100.00
	0.75	0.0	100.00
Benlate	1.50	0.0	100.00
	3.00	0.0	100.00
	1.50	0.0	100.00
Delsene M	3.00	0.0	100.00
	6.00	0.0	100.00
	3.20	5.0	44.44
Rovral	6.40	4.7	47.78
	12.80	3.6	60.00
	No Fungicide	9.0	
Control	"	9.0	
	"	9.0	

Reduction or complete inhibition of mycelial growth of many fungi in-vitro by different concentrations of various fungicides has been reported by inumerable workers. However, it has been well established that the efficiency of a fungicide in-vitro may not hold in the field (in-vivo) (Davis and Dimond, 1952). Rather the results provide simple clues to what may probably occur in the field.

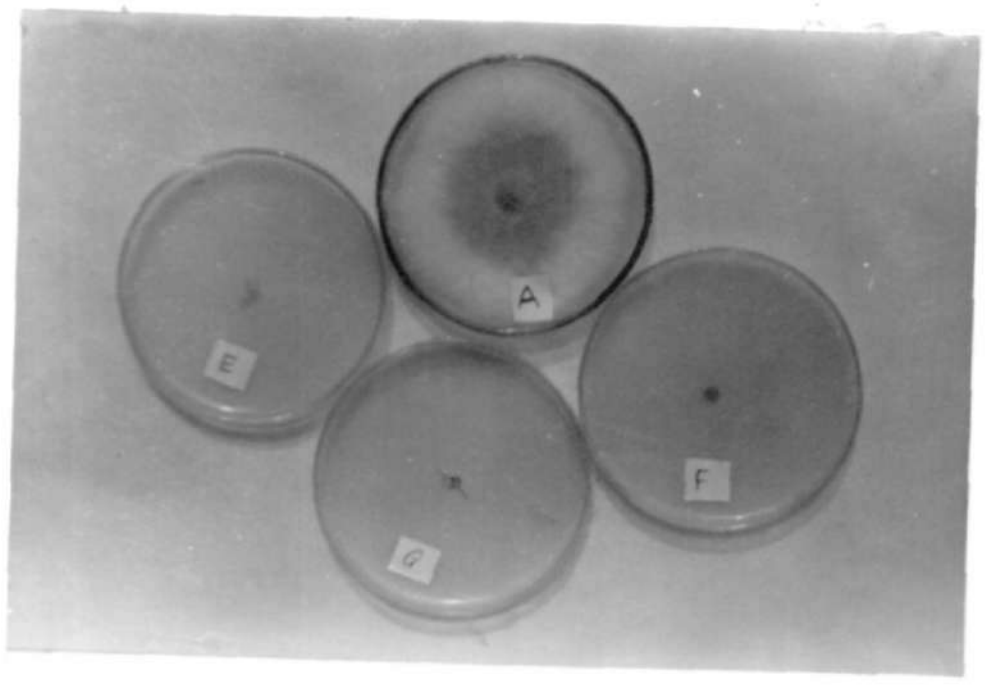


Plate 8: Effect of different concentrations of Delsene M on the mycelial growth of U. virens on potato dextrose agar (PDA).

A = control, E = 0.5x
F = x and G = 2x

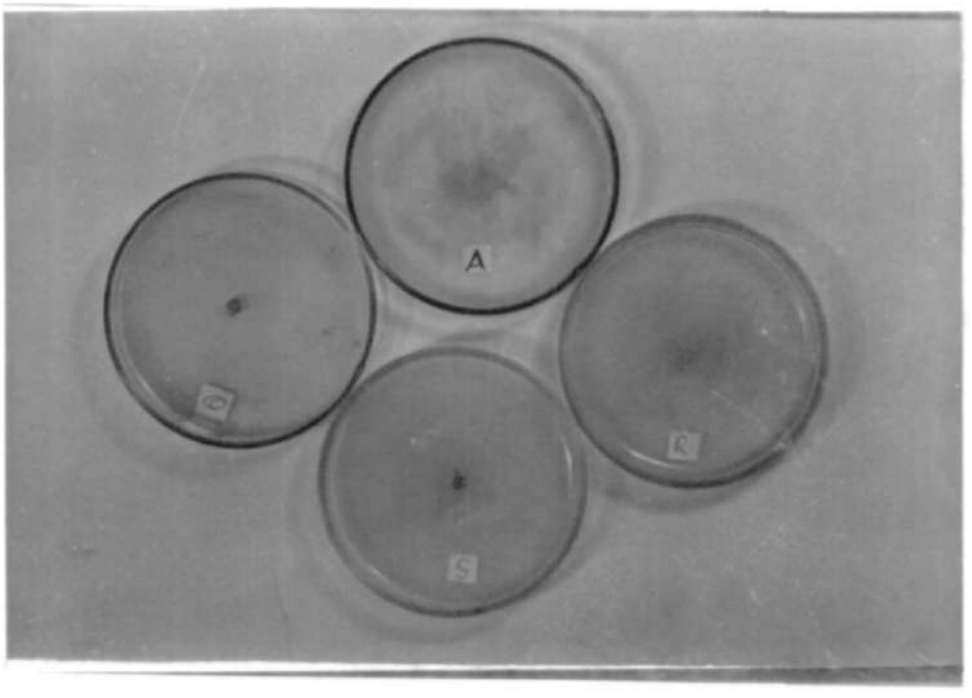


Plate 9: Effect of different concentrations of Benlate on the mycelial growth of _U. virens_ on potato dextrose agar (PDA).

A = control, Q = 0.5x, R = X and S = 2x

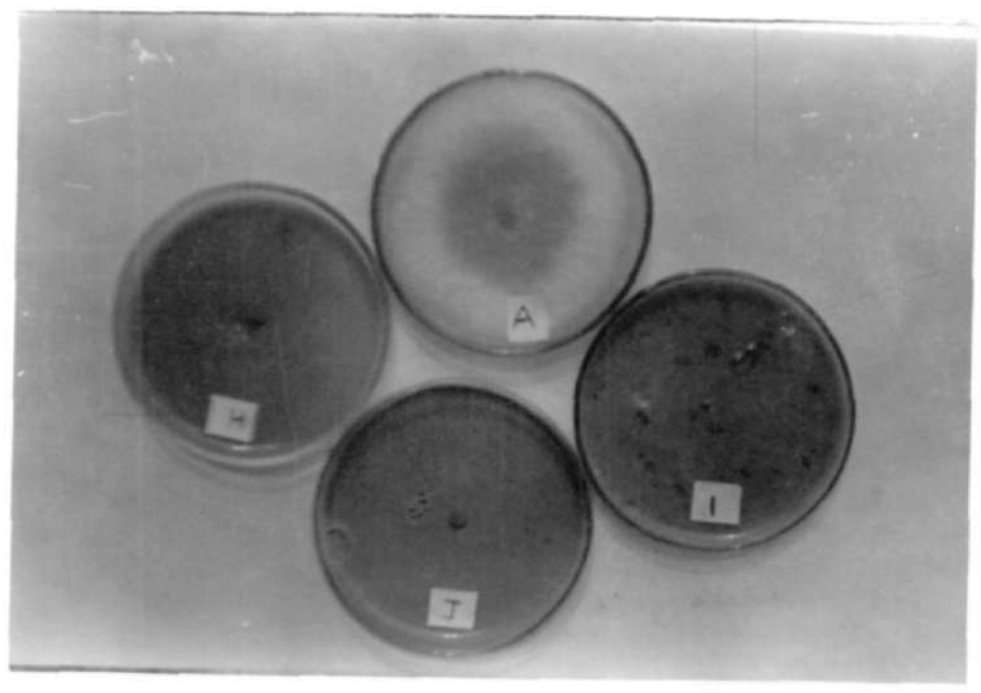


Plate 10: Effect of different concentrations of cupravit on the mycelial growth of U. virens on PDA medium.

A = control, H = 0.5x, I = X and J = 2x

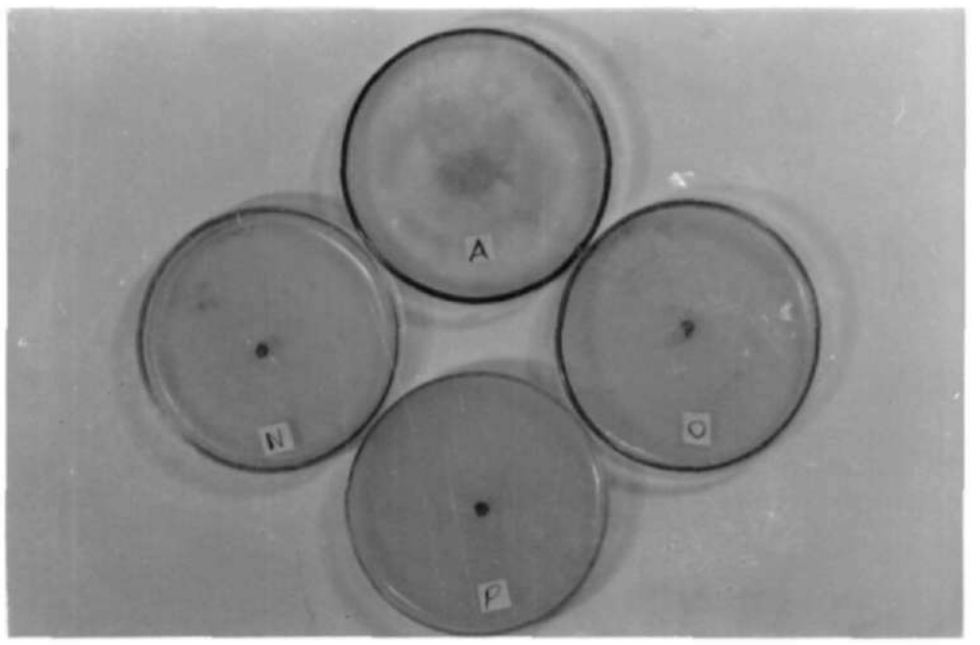


Plate 11: Effect of different concentrations of Tecto on the mycelial growth of _U. virens_ on PDA medium.

A = control, N = 0.5x, O = X and P = 2x

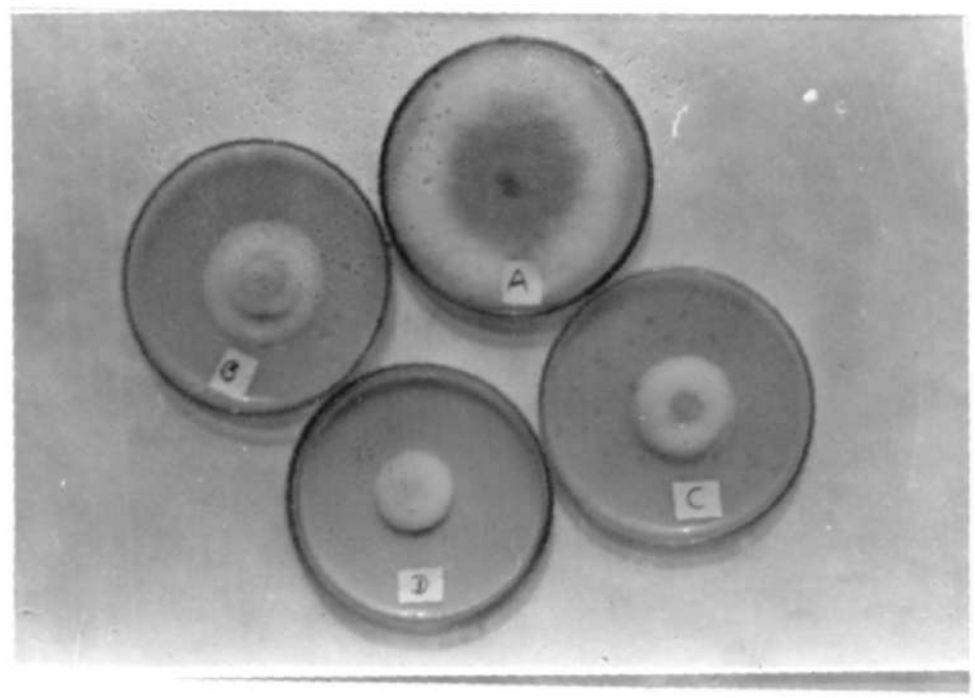


Plate 12: Effect of different concentrations of Dithane M-45 on the mycelial growth of _U. virens_ on PDA medium.

A = control, B = 0.5x, C = X and D = 2x

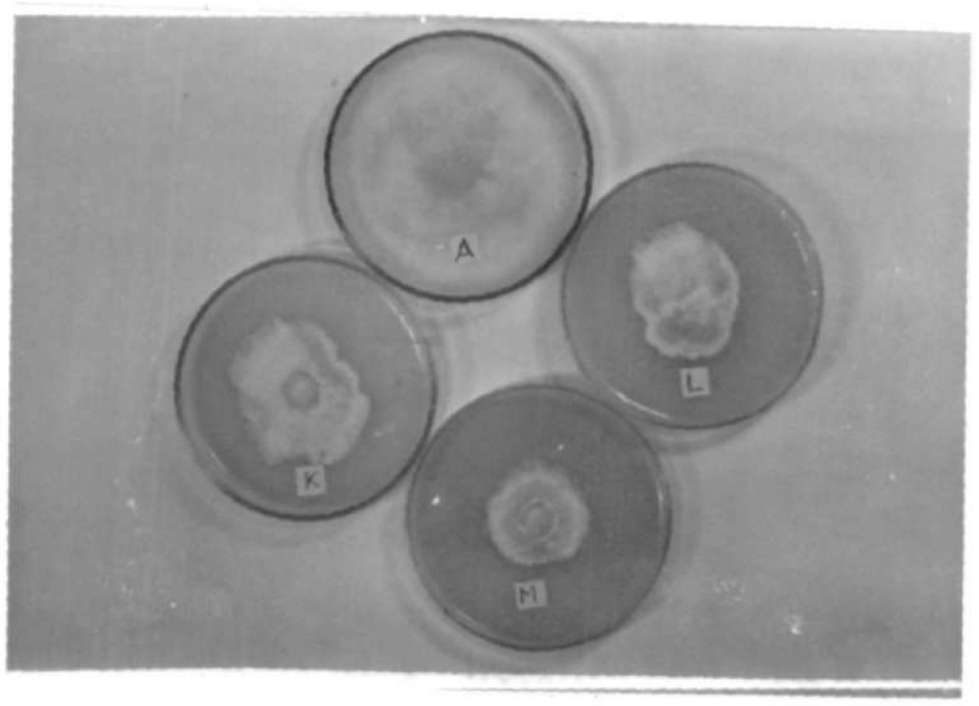


Plate 13: Effect of different concentrations of Rovral on the mycelial growth of _U. virens_ on PDA medium.

A = control, K = 0.5x, L = X and M = 2x

8.3.2 Evaluation of effects of six fungicides on the control of false smut of rice under field conditions

The results (Table 11) showed that there was a high significant difference (P = 0.01) between the percentage severity in plots sprayed with any of the six fungicides screened and that in the control plot.

Delsene M apparently gave the highest percentage disease control of (100%). However, it was phytotoxic to the test plants at the field concentration (1.2kg/ha) used. The rice plants were scorched and killed (Plate 14) due to Delsene M sprays. Only few tillers survived, producing panicles that were completely free from false smut infection.

The fungicide that was most effective in controlling false smut on rice, therefore was Cupravit; followed by Dithane M-45. Cupravit gave 75.9% disease control and significantly increased grain yield by 43.2% as compared with the control. Spraying the crop with Dithane M-45 resulted in 47% disease control but the resulting increase in grain yield was not significant (P = 0.05) statistically.

Tecto, and Benlate though reduced percentage disease severity of false smut, the percentage disease control (33.8% and 26.6% respectively) achieved can not be compared with the percentage reduction in mycelial growth (100% respectively) of U. virens in the laboratory. This could probably be explained on the basis of the mode of action of the two fungicides. Both fungicides are systemic in their action. To be effective, in-vivo, they need to be absorbed into the plant tissue, and

Table 11: Effect of foliar application of six fungicides on the incidence and severity of false smut of rice in Ovbiowun-Emai, During the 1994 cropping season

Fungicide	Mean Disease Incidence (%)	Mean Disease Severity (%)	Mean Grain yield (t/ha)	Percentage Disease control (PDC)	Percentage Deviation in yield (PDY)
Dithane M-45	38.88[b]	18.51[c]	1.370[b]	47.02	8.04
Delsene M	0.00[d]	0.00[e]	0.250[c]	100.00	-80.28
Tecto	47.36[a]	23.14[bc]	1.289[b]	33.77	1.66
Cupravit	22.41[c]	8.41[d]	1.816[a]	75.93	43.22
Rovral	50.26[a]	24.47[b]	1.46[ab]	30.00	15.46
Benlate	50.79[a]	25.66[b]	1.370[b]	26.56	8.04
Control	59.52[a]	34.94[a]	1.268[b]	-	-
SED±	5.69	2.70	0.13		
LSD (0.05)	12.39	5.88	0.28		

* The plants were scorched and killed by the fungicide at the field concentration (1.2Kg/ha) used. However, the few tillers that survived, produced panicles that were completely free from infection.

Figures followed by the same letter in the same column are not significantly different at P = 0.05



Plate 14: A rice plot scorched and killed by spraying with Delsene M at 1.2Kg/ha during the 'booting' stage of the plants

translocated acropetally to the meristematic tissues where they become active (Hill and Waller, 1990). It is therefore possible that most of the panicle primodia had been formed before the spray schedule started. Another explaination could be that, false smut being an air-borne disease of inflorescence has little or no contact with the plant meristematic tissues, or transport system, and therefore contact with the fungicides.

Dithane M-45 (mancozeb) has been reported by Awoderu (1974) to be effective in the control of rice blast (Pyricularia oryzae) and brown spot (Cochliobolus miyabeanus), both in the laboratory and in the field.

Cupravit (copper oxychloride) has also been reported earlier to be effective in reducing the incidence of false smut on rice (Kannaiyan and Rao, 1976). However, contrary to the results obtained in this present study, the reduction in incidence observed by Kannaiyan and Rao, did not result in any yield increase.

SUMMARY AND CONCLUSIONS

Symptomatological studies carried out in 1992, revealed that the first visible sign of false smut infection was noticed as a small whitish-yellow ball of mycelium between the glumes (lemma and palea) at about two weeks of 'heading' ('flowering'). This later developed into large greenish-yellow smut ball of a velvety appearance. The smut ball consisted of an outer greenish and powdery layer, central yellow layer, and inner whitish core, both of which were highly sclerotic in nature.

Chlamydospores produced in the field, germinated on PDAS medium and produced white fluffy, cottony mycelium which later developed an orange-yellow colouration at the centre, with a dark brown background. Hyphae were profusely branched, and septate, bearing pear-shaped, hyaline, minute conidia at their tips in clusters.

Reproduction of the disease symptoms by artificial inoculation was achieved by spraying young panicles to run-off with both chlamydospores suspension, and condidia suspension respectively, using a hand sprayer. Injecting spore suspension into the leaf-sheaths enclosing young panicles, at 'booting' stage, as well as spraying developing grains with spore suspension at the 'milk-grain' stage did not cause any infection.

Reproduction of the typical symptoms of false smut only by spraying young panicles with spore suspension indicated that infection occurs at 'flowering' (or 'heading') stage of the rice plant.

Furthermore, reproduction of false smut disease by spraying young panicles with a suspension of conidia obtained from pure

culture of the isolate (U. virens), confirms the organism as the pathogen of false smut of rice.

Field surveys carried out in 1992 revealed that there was significantly (P = 0.01) higher incidence and severity (17.8% to 53.8% and 7.7% to 29%, respectively) of false smut of rice in mid-August to mid-September, than in late September to mid-October. Although the disease was found to be present in all the rice producing areas of Edo State surveyed; it was most prevalent in Ovbiowun-Emai and Uokha, both in Owan east local government area of Edo State. This is likely so, because most farmers in the area grow a local variety 'Agbede' which is highly susceptible to the disease, while few others grow ITA 150 variety which is also susceptible.

Sowing date trials conducted in 1993 cropping season, showed that the disease was most severe on rice sown between mid-May and early-June. This was followed by those sown at later dates. On the otherhand, rice sown with the early rains (between early-April and early-May) were virtually free from false smut infection.

Weather conditions during flowering period, appeared to be the major factor influencing the development of the disease. Rice sown between mid-May and early-June flowered between late-August and early-September, when the weather was really damp; hence the significantly (P = 0.01) higher percentage disease incidence and severity on these crops. Towards ending of September when the later-sown rice flowered, the dampness of weather had reduced hence the corresponding decrease in the incidence and severity of the disease. There was little

overcast, between late-June and early-August when the early-sown rice flowered; hence the escape. Thus, sowing susceptible cultivar early in the season, to avoid flowering during damp weather, will be a good method of controlling false smut of rice.

Plant density trial conducted in 1994 showed that percentage incidence of false smut increased with increase in plant density. However, the severity of the disease was not significantly affected by plant density, at the three spacing levels tried. Grain yield was found to increased with increase in plant population. Wide spacing of rice to reduce false smut infection, may not be an effective control of the disease on the field.

Varietal resistance screening carried out in 1993 cropping season under field conditions, revealed that the seven cultivars screened reacted differently to false smut infection. It was found that ITA 315, ITA 335 and 'Agbede' (the 'check') cultivars were highly susceptible. On the other hand, ITA 316, Ex-China and IRAT 170 cultivars were moderately resistant, resistant, and highly resistant respectively. The results also showed that ITA 150 cultivar was susceptible. The study has indicated ITA 316, Ex-china, and IRAT 170 as the most promising genotypes for resistance to false smut of rice.

Laboratory evaluation of six fungicides (Dithane M-45, Delsene M, Rovral, Cupravit, Tecto, and Benlate) at three concentrations, on the control of false smut of rice, showed that all the six fungicides at the three concentrations, significantly inhibited, or reduced mycelial growth of the pathogen, U. virens on agar medium to various extents. The six fungicides were also evaluated in the field at their recommended rates. The results

showed that four fungicidal sprays at an interval of 7-days, starting at the sighting of the first 'boot', significantly (P = 0.01) reduced the severity of the disease. Although all the six fungicides were effective in reducing infection; the best result was obtained by spraying with Cupravit. This resulted in 75.9% disease control, and significantly (P = 0.01) increased grain yield by 43.2%. Spraying crops with Dithane M-45 also resulted in 47% disease control, but did not increase grain yield significantly. Delsene M completely controlled the disease but was highly phytotoxic at the recommended field concentration (1.2Kg/h) used.

From this study, the following concluding remarks could be made:-

False smut of rice induced by Ustilaginoidea virens (Cke.) Tak. is an inflorescence disease of rice. It is the most common, and most damaging disease of rice grains in the humid areas of Edo State, Nigeria. Though the disease was present in all the rice producing areas of the state surveyed, it appeared to be more prevalent in some areas (Ovbiowun and Uokha specifically) than others.

False smut occurrence is favoured by damp weather, therefore rice flowering under this condition (which normally occurs at the end of August and early to mid-September) are most vulnerable. Appropriate timing of sowing would be a very good method of avoiding severe infection.

Plant density trial did not show any significant effect on the severity of false smut on rice and therefore might not be of any use in the control programme.

While some rice cultivars appeared to be resistant to the disease, others differ in their degree of suceptibility. This indicates that the use of host resistance may be a good control measure that could be exploited.

Four fungicidal sprays of cupravit (copper oxychloride) during the 'booting' stage of rice at 7-days intervals, at the rate of 4.0kg/ha, significantly controlled false smut and increased grain yield. Thus, this fungicidal control method, could be employed when a severe infection is envisaged. However, the comparative cost benefit ratio of the use of the chemical needs to be determined.

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APPENDICES

Appendix 1: Description of some terms as Used by the Author

'Booting' stage: This is the stage of rice plant at which the young panicles (or panicle prmordium) develops from the apical part of a tiller and is enclosed by the last and specially modified leaves that form a tube or sheath around it in the form of a cone.

'Boot': This is cone-shaped tube or sheath in which the yong panicles is enclosed and develops, during the booting stage of the rice plant.

'Heading' stage: ('Flowering' stage): Heading stage is the stage of the rice plant at which the leafsheaths or tubes enclosing the young panicles had opened up, and the young panicles emerged. i.e at this stage the young panicles are carried aerialy either fertilized or yet unfertilized. It is synonymous to 'flowering' which refers to the emergence of young panicles from the boots.

'Milk-grain' stage: At this stage, the flowers had been fertilized and the glumes filled up with milky sustance that later solidifies into the grain.

'Smut' ball and 'spore' ball: 'Smut' ball refers to the mass of mycelia and spores of the smut fungus, with the 'grain' smutted. On the other hand, 'spore ball refers to a mass of spores of the

smut fungus. However, smut ball is also sometimes referred to as spore ball, thus they are used interchangeably.

Appendix 2: Analysis of variance of the incidence and severity of false smut of rice in some major rice producing areas of Edo State, from a survey carried out between September 2nd - 16th, 1992

(a) Incidence

Source	DF	SS	MS	F
Replicates	2	5.4	2.7	0.01NS
Treatments	7	2950.0	421.4	2.84*
Residual	14	2073.5	148.1	
Total	23	5028.9		

(b) Severity

Source	DF	SS	MS	F
Replicates	2	3.84	1.92	0.03NS
Treatments	7	1008.54	144.08	2.74*
Residual	14	736.29	52.59	
Total	23	1748.67		

NS = Not significant at 5% level
* = Significant at 5% level

Appendix 3: Analysis of variance of the incidence and severity of false smut of rice in some major rice producing areas of Edo State from a survey carried out between October 3rd - 13th, 1992

(a) Incidence

Source	DF	SS	MS	F
Replicates	2	166.68	83.34	3.24*
Treatments	7	548.39	78.34	3.04*
Residual	14	359.85	25.70	
Total	23	1074.92		

(b) Severity

Source	DF	SS	MS	F
Replicate	2	31.22	15.61	1.65NS
Treatments	7	189.52	27.08	2.86*
Residual	14	132.14	9.44	
Total	23	352.88		

NS = Not significant at 5% level
* = Significant at 5% level

Appendix 5: Analysis of variance of the incidence and severity of false smut of rice, and the grain yield from plant density experiment conducted in 1994

(a) Incidence

Source	DF	SS	MS	F
Replicates	2	292.02	146.01	
Treatments	2	376.27	188.13	4.04*
Residual	4	188.99	46.50	
Total	8	854.27		

(b) Severity

Source	DF	SS	MS	F
Replicate	2	721.61	360.80	
Treatments	2	1749.00	874.50	11.32*
Residual	4	308.86	77.22	
Total	8	2779.47		

(c) Grain yield

Source	DF	SS	MS	F
Replicates	2	0.1419	0.0709	
Treatments	2	0.8129	0.4064	32.82**
Residual	4	0.0481	0.0120	
Total	8	1.0028		

NS = Not significant at 5% level
* = Significant at 5% level
** = Significant at 1% level

Appendix 6: Analysis of variance of the incidence and severity of false smut of rice from varietal resistance screening carried out in 1993

(a) Incidence

Source	DF	SS	MS	F
Replicates	2	346.4	173.2	
Site	1	1315.37	1315.3	11.15**
Residual[A]	2	235.8	117.9	
Varieties	6	14098.7	2349.8	12.72**
Varieties.Site	6	980.1	163.3	
Residual[B]	24	4432.1	183.7	
Total	41	21408.4		

(b) Severity

Source	DF	SS	MS	F
Replicates	2	160.36	80.18	
Site	1	581.21	581.21	12.06**
Residual[A]	2	96.35	48.17	
Varieties	6	5298.53	883.09	14.82**
Varieties.Site	6	631.20	105.20	1.76NS
Residual[B]	24	1429.66	59.57	
Total	41	8197.32		

NS = Not significant at 5% level
** = Significant at 5% level

Appendix 7: Analysis of variance of the incidence and severity of false smut of rice and grain yield from fungicidal evaluation experiment conducted in 1994

(a) Incidence

Source	DF	SS	MS	F
Replicates	2	143.94	71.97	
Treatments	6	9290.25	1548.38	21.87**
Residual	12	849.49	70.79	
Total	20	10283.68		

(b) Severity

Source	DF	SS	MS	F
Replicate	2	49.40	24.70	
Treatments	6	3540.06	590.01	35.33**
Residual	12	200.39	16.70	
Total	20	3789.85		

(c) Grain yield

Source	DF	SS	MS	F
Replicates	2	0.0065	0.0032	
Treatments	6	4.1833	0.6972	24.92**
Residual	12	0.3356	0.0280	
Total	20	4.5254		

** = Significant at 1% level