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by

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CAB International Mycological Institute
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Contents

Preface

A few years ago the Institute of Seed Pathology in Denmark decided to produce a series of publications dealing with seed-borne diseases of individual crops. The main objective was to compile and collate information of practical value which plant pathologists and seed technologists could use in handling such diseases both in the field and in the laboratory. The first publication in the series was "Seed-Borne Diseases of Chickpea" by Haware, Nene & Mathur (1986). The 32-page publication was the result of a fruitful co-operation between our Institute in Denmark and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in Hyderabad, India. The present publication deals with seed-borne diseases of rice.

Rice is among the most important cereals in the world. Efforts should be made to keep its seed health at a high level in order to meet the challenge posed by the growing population. The rice crop is known to be attacked by many seed-borne diseases of major and minor importance. Twenty of these are described here. Some are well known, widely distributed diseases of economic significance (blast, brown spot, bakanae), others are of restricted distribution, important in certain special areas, but which are becoming increasingly known in recent years (leaf scald, sheath rot, false smut, kernel smut) and lastly a few bacterial diseases which have become known only recently (bacterial stripe, bacterial sheath brown rot, bacterial grain rot). A short description of each disease consists of information on its geographical distribution, losses incurred, characteristics of the causal organism, location of the organism in or on the seed, damage caused to seeds, seedlings and plants, transmission of the organism through seeds and other means, laboratory testing of seed for health, and control of seed-borne infection and to a very limited extent the diseases in the field. Comments have been made, wherever known, on seed certification and quarantine. Knowledge of these aspects is essential in multiplication programmes and in plant quarantine laboratories where seed is tested for quality and freedom levels from dangerous organisms. This can be achieved easily by following the seed health testing methods described in this publication. The testing methods described are either those which have been evaluated internationally and recommended by the International Seed Testing Association or tests that have been used at our Institute for a number of years with success.

Seed-Borne Diseases and Seed Health Testing of Rice is a joint publication between our Institute and the CAB International, United Kingdom. We thank *Professor David Hawksworth*, Director, CAB International Mycological Institute for this arrangement, and for providing us CMI Maps, and *Drs B.C. Sutton* and *J.F. Bradbury* of the same institute for editing the chapters.

The authors are grateful to various colleagues who provided colour photographs of field symptoms for this publication: *Dr Y.H. Lee*, Lecturer, Agricultural Institute, Rural Development Administration, Suweon, Republic of Korea; *Dr A.S. Prabhu*, Rice Plant Pathologist, Centro Nacional de Pesquisa-Arraz, Feijao (CNPAF), Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), Goias, Brazil; *Dr Robert S. Zeigler*, Leader Rice Programme, International Center for Tropical Agriculture (CIAT), Cali, Colombia; *Dr V.T. John*, Rice

Research Programme, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; *Dr M. Agyew-Sampong,* West Africa Rice Development Association (WARDA), Monrovia, Liberia; *Dr H. Kato,* Division of Microbiology, National Institute of Agro-Environment Studies, Ibaraki, Japan; *Dr L.P. Kauraw,* Rice Plant Pathologist, Central Rice Research Institute (CRRI), Cuttack, India; *Mr Jusuf Soepriaman,* Rice Plant Pathologist, Bogor Research Institute for Food Crops (BORIF), Bogor, Indonesia, *Dr Shizuo Mogi,* Indonesia-Japan Joint Programme on Food Crop Protection, Ministry of Agriculture, Jakarta, Indonesia; and *Dr Sergia P. Milagrosa,* Benquet State University, Philippines.

We wish to thank *Ms Rigmor Hjælmhøj* for efficient secretarial help and *Mr Magd El-din Ragab* and *Ms Hanne Petersen* Laboratory Technicians of our Institute for technical assistance and photography.

A number of chapters were critically examined by *Drs A.S. Prabhu, V.T. John, Y.H. Lee, Robert S. Zeigler, D.D. Shakya,* Lecturer, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, and *Professor Masao Goto,* Faculty of Agriculture, Shizuoka University, Japan, and we thank them for their suggestions. The English text has been checked by *Dr Mary Noble,* Scotland, and we are indeed very thankful to her.

Copenhagen, November, 1989

Authors

Chapter 1

Blast

Widespread in Africa, Asia, Australasia and Oceania, Europe, Central America and West Indies, North and South America (CMI Distribution Maps of Plant Diseases No. 51, ed. 6, 1981; Fig. 1.1).

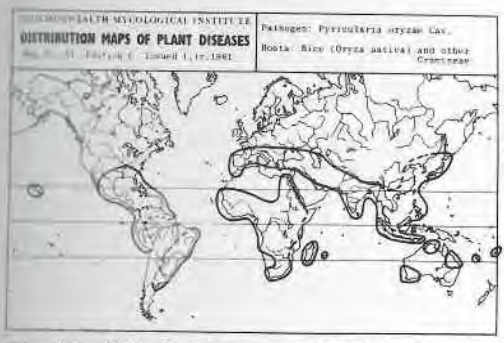

Fig. 1.1. Distribution map of blast disease published by the CAB International Mycological Institute, Kew, U.K. in 1981

Blast is the most serious disease of rice because of its devastating nature, widespread distribution and existence of several physiologic races of the causal organism. The disease has occurred in epiphytotic condition in many countries. In India, 75% loss of grain occurred in 1950 in susceptible cultivars (Padmanabhan, 1965), while in the Philippines several thousand hectares suffered more than 50% loss in yield (Ou, 1985). Forty per cent loss in yield has been reported both from Nigeria (Awoderu & Esuruoso, 1975) and Liberia (Carpenter, 1977). Blast is the major yield constraint for upland rice production in Brazil (Prabhu & Morais, 1986). These are only a few examples of many reported in the literature on the seriousness of the disease.

Symptoms

The fungus can infect rice plants at any growth stage although it is more frequent in the nursery (Fig. 1.2) and flowering stages. Spots or lesions are produced on leaves, nodes, different parts of the panicle and the grains. Symptoms on the leaves first appear as small whitish or greyish specks which enlarge quickly under favourable conditions such as drought stress, dew and high nitrogen levels in the soil.

Fully developed lesions on susceptible cultivars are spindle shaped and measure 1 to 1.5 cm in length and 0.3 to 0.5 cm in width (Fig. 1.3), but under very favourable conditions for disease development lesions may be as large as 8 cm long and 0.5 to 0.9 cm wide (L.H. Lee, pers. comm., 1988). Heavi-

Fig. 1.2. Severe blast in a rice nursery (Photo: Courtesy Dr M. Agyew-Sampong, WARDA, Liberia)

ly infected leaves soon dry up and plants die. In resistant cultivars, no symptoms or only minute brown pin head spots are observed. At this stage they can be confused with the symptoms of brown spot produced by *Bipolaris oryzae* (Breda de Haan) Shoem. Cultivars of intermediate reaction show small, circular or elliptical lesions with a brown margin (Ou, 1985).

When the base of the panicle (neck) is attacked (Fig. 1.4) the tissue shrivels and becomes black. Infected panicles often break at the neck and hang down. Such symptoms called 'neck rot' result in higher crop losses.

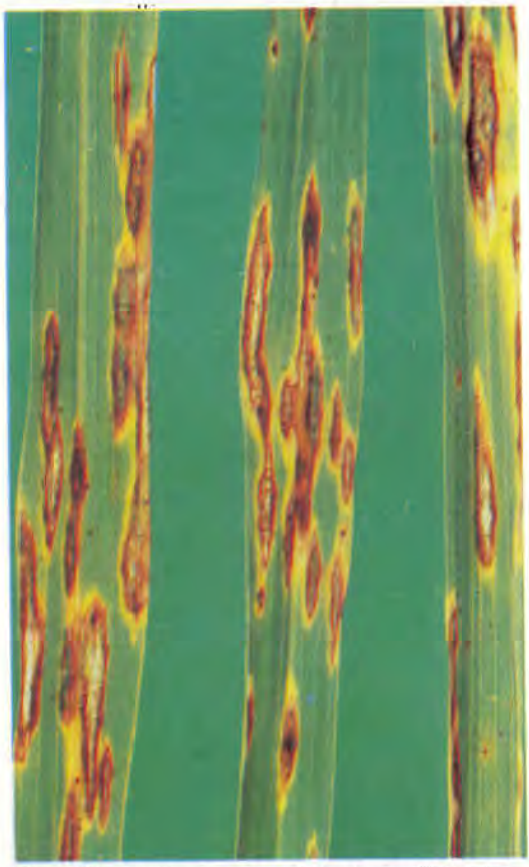

Fig. 1.3. Fully developed lesions of blast on rice leaves
(Photo: Courtesy Dr A.S. Prabhu EMBRAPA, Brazil)

Fig. 1.4. Panicle infection due to *Pyricularia oryzae*
(Photo: Courtesy Dr Y.H. Lee, South Korea)

In nodal infection, the nodal region of the flowering axis rots and turns black (Fig. 1.5). Blackening may extend both ways up to 1–2 cm. The flowering axis often breaks apart at such nodes (Figs. 1.5 and 1.6) but remains connected only by the nodal septum.

Pathogen

Pyricularia oryzae Cav.

Growth of the fungus on PDA is greyish. Mycelium is septate, branched and hyaline to olivaceous. Conidiophores arise singly or in fascicles, simple, rarely branched, slightly thickened at the base. Conidia are borne sympodially, pyriform to obclavate, hyaline to pale olive, usually 2-septate, rarely 1- or 3-septate, apex narrow, base

Fig. 1.5. Nodal infection due to *Pyricularia oryzae*
(Photo: Courtesy Dr A.S. Prabhu, EMBRAPA, Brazil)

Fig. 1.6. Severe nodal infection. Breaking of nodal tissue in cv. Bong Kwang
(Photo: Courtesy Dr Y.H. Lee, South Korea)

rounded with a basal appendage or hilum (Fig. 1.7). Size of conidia varies considerably depending upon the conditions under which they are produced. Average size is 19-23x7–9 μm (Cavara, 1891; Nisikado, 1917). Thick walled chlamydospores measuring 5-12 μm in diameter are often produced in culture. The fungus sporulates extremely well on rice-straw-decoction agar (V.T. John, unpubl.) and also on polished rice agar (Yeh & Shu, unpubl.).

A large number of physiologic races have been reported, 250 from Philippines, 18 from Japan, 27 from Taiwan and 18 from South Korea, etc. Isolates from Japan, USA and Taiwan were categorised into 32 race groups. The geographical difference in virulence pattern has also been studied (Ou, 1985). Matsumoto, Kozaka & Yamada (1969) grouped isolates from 13 countries into 3 groups, namely Japan, Philippines and India, which Matsumoto (1975) later divided into four groups, Japan, Philippines, India and America.

Fig. 1.7. Conidia of *Pyricularia oryzae*, x 750

Location of the pathogen in the seed

Conidia of the fungus may be present on the seed surface and resting mycelium in the tissues of embryo, endosperm, bran layers and glumes, and also between the glumes and the kernel (Mathur, 1981). The healthy appearance of seeds must not be taken as proof that they are free from infection.

Seed infection and plant infection

According to Ou (1985) air-borne conidia are the most important means of dissemination of *P. oryzae,* although the fungus may also be disseminated by infected seeds and straw, and by conidia which fall into the irrigation water. Transmission of the pathogen from seed to plant under upland conditions was observed in an isolated, virgin field in Goiania, Goias, Brazil (A.S. Prabhu, pers. comm., 1989). Seeds of cv. IAC 165 were machine drilled. The blast was first observed only in those seedlings which developed from seeds that fell on the ground and were not covered by soil. The seedling infection resulted in a blast epidemic during the vegetative phase. The inoculum from the diseased plants caused severe panicle infection and the yield was reduced to half. Dr Prabhu strongly believes that the rate of transmission of *P. oryzae* from seeds to seedlings is much greater in situations where seeds are not covered by soil. This is probably the reason for high transmission rate in fields transplanted from seedlings raised in nursery boxes. The blast epidemic of 1967 in Japan was attributed to seed-borne infection of *P. oryzae* (H. Suzuki, pers. comm., 1969).

A seed sample of cv. 'Jinheung' collected from heavily infected fields in Korea, on laboratory examination, showed 65% infection on the hulls, 25% on pericarp and 4% in embryo. The same seed lot when grown on water agar resulted in 7 to 8% of young seedlings with typical lesions while approximately 90% seedlings showed no apparent symptoms (Chung & Lee, 1983). In another study in the U.S.A., seed samples with 40% surface infection resulted in 7 to 13% diseased seedlings (Lamey, 1970). Aulakh, Mathur & Neergaard (1974b) reported that moderate infection in the field could result in 7 to 18% of seed infection.

Seed health testing

According to the definition of the International Seed Testing Association (ISTA, 1985), health of seed refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests, such as eelworms and insects but physiological conditions such as trace element deficiency may be involved.

International comparative tests have been conducted to standardize testing methods for important seed-borne fungal pathogens of rice. In 1985 the blotter method was recommended in the International Rules for Seed Testing as a testing method for detecting *Pyricularia oryzae* in rice seed samples. The method, and the procedural details as followed at the Institute of Seed Pathology in Denmark are:

- A working sample of 400 seeds is tested in replicates of 25 seeds per dish of 9 cm diam. Since the seeds are later incubated in light, the dishes should be of such a material that allows light to pass through, e.g. Petri dishes made from clear plastic, and glass dishes of Pyrex or Corning. Dishes made from clear plastic are now widely used, but they are not readily available in many developing countries.

  If the seed health testing is conducted at a Seed Testing Station for issuing International Seed Lot Certificate, the working sample must be a well-mixed, representative sample of the seed lot for which the health report is required. In plant quarantine laboratories, sometimes the samples received for testing contain less than 400 seeds. In such

Fig. 1.8. Dripping off of the excess water from the well soaked three pieces of filter papers. The filter papers should be set in the Petri dish the minute running of water stops

Fig. 1.9. Plating of rice seed on well water-soaked filter papers

cases the whole sample or part of it may be tested. Only healthy seedlings, free from infection, must be released and grown under strict control of the plant quarantine inspector.

- Label each dish properly with the accession number of the seed sample, date of examination and the dish number.
- Before plating the seeds in the Petri dishes, each dish should be lined with three filter papers (blotters), well soaked in water. The water soaked filter papers for the dishes can be prepared in the following way: Count three filter papers at a time, dip them in water (tap water or distilled water) for a few seconds, lift and let the excess water drip off (Fig. 1.8) before setting the soaked blotters in the dish. Prepare 16 such dishes for the 400 seeds to be tested, 25 seeds per dish.
- Plate 25 seeds in each dish as shown in Fig. 1.9, 15 seeds in the outer ring, 9 in the middle and one in the centre.
- Incubate the 16 dishes (Fig. 1.10) at 20-

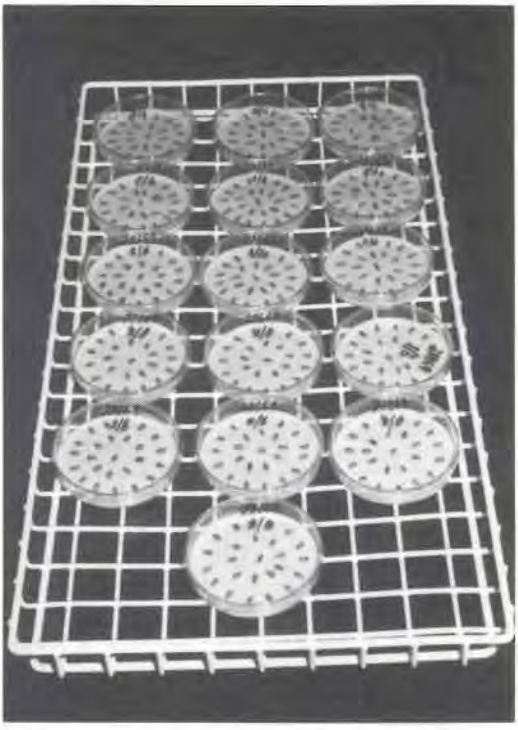

Fig. 1.10. A sample of 400 seeds, 25 seeds in each Petri dish, ready to be incubated

22°C for 7 d in alternating cycles of 12 h darkness and 12 h light. The common source of light used at present is the near ultraviolet (NUV) supplied by black light tubes (e.g. Philips TLD 36W/08) or daylight provided by cool, white fluorescent tubes (e.g. Philips TLD 36W/84). In either case, light should be supplied by two tubes hanging horizontally, 20 cm apart from each other and the distance between the light tubes and the dishes should be 40 cm.

- After 7 days' incubation, start examination of seeds under a stereoscopic microscope with magnification at least up to × 50 or × 60. All seeds of the outer ring must be examined first, then the seeds of the second ring and finally the seed in the centre of the dish. Examination of seeds in sequence becomes easier when a line is drawn with a coloured pencil (Fig. 1.11). The first seed to be examined is the one which lies on the right hand side of the line in the outer circle. Examine thoroughly the whole seed at different magnifications before proceeding to the next seed.

  Once the examination of the first seed is finished rotate the dish gently clockwise with the middle finger and the thumb of the left hand (for a right handed person) while still looking into the microscope. Follow this procedure in moving from one seed to the other.
- Whenever the growth of *P. oryzae* is seen on a seed, mark the seed infected by writing letters such as Py (Fig. 1.11), near to the infected seed.

  Usually, the growth of *P. oryzae* is small, inconspicuous, grey to greenish-grey on glumes, consisting of short, delicate conidiophores carrying clusters of conidia (Fig. 1.12). Each cluster consists of a few conidia. Rarely, the growth of the fungus may cover the whole seed.

  Under a stereoscopic low-powered microscope the growth of *P. oryzae* can sometimes be confused with that of *Cladosporium* (Fig. 1.13) which is a common saprophyte. In *Cladosporium*, conidia are grouped in brush-like structures on comparatively long, dark conidiophores while in *Pyricularia*, each conidium in a cluster ends in an acute tip which is clearly seen at 50 times or higher magnifications.
- Identification of *P. oryzae* under the stereoscopic microscope needs experience in seed health testing. Whenever a growth is suspected to be of *Pyricularia* or a *Cladosporium*, mount the conidia in water on a glass slide and confirm the conidial morphology of *P. oryzae* under higher magnifications of a compound microscope as in Fig. 1.7.
- Count the total number of seeds infected by *P. oryzae* in each dish and enter the figures in a recording sheet, and calculate per cent seed infection.

Fig. 1.11. Examination of seeds under stereoscopic microscope done after drawing a helping line as shown in the photo. Py stands for infection of *Pyricularia oryzae*

Fig. 1.12. Growth of *Pyricularia oryzae* at the embryonal end of a rice seed incubated in the blotter test, x 50

Fig. 1.13. Growth of *Cladosporium cladosporioides* at the embryonal end of rice seed incubated in the blotter test. Note the difference between this and Fig. 1.12, x 50

Control

To control seed-borne inoculum a number of fungicides and antibiotics and a combination of fungicides and antibiotics have been used. Effective control was achieved with Aureofungin (Rahalkar & Neergaard, 1969), Kasugamycin (Dekker, 1971), Rhodane (Polyakov & Petrova, 1962), Dithane M-45 (Park & Cho, 1972), Blastidin S + phenyl mercury acetate (Ishii, 1962). More recently CGA 49104 50w.p. (Ciba-Geigy) and PP 389 (ICI) (Bandong, Nuque, Torres & Crill, 1979), Benomyl + Thiram and Topsin M + Thiram (Mogi, 1979) were found to be very effective.

Application of Tricyclazole, a systemic fungicide as a seed treatment, foliar spray, soil drenching of nursery bed and bare root soak can give long term effective control (Froyd, Paget, Guse, Dreikorn & Pafford, 1976).

Some non-mercuric fungicides developed and widely used in recent years are Kitazin P, Hinosan, Oryzemate, Fuji-one, Rabcide (Mogi, 1979). Phthalide 30 W.P. (Zafar, 1986), isoprothiolane 40 EC (Verma & Kumar, 1985) and pyroquilon (Prabhu, 1985) have also been found to be effective in blast control.

Early planting and restricted nitrogen fertilizer application help in avoiding serious outbreaks of blast. Raising seedlings in drought-prone upland, deep transplanting, close spacing and cold irrigation water (20°C or below) increase blast incidence.

According to Prabhu (pers. comm., 1989) in Brazil one foliar fungicide spray at heading is recommended as a safeguard in upland rice where the yields are low. The seeds are generally contaminated with spores and there is no legislation for seed treatment. However, some seed producers treat seed with Thiram and thiabendazol.

Quarantine

While categorizing quarantine objects

Neergaard (1979) considered *Pyricularia oryzae* under category A. In this category, dangerous plant pathogens, which are not present in the region of introduction and which have a high or considerable epidemic potential or possess a large number of races, are included. Neergaard (1981) stated that since *P. oryzae* has several hundred pathogenic races, seeds imported to the European and Mediterranean Plant Protection Organization (EPPO) region should not be infected with this serious pathogen. He also maintained that introduction of new races to rice growing countries in the EPPO area could be just as detrimental as it became to rice cultivation in Burkina Faso, Nigeria and South Korea. In 1971 the Government of Greece (FAO, 1972) prohibited the introduction of seed for sowing of the genus *Oryza* infected with *P. oryzae*. Similar restrictions were also imposed in Chile (Neergaard, 1981). The Plant Quarantine Station at Muguga in Kenya used post-entry cultivation of rice in greenhouses for seed introduced into Kenya, Tanzania and Uganda. Only seeds from healthy plants were released (Neergaard, 1970).

Chapter 2

Brown spot

Widespread, reported in all rice growing countries of Asia, America and Africa *(CMI Distribution Maps of Plant Diseases* No. 92, ed. 4, 1973a; Fig. 2.1).

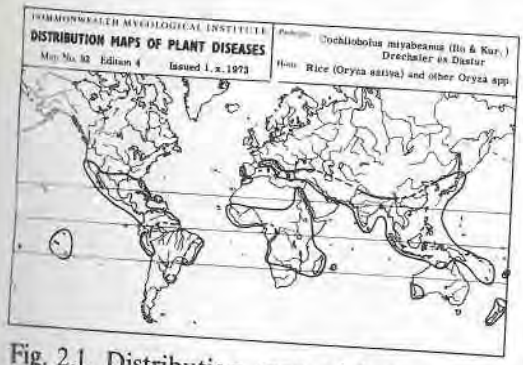

Fig. 2.1. Distribution map of brown spot published by the CAB International Mycological Institute, Kew, U.K. in 1973

Brown spot was one of the principle causes of the Bengal famine of 1942 in India. Estimates show that two million people died of starvation. Nothing as devastating as this epiphytotic has been recorded in plant pathological literature, the only comparable instance being that of the Irish potato famine of 1845. Crop loss during the epidemic year of 1942 ranged from 50 to 90% (Ghose, Ghatge & Subramanyan, 1960; Padmanabhan, 1973). In Nigeria, Aluko (1975) estimated crop losses between 12 and 43%. Loss in grain weight ranging from 12 to 30%, as well as loss in filled grain from 18 to 22%, depending upon the degree of cultivar susceptibility, was recorded by Prahbu, Lopez & Zimmermann (1980) in the State of Para, Brazil. Considerable crop losses have been reported from Japan and Surinam (Ou, 1985).

Symptoms

Typical symptoms of the disease on the leaves are small, circular, dark brown or purplish dots (Fig. 2.2). Fully developed lesions are oval, brown, with grey or whitish centres and are up to 1 cm long in susceptible cultivars (Fig. 2.3). Most spots have a light-yellow halo around their margins. Spots are relatively uniform and evenly distributed. Spots on the glumes are black or dark brown. Under favourable conditions, conidiophores and conidia develop in the spots. In severe cases, the spots may cover the entire panicle, causing direct grain loss. When the infected seed is planted on a substrate, small, brown, circular or oval spots may appear on the coleoptile, sometimes killing it (Fig. 2.4); roots may also show brown to blackish lesions (Fig. 2.5).

Fig. 2.2. Typical symptoms of brown spot *(Bipolaris oryzae)* on flag leaves of a susceptible cultivar. Note pronounced yellow halo. The black grains in the foreground are probably affected by the same pathogen
(Photo: Courtesy Dr Robert S. Zeigler, CIAT, Colombia)

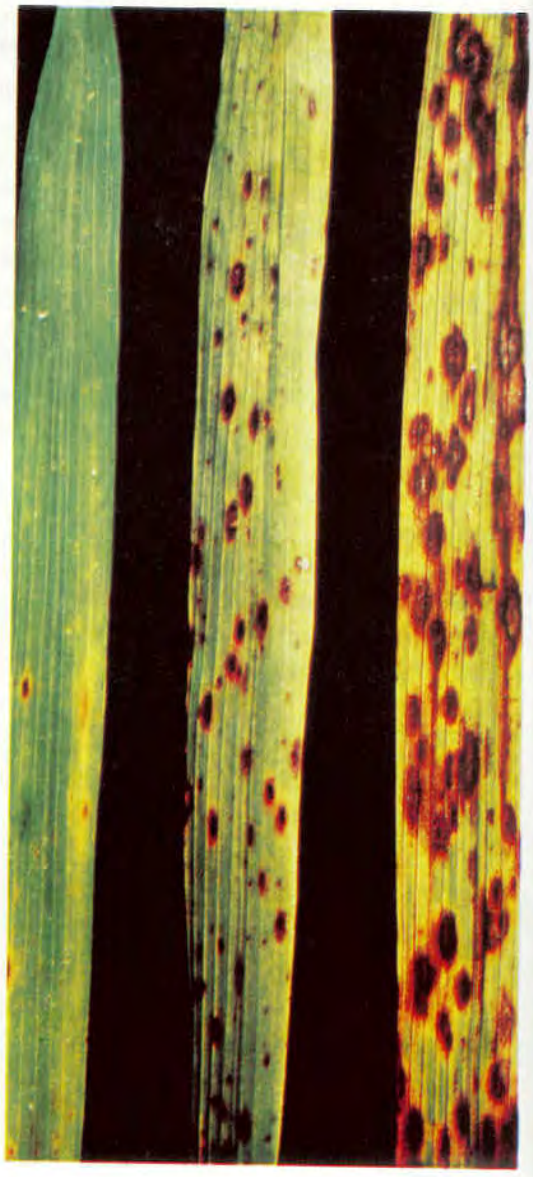

Fig. 2.3. Brown spots caused by *Bipolaris oryzae* showing varietal differences in lesion size and number
(Photo: Courtesy Dr A.S. Prabhu, EMBRAPA, Brazil)

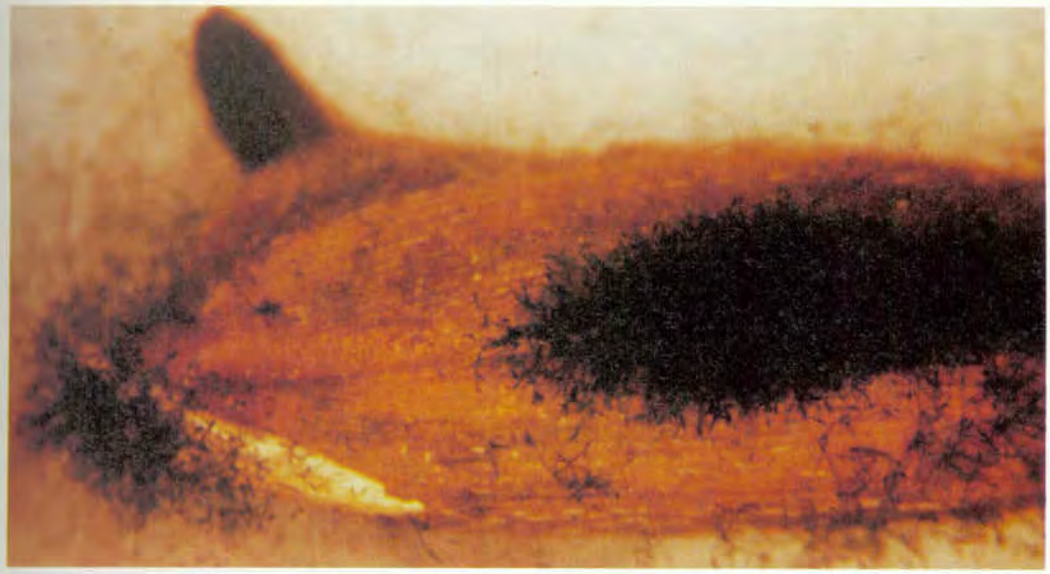

Fig. 2.4. Growth of *Bipolaris oryzae* on an incubated rice seed in the blotter test. Note the highly necrotic coleoptile emerging from the seed

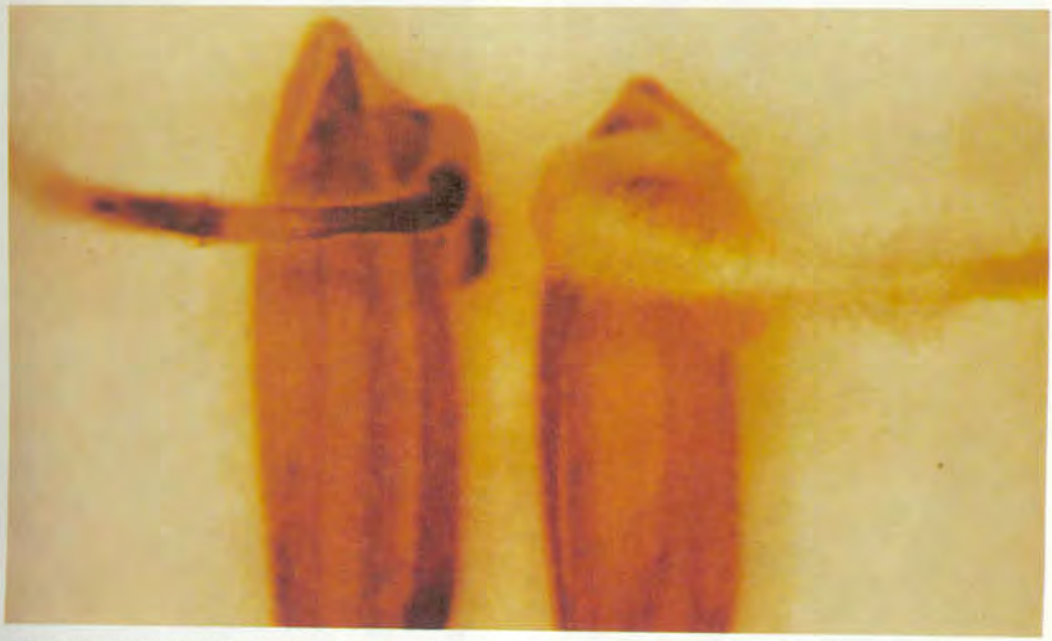

Fig. 2.5. Brown discoloration caused by *Bipolaris oryzae* infection in the radicle (left). Healthy looking radicle (right)

Pathogen

Bipolaris oryzae (Breda de Haan) Shoem.

(syn. Drechslera oryzae (Breda de Haan) Subram. & Jain).

Growth of the fungus on PDA is grey to dark greenish-grey. Mycelium is usually fluffy, aerial, cottony, and sporulation generally sparse; conidiophores solitary or in small groups, olivaceous to light fuliginous. Conidia are fuliginous to olivaceous brown, usually curved, widest at the middle or just above the middle, tapering to rounded ends (Fig. 2.6), occasionally cylindrical and almost straight, 6-14 distoseptate, 63-153(109)x14-22(17)μm; hilum in most cases inconspicuous (Chidambaram, Mathur & Neergaard, 1973; CMI Descriptions of Pathogenic Fungi and Bacteria, No. 302, 1971b).

Cochliobolus miyabeanus (Ito & Kuribay.) Drechsler ex Dastur is the teleomorph of the fungus. Initially it was reported in culture by Ito & Kuribayashi (1927) in Japan, and later Ueyama, Tsuda & Akai (1973) found it on rice glumes.

In Japan, Sakamoto (1934) separated 132 monosporic strains into ten growth types based on cultural, morphological characters and variation in pathogenicity. Nawaz & Kausar (1962) reported similar findings from Pakistan. In India, Padmanabhan (1953) considered that there was no specialization in pathogenicity. Misra & Chatterjee (1963), however, found great differences in sporulating ability and pathogenicity between two isolates.

Location of the pathogen in the seed

Resting mycelium of *Bipolaris oryzae* may

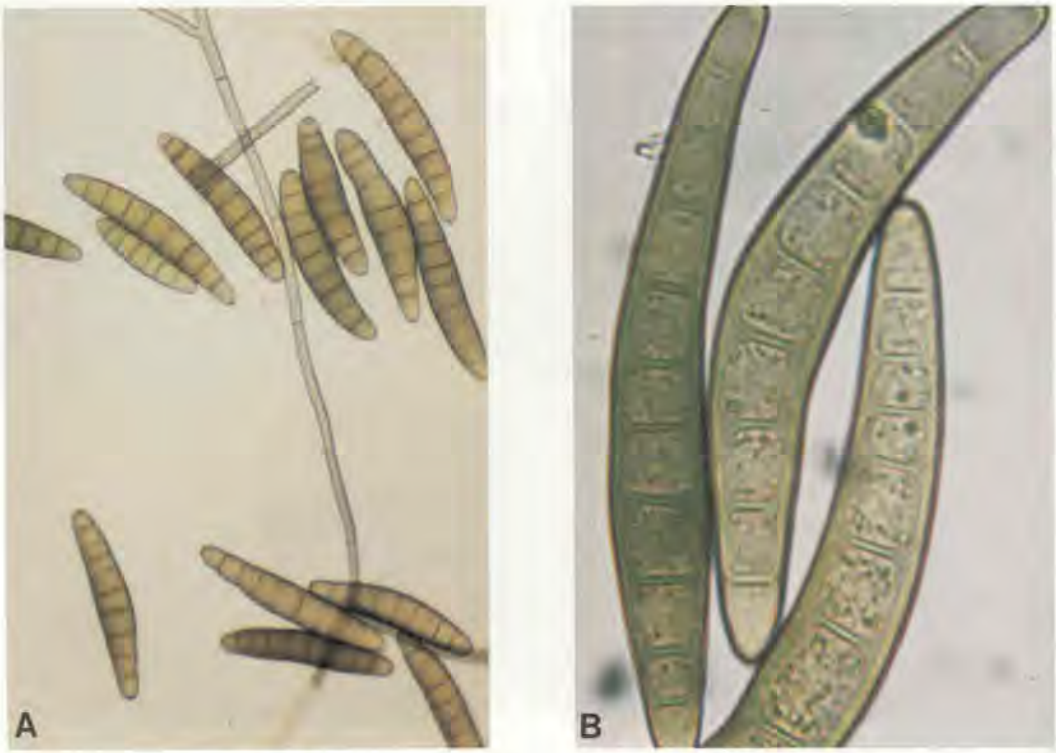

Fig. 2.6. Conidia of *Bipolaris oryzae*, x 750

be present in the seed coat, pericarp and glumes and sometimes in the endosperm (Ocfemia, 1924; Nisikado & Nakayama, 1943; Fazli & Schroeder, 1966). Infected grains show brown stains ('pecky' rice) and sometimes dark brown or black masses of conidia and conidiophores (ISTA, 1964). Apparently healthy looking seed should not be accepted as free from infection (Hiremath & Hegde, 1981).

Seed infection and plant infection

Ou (1985) writes that the primary infection through diseased seed is probably most common, although diseased seeds do not always give rise to infected seedlings. The coleoptile and sometimes roots are often infected from diseased seeds but lesions may not be produced on subsequently developed leaves on account of rapid growth of the leaves under normal conditions. Infection may also take place from the soil (Thomas, 1940; Ganguly, 1946b).

The fungus can survive in the infected grains for varying lengths of time depending on the storage conditions. An average of 2 years was reported from Hokkaido, Northern Japan by Kuribayashi (1929).

The fungus was found not only within discoloured seeds but also in apparently healthy ones by Suzuki (1930). He also found that the fungus can survive in the seed for at least 4 years, and considered that in Japan infected seed cause the first occurrence of the disease the following spring. In India, Padmanabhan (1953) tested various infected plant parts and showed that the fungus was viable from one year to the next growing season on the seed only.

Germination of seed infected by *Bipolaris oryzae* is lower than that of healthy seed. Singh, Singh & Shukla (1979) observed 11–29% reduction in germination, Herrera & Seidel (1978) up to 66%, and Aluko (1970) 29%. Similar observations were also made by Kulkarni, Ramakrishnan & Hegde (1980) in India and Kulik (1977) in the U.S.A. A severely infected seed lot of cv. IAC 899 showed only 17.5% germination, with transmission of 59.4% of *B. oryzae* from seed to plants (Prabhu & Vieira, 1989). They further showed that in naturally infected seed lots, predominantly associated with *B. oryzae* and *Phoma sorghina*, significant, negative correlation exists only between germination and seeds infected with *B. oryzae*.

Aulakh, Mathur & Neergaard (1974a) investigated death of seedlings infected with *B. oryzae* in the blotter test and in direct sowings of seed in pots. In the blotter test they observed up to 100% loss while the ratio of seed infection to seedling loss in pots was 1:0.8. In Nigeria, Aluko (1969) observed that 81.9% seed infection can give 90% loss in seed viability as recorded in the laboratory tests. When these seeds were sown in the field, about 45% germinated; six weeks later, the remaining seedlings developed blight and died. In India, Hiremath & Hegde (1981) detected *B. oryzae* in 60-72.5% of the partially developed grains. Seedlings from such seeds died before maturity. Aulakh *et al.* (1974b) found a relationship between field incidence of *B. oryzae* and seed infection recorded in laboratory tests.

Guerrero, Mathur & Neergaard (1972) made two interesting observations, 1) *B. oryzae* was detected in 60% of abnormal seedlings, and 2) 78% of the seedlings having root and shoot decay had infection of *B. oryzae*. Kulik (1977) recommended the towel test (between paper) at 15 or 20°C to predict the field emergence of *B. oryzae* infected rice seeds.

Air-borne spores are responsible for secondary infection in the field. Sreeramulu & Seshavataram (1962) and Chandwani, Balakrishnan & Padmanabhan (1963) showed that the numbers of air-borne con-

idia over rice fields in India to be highest during October to January.

Seed health testing

Seed samples must be tested by the blotter method as recommended in the International Rules for Seed Testing (ISTA, 1985). Details of the method are the same as described in Chapter 1 for *Pyricularia oryzae*.

- Examine each seed at 12-50 x magnification under a stereoscopic microscope for the growth of *Bipolaris oryzae*. Two types of growth may be observed: 1) Mycelium absent or scanty, conidiophores straight or flexuous, brown, bearing dark brown conidia acropleurogenously. Conidia curved and tapering towards the ends, olivaceous to dark brown (Fig. 2.7), 2) Greyish black fluffy mycelial growth with slender conidiophores and conidia covering part or whole seed, often extending on to the blotter (Fig. 2.8) (Chidambaram *et al.*, 1973). In doubtful cases confirmation may be made by preparing slides and examining them under a compound microscope (Fig. 2.6). See further details under pathogen.
- Count the total number of seeds infected by *Bipolaris oryzae* in each dish and enter the results in a recording sheet.

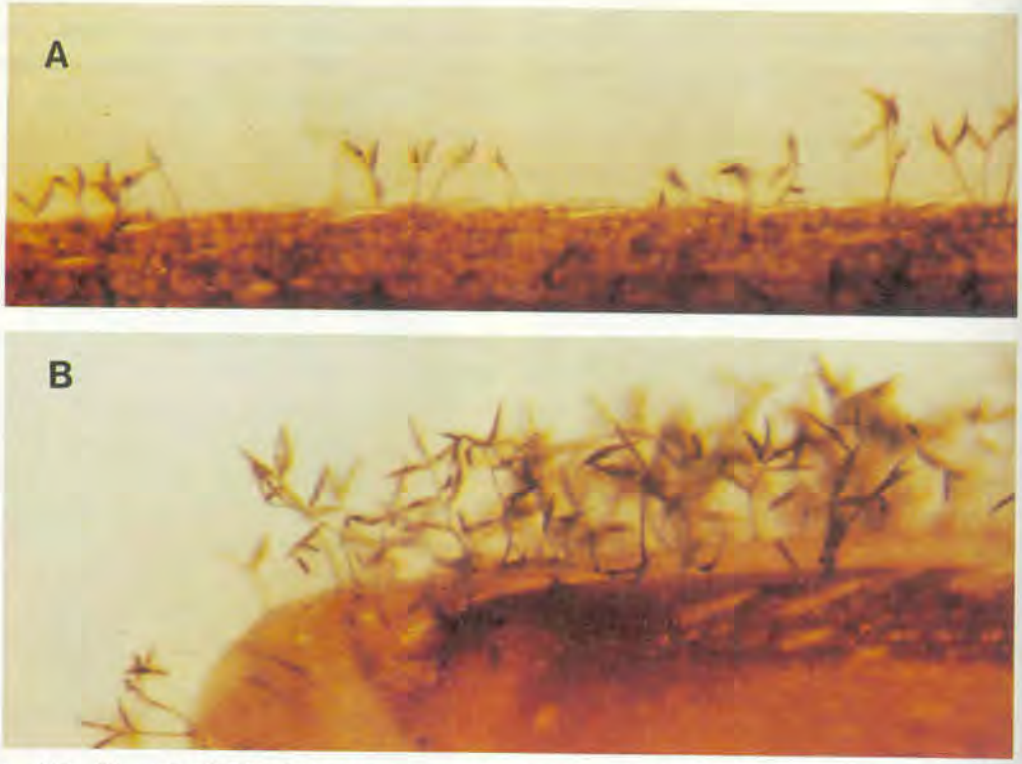

Fig. 2.7. Growth of *Bipolaris oryzae* on a rice seed incubated in the blotter test for 7 d.
A.: Young growth, each condiophore bears only one or two conidia, x 50
B.: An advanced stage of growth, x 50

Fig. 2.8. An ungerminated rice seed in the blotter test covered extensively by the profuse growth of *Bipolaris oryzae*. Note the growth of the fungus on the filter paper

Control

Seed treatment is effective in controlling seedling blight. Dithane M-45 and Ceresan can give complete control (Dharam Vir, Mathur & Neergaard, 1970; Park & Cho, 1972). The fungus can be eradicated from all parts of the seed following immersion in a mixture of guazatine and dichloromethane for 1 h (Vidhyasekaran, 1980). In Japan, chemicals recommended for seed treatment are Benlate T and homai (Fujii, 1983). Thiabendazole, a systemic fungicide, has been registered for seed treatment in the U.S.A. (Neergaard, 1977). Effective control can also be achieved with Aureofungin (Thirumalachar, 1967) and seed treatment by 50% copper oxychloride (Chattopadhyay & Bose, 1979).

Treatment of seeds of 10 rice cultivars with carboxin plus thiram (112.5 g + 112.5 g of a.i./100 kg seed) resulted in 97 to 100% control of *B. oryzae* and *Phoma sorghina* (Prahbu & Vieira, 1989).

Kulkarni *et al.* (1980) could not control seedling blight by hot water treatment. Hiremath & Hegde (1981), however, controlled seed infection by treating the seed at 52°C for 10 min in the case of fully developed grains but not in partially developed grains.

Efforts should be made to minimize the establishment of seed infection in the field. A good method is to spray with Brestan (fentin acetate) + Dithane M-45 (mancozeb) in proportions of 1:5 at 0.2% at heading and grain maturation which gave excellent control in India (Kulkarni *et al.*, 1980).

Brown spot is generally present under poor soil conditions. Proper application of fertilizers, good water management and soil

amendments can help in reducing disease severity.

Seed Certification

In Portugal, since 1955, seed intended for multiplication is officially certified by the National Seed Testing Station for *Bipolaris oryzae*. Both field inspection and laboratory tests are carried out (Florencio, 1962). In 1980, the Government of the Philippines introduced seed health testing of rice in addition to field inspections for breeder and foundation seed. *B. oryzae* was one of the pathogens nominated. Later, in 1982, it was made essential to carry out post control tests on all seeds intended for certification (Sevilla & Guerrero, 1983). However, no tolerance levels have so far been established.

Grain discoloration and spotting due to *B. oryzae* constitute a serious problem for healthy seed production in Brazil (A.S. Prabhu, pers. comm., 1989). Tonnes of rice seed are being rejected annually due to low seed germination. Seed lots showing germination lower than 80% increased to 92–99% when they were treated with a formulated mixture of carboxin plus thiram (112.5 g + 112.5 g of a.i./100 kg seed). The resultant seedlings were free of brown spot symptoms. Dr Prabhu has suggested seed treatment to reach the established germination standard, which at the same time gives control of the disease in the field.

Quarantine

The Government of Chile has included *Bipolaris oryzae* in its import regulations (Neergaard, 1980). Although *B. oryzae* is not a strict quarantine object in the EPPO region, because of its widespread prevalence, seeds intended for import should not be infected with this serious pathogen (Neergaard, 1981). In India, imported seed samples of rice infected with *B. oryzae* in routine seed health testing have been rejected and destroyed at the Plant Quarantine Laboratory of the National Bureau of Plant Genetic Resources, New Delhi. However, attempts are now being made to salvage infected material and make it available for crop improvement programmes in the country.

Chapter 3

Narrow Brown Leaf Spot

Widespread in Africa, Asia, Australasia and Oceania, North America, Central America, West Indies and South America (CMI Distribution Maps of Plant Diseases No. 71, ed. 5, 1985; Fig. 3.1).

Crop losses as high as 40% were reported from Surinam during 1953-54 (Overwater, 1960). Heavy losses generally occur in highly susceptible cultivars.

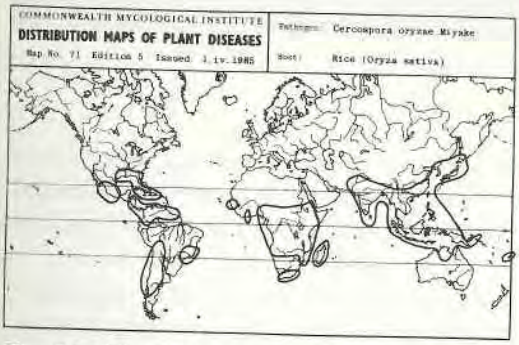

Fig. 3.1. Distribution map of narrow brown leaf spot published by the CAB International Mycological Institute, Kew, U.K. in 1985

Symptoms

Infected plants show brown, short, linear lesions about 2-10 mm long and 1 mm wide on the leaves, also on leaf sheaths and glumes (Fig. 3.2). Size and colour of lesions depend on the resistance and susceptibility of cultivars.

Pathogen

Cercospora janseana (Racib.) O. Const. (syn. *Cercospora oryzae* Miyake) (Teleomorph *Sphaerulina oryzina* Hara)

Conidia cylindrical to clavate , 3- 10-septate and generally 20-60 x 5 μm (Fig. 3.3);

Fig. 3.2. Symptoms of narrow brown leaf spot caused by *Cercospora janseana* (Photo: Courtesy Dr A.S. Prabhu, EMBRAPA, Brazil)

Fig. 3.3. Conidia of *Cercospora janseana*, x 750

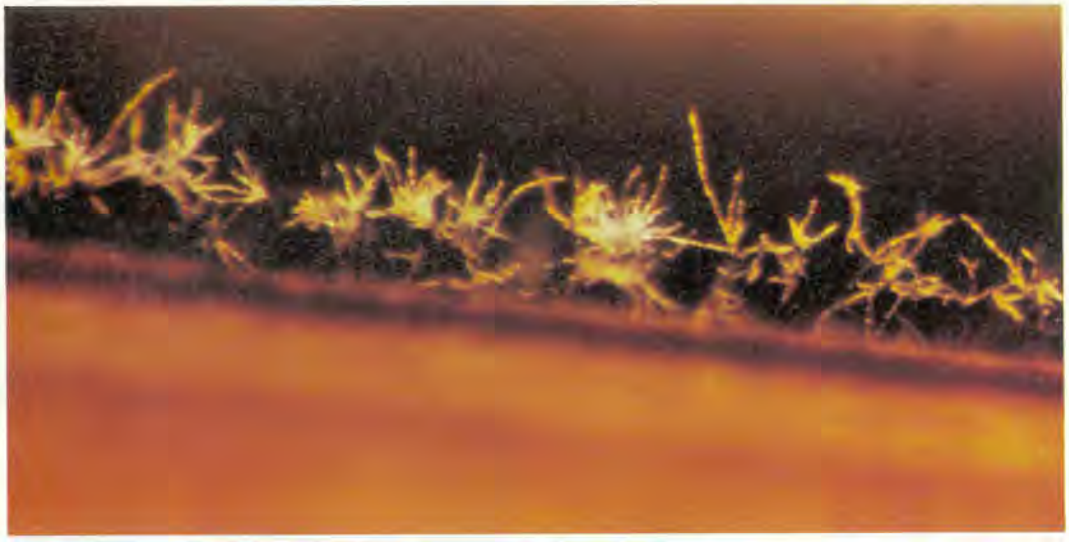

Fig. 3.4. Growth of *Cercospora janseana* on a rice seed tested by the blotter method, x 100

size may vary on the host plant and in culture. Secondary conidiophores are formed in culture. Conidia may also be catenulate (Ganguly, 1946a).

The teleomorph was described on the host plant by Hara (1918). Asci cylindrical or club-shaped, 50-60 x 10-13 μm. Ascospores spindle-shaped, 3-septate, hyaline, 20-23 x 4-5 μm.

Based on the reaction of 8 different cultivars, 10 races have been reported (Ryker, 1943, 1947; Chilton & Tullis, 1946; Ryker & Cowart, 1948). Fortythree races in six physiologic race groups were recently identified among 76 isolates collected from the States of Louisiana, Arkansas and Texas in the United States of America by Sah & Rush (1987).

Seed health testing

It is easy to detect *Cercospora janseana* in the blotter method. Follow the details of the method described in Chapter 1.

- Examine each seed carefully after 7 days' incubation as the growth of the fungus is rather limited, generally comprising only a few conidiophores.
- *C. janseana* produces hyaline, cylindrical conidia on dark brown to black filiform conidiophores which generally shine in the light falling on them from the lamp of the stereoscopic microscope (Fig. 3.4). Confirm the identification by examining conidia under compound microscope (Fig. 3.3).

Sittichai (1969) recorded an increase in infection counts of approximately 60% when seed samples were incubated in daylight at 28°C compared with incubation under NUV at 20°C.

Control

Cherewick (1954) reported that seed treatment with organomercurial powder was effective in controlling the disease in Malaysia.

Chapter 4

Stackburn

Widespread in Africa, Asia, Australasia and Oceania, and North and South America (CMI Distribution Maps of Plant Diseases No. 314, ed. 4, 1984b; Fig. 4.1).

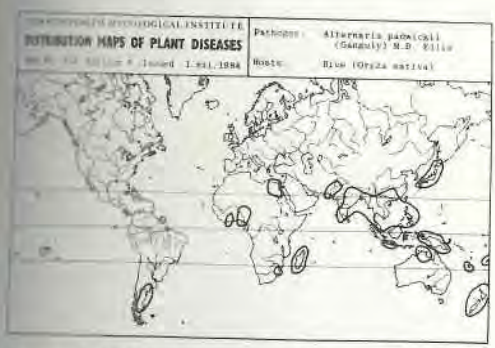

Fig. 4.1. Distribution map of stackburn published by the CAB International Mycological Institute, Kew, U.K. in 1984

Stackburn disease of rice is generally underestimated because leaf spots usually do not cause much damage. The damage is considerable when the seeds are attacked. A review of the literature shows that stackburn has caused considerable losses in the states of Kerala and West Bengal in India (Rangaswami, 1975). The extent of loss is, however, not mentioned by the author. A number of workers have reported very high percentages of seed infection. In India, Padmanabhan (1949) recorded 51-76%, Cheeran & Raj (1966) up to 80%, and 40-46% by Sharma & Siddiqui (1978) and Reddy & Khare (1978). In a survey from 11 countries in Asia and Africa, Mathur, Mallya & Neergaard (1972) observed seed infection up to 80%.

Symptoms

Symptoms of stackburn appear on the seedlings, leaves and the grains. When seedlings are infected, necrotic spots develop in the roots and coleoptile. The spots often coalesce to form larger spots of 1-2 mm diam. Heavily infected seedlings eventually die. Spots on the leaves are oval, 0.3 to 1 cm long with pale brown centres and dark brown margins. Later, the lesions become almost white bearing black dots, the sclerotia of the fungus. Infection may reach the kernel causing kernel spot, discoloration and shrivelling. On the affected glumes, pale brown to whitish spots occur.

Pathogen

Alternaria padwickii (Ganguly) M.B. Ellis
(syns. *Trichoconis padwickii* Ganguly; *Trichoconiella padwickii* (Ganguly) Jain)

Growth of the fungus on PDA is greyish, often deep pink or purple in reverse, sporulating freely under alternating cycles of 12 h light (NUV) and darkness. Mycelium is septate, profusely branched, hyaline to creamy yellow. Conidiophores not sharply distinguishable from mature hyphae, and often swollen at the apex. Conidia straight or curved, fusiform to obclavate and rostrate, 3- to 5-septate with a long appendage (Fig. 4.2), at first hyaline, later straw-col-

Fig. 4.2. Conidia of *Alternaria padwickii*, x 750

oured to golden brown, thick-walled, second or third cell from the base larger than the rest, 95-170 (130) μm long (including appendages), 11-20 (15.7) μm wide in the broadest part, and 1.5-5(2.7) μm wide in the centre of the appendage. Small black sclerotia with very distinct reticulate walls are often formed in old cultures (Ganguly, 1947; CMI Descriptions of Pathogenic Fungi and Bacteria No. 345, 1972).

Location of the pathogen in the seed

Conidia of the fungus may be present on the seed surface and the resting mycelium in the tissues of endosperm, embryo, bran layers and glumes (Ou, 1985; Cheeran & Raj, 1966). Sclerotia of the fungus were observed in the endosperm (Ganguly, 1947).

Seed infection and plant infection

In the blotter test, seed infection with *Alternaria padwickii* results in poor germination, rotting of seed, root and coleoptile, and in the ultimate death of young seedlings. Percentage loss of seedlings in pots is about half of the seed infection recorded in the blotter test (Mathur *et al.*, 1972; Cheeran & Raj, 1966). Kulik (1977) in the U.S.A., however, observed that field emergence is closely correlated with laboratory germination test results but not with infection levels of *A. padwickii*. Guerrero *et al.* (1972) observed *A. padwickii* in 23% of the abnormal seedlings and 15% of seedlings with decaying roots and shoots.

Tisdale (1922) stated that the fungus doubtless lives through the winter in the soil and on old rice grains, and infects the rice plants the following season. Ou (1985) mentioned that the fungus was isolated from 60% of discoloured Thai grains which can be another important source of primary infection.

Seed health testing

The International Seed Testing Association (1985) has recommended the blotter method for the detection of *Alternaria padwickii* in rice seed. The method is the same as described for *Pyricularia oryzae* in Chapter 1.

- After 7 days' incubation, examine each seed carefully under the stereoscopic microscope.
- *A. padwickii* produces two types of growth on the infected seeds, one with more conidia and less mycelium (Fig. 4.3A) and the other with more mycelium

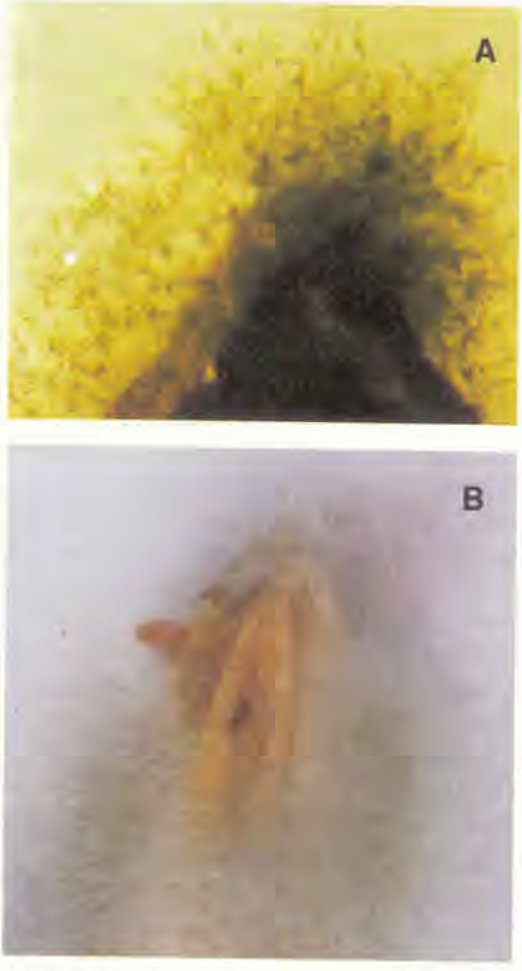

Fig. 4.3. Growth of *Alternaria padwickii* on rice seed; (A) less mycelium, more conidia, x 25; (B) more mycelium, fewer conidia, x 25

Fig. 4.4. Rice seeds infected with *Alternaria padwickii* in the blotter method are often surrounded by pink colour

and fewer conidia (Fig. 4.3B). The first type is generally common, a seed may be covered completely or partly. When more mycelium is present it becomes difficult to see the conidia but they can be observed when the mycelium is examined under compound microscope. The mycelium of *A. padwickii* is usually sticky and this property is detected when slide preparations are made with the help of needles.

- Infected seeds are often surrounded by a characteristic pink to purple stain which diffuses out into the blotter (Fig. 4.4).

Control

Seed treatment with Dithane M-45 and Ceresan at the rate of 0.3% of seed weight was found to eradicate infection of *Alternaria padwickii* as recorded by the blotter tests in the laboratory (Dharam Vir, Mathur & Neergaard, 1971). Hot water treatment at 50°C for 15 min is effective in eradicating seed infection but this treatment has been reported to reduce germination by Suryanarayana, Ram Nath & Lal (1963).

Quarantine

The Governments of Australia, Greece and Japan have imposed quarantine regulations to prohibit the entry of rice seed infected with *Alternaria padwickii* (Neergaard, 1980). In Europe, though the pathogen is not a strict quarantine object, Neergaard stated in 1981 that seed samples introduced in the EPPO region should not have the *A. padwickii* infection.

Leaf Scald

Reported from Africa, Asia, Australasia and Oceania, Europe, Central America and West Indies, and North and South America (CMI Distribution Maps of Plant Diseases No. 492, ed. 2, 1982c; Fig. 5.1).

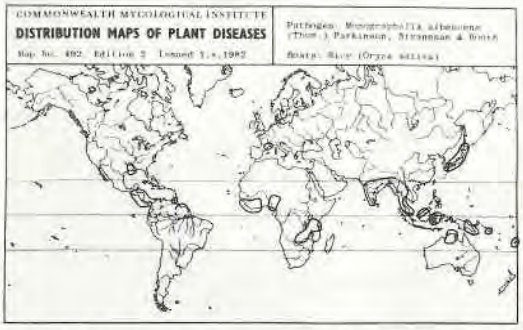

Fig. 5.1. Distribution map of leaf scald published by the CAB International Mycological Institute, Kew, U.K. in 1982

Leaf scald reached epidemic level in Japan in 1967 (Matsuyama & Wakimoto, 1977). In recent years, it has become common and sometimes serious in other parts of Asia and Africa. Lamey & Williams (1972) reported that the disease could sometimes be as devastating as rice blast in West Africa. In Bangladesh, Bakr & Miah (1975) estimated a yield loss of 20 to 30% in high yielding dwarf cultivars. The disease has also caused considerable losses in Latin America (Ou, 1985).

Symptoms

Leaf scald is usually seen on mature leaves of older plants. Characteristic symptoms are zonate lesions starting in the form of oblong or diamond-shaped water soaked blotches, eventually developing into large areas encircled by dark brown bands with light brown haloes. Lesions usually develop at leaf apices and spread downwards or from margins towards the centre. In all cases, the dark brown bands are characteristic. Individual lesions are 1-5 cm long and 0.5-1 cm broad.The continuous enlargement and coalescing of lesions result in the blighting of the leaf blade (Fig. 5.2), finally leading to the drying of the whole leaf. The fungus can also attack grains, causing glume discoloration and sterility. Varieties with

Fig. 5.2. Severe symptoms of leaf scald caused by *Microdochium oryzae*
(Photo: Courtesy Dr A.S. Prabhu, EMBRAPA, Brazil)

...wood leaves are more susceptible to leaf ...ald than those with narrow leaves (V.T. ...ho, unpubl.).

Pathogen

Microdochium oryzae (Hashioka & Yokogi) Samuels & Hallett
(syns. *Gerlachia oryzae* (Hashioka & Yokogi) W. Gams; *Rhynchosporium oryzae* Hashioka & Yokogi)

Growth of the fungus on the medium is white to buff. Mycelium is septate, branched and light yellow in colour. Conidia (Fig. 5.3) are sickle-shaped, single-celled when young and two-celled when mature, occasionally 2- or 3-septate, not constricted at the septum, epispore very thin, pink in mass and individually hyaline, 9-14 x 3-4.5 µm (Ou, 1985).

Monographella albescens (Thüm.) Parkinson, Sivanesan & Booth (syn. *Metasphaeria albescens* Thüm.) is the teleomorph. It is reported both in diseased tissue and in culture.

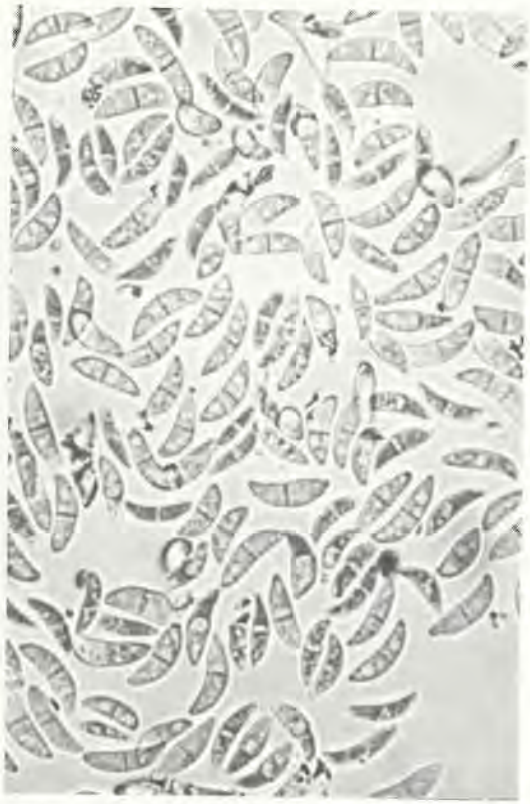

Fig. 5.3. Conidia of *Microdochium oryzae*, x 750

Location in the seed

Singh & Sen Gupta (1981) found *Microdochium oryzae* to be only externally seed-borne. However, Mia, Safeeulla & Shetty (1986) demonstrated it to be both externally and internally seed-borne. They detected the fungus in the husk, endosperm and embryo with as high as 35, 21 and 10% infection, respectively, in the three components of some rice samples. Yu & Mathur (unpubl.) obtained almost similar figures for the three components, i.e. 29.5, 30.0 and 9%.

The fungus survived in seed, stored at 5°C, for more than 11 years (Mia, Mathur & Neergaard, 1985).

Seed infection and plant infection

Singh & Sen Gupta (1981) failed to record diseased plants when seeds collected from naturally infected fields or after artificial inoculations were sown. However, Mia (pers. comm.) observed that 50% of infected seeds transmitted the disease to the seedlings. Yu & Mathur (unpubl.) obtained up to 31 and 41% infected seedlings when infected seeds and seedlings from a blotter test were transferred to water agar and soil, respectively.

Seed health testing

Rice seed samples should be tested by the blotter method (see Chapter 1 for details).

- After incubating the seeds for 7 d, examine each seed at 12-50 magnification for the growth of *Microdochium oryzae*. The fungus forms small, isolated pionnotes of conidia on the seed surface as seen in Fig. 5.4. The colour of the pion-

Fig. 5.4. Orange coloured pionnotes (group of conidia) of *Microdochium oryzae* on a rice seed. Although rare, pionnotes may develop on blotters as seen in this photograph

notes ranges from dull orange to bright orange and sometimes light pinkish. In most cases, mycelium is scanty, only few hyphal strands are seen.

- Confirm identification of *M. oryzae* by examining conidia from the pionnotes (Fig. 5.3) at higher magnification of a compound microscope.

Control

Traoré (1987) investigated 12 seed samples of rice from Cote d'Ivoire for fungicidal seed treatment against *Microdochium oryzae*. Samples had 0.2 to 50.5% seed infection. Treatment of these samples with Dithane M-45 (0.15% by seed weight) + Benlate (0.15%) eradicated the pathogen from the seed as verified by the blotter method.

Chapter 6

Bakanae

Widespread in all rice growing countries (CMI Distribution Maps of Plant Diseases No. 102, ed. 6, 1984a; Fig. 6.1).

Fig. 6.1. Distribution map of bakanae published by the CAB International Mycological Institute, Kew, U.K. in 1984

The disease in general, renders severe damage in specific localities or seasons. Yield losses, as high as 20 to 50%, have been reported in Japan, 15% in India, 3.7 to 14.7% in Thailand (Ou, 1985). From Bangladesh, a loss of 21% has been reported in a susceptible variety (Anonymous, 1976).

Symptoms

Bakanae, which means abnormal elongation of the plants, may take place in the nursery (seedbed) or in the main field. In the nursery the affected seedlings are yellowish-green, thin and abnormally elongated or rarely dwarf. Seedlings with severe infection die before transplantation and those that survive may die after transplantation.

Fig. 6.2. Abnormal elongation of rice seedlings in a nursery (Photo: Courtesy Dr H. Kato, Japan)

Fig. 6.3. A rice plant, infected with *Fusarium moniliforme*, showing early flowering
(Photo: Courtesy Dr Y.H. Lee, South Korea)

Fig. 6.4. Development of adventitious roots on nodes of rice plants infected with *Fusarium moniliforme*
(Photo: Courtesy Dr Robert S. Zeigler, CIAT, Colombia)

In general, severely infected plants in the field develop tall tillers (Fig. 6.2) which are abnormally elongated and flower earlier than healthy ones (Fig. 6.3), but weakly infected plants sometimes recover after transplantation (Lee, 1983). Highly diseased plants show collar infection and die within two to six weeks. There is profuse branching in the roots and adventitious roots may develop from the lower nodes (Fig. 6.4). Leaves dry up starting from below. At this stage a pinkish growth may be observed at the base of the plant and whitish cottony mycelium may be seen on splitting the til-

ers. In some cases plants survive till maturity but infected plants bear mostly sterile panicles (Ou, 1985). Differences in symptom development are due to relative amounts of gibberellic acid and fusaric acid produced by the fungus, and to varietal resistance (Lee, 1983).

Pathogen

Fusarium moniliforme Sheld.
(Teleomorph *Gibberella fujikuroi* (Saw.) Wollenw.)

Growth of the fungus on PDA is powdery, light pink and the reverse of cultures is typically dark violet but occasionally pale lilac, vinaceous or even creamy. Microconidia formed in chains, usually one- but occasionally two-celled, 5-12 x 1.5-2.5 μm, fusiform to clavate in shape and slightly flattened at each end (Fig. 6.5). Macroconidial formation is rare in many strains. Macroconidia are inequilaterally fusoid, delicate, thin-walled, with an elongate, often sharply curved apical cell and pedicellate basal cell, 3- to 7-septate, 25-60 x 2.5-4 μm (Fig. 6.5). Chlamydospores absent. Dark blue irregularly globose sclerotia are frequently formed. Perithecia usually occur on dead plant material and are superficial. Ascospores hyaline, elliptical, often 1-septate and occasionally 3-septate (Booth, 1971).

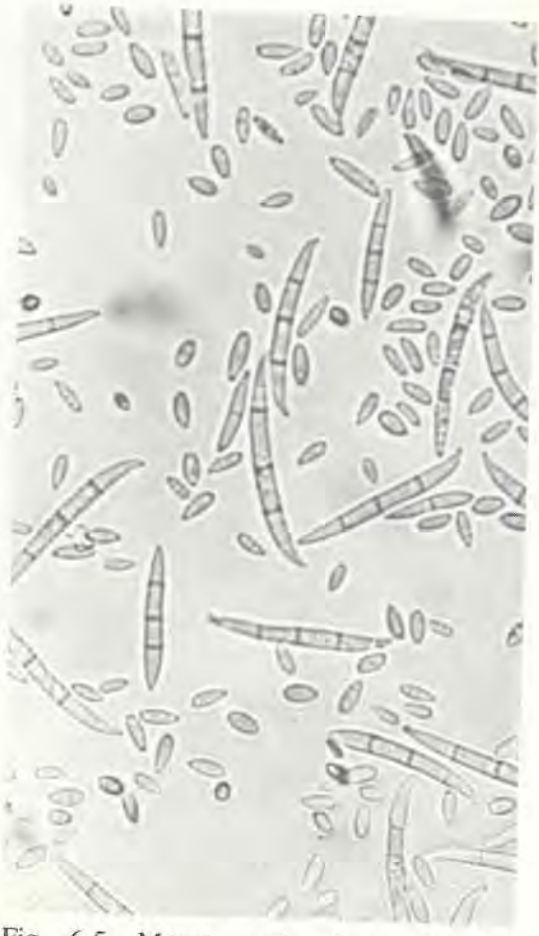

Fig. 6.5. Macro- and microconidia of *Fusarium moniliforme*, x 750

Location of the pathogen in the seed

The fungus has been found to be harboured mainly in the embryo (Vidhyasekaran, Subramanian & Govindaswamy, 1970: Hino & Furuta, 1968), but also in the empty glume, pedicel, palea and lemma. Severely infected grains are discoloured, sometimes pink due to the presence of conidial masses. Healthy looking seeds may also carry the fungus.

Seed infection and plant infection

Yu & Sun (1976) reported from Taiwan that in moderately diseased rice fields, 100% of the seeds yielded *Fusarium moniliforme* on agar plates and 30% of them showed bakanae symptoms when planted. In Thailand, Kanjanasoon (1965) found 1-31.2% seedling infection from healthy looking seeds collected from diseased fields. The copious production of conidia and perithecia on diseased culms in the field during the latter part of the growing season coincides with flowering and maturation, thus facilitating the infection or contamination of seeds.

Seed health testing

The blotter method, as described for

Pyricularia oryzae in Chapter 1, should be followed for detecting *Fusarium moniliforme* in rice seed.

- After 7 days' incubation, examine each seed very carefully under the stereoscopic microscope for the characteristic chains of microconidia of *F. moniliforme*. These chains can be very clear in poorly developed colonies (Fig. 6.6).

Fig. 6.6. Microconidial chains of *Fusarium moniliforme* on a rice seed, incubated for 7 d in the blotter test, x 100

In well-developed colonies, abundant whitish, purplish white or very light orange-white mycelium is observed. Such colonies under a stereoscopic microscope also show numerous short or long chains, and small heads of microconidia (Fig. 6.7). The colonies have a powdery appearance.

For confirmation, make dry mounts of chains and examine under a compound microscope. This can be done by scraping the powdery growth of the fungus with a dry needle, tapping the tip of the needle on to a glass slide a couple of times and examining the slide under a compound microscope without using a cover glass. If, by mistake, water is added, the conidia are dispersed in the water and the chains

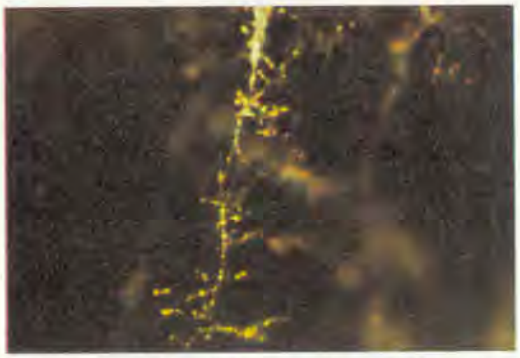

Fig. 6.7. Mycelial growth of *Fusarium moniliforme* on a rice seed, incubated for 7 d in the blotter test, x 100.
Note false heads of microconidia

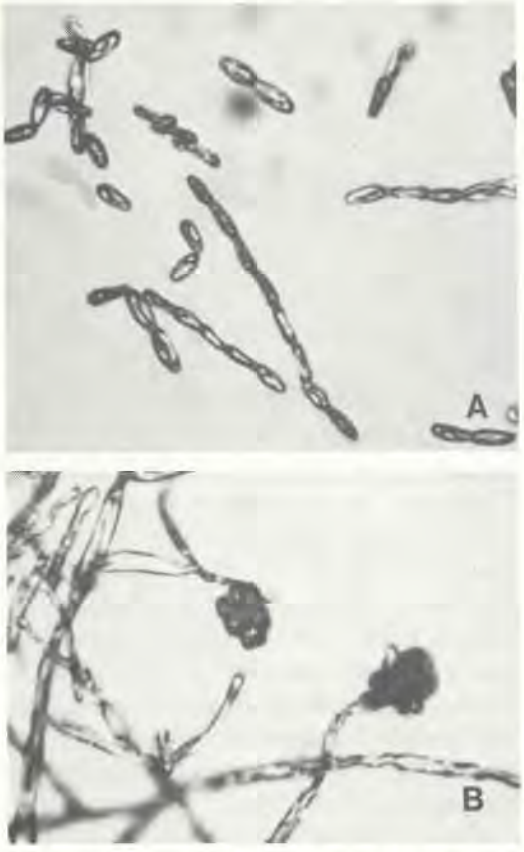

Fig. 6.8. In a sticky tape preparation, microconidia of *Fusarium moniliforme* in chains (A) and in false heads (B), × 750

Fig. 6.9. Pionnotes of *Fusarium moniliforme* on a rice seed, x 100

cannot be observed. Microconidia in chains and in false heads (Fig. 6.8) can also be seen if the powdery growth of the fungus is touched by a thin strip of ordinary sticky tape and the tape is examined under a compound microscope after it has been mounted on a glass slide.

- In some cases, the growth of *F. moniliforme* is accompanied by orange or purplish red pionnotes of irregular shape and size (Fig. 6.9). In other cases only the pionnotes are observed on the incubated seed.
- Occasionally, brown or chocolate coloured, globose perithecia of *Gibberella fujikuroi* may develop on seed (Ram Nath, Neergaard & Mathur, 1970).

Control

Seed treatment with organo-mercury compounds either as dust or in liquid form is effective in controlling *Fusarium moniliforme* infection in seed. Soak the seed for 16-24 h in 0.1% solution or for 2 h in 0.25% solution. Dry seed treatment at 0.2% is, however, easy to apply. Due to a ban on the use of mercury compounds in several countries fungicides of other groups have been used in recent years. Benomyl or benomyl + thiram have been used extensively in Japan and Taiwan. Dry seed coating with benomyl or benomyl-T, 1-2% fungicide solution for 1 h or a 1:2000 solution for 5 h gives good control (Ou, 1985). According to Kang, Heo & Heo (1986) seed treatment with Proraz-EC is best for controlling blast and bakanae. This treatment is used very widely in Korea, where 10 kg of rice seed is soaked in a 1:2000 solution for 24 h, rinsed 1 or 2 times in fresh water and dried in the shade before use.

Chapter 7

Sheath Blight

The disease is reported from Bangladesh, Brazil, China, India, Japan, Korea, Madagascar, Nepal, Nigeria, Philippines, Sri Lanka, Surinam, Taiwan and Venezuela.

Sheath blight is considered to be of major economic importance, only second to blast in Japan, China, Taiwan, Sri Lanka, and U.S.A. (Gangopadhyay & Chakrabarti, 1982; Lee & Rush, 1983). In Japan, losses to the extent of 24,000 to 38,000 tons of rice occur almost every year due to infection of 120,000 to 190,000 hectares of the crop (Ou, 1985). Kozaka (1970) reported losses up to 30-40% in cases of severe infection of the sheath and leaf blades. The disease outbreak covered an area of 1.4 million hectares in Japan during 1967 (Yamaguchi, 1980). Rangaswami (1975) estimated that in the Philippines 25-50% rice production is damaged annually by this disease. In the U.S.A., Lee & Rush (1983) reported yield losses up to 50% in susceptible cultivars.

Symptoms

Under normal conditions, the fungus restricts itself to the lower leaf sheaths of older leaves and without any visual damage to the crop. However, under favourable combinations of host susceptibility, high nitrogen, shade and high humidity, the disease gradually moves up to the leaves, sometimes even

Fig. 7.1. Symptoms of 'banded blight' in rice caused by *Rhizoctonia solani* (Photo: Courtesy Dr V.T. John, IITA, Nigeria)

appearing on the panicles. When severely infected, the whole hill, or part of the tiller die and fungal hyphae and sclerotia can be seen growing out of the affected parts including leaves (Gangopadhyay & Chakrabarti, 1982; Ou, 1985; V.T. John, unpubl.). On leaves the symptoms usually occur as bands and the term 'banded blight' (Fig. 7.1) is applied to this syndrome (Saksena, 1973).

Sclerotia may also form between the leaf sheath and culm, within the lumen of the damaged culms and also within the larger cells of the leaf sheath tissues (Lee, 1983).

Pathogen

Rhizoctonia solani Kühn
(Teleomorph *Thanatephorus cucumeris* (Frank) Donk)

Colonies on potato dextrose agar are colourless to brown. Sclerotia develop in culture, on rice plants and on the soil surface; they are brown to dark brown, roughly spherical or somewhat flattened and irregular. Sclerotia are generally found on the host surface along with mycelium.

Basidia measuring 10-15 μm long and 6-9 μm wide are produced on stems or leaves just above the soil surface or on soil particles under extremely moist conditions. Basidiospores are hyaline, oblong to broad ellipsoid and unilaterally flattened, prominently apiculate, smooth, thin-walled, 6-14 x 4-8 μm (CMI Descriptions of Pathogenic Fungi and Bacteria No. 406, 1974).

Rhizoctonia solani is probably not a single species but is composed of groups based on hyphal anastomosis patterns. Four main groups, each a non-interbreeding population, were recognized by Talbot (1970). Chien & Chung (1963) from Taiwan separated 300 isolates into 7 cultural types and 6 physiologic races based on the degree of pathogenicity on 16 rice cultivars (for more details see Ou, 1985; Gangopadhyay & Chakrabarti, 1982).

Location of the pathogen in the seed

It seems likely that the disease is seed-borne when infection reaches panicles and also when basidiospores initiate flower infection (Ou, 1985).

Seed health testing

- Sclerotia of the fungus mixed with seeds can be detected by visual inspection of the seed sample. However, for broken pieces of sclerotia a stereoscopic microscope may be used.

Fig. 7.2. Mycelium of *Rhizoctonia solani* spreading out on filter paper from an infected seed of rice in the blotter test

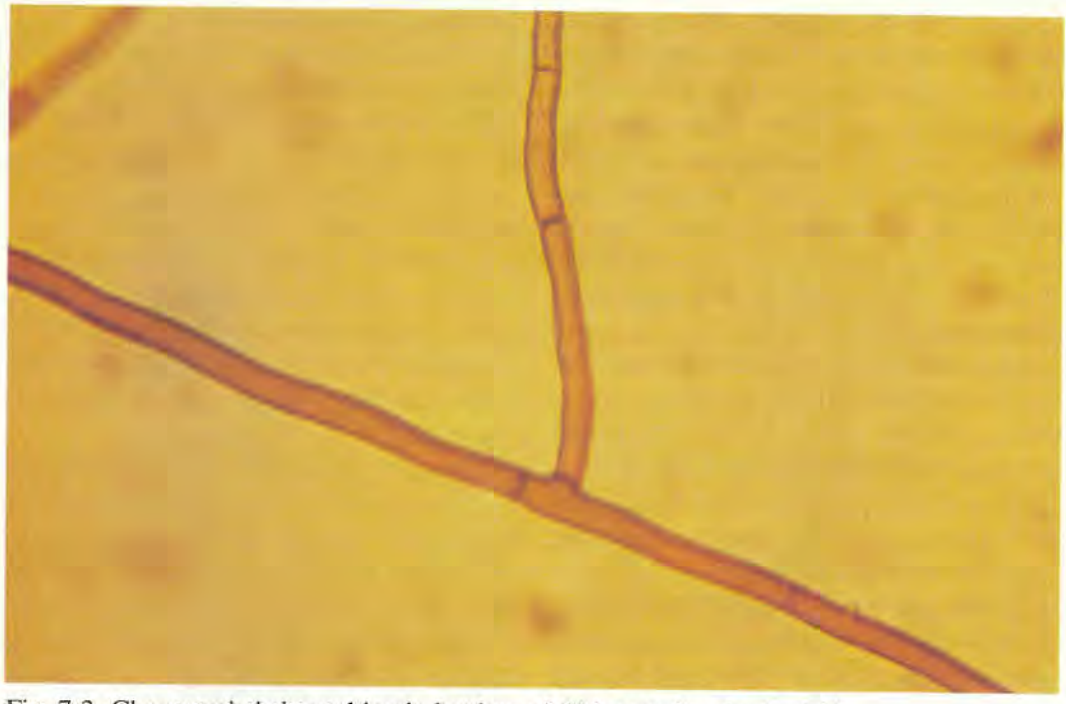

Fig. 7.3. Characteristic branching in hyphae of *Rhizoctonia solani,* x 750

- For the detection of mycelial infection in seed use the blotter test as described for blast disease in Chapter 1.
  After 7 days' incubation, dark brown mycelium spreads from the seed to the filter paper and may even cover the entire plate (Fig. 7.2). A slide preparation of the mycelium under higher magnification shows the characteristic branching in the hyphae of *R. solani* (Fig. 7.3).
- Sclerotia in some cases may be observed after 12-15 days' incubation.

Control

Seed treatment with Arasan (Thiram 75 a.i.) or Terracoat (Quintozene 23.2% PCNB) at the rate of 100g/100 kg of seed can control sheath blight and increase the germination percentage (Marcos, 1975).

Foliar sprays with 0.5% carbendazim (Bavistin), or 0.5% edifenphos (Hinosan), or 0.5% carboxin (Vitavax), or IBP (Kitazin), or Benomyl (Benlate), twice at intervals of 10-15 days after the first appearance of disease can give good control. Polyoxin and Validamycin antibiotics developed in Japan were tested extensively and found to be very effective.

Soil application of edifenphos, IBP, and carboxin can completely inhibit sclerotial germination and inactivate the mycelium of *Rhizoctonia solani* (Kannaiyan & Prasad, 1979).

Chapter 8

Sheath Rot

The disease is present in Taiwan, Japan, very common in Southeast Asia and the Indian subcontinent and USA (Ou, 1985). In the CMI Descriptions of Pathogenic Fungi and Bacteria No. 673 (1980) the disease is also reported to be present in Kenya and Nigeria. Mathur (1981) detected sheath rot in Brazil and concluded that the disease was introduced through seed.

Sheath rot was once considered to be a minor disease of rice but it is becoming increasingly destructive and widely distributed. Ou (1985) wrote in his book that Dr Buddenhagen considered it a major problem in upland rice in West Africa on rice cultivars introduced from Asia. No date of this personal communication is given. Seed transmission of the causal pathogen, *Sarocladium oryzae*, has now been shown experimentally (see section on seed infection and plant infection).

Since sheath rot affects uppermost leaf sheaths which enclose young panicles the disease can result in shrivelled grains, brown discoloration of various intensities and even sterility. The damage thus caused by this disease can sometimes be high. Chen (1957) reported 3-20% damage and it may be as much as 85% as recorded in Taiwan by Chien & Huang (1979). In India, Chakravarty & Biswas (1978) recorded 9.6-26% yield reduction amongst the seven cultivars they examined, with an average of 14.5%. Severe infection was observed on semi-dwarf rice varieties by Raina & Singh (1980). Muralidharan & Venkata (1980) observed sheath rot at panicle initiation stage, panicle either remained within the leaf sheath or emerged only partially causing

Fig. 8.1. Sheath rot affected rice plants. Note discoloration in upper parts of leaf sheaths (Photo: Courtesy Dr Sergia P. Milagrosa, Benquet State University, Philippines)

85% loss in yield. Losses caused by sheath rot in 16 cultivars grown under Coimbatore conditions were estimated by Mohan & Subramaniam (1979) at a maximum of 57%, although wide variation among different cultivars was noticed. Up to 50% loss was recorded by Kang & Rattan (1983). A total of 52.8% yield loss was noticed by Estrada, Torres & Bonman (1984) in the Philippines. In a personal communication, Buddenhagen informed Ou (1985) that in West Africa he found infected husks which resulted in infection of the caryopses and in shrivelled grains with resultant losses in milling.

Symptoms

Symptoms of the disease become apparent only when plants reach maturity stage (Fig. 8.1). Sheath rot occurs on the uppermost leaf sheath enclosing the young panicles and starts as oblong or irregular lesions with brownish margins and greyish centres, 0.5-1.5 cm long, which later enlarge, coalesce and may cover most of the leaf sheath (Fig. 8.2). Whitish mycelium may be seen in the centre of the lesions (Fig. 8.3), and generally, also on the inside surface by removing the outermost sheath. The young panicles remain within the sheath or emerge only partially.

The disease is associated with reduced spikelets per panicle as well as grain weight. Extensive rotting of the sheaths enclosing the panicles caused significant losses and glume discoloration in India (Upadhay & Diwakar, 1984). The quality of rice grains from sheath rot affected plants was low, grains were poorly filled and the percentage germination was reduced from 94 to 58% in one sample and from 97 to 63% in another

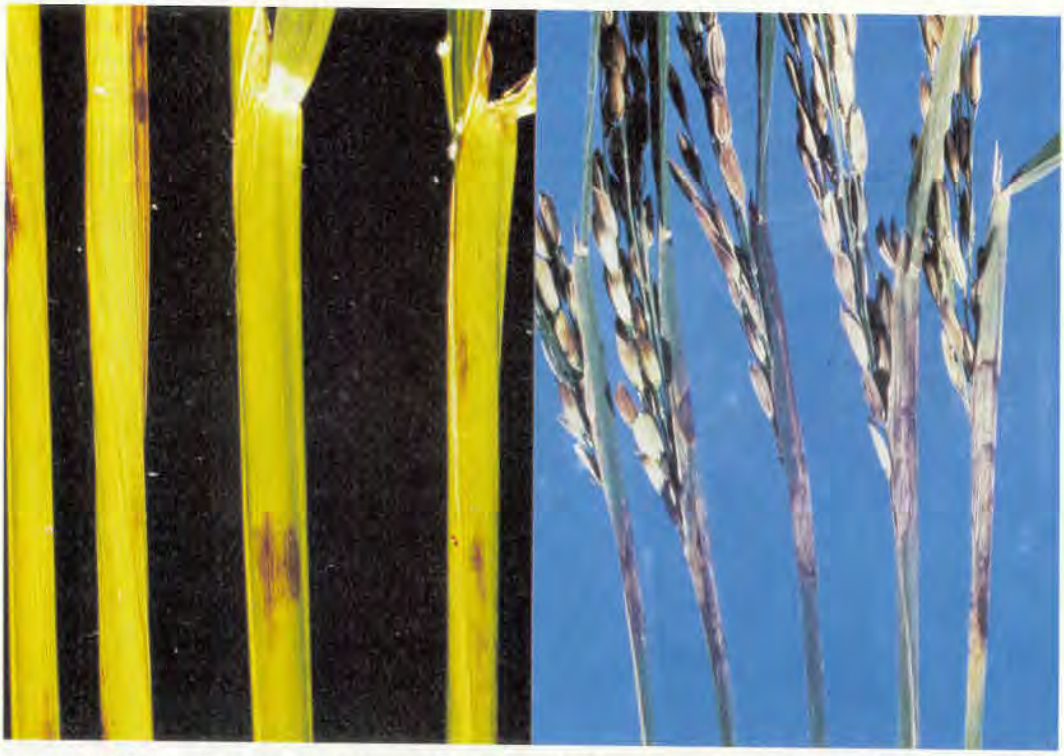

Fig. 8.2. Mild and severe symptoms of sheath rot. Many seeds produced in the panicle show discoloration of different intensities
(Photo: Courtesy Dr Sergia P. Milagrosa)

Fig. 8.3. White mycelium of *Sarocladium oryzae* on rotted parts of uppermost leaf sheaths. These symptoms were observed in Sept., 1988 at the Institute of Seed Pathology in Denmark in plants raised from infected seeds received from India

sample (Vidhyasekaran, Ranganathan, Rajamanickam & Radhakrishnan, 1984). Protein contents went down from 8 to 2.2%.

Pathogen

Sarocladium oryzae (Sawada) W.Gams & D.Hawksw. (syn. *Acrocylindrium oryzae* Sawada)

Mycelium white, sparsely branched and septate. Conidiophores arising from the mycelium, slightly thicker than the vegetative hyphae, branched once or twice, each time with 3 or 4 branches in a whorl. Conidia formed singly, cylindrical with rounded ends (Fig. 8.4), sometimes slightly curved, hyaline, smooth, one celled, 3.5-9 × 0.8-2.5 μm (Ou, 1985; CMI Descriptions of Pathogenic Fungi and Bacteria No. 673, 1980), collecting in slimy masses at the tips of the phialides.

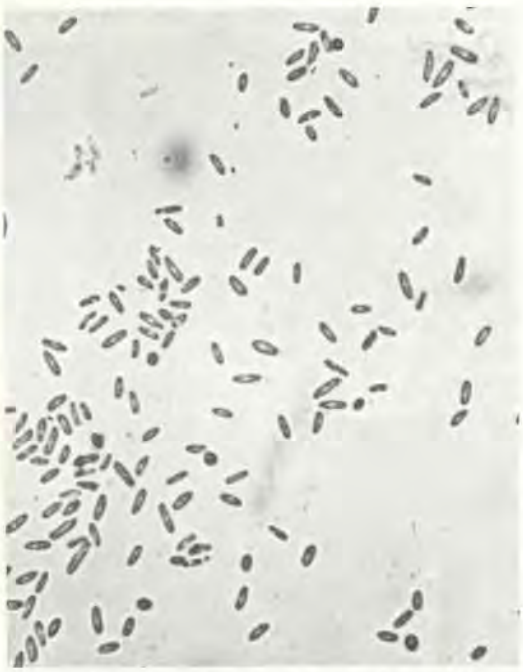

Fig. 8.4. Cylindrical conidia of *Sarocladium oryzae*, x 750

Location of the pathogen in seed

In recent investigation, Milagrosa (1987) isolated the fungus from the husks and kernels of both discoloured and non-discoloured seeds from panicles of sheath rot affected plants. Discoloured seeds yielded the fungus more readily than the non-discoloured ones. Seeds collected from apparently healthy panicle also showed *Saro-*

cladium oryzae infection, but the infection was considerably lower.

Seed infection and plant infection

Hsieh, Shue & Liang (1980) found no evidence of seed transmission of *Sarocladium oryzae* in Taiwan when infected seeds were planted. Seed transmission was, however, demonstrated a few years later by Chuke (1983) at the University of the Philippines, Los Banos, when naturally infected seeds of TN 1 and RGS 20 were sown in sterilized soil. Seed transmission of the pathogen was further substantiated by the studies of Milagrosa (1987) working at the International Rice Research Institute (IRRI) in the Philippines. Seed of cv. IR 36, collected from panicles of sheath rot infected plants, were incubated on wet filter papers in plastic Petri dishes (incubation conditions: near ultraviolet light (12 h), 20°C for 7 d) and those found infected with *S. oryzae* were transferred to plastic trays having sterilized soil. The experiment was conducted in a glasshouse. *S. oryzae* was isolated from mature plants showing typical sheath rot symptoms, as well as from the leaf sheath of young seedlings which did not exhibit any symptom. Transmission of the pathogen from seed to plant was also seen in Denmark when seeds of cv. CR333-1-2 received from the Central Rice Research Institute of India were sown in peat soil in a room at 25°C where light was provided for 12 h each day by white daylight fluorescent tubes. Symptoms were seen only at maturity (Fig. 8.3). The fungus was isolated from all parts of the diseased plants.

Seed health testing

Follow the blotter method as described for *Pyricularia oryzae* in Chapter 1.

Examine each of the incubated seed carefully as the growth of *Sarocladium oryzae* is usually very sparse, generally confined to the embryonal end of the seed. It consists of

Fig. 8.5. Growth of *Sarocladium oryzae* at the embryonal end of an infected seed in the blotter method, x 50. The fungus can develop on other parts of the seed also

small, hyaline conidiophores in whorls (verticils) having circular watery drops at their tips (Fig. 8.5). The conidiophores usually emerge from the seed surface singly, sometimes in two's and rarely in three's. Whenever such a growth is present on a rice seed, examine carefully the angle between the conidiophores. In *S. oryzae* the angles are not wide and if they are the fungus in question could be a species of *Verticillium*. One should therefore make slide preparations of the watery drops and examine the conidia at higher magnifications (e.g. x 400). Conidia of *Sarocladium* are cylindrical in shape (Fig. 8.4), while those of *Verticillium*, oval to oblong. Since the latter has not been shown to be pathogenic to rice seedlings and plants it is important to avoid misidentification of these two fungi on the seed. One needs experience which only comes after testing and examining many rice seed samples under the stereomicroscope and subsequent confirmation of conidial morphology at higher magnifications under the compound microscope. According to Milagrosa (pers.comm., 1988), *Verticillium* occurs more on stored rice seed than in seed tested soon after harvest.

Chapter 9

False Smut

Widespread in Africa, Asia, Australasia and Oceania, North America, Central America, West Indies and South America. In Europe it has been reported only from Italy (CMI Distribution Maps of Plant Diseases No. 347, ed. 3, 1982a; Fig. 9.1).

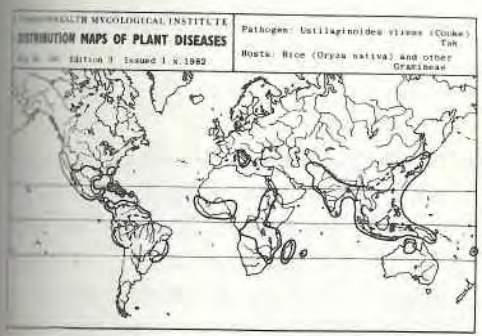

Fig. 9.1. Distribution map of false smut published by the CAB International Mycological Institute, Kew, U.K. in 1982

This has generally been regarded as a minor disease, but heavy losses have been reported from some countries. Reinking (1918) mentioned severe infection in the Philippines and Seth (1945) reported an epidemic in Burma in 1935. A 20% loss in yield has been reported from Cauca Valley in Colombia by Martinez (1953), over 25% loss in the Tumbes Valley of Peru (Revilla, 1955), 10% loss in Fiji by Morwood (1966), and up to 44% in India by Singh & Dube (1978). According to Dr V.T. John (pers.comm., 1988) false smut is a serious problem in the high humid areas of several West African countries.

Symptom

Symptoms are seen only in maturing panicles. Usually few, occasionally several, grains are affected in each panicle (Fig. 9.2). Affected grains are transformed into masses of spores that are greenish outside and yellowish orange inside, and eventually turn dark. The spore masses may be 1 cm or more in diameter. Chlamydospores can not be freed easily from the smut balls because of the presence of some sticky material.

Fig. 9.2. Panicle of rice infected with false smut, *Ustilaginoidea virens*. (Specimen courtesy of Dr L.P. Kauraw, India)

Pathogen

Ustilaginoidea virens (Cooke) Tak.
(syns. *Ustilago virens* Cooke; *Tilletia oryzae* Patouillard)
(Teleomorph *Claviceps oryzae-sativae* Hashioka)

Chlamydospores are olivacious, spherical to elliptical, 3-5 x 4-6 μm, younger spores are pale and smooth, warty when mature (Fig. 9.3). Chlamydospores germinate in culture and produce conidia. Some of the green spore balls develop one to four sclerotia in the centre. Ascospores are hyaline, filiform, unicellular, 120-180 x 0.5-1 μm (Ou, 1985; CMI Descriptions of Pathogenic Fungi and Bacteria No. 299, 1971a).

Singh (1984) working with 8 isolates of the fungus, collected from different parts of India, described 4 groups based on symptoms, colony characters, spore morphology and sporulation.

Location of the pathogen in the seed

Spores present on the seed surface as contaminants.

Recurrence of the disease

Primary infection of plants in the field is known to occur by ascospore infection of flowers (Ikegami, 1960). Once the smut balls develop in the infected panicles secondary infection of flowers takes place by chlamydospores formed in the primary smut balls (Ou, 1985).

Seed health testing

No seed health testing method has so far been established for detecting *Ustilaginoidea virens* in rice seed samples. However, it is imperative that the whole submitted sample should be subjected to visual inspection for locating false smutted grains as seen in Fig. 9.2.

If false smutted grains are not found it does not mean that the clamydospores of the fungus may not be present as surface contaminants. In such cases, a part of the submitted sample, at least 400 seeds, must be tested by the washing method. In the case of germplasm, the whole sample can be tested by this method which is nondestructive; the tested seeds can be used later for other tests or for raising plants. The washing test consists of the following steps:

- Place the seeds in a conical flask and cover them with water. Add 1-2 drops of a detergent.
- Shake the seeds over a mechanical shaker for 10 min.
- Centrifuge the water at 2500 to 3000 rev.min^{-1} for 20 min.
- Decant the water and add a few drops of glycerol (2%) or lactophenol to the remaining sediment in the centrifuge tube. Scrape the sediment gently, examine a number of drops in a compound microscope and look for the clamydospores of *U. virens* (Fig. 9.3).

Since no information is available on the spore load required to induce disease development in the field, it is enough to record presence or absence of the pathogen in the sample tested.

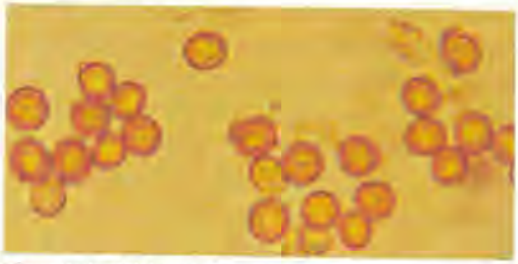

Fig. 9.3. Chlamydospores of *Ustilaginoidea virens*, x 750

Control

Hashioka (1952) reported that it is possible to combat false smut by spraying or dusting the plants with a fungicide a few days before heading. Kannaiyan & Rao (1976) found that copper oxychloride reduced false smut effectively.

Chapter 10

Kernel Smut

Kernel smut, also known as bunt, is known to occur in Africa (Sierra Leone), Asia (Burma, China, India, Indonesia, Japan, Korea, Malaysia, Pakistan, the Philippines), Australasia and Oceania (Australia and Fiji), Europe (Greece) and America (Mexico, U.S.A. and Trinidad) (CMI Distribution Maps of Plant Diseases No. 75 ed. 3, 1976; Fig. 10.1).

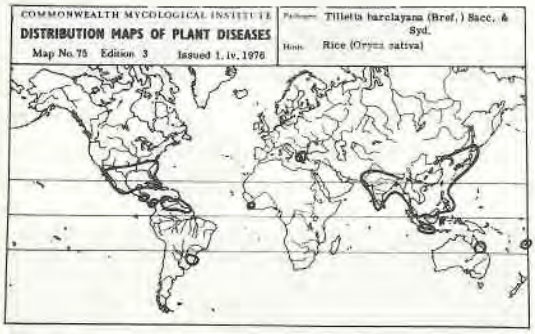

Fig. 10.1. Distribution map of kernel smut published by the CAB International Mycological Institute, Kew, U.K. in 1976

Kernel smut, which is usually considered a minor disease, has at times caused considerable economic losses. Fulton (1908) reported 3-4% infected grains in South Carolina, which caused serious damage to rice flour due to the dark colour from the smutted grains. A loss of 2-5% has been reported from Mandalay, Burma by Su (1933). Reyes (1933) from the Philippines reported stunting of seedlings and a reduction in the number of tillers when smutted seeds were sown. The disease also caused concern in the U.S.A. (Whitney & Frederiksen, 1972). In Pakistan, Hassan (1971) reported panicle infection up to 87%.

Symptoms

The disease can be seen at crop maturity. Usually only a few grains in an ear are attacked, and these may be either partly or wholly infected. Infected grains show minute, black pustules or streaks bursting through the glumes. In the field, spores shed from the grains settle on to other grains or leaves and form a characteristic black covering that helps in the detection of the disease.

Pathogen

Tilletia barclayana (Bref.) Sacc. & Syd. (syns. *Neovossia barclayana* Bref.; *Tilletia horrida* Tak.; *Neovossia horrida* (Tak.) Padw. & Kahn)

Smut sori are produced within the ovaries which remain covered by the glumes. They may burst at maturity. Spore mass granular and black, sterile cells intermixed with the spores, few to many, usually globose,

Fig. 10.2. Teliospores of *Tilletia barclayana* (black) and sterile cells obtained in seed washings, × 750

hyaline to yellowish, wall 2-4 μm thick, 10-30 μm diam. Spores globose to subglobose, first light brown, becoming dark brown at maturity (Fig. 10.2), enclosed in a tinted or hyaline sheath with or without a short apiculus, mostly 17–25 μm diam (occasionally up to 35 μm).

Location of the pathogen in seed samples

A seed sample may have infected rice seeds with smut sori full of teliospores, as shown in Fig. 10.3 or loose teliospores may be present on seed surface (Fig. 10.4). The surface-borne inoculum may be as high as 40.55×10^5 spores per gram of seed (Shetty & Shetty, 1986).

Seed infection, contamination and plant infection

Teliospores of the pathogen are transported with the infected and contaminated seed to the soil. Once the spores reach the soil they germinate producing primary and secondary sporidia. The secondary sporidia are sickle-shaped and are forcibly discharged. In this way the pathogen is disseminated and infection spreads (Vanterpool, 1932). Floral infection by sporidia has been confirmed by Chowdhury (1946), Templeton, Johnston & Henry (1960) and Templeton (1961).

According to Ou (1985) teliospores live for a year or more under normal conditions but have been found viable in stored grains after 3 years.

Seed health testing

1. *Detection of teliospores on seed surface*

- Contaminated seeds can be detected by examining seeds under a stereoscopic microscope (Fig. 10.4).
- Sometimes, rice seeds may also be contaminated with conidia of *Nigrospora oryzae* which makes identification slightly difficult. Conidia of *N. oryzae* are jet black (deep, glossy black); they shine in

Fig. 10.3. Kernel smut infected rice seeds with ruptured glumes containing black powdery mass of smut spores

Fig. 10.4. Rice seeds contaminated with surface borne teliospores of *Tilletia barclayana*, × 100

the light of the microscope while teliospores of *Tilletia barclayana* are dull black and slightly bigger in size. It is advisable to confirm the identity. For this, slide preparations can be made by rolling the contaminated seed in a drop of water and examining the spores at higher magnification of a compound microscope. Spores of *T. barclayana* are shown in Fig. 10.2 and conidia of *N. oryzae* in Fig. 10.5.

Fig. 10.5. Conidia of *Nigrospora oryzae*, × 750

2. *Detection of infected seed*

Two types of infected seeds can be found in a seed sample.

- Infected seeds with ruptured glumes containing a black powdery mass of smut spores can easily be identified visually or under a stereoscopic microscope (Fig. 10.3).
- Infection in seeds with unruptured glumes can be detected by the method of Agarwal & Srivastava (1981, 1985):

  Soak the seeds (2000 seeds in 2 replicates of 1000 each) in 0.2% solution of sodium hydroxide (NaOH) for 24 h at 18-25°C. Decant the solution; spread the swollen seeds on a filter paper. Examine the seeds visually. Infected seeds will show shiny, jet-black discoloration while seeds with only brown or dull black discoloration are not infected. The infection can be confirmed by puncturing discoloured seed with a needle in a drop of water. Bunt spores will be released from the infected, shiny, jet-black seeds (Fig. 10.6), while

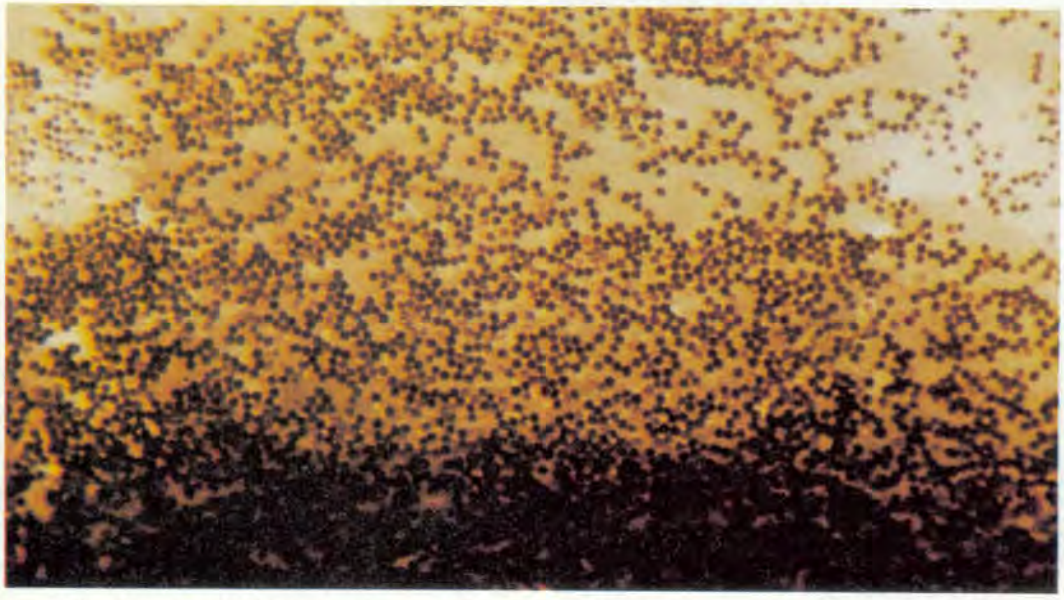

Fig. 10.6. Spores of *Tilletia barclayana* released from kernel smut infected seed after soaking for 24 h in sodium hydroxide solution

no spores will come out from healthy seeds or those with dull black discoloration.

It is useful to follow the method of Agarwal & Srivastava when certification standards are based on recording of bunted grains. This is the case for seed certification in India for domestically produced rice seed. A situation can, however, arise where bunted seeds may not be found in the sample. This may happen particularly in small rice samples which are generally used for germplasm purposes. In such cases, the whole sample must be subjected to the washing test (Shetty & Shetty, 1986). Shake the sample well for a few minutes with enough water to cover the seeds, centrifuge at about 2500 rev. min^{-1} and examine the sediment for teliospores of the fungus. The washed seeds can be used for other seed health tests, such as the blotter method, growing-on test, etc.

Control

Reyes (1950) recommended hot water treatment of seeds to control kernel smut disease. Singh (1975) found that crop rotation is more successful. Ospischev (1980) suggested effective prophylactic measures such as spraying with fungicides like Benlate or Thiophanate methyl during anthesis.

Seed certification

The Central Seed Certification Board in India has fixed standards for rice kernel smut. For foundation seed the tolerance level is 0.1% and for certified seed it is 0.5%.

Quarantine

Tilletia barclayana is a quarantine object in Australia (Neergaard, 1980).

In India, rice seed samples found infected/contaminated with *T. barclayana* are rejected by the National Bureau of Plant Genetic Resources during quarantine inspection.

Chapter 11

Udbatta Disease, Incense Rod

The disease has been reported from India, China, Hong Kong, New Caledonia and West Africa (CMI Descriptions of Pathogenic Fungi and Bacteria No. 640, 1979). In India it occurs particularly in the hilly regions of Orissa State. Usually 2-3% of ear head infection is observed; but in years of severe incidence, losses up to 10-11% are common in susceptible cultivars. In Karnataka State losses up to 30% were noticed in cultivars Madhu, IR-20, PTB-20 and Intan (H.S. Shetty, pers.comm., 1986-88).

In China, panicle infection of 5-20%, occasionally 30%, has been reported (Ou, 1985). In India also, Kamat & Patel (1951) observed panicle infection up to 11%, and later Govindu (1969) reported 10% infection on cultivar IR-8. Shivanandappa & Govindu (1976) considered Udbatta as an important disease in some areas of Bangalore, causing direct and indirect losses, 1.75-3.69% in different rice cultivars.

Symptoms

The disease is seen only at the time of panicle emergence. An erect greyish white cylindrical rod much like 'Agarbatti' (incense rod) emerges from the boot leaf sheath instead of a normal earhead, covered with white mycelium; no grain is formed (Fig. 11.1). The affected panicles eventually become hard and sclerotium-like, bearing many black dots. The infected plants are usually stunted. Sometimes before panicle emergence, white mycelium and conidia form narrow stripes on the flag leaves along the veins. Usually all tillers in a plant are infected, indicating a systemic infection (Mohanty, 1964; Ou, 1985).

Fig. 11.1. A healthy panicle of rice (left), and a diseased panicle infected by *Ephelis oryzae* (right)

Pathogen

Ephelis oryzae Syd.
(syn. *Ephelis pallida* Pat.)
(Teleomorph *Balansia oryzae-sativae* Hashioka,
syn. *Balansia oryzae* (Syd.) Narasimhan & Thirum.)

Conidia hyaline, filiform to needle-shaped, aseptate, straight or curved, 12–22(-40)×1.2-1.5 μm (Figs. 11.2 and 11.3).

Perithecia, embedded in the periphery of the head of the stroma, are rounded ovate to pyriform, 125-200×85-100 μm diam. Asci 8-spored, cylindrical, hyaline. Ascospores filiform, straight or curved, 12-27 × about 1 μm wide, septa not apparent (Ou, 1985; CMI Descriptions of Pathogenic Fungi and Bacteria No. 640, 1979).

Fig. 11.2. Conidia of *Ephelis oryzae* oozing out from a seed which has been put in a drop of water. The seed was taken from an infected panicle, × 12

Location of the pathogen

Mycelium of *Ephelis oryzae* is present in the embryo (H.S. Shetty, pers.comm., 1988), as well as conidia on the seed surface (Mohanty, 1977). Both mycelium and conidia have been shown to initiate infection in plants.

Presence of the mycelium in all parts of the embryo was demonstrated at the University of Mysore, India by examining embryos of all available normal looking seeds, collected from the Udbatta affected plants (see Seed health testing).

Fig. 11.3. Conidia of *Ephelis oryzae*, × 750

Transmission

In the CMI Descriptions of Pathogenic Fungi and Bacteria No. 640, (1979) Booth states that *Ephelis oryzae* is seed-borne. This statement is based on the work done in India by Mohanty (1964) where the author made inoculation experiments and found that the fungus is internally seed-borne and systemic in the plant. Mohanty also showed that the fungus is not soil-borne. The incidence of the disease was found to be comparatively less in very early or very late sown

crops than in those sown at the normal time for period-bound varieties.

In 1977, Mohanty showed that conidia of *E. oryzae* can induce Udbatta disease in plants. Inoculation of dry seeds with a conidial suspension or of soaked seeds with conidial dust induced the disease more effectively than inoculation of germinated seeds. Seedling infection seems to be the principal mode of infection.

At the University of Mysore in 1987, normal looking seeds collected from Udbatta affected plants (cv. Madhu, IR-20, PTB-20 and Intan) were sown in sterilized soil in a greenhouse. Minimum distance between the greenhouse and any nearby rice field was more than two kilometres. According to Professor H. S. Shetty (pers.comm., 1988) a number of rice plants developed Udbatta disease. The work is being continued in order to establish correlation between seed-borne infection (embryo infection) and infection in plants raised from normal looking, infected seeds, as well as varietal response to seed infection.

Seed health testing

No single testing method has been standardized so far, but the following can be tried.

1. *Inspection of dry seed.*

- The first indication of the presence of seed-borne infection of *Ephelis oryzae* in a seed sample can be obtained if the seeds are examined visually and under lower magnifications of a stereoscopic microscope very carefully for diseased seeds which may have become mixed up with normal seeds at the time of threshing. The diseased seeds are comparatively small, disfigured, often empty, covered with whitish-gray, dry conidial masses of *E. oryzae* (Fig. 11.4) or may even have black perithecia of *Balansia oryzae-sativae*. Make slide preparations and examine the fungal structures under

Fig. 11.4. One diseased rice seed infected with *Ephelis oryzae*, circled (top); magnified (bottom).

higher magnifications of a compound microscope (see Fig. 11.3).

The whole seed sample or part of a sample can be subjected to such an inspection, and it appears the method will work. In the First International Training Course on Seed Health Testing for Rice, conducted at the International Rice Research Institute in the Philippines (Oct. 17-Dec. 2, 1988), all 19 participants were able to detect the diseased seed in a rice sample, each participant having been provided with a small sample which contained 99 normal seeds and one diseased seed from a diseased panicle. Participants confirmed their identification by examining conidia under compound microscopes.

2. *The washing test*

Follow the steps as described for the test in Chapter 9. For conidial morphology, see Fig. 11.3.

3. *The whole-embryo count method*

In a personal communication Professor H. S. Shetty (1988) informed us that he and his students have been able to demonstrate that the mycelium of *Ephelis oryzae* is present in all parts of the infected embryos and that it can be detected by the whole-embryo count method. The whole-embryo count method is routinely used in seed health testing laboratories to detect the mycelium of the loose smut fungus in wheat and barley (Rennie & Seaton, 1975; Khanzada, Rennie, Mathur & Neergaard, 1980; Rennie, 1981).

Since the infection of loose smut fungus in wheat and barley is usually in trace amounts, 2000 to 3000 embryos are examined per sample. Such a high number was not available to Professor Shetty and his co-operators. They used only those seeds which were available from Udbatta diseased plants in their experimental fields. Although the testing method is yet not standardized one can follow the following steps:

- Remove the husks manually.
- Soak kernels in 5% sodium hydroxide solution (NaOH) containing 0.01% trypan blue stain for 24 h at 25-28°C.
- Agitate the soaked kernels gently with a stirrer for about 2-3 min.
- Drain the solution and with a fine brush collect the embryos in lactophenol.
- Clear the embryos by boiling in lactophenol for 2-3 min and finally examine them under stereoscopic microscope.
- Mycelium of *E. oryzae* is thin, septate and knotty.

Control

The disease was effectively controlled by hot-water treatment of seed at 54°C for 20 min, and fungicidal seed treatment with Tecto-60 and Granosan L at 1:444 (dry seed treatment) and 1:2000 (slurry) by seed weight respectively, also gave equally good results (Mohanty, 1975).

Chapter 12

Scab

A widely distributed disease of rice in the tropics (CMI Descriptions of Pathogenic Fungi and Bacteria No. 384, 1973b).

The disease normally does not cause heavy damage but it may be severe under favourable environmental conditions such as high humidity (Ou, 1985).

Symptoms

Infected seeds show white, yellow or reddish discoloration or spots over a part or whole of the surface. Such lesions may bear masses of conidia. Infected grains are lighter in weight, shrunken and brittle and often do not germinate. If germination occurs diseased seedlings are produced. The fungus may also attack nodes. Infected stems wilt and break.

Pathogen

Fusarium graminearum Schwabe
(Teleomorph *Gibberella zeae* (Schw.) Petch)

Growth of the fungus on potato sucrose agar is greyish or greyish-yellow or white. Macroconidia of the fungus range from falcate, with or without an elongated apical cell, to sickle-shaped and have a well-marked foot cell which in certain strains is conspicuously pedicellate, 3- to 7-septate measuring 25-50×2.5-3.5 μm (Fig. 12.1). Microconidia absent. Chlamydospores, when present, are intercalary, globose, thick-walled and hyaline to pale brown. Perithecia ovoid, 140-250 μm diam; asci clavate, 60-85×8-11 μm; with 8 or occasionally 4-6 ascospores. Ascospores 3-septate, light brown and fusiform (CMI Descriptions of Pathogenic Fungi and Bacteria No. 384, 1973b; Booth, 1971; Ou, 1985; Ram Nath *et al.*, 1970).

Ikeya (1933) observed that isolates from rice and wheat seemed to be morphologically and physiologically alike. Chung *et al.* (1964) found that an isolate from wheat infected rice and caused post emergence blight.

Fig. 12.1. Macroconidia of *Fusarium graminearum*, × 750

Seed health test

The standard blotter method as described in Chapter 1 for *Pyricularia oryzae* should be used for the detection of *Fusarium graminearum*. Observe whitish, loose mycelium and clusters of shiny macroconidia (Fig. 12.2). On such infected seeds small, wet, pale pionnotes of macroconidia may be seen. In a few severely attacked seeds (Fig. 12.3) the growth may be more pronounced. Here the pionnotes of macroconidia are slightly larger, wet, orange to pinkish in colour.

Rarely, in some seed samples, perithecia of *Gibberella zeae*, the teleomorph of *F. graminearum* develop (Fig. 12.4A). The perithecia can occur singly or in groups, generally black, sometimes having a greenish

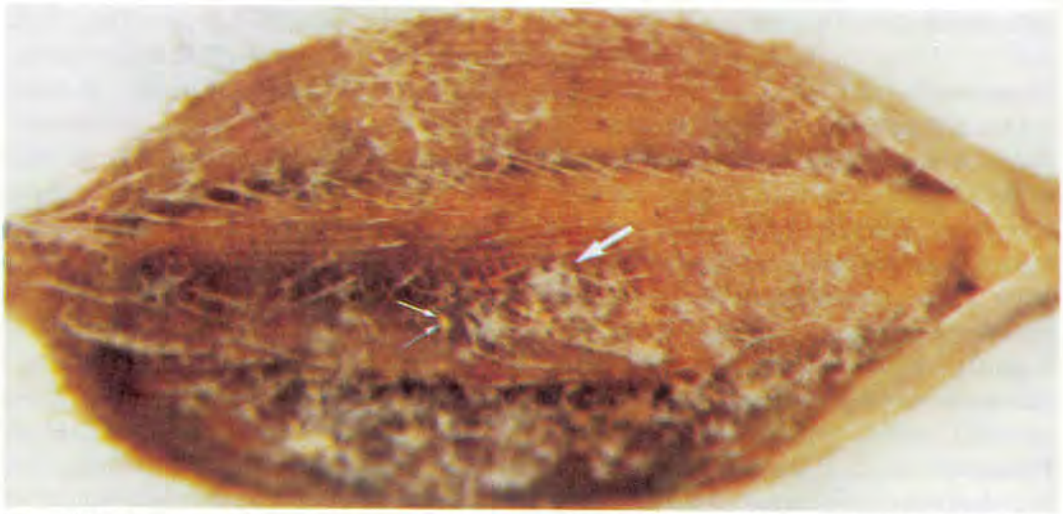

Fig. 12.2. Growth of *Fusarium graminearum* on a rice seed in the blotter test. Note the white clusters of free macroconidia (one arrow), and small, wet, pale pionnotes full of macroconidia (two arrows)

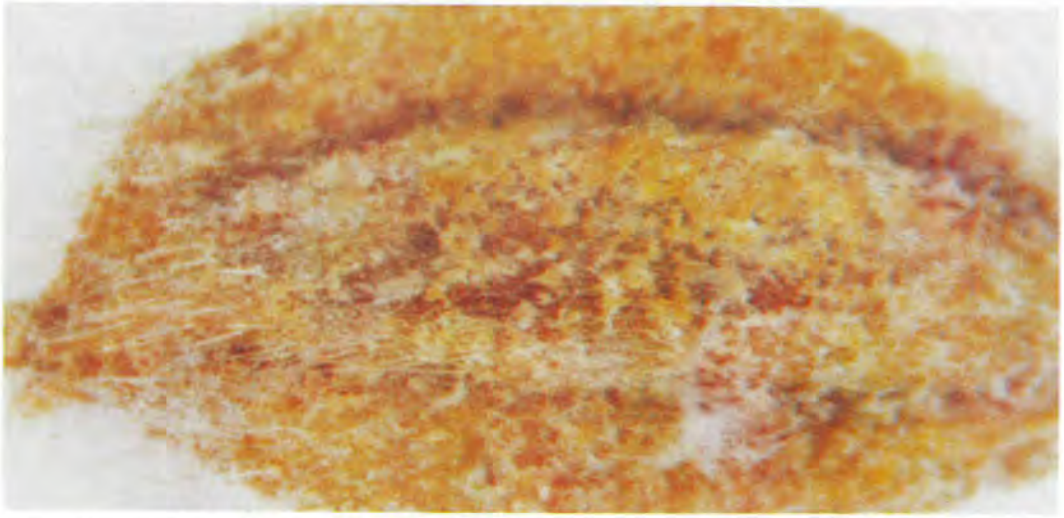

Fig. 12.3. Well-developed orange coloured pionnotes, full of macroconidia of *Fusarium graminearum* on a rice seed in the blotter test

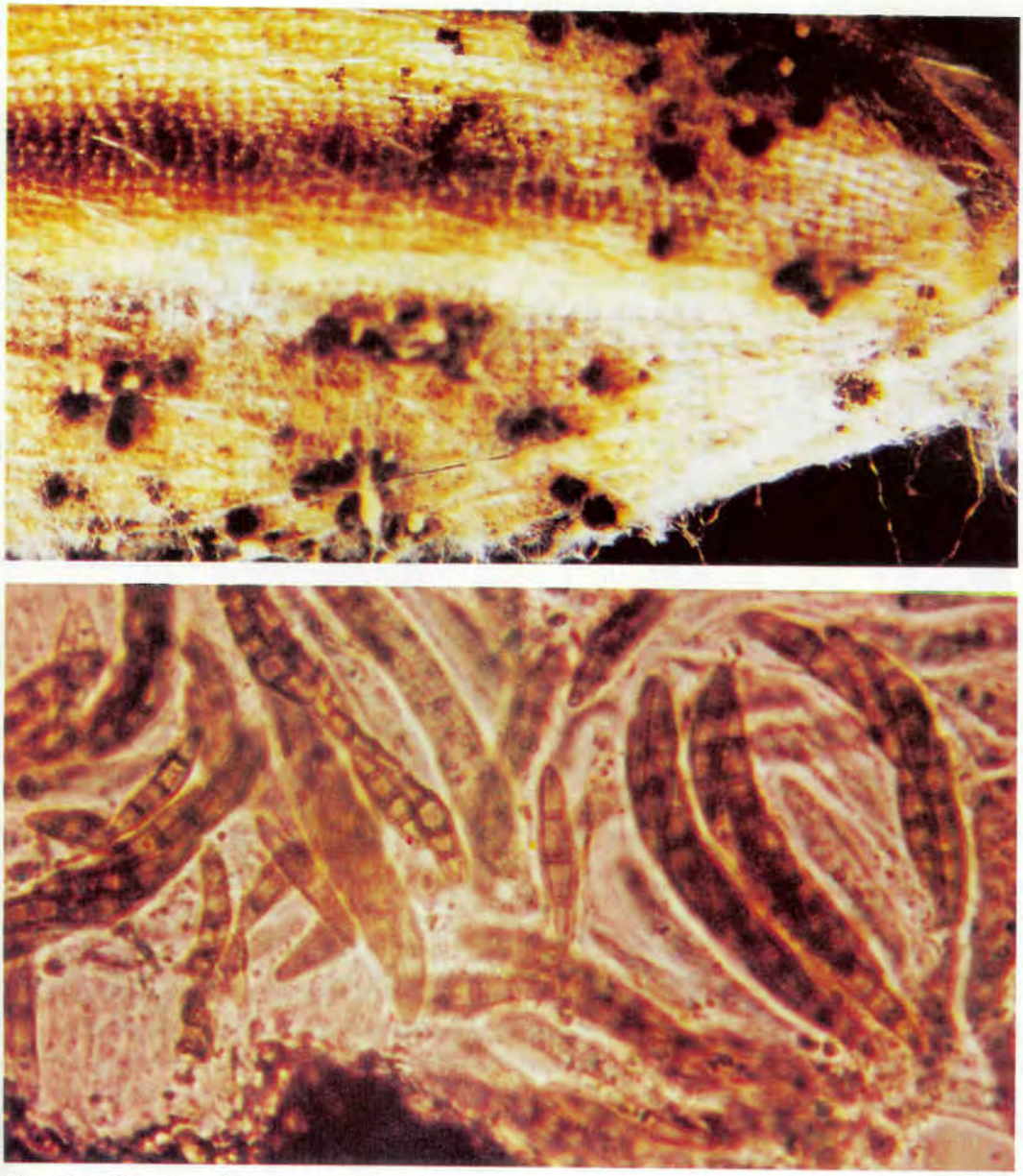

Fig. 12.4. Black perithecia of *Gibberella zeae* on a rice seed in the blotter test (top); asci and ascospores (bottom), × 750

tinge leaving a reddish brown colour on the seed. For confirming the identity of the fungus perithecia must be mounted in water and studied under the compound microscope for the characteristic ascospores as shown in Fig. 12.4B. Whitish pale, pale or pale brown pionnotes can be observed within the mycelial growth. Often dark blue perithecia of *G. zeae* can be observed on the seed.

Control

Treatment of moistened seed with 2.3% Rhodane at 500 g/100 l/1000 kg was recommended by Mironenko (1960).

Chapter 13

Grain Discoloration

Discoloration of rice grain is generally due to infection by certain micro-organisms on the glumes or kernels, or both. Infection may occur before or after harvest. Certain factors other than micro-organisms are sometimes also responsible for grain discoloration. Rice grown in highly acid upland soils or flooded acid soils with high iron content resulted in severe grain discoloration (Zeigler, Aricapa & Hoyos, 1987). Higher plant density, higher dosages of nitrogenous fertilizers and stacking of rice in the field after harvest followed by wet weather induce discoloration. High moisture content in grains (>15%) and high relative humidity (>65%) also favour discoloration. Rice grains may also show discoloration because of virus infection *(hoja blanca)*. Zeigler *et al.* (1987) isolated pathogenic *Pseudomonas* spp. from discoloured grains (see chapters 16-19).

Fungi that are associated with discoloured grains can be divided into two major groups. One consists of field fungi (infects the grain before harvest) and the other includes storage moulds. Most common among the former group are *Bipolaris oryzae, Alternaria padwickii, Pyricularia oryzae, Fusarium moniliforme, F. graminearum, Nigrospora oryzae, Epicoccum nigrum, Curvularia* spp., *Phoma sorghina, Dichotomophthoropsis nymphacearum, Heterosporium echinulatum,* etc. Vidhyasekaran *et al.* (1984) reported that the sheath rot pathogen, *Sarocladium oryzae,* caused discoloration in grains. Visual inspection of over 3.500 seed samples from 53 countries, at the Institute of Seed Pathology in Denmark, showed generally discrete brown to dark brown spots on glumes or diffused type of discoloration of different shades covering part of the seed or rarely whole seed (Fig. 13.1). Grey discoloration of tips or whole seed was also observed. *Alternaria alternata, Curvularia lunata, Bipolaris oryzae, Fusarium* spp., *Nigrospora oryzae* and *Alternaria padwickii* were mainly isolated. *Pyricularia oryzae* and *Sarocladium oryzae* were also occasionally found associated with discoloured seeds.

Grain discoloration caused mainly by fungi such as *Bipolaris oryzae, Phoma sorghina, Nigrospora oryzae* and *Curvularia* spp. and to a limited extent by bacteria, species of *Pseudomonas,* pose a serious problem in the humid tropics of Northern Brazil (A. S. Prabhu, pers. comm., 1989). In Central Brazil, however, grain discoloration is primarily caused by *Phoma sorghina* and *Bipolaris oryzae* and it is a major problem in seed production.

Most of the storage moulds are species of *Aspergillus, Penicillium, Absidia, Mucor, Rhizopus, Chaetomium, Monilia, Streptomyces,* etc.

Infected kernels show red, pink, blue, yellow and green discoloration depending upon the organisms involved. Species of *Aspergillus* and *Penicillium* give green, blue and yellow coloration to the grain while species of *Fusarium* impart pink colour and *Erwinia herbicola* causes black rot. Duraiswamy & Mariappan (1983a) observed *Bipolaris oryzae* predominantly in purple and brown coloured grains.

Discoloration of grain causes deterioration in grain quality (Vidhyasekaran & Govindaswami, 1968; Duraiswamy & Mariappan, 1983b), reduction in seed viability and such grains on planting usually exhibit pre-emergence or post-emergence death of seedlings. Uraguchi (1942) and Iizuka (1958) reported toxin production by storage moulds associated with discoloured grains.

Fig. 13.1. Rice seeds showing spots and discoloration

Detection methods

The blotter method described in chapter 1 should be followed to detect field fungi while storage fungi can be detected by plating seeds on a high salt agar medium (e.g. 7.5-10% NaCl agar medium).

Control

Application of edifenphos and copper oxychloride before harvest reduced grain discoloration (Govindarajan & Kannaiyan, 1982). Good control was found in a field trial in India with IBP, mancozeb and quazatine (V. S. Duraiswamy, pers.comm., 1988). Singh & Chand (1985) found similar results with edifenphos and mancozeb. It is recommended that these chemicals should be applied twice, before flowering and after flowering. Moisture content of grain should be reduced to less than 14% before they are stored.

Infrared drying and treatment with sodium propionate has been found to reduce the infection by field fungi (Schroeder, 1964).

Chapter 14

Bacterial Leaf Blight

The disease is widespread in Asia, and has been reported from Australia (CMI Distribution Maps of Plant Diseases No. 304, ed. 5, 1987; Fig. 14.1). Its presence has been recorded in Latin America, Caribbean region, North America, Malaysia, West Africa, Mali, Niger and Senegal. However, it has not been reported from Europe, with the exception of U.S.S.R.

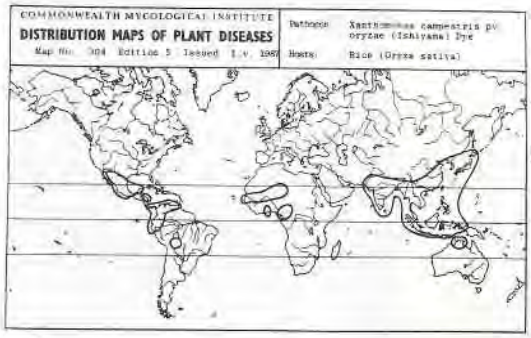

Fig. 14.1. Distribution map of bacterial leaf blight published by the CAB International Mycological Institute, Kew, U.K. in 1987

Bacterial Leaf Blight is the most serious disease of rice in South East Asia, particularly in Japan, Philippines, Indonesia and India. According to Ou (1985) yield losses in Japan normally range between 20-30%, occasionally increasing up to 50%. In the Philippines and Indonesia losses are even higher. In India, Srivastava (1967) reported losses between 6 and 60%, later, Ahmed & Singh (1975) up to 74% and Singh, Srivastava, Singh & Singh (1977) even to 81.3% in some cultivars. The disease reached epidemic proportions in Bihar State and parts of North India in 1975 as reported by Rangaswami (1975).

Symptoms

The disease commonly appears as yellow to white water-soaked stripes at the margins of infected leaves. Under favourable conditions these stripes can enlarge and coalesce to give the characteristic yellowish lesions with wavy edges mainly in the upper part of the affected leaves (Fig. 14.2). Sometimes the entire leaf may turn whitish to greyish and die. In the case of highly susceptible varieties even the leaf sheath exhibits such symptoms.

Fig. 14.2. Symptoms of bacterial leaf blight in rice caused by *Xanthomonas campestris* pv. *oryzae*
(Photo: Courtesy Mr Jusuf Soepriaman, Indonesia)

Kresek, which occurs in tropical regions, is a strong systemic infection, in which leaves or entire plants wilt during the seedling and early tillering stage and die. In older plants the leaves become pale yellow.

Pathogen

Xanthomonas campestris pv. *oryzae* (Ishiyama) Dye
(syns. *Xanthomonas oryzae* (Ishiyama) Dowson; *Xanthomonas kresek* Schure; *Bacterium oryzae* (Ishiyama) Elliott)

The bacterium is a Gram-negative, aerobic, capsulate rod, occurring singly, rarely in pairs, but not in chains, 0.5-0.8×1.3-2.2 μm, and slightly smaller in the host, motile with a single polar flagellum. On nutrient agar, colonies are circular, entire, smooth, convex, opaque, whitish yellow at first, straw yellow later. For further details see CMI Descriptions of Pathogenic Fungi and Bacteria No. 239, 1970c.

Ou (1985) has summarized variability in *Xanthomonas campestris* pv. *oryzae*. Horino, Mew, Khush & Ezuka (1980, 1981) reported that pathotypes from Japan and the Philippines are quite different. Mew (1978) has also stated that there appear to be two biotypes or ecotypes of the bacterium.

Location of the pathogen in the seed

The pathogen has been found in the glumes and occasionally within the endosperm of seed collected from heavily diseased fields (Fang, Lin & Chu, 1956; Srivastava & Rao, 1964).

Seed infection and plant infection

It has been stated by Fang *et al.* (1956) that *Xanthomonas campestris* pv. *oryzae* within the glumes of rice grain appears to be the primary source of inoculum, seedlings grown from infected grains usually being diseased. Srivastava & Rao (1964) from India reported 50 to 100% seed infection. The infection was present not only in the husk of the seeds but also in the endosperm; infected seeds transmitted the disease to seedlings when raised under conditions of high humidity. Also from India, Durgapal, Singh & Pandey (1980) reported that active inoculum of the bacterium is present in 90% of infected seeds immediately after harvest. It has been claimed by Singh, Banerjee, Rai & Srivastava (1980) that seeds can retain enough inoculum until the next season to cause an epidemic under favourable conditions. The importance of seed transmission has been sufficiently emphasized in India and China, but not in Japan. This anomaly could be due to the genetic differences in the rice varieties grown (Mizukami & Wakimoto, 1969).

Seed health testing

Singh & Rao (1977) developed a method for the detection of the pathogen. The technique, roll towel method, with minor modifications as carried out at the Institute of Seed Pathology, is as follows:

- Count 400 seeds and plate them equally spaced between two paper towels, 45 ×28 cm size which have previously been soaked in tap water; prepare 8 replicates of 50 seeds in each towel (Fig. 14.3).
- Roll the towels and close the ends with rubber bands (Fig. 14.3) and place them in an upright position from 5 up to 9 d at 30±2°C and in a light regime of 12 h each day supplied by ordinary white fluorescent tubes.
- Remove the bands, unroll the towels and examine the seedlings carefully for symptoms. Small pieces of coleoptile, leaf sheath and leaf of 4-5 seedlings showing symptoms of water soaking, brown or yellow discoloration are examined under compound microscope for bacterial ooze. Only if bacterial ooze is observed proceed with the isolation and testing for pathogenicity.

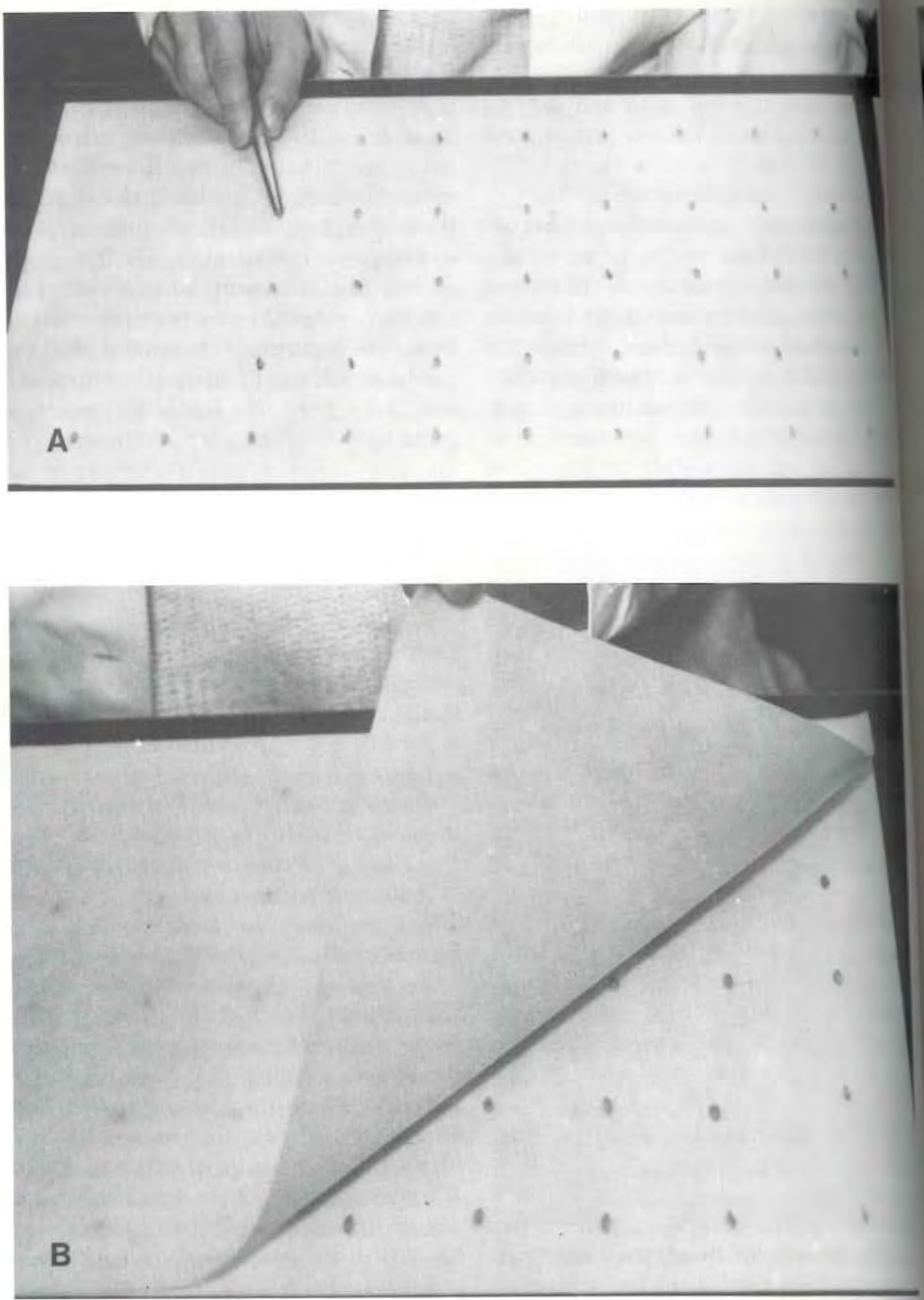

Fig. 14.3. Roll towel method. Plating of rice seeds on wet paper towel (A), covering with another wet paper towel (B)

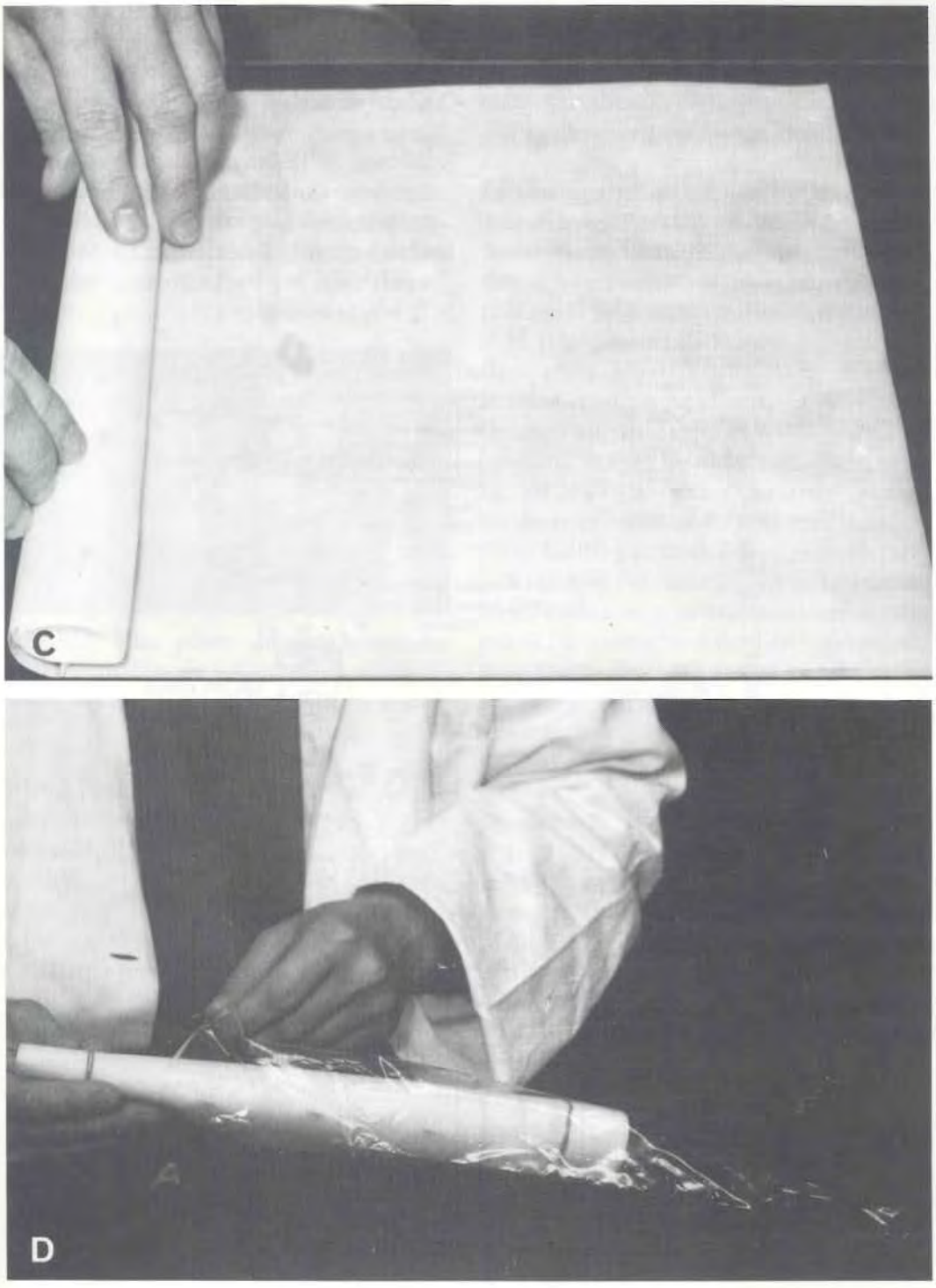

Fig. 14.3. Roll towel method. Rolling of towels (C) and finally placing of the rolled towels in plastic bag after putting rubber band at the ends (D)

- Take out all seedlings showing infection and cut them into small pieces (except roots) in a beaker having enough sterile water to immerse them (Fig. 14.4). Wait for 15 min to obtain bacterial cells in the water.
- The water containing bacteria can either be used directly for the pathogenicity test or the pathogenicity tests performed using pure colonies obtained after usual isolation.
- The pathogenicity test can be conducted in three ways. 1) Dip a pair of scissors in the bacterial suspension and cut the tips of 30-40 leaves. 2) Cut the tips of the leaves with the scissors while still immersed in the bacterial suspension. 3) Spray the clipped leaves with the bacterial suspension.
- Cover the inoculated plants for 24 h with a polythene bag and incubate at 30°C with 12 h light cycle.

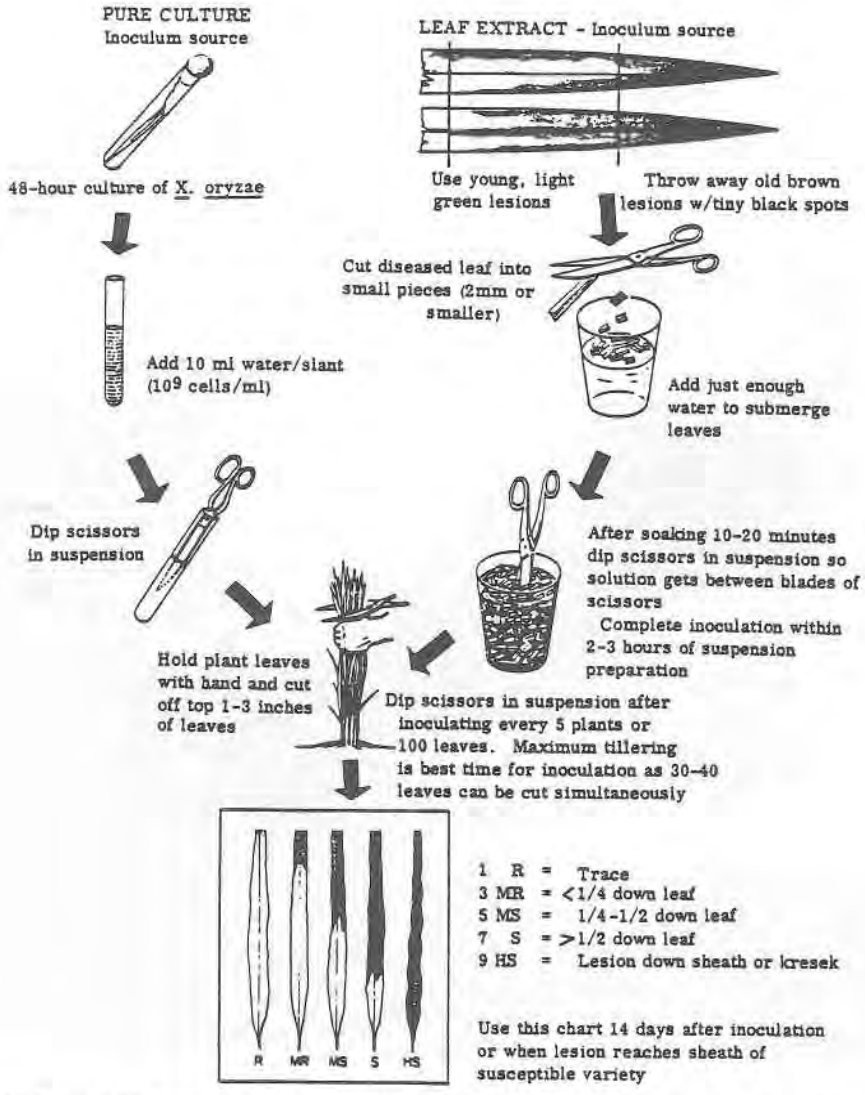

Fig. 14.4. Bacterial leaf blight inoculation method (After Kauffman, Reddy, Hsieh & Merca, 1973)

- Observe plants with symptoms within 48-72 h. Check for water soaked areas in the inoculated leaves, usually beginning from the inoculated ends as water soaked stripes. Lesions enlarge and may turn yellow within a few days (Fig. 14.5). Milky opaque drops of bacterial exudate can be observed. Make sure that the symptoms are not just localized, but extend downwards.

Fig. 14.5. Rice plants showing symptoms after inoculation with *Xanthomonas campestris* pv. *oryzae* by the leaf 'clipping' method

The presence of the bacterium in rice seed can also be detected by conventional methods such as direct plating of surface disinfected seeds on general bacteriological media, e.g. nutrient agar and sucrose peptone agar. The bacterium can also be isolated from seed extract obtained from seed flour.

In China, the bacterium has been isolated from glumes of a 5 g rice seed sample incubated in 20 ml of sterile water for 2 h. The sample was centrifuged and the pellet tested by indirect immunofluorescence antibody technique (IF) (Di Yuan-bo, pers.comm., 1985).

Control

The bacterium can be eradicated from seed by soaking for 12 h in an aqueous solution of Ceresan (500 ppm) + agrimycin 100 (250 ppm) followed by hot-water treatment at 53°C for 30 min (Shekhawat, Srivastava & Rao, 1969). Singh & Rao (1982) reported that infection can be eliminated by treating the seed with Brestanol 45 WP (triphenyltin chloride) at the rate of 0.25 g/100 g of seed or HPMTS 80 EC (2-hydroxy propylmethane-thiosulphonate) at the rate of 0.098 ml/100 g of seed, in a slurry form.

To reduce the disease, one to two sprayings with mercury compounds or antibiotic solutions (having bactericidal effect) should be applied at the late nursery stage. Similar sprayings, should be made two to three times at the maximum tillering stage, and as soon as possible after flowering.

Quarantine

Lozano (1977) indicated that the disease has been introduced to the Caribbean region and South America through the import of infested or infected seed, or both.

Xanthomonas campestris pv. *oryzae* has been included in the import regulations of Chile, Greece and Netherlands New Guinea (Neergaard, 1970).

Chapter 15

Bacterial Leaf Streak

The disease is widespread in tropical areas. It has been reported from Australia, Bangladesh, Cambodia, China, India, Malaysia, Nepal, Philippines and Thailand (CMI Distribution Maps of Plant Diseases No. 463, ed. 2, 1982b; Fig. 15.1) but not from Japan and other temperate areas (Ou, 1985).

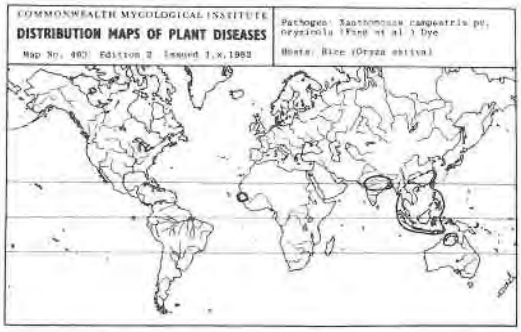

Fig. 15.1. Distribution map of bacterial leaf streak published by the CAB International Mycological Institute, Kew, U.K. in 1982.

As high as 17% yield loss was recorded in a susceptible cultivar due to bacterial leaf streak in the wet season by Opina & Exconde (1971) in the Philippines. The loss was 5.9% in the dry season and none in the resistant cultivar used in the experiment. In India, 32.3% loss based on 1000 grain weight, was observed in cv. IR 20 and 28.6% loss in Pusa 2-21 by Naik, Nema, Kulkarni & Srivastava (1973). No loss was seen in cv. BC 5 and PI 180061. However, Ho (1975) indicated that losses due to bacterial leaf streak in Malaysia are insignificant even though severe symptoms may appear at certain stages of growth. Losses due to the disease have also been reported from Nepal but no figures given (Manandhar, 1987).

Symptoms

Bacterial leaf streak is a foliar disease. Small water soaked streaks appear first and later they elongate and darken. They tend to be delimited by the veins (Fig. 15.2). Under humid conditions exudation is common, often being found in tiny yellow beads. The narrow, long or short translucent lesions may coalesce forming large patches. In severe cases, the rice plots seem to acquire a burnt appearance. At this stage leaves wither, turn brown and the disease is indistinguishable from bacterial blight.

Fig. 15.2. Rice plants showing bacterial leaf streak symptoms caused by *Xanthomonas campestris* pv. *oryzicola*

Pathogen

Xanthomonas campestris pv. *oryzicola* (Fang *et al.*) Dye
(syns. *X. oryzicola* Fang *et al.*;
X. translucens f.sp. *oryzicola* (Fang *et al.*) Bradbury)

The bacterium is a Gram-negative, non-spore forming, capsulate rod with single polar flagellum, 0.4-0.6×1.0-2.5 μm. On nutrient agar, colonies are whitish at first, becoming straw to pale yellow later; about 1 mm diam in 3 d. For further details see CMI Descriptions of Pathogenic Fungi and Bacteria No. 240, 1970d. Growth is rapid, gelatin and starch are hydrolyzed; these characters distinguish it from *X. campestris* pv. *oryzae* (Bradbury, 1986).

No pathogenic races have been recorded but there have been reports of many isolates varying in pathogenicity (Vera Cruz, Gosselé, Kersters, Segers, van den Mooter, Swings & Ley, 1984).

Seed infection and plant infection

The causal bacterium hibernates under the glumes. Shekhawat *et al.* (1969) have reported that seeds from an infected crop carry the bacterium to the next season and are a potential source of dissemination to distant places. The bacterium also infects wild species of *Oryza*. During germination of the seed, the plumule is infected by bacteria located under the glumes, secondary infections occurring through wounds and stomata (Singh, 1978). The bacterium mainly infects the parenchyma cells of the leaves but is not systemic. Later the parenchymatous tissues may be replaced by bacterial masses (Ou, 1985). Exudate from the lesions spreads to other plants.

Seed health testing

No testing method has been published for the detection of the bacterial streak pathogen from rice seed. However, the bacterium can be isolated by conventional bacteriological techniques and can be further identified by biochemical and pathogenicity tests. *Xanthomonas campestris* pv. *oryzicola* is differentiated from *X. campestris* pv. *oryzae* by its rapid growth and ability to hydrolyse starch and gelatin.

Control

Effective eradication of the pathogen from the seed has been reported by soaking them in 0.025% streptocycline for 30 minutes (Shekhawat & Srivastava, 1971). Foliar sprays with 0.15-0.3% of vitavax have also been effective. Singh (1978) reported control of the disease with Sankel, Captan and Fytolan. Agrimycin and Streptocycline at the rate of 100 ppm have also been shown to control the disease (Banerjee, Rai, Srivastava & Singh, 1984).

Bacterial Stripe

The disease is reported from Japan, Taiwan, the Philippines (Ou, 1985), Korea (Shakya & Chung, 1985) and Iran (Rahimian, 1986). Shakya, Vinther & Mathur (1985) reported that the pathogen is present in the major rice producing countries of Africa, Asia, Latin America and Portugal in Europe, based on symptoms developed in rice seedlings raised in Denmark from rice seed samples received from a number of countries. Out of 62 samples tested from 29 countries, 55 from 28 countries showed infection of *Pseudomonas avenae* not until now known to occur there. Infection ranged from 1 to 75%. The identity of the bacterium was confirmed serologically. It was even found in 8-year-old seed samples which had been stored at 5°C. The bacterium infects other species of graminaceous hosts.

Symptoms

The disease appears on seedlings and young plants in the nursery. Symptoms first appear on the lower part of the leaf sheath, where water soaked dark green longitudinal stripes are formed. They soon turn dark brown (Figs. 16.2 and 16.6), can measure up to 10 cm in length and 1 mm in width, sometimes coalescing to form wider lesions. Severe infection may cause stunting and seedling death. Young unfolding leaves may also be attacked leading to bud rot; such parts eventually die. Seeds may show moderate to severe discoloration of the husk and endosperm. In extreme cases the grains may rot completely and may remain unfilled.

Pathogen

Pseudomonas avenae Manns
(syn. *Pseudomonas alboprecipitans* Rosen; *Pseudomonas setariae* (Okabe) Savulescu; *Bacterium setariae* Okabe)

The bacterium is a Gram-negative rod, 0.4-0.8×1.8-4.4 μm, occurring singly or in pairs, occasionally in short chains, usually with a single polar flagellum, no capsules or endospores. On nutrient agar, colonies are circular, entire, smooth, glistening and raised. For further details consult CMI Descriptions of Pathogenic Fungi and Bacteria No.237, 1970b.

Location of the pathogen in the seed

Shakya, Chung & Vinther (1986) reported that the bacterium may be located between the glumes and the pericarp, or deeper in the seed.

Seed infection and plant infection

Baraoidan (1981) in the Philippines observed bacterial stripe in seedlings raised from inoculated seeds, and from seeds produced from plants inoculated at the boot and flowering stages, and in seedlings grown in artificially infected soil. The author observed 0.5 to 8% disease incidence on sixteen rice varieties in the field, but no information is given on the extent of seed infection.

Transmission of *Pseudomonas avenae* from rice seed to seedling and from plant to seed was shown by Shakya *et al.* (1986). They also showed that the bacterium can be transmitted internally from plants to seed in latently infected plants.

In a recent investigation Shakya *et al.* (1988) made a correlation between laboratory assay and field incidence of bacterial stripe disease in Nepal. Out of the 308 seed lots assayed 260 seed lots of different cultivars were found infected, indicating frequent occurrence of the disease in the country. The incidence of bacterial stripe in the field nurseries was higher in the field plots

which initially contained more than 50% infected seeds while low in plots with less than 10% infected seeds. The field incidence of the disease ranged from 1 to 28%. The brown stripes were distinct at the base of the stem of plants at an early stage of tillering. The tillering was poor and plants were stunted in about 30% of the hills. This investigation indicated positive correlation between the laboratory test method and field incidence of bacterial stripe and supports the use of the suggested method (see the details of the method under seed health testing) in bacterial stripe seed health programmes.

Seed health testing

Shakya & Chung (1983) developed a testing method to detect seed-borne *Pseudomonas avenae* in rice seed by recording symptoms in seedlings grown in 230 ppm of nitrogen solution. The test was originally conducted in Petri dishes but more recently the test is conducted using slide cassette holders. The details of both procedures are described below.

Seedling symptom test using Petri dishes

- Test 100 seeds, in four replicates of 25.
- Plate 25 seeds in one Petri dish of 9 cm diam having three layers of filter paper moistened with 230 ppm of nitrogen (urea) solution (H_2NCONH_2).
- Incubate the dishes at 27-30°C and 12 h daylight or light provided by white fluorescent lamps.
- Add nitrogen solution 2 to 3 times during the first week to keep the filter papers well moistened.
- Remove the lids when the seedlings start to push them up. From now onwards incubate the dishes in a humid chamber (Fig. 16.1).
- Record symptoms between 10 and 14 d. The characteristic symptom produced by *P. avenae* is the development of brown

Fig. 16.1. Seedling symptom test using Petri dish. Incubation of rice seedlings in a polythene bag

Fig. 16.2. Typical bacterial stripe symptom (arrow), and yellow discoloration of seedlings

stripe, usually in the coleoptile and sometimes also in the leaves (Fig. 16.2). The stripe can be continuous or broken. Other symptoms are diffused browning in the coleoptile, water soaked areas and yellowing of the leaves. Leaf tips may sometimes show browning and drying.

- Count all seedlings with stripes and other symptoms to obtain percentage of infection in the seed sample.

Seedling symptom test in slide cassette holder

- In this procedure, instead of circular papers, filter papers cut in 4.5^2 cm are used. Two filter papers are put together in every second slit of the slide holder (Fig. 16.3).
- The cassette having the filter papers is placed in a tray filled with 230 ppm nitrogen solution so as to cover 2 cm of the cassette.
- Once the filter papers become moist place 4 seeds between the two layers of the filter papers (Fig. 16.4).
- Incubate the trays under same conditions as mentioned earlier for the Petri dishes. Make sure that the tray or trays are kept in high humid conditions. This can be achieved by putting them under plastic as shown in Fig. 16.5.

Fig. 16.3. Seedling symptom test using cassette holders. Two filter papers (4.5^2 cm) are put in alternate slits of a slide cassette holder

Fig. 16.4. Placing of seeds between the two layers of the filter paper in the cassette method. The cassette is placed in a tray having 230 ppm of nitrogen solution (urea)

Fig. 16.5. A humid chamber for the seedling symptom test using cassette holders

Fig. 16.6. Symptom of bacterial stripe (arrow) in a rice seedling raised in cassette holder

- Record infection of *P. avenae* as described earlier (Fig. 16.6).

Isolation and identification of bacteria from seedlings

Since symptoms such as water soaking and yellowing observed in seedlings raised in Petri dishes or slide cassettes can be due to *P. avenae* as well as other bacteria in the sample, isolations must be made from infected parts and bacterial colonies should be identified with the help of pathogenicity, serological and/or biochemical tests. Descriptions of these tests are included in Chapter 20. Only those colonies which are small, non-fluorescent, whitish-grey, translucent on nutrient agar and which are Gram-negative, give positive reaction in serological slide agglutination or Ouchterlony double diffusion tests belong to *P. avenae*.

If antiserum against *P. avenae* is not available, the bacterial cultures can be tested for oxidase reaction, pathogenicity on 15-20-d-old rice plants of cv.taichung native 1 and by hypersensitive reaction test. Non-fluorescent colonies, oxidase positive, provoking bacterial stripe symptoms after 4-5 d and inducing hypersensitive reaction on tobacco leaves are *P. avenae*.

Control

Seed treatment with kasugamycin effectively controls damage to seedlings in nursery boxes (Yaoita & Fujimaki, 1984). Heat therapy at 65°C for 6 d eradicates *Pseudomonas avenae* in the rice seed (Zeigler & Alvarez, 1988).

Quarantine

Shakya *et al.* (1985) suggested that infected seed may have acted as an important source of dissemination of the bacterium from one geographical area to another. However, given the already wide distribution of the pathogen, it should not be considered a serious quarantine object unless pathogenic races of different virulence are identified through future research.

Chapter 17

Bacterial Sheath Brown Rot

Widely distributed in Latin America (Brazil, Colombia, Guatemala, Mexico, Panama, Peru and Surinam) and Northern Japan (Zeigler *et al.*, 1987). There is also a report of its existence in Central Africa (Burundi) (Autrique & Maraite, 1983). The causal organism, *Pseudomonas fuscovaginae*, has also been isolated at CIAT, Cali, Colombia from rice seed received from Asia, and at the Institute of Seed Pathology from seed received from the Philippines and Indonesia. The bacterium has been found to infect other species of Gramineae and non graminaceous hosts (Miyajima, Tanii & Akita, 1983; Zeigler, Hoyos & Aricapa, 1986).

Information on economic losses is not available, perhaps because the disease has not yet been well investigated. *P. fuscovaginae* may be an important component of the dirty panicle disease, or 'manchado del grano'. This syndrome has been ascribed to soil nutritional problems and a host of fungal pathogens (Zeigler & Alvarez, 1987).

Symptoms

Infected seedlings show brown water-soaked necrotic areas on the leaf sheath (Fig. 17.1). Occasionally, necrotic brown stripes can be observed on leaves. Such seedlings may die. At subsequent stages longitudinal brown to reddish brown (maroon) necrotic areas 2-5 mm wide develop on the sheath and may extend along the midrib of the leaf lamina.

Affected sheaths enclosing the panicle show extensive water soaking and necrosis with poorly defined margins (Fig. 17.2). Glumes discolour before emerging from such panicles. Panicles may not emerge from affected sheaths. Grains from infected tillers may be completely discoloured (Figs. 17.2 and 17.3) and sterile or have very little discoloration. Similar discoloration in grains is also caused by other seed-borne bacterial pathogens such as *Pseudomonas syringae* pv. *syringae*, *P. glumae* and *P. avenae*. Roots may also show discoloration.

Fig. 17.1. Symptoms on rice seedlings caused by *Pseudomonas fuscovaginae*

Fig. 17.2. Grain and sheath rot symptoms caused by *Pseudomonas fuscovaginae*. (Photo: Courtesy Dr Robert S. Zeigler, CIAT, Colombia)

Fig. 17.3. Grain discoloration in rice caused by *Pseudomonas fuscovaginae*. These seeds were received at the Institute of Seed Pathology, Denmark from Dr Robert S. Zeigler, CIAT, Colombia

Pathogen

Pseudomonas fuscovaginae Miyajima, Tanii & Akita

The bacterium is aerobic, Gram-negative, rod-shaped (0.5-0.8×2-3.5 μm), occurs singly or in pairs, is motile by means of 1 to many polar flagella. Growth on nutrient agar is moderate (3-5 mm diam), circular, raised, smooth, and white to light brown in colour. A green fluorescent diffusible pigment is produced on King's medium B. The bacterium is positive for arginine dihydrolase and oxidase reactions (for further reference see Miyajima *et al.*, 1983).

Miyajima (1980) divided isolates from various localities in Japan into 4 lysotypes, based on their sensitivity to 3 phage strains. Differences in 16 strains of *P. fuscovaginae* based on biochemical characters have been observed by Miyajima *et al.* (1983).

Location of the pathogen in the seed

There are no specific reports concerning the location of the bacterium in different parts of the seed. However, the bacterium appears to be internally seed-borne.

Seed infection and plant infection

Zeigler & Alvarez (1986, 1987) reported that the rate of disease transmission can be very high, since 30 per cent seed infection was found in a small sample of 25 seeds, and up to 100 per cent of the seed from affected plants yield fluorescent bacteria in artificial inoculation experiments. There is no information available which indicates correlation between seed infection levels and infection in the field. Severely affected seedlings may die, but usually the plants survive to the flowering stage. Symptomless plants carry the pathogen epiphytically until the reproductive stage. If environmental conditions are appropriate the pathogen infects the root and flag leaf sheath. Seed transmission has exceeded 75% in many inoculation experiments.

Seed health testing

- Collect 400 seeds in a cheese cloth, wash them in running tap water for 1-2 h and then dry them over filter papers. (If discoloured seeds are present in the sample use as many of them as possible while selecting seeds for the test).
- Plate the washed seeds partially embedded in the King's medium B (see Chapter 20); 25 seeds can conveniently be placed in Petri dishes of 9 cm diam, and 40 seeds in dishes of 14 cm as is done presently at CIAT, Colombia.
- Incubate the plates at 27°C for 24-48 h.
- Place the plates under NUV light and record seeds with bacterial colonies showing fluorescence.
- Transfer a loopful of bacteria from the fluorescent bacterial growth to nutrient broth medium and incubate for 2 h at 27°C on a rotary shaker.
- Inject the stems of 15-20-d-old rice plants of the cv. oryzica 1 and tobacco leaves with the 2-h-old broth culture.
- Incubate the inoculated rice plants in a growth chamber at 25-27°C, 100% relative humidity and 12 h daylight cycle for 2 d before transferring to the greenhouse. In the case of tobacco leaves, incubate as mentioned in Chapter 20.
- Test the pure fluorescing colonies for Gram reaction and tube agglutination. If the bacterium tested gives Gram-negative reaction, shows agglutination, provokes stripes on the injected stems measuring around 10 mm from the inoculation point after 5 d and/or induces hypersensitive reaction it is *P. fuscovaginae*. (For conducting the different tests consult Chapter 20).
- If antiserum against *P. fuscovaginae* is not available, isolates can also be tested biochemically for oxidase reaction, levan formation from sucrose and arginine dihydrolase (See Table 1 in Chapter 20). If the bacterium is Gram negative, levan positive and arginine dihydrolase positive it is *Pseudomonas fuscovaginae*.

Control

Heat treatment at 65°C for 6 d has been recommended for the eradication of the bacterium from infected seed lots (Zeigler & Alvarez, 1986, 1987, 1988; Zeigler, Hoyos & Aricapa, 1986). This treatment is given to rice samples which are introduced to Colombia via CIAT and to material to be sent to other co-operating institutions. According to Zeigler & Alvarez (1986), heat treatment of seed and monitoring of samples to ensure freedom of the pathogen should be adopted in international seed exchange.

Chapter 18

Bacterial Grain Rot

Grain rot occurs in many countries (Kaku, 1988). It was first found in Kyushu district of Japan (Goto & Ohata, 1956) and since then in Latin America (Zeigler & Alvarez, 1988) and Taiwan (Chien, Chang, Liao & Ou, 1983). In Denmark, *P. glumae* has been recorded in a rice seed sample received from the Philippines. (Mortensen, 1988, unpubl.).

The disease causes the death of seedlings which rot in nursery boxes prepared for machine transplanting. Serious losses in yield are due to sterility or poor ripening of the grain (Wakimoto, Akaki & Tsuchiya, 1987).

Symptoms

The pathogen attacks both seedlings and grains. (Wakimoto *et al.*, 1987; Figs. 18.1 and 18.2). Initial symptoms include discoloration or pale-yellowing at the basal part of the husk, which rapidly advances over the entire husk, becoming greyish-white to yellowish-brown or reddish-brown. Infected grains are scattered in the panicle but in severe cases more than half the grains may be attacked (Ou, 1985).

Pathogen

Pseudomonas glumae Kurita & Tabei

Colonies are slow growing, circular, raised, smooth, greyish white producing aerobic Gram-negative rods with 1-3 polar flagella, encapsulated, without spores, 0.5-0.7×1.5-2.5 μm. *P. glumae* occasionally produces a diffusible yellow-greenish, non-fluorescent pigment even on King's medium B. Gelatin hydrolyzed, nitrate reduced, milk coagulated and litmus reduced, margarine and Tween 80 hydrolysed, oxidase and arginine negative, acid produced from

Fig. 18.1. Panicles with grains infected by *Pseudomonas glumae*
(Photo: Courtesy Dr Shizuo Mogi, Indonesia)

Fig. 18.2. Grain discoloration and formation of brown bands across the endosperm caused by *Pseudomonas glumae*
(Photo: Courtesy Dr Shizuo Mogi, Indonesia)

sugars. For more information consult Ou (1985) and Bradbury (1986).

Location of the pathogen in the seed

Pseudomonas glumae cells are present on the surface of the basal part of lodicule and inner surface of lemma. The bacterial cells invade the interspaces of host cells of outer epidermis and spongy parenchyma of lemma (Tsushima, Tsuno, Mogi, Wakimoto & Saito, 1987).

Seed infection and plant infection

Rice grains are most susceptible to *P. glumae* during flowering and less susceptible before flowering and about 6 d or more after flowering. The total population of the pathogen in the grain at heading is less than 10^7 cfu/ml fresh weight of grain, increasing to 10^8-10^9 cfu/g 6 d after heading (Tshushima, Tsuno, Mogi, Wakimoto, Saito & Naito, 1988).

Pseudomonas glumae has been obtained from grains of diseased rice plants (Goto, Nishiyama & Ohata, 1987). Axes and branches of an infected panicle may not show symptoms while grains turn yellow or light pink, yellowing starting from the base. The endosperm ceases to develop and the grain becomes empty. An infected panicle may stand erect at maturity and the infected grains may show a brown stripe across on the endosperm (Mogi, 1988; Fig. 18.2). Seedlings raised from infected seeds can rot, but the pathogen usually does not attack any other part of adult plants or cause symptoms during vigorous growing stages (Wakimoto *et al.*, 1987).

Seed health testing

A semi-selective medium S-PG (recipe in Chapter 20) has been developed for isolation and identification of *P. glumae* by Tsushima, Wakimoto & Mogi (1986). This medium has been used by Mogi (1988) for the detection of the bacterium in rice seed. The S-PG medium has been used also at the Institute of Seed Pathology with success. Details of the procedure as followed in Denmark are:

- Take 400 seeds and grind them to coarse flour.
- Collect the flour in a polythene bag and add 200 ml of sterile 0.85% NaCl and incubate for 2 h at room temperature. Shake the bag every 30 min.
- Prepare 3×1:10 serial dilutions in nutrient broth from the seed suspension. Pipette 0.05 ml of the concentrated suspension and from the serial dilutions on to three plates of S-PG medium, spread with a L-shaped glass rod and incubate the plates in an inverted position at 26°C for 3-5 d.

Pseudomonas glumae produces two types of colonies on the S-PG medium; Type A colonies are circular, entire and

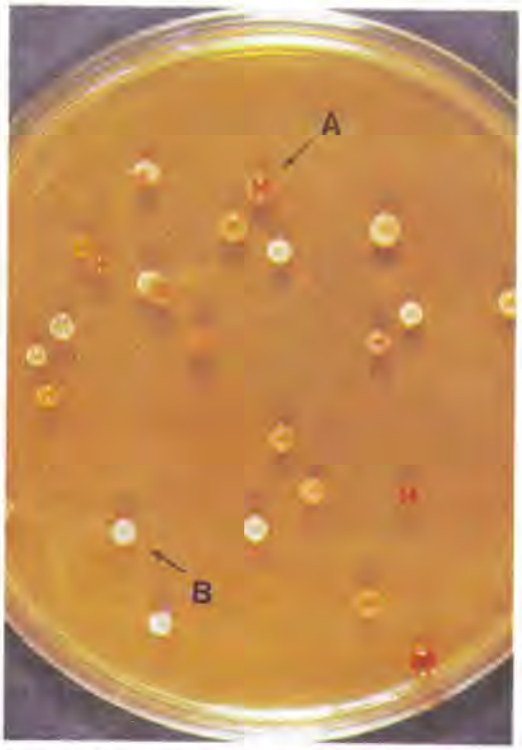

Fig. 18.3. Colonies of *Pseudomonas glumae*, Type A and Type B, on the S-PG medium

brown-red (Fig. 18.3), while Type B are opalescent purple to purple-red.

Since *P. avenae* has also been found to produce colonies on the S-PG medium similar to Type B of *P. glumae*, suspected colonies of *P. glumae* should be purified for further identification and confirmation of the organism.

- Transfer the suspected colonies to nutrient agar and incubate at 26°C for 1 d.
- Test the purified colonies for Gram reaction, slide agglutination or Ouchterlony double diffusion test, pathogenicity (15-20-d-old rice plants, cv. taichung native 1) and/or hypersensitive reaction (consult Chapter 20).

Colonies of *P. glumae* are whitish-grey, circular, raised, smooth, produce occasionally a diffusible greenish-yellowish nonfluorescent pigment, Gram-negative, react positive in slide agglutination or Ouchterlony double diffusion test, pathogenic to rice plants after 3 d (Fig. 18.4) and induce hypersensitive reaction in tobacco leaves.

- If antiserum is not available, test for oxidase reaction, production of fluorescent pigment and pathogenicity in rice plants. Colonies which are oxidase positive, non-fluorescent under UV light and which induce seedling rot are those of *P. glumae*.
- *Pseudomonas glumae* can also be detected from seeds by the method described by Shakya & Chung (1983) for *P. avenae*. Identification of the bacterium involved must be confirmed by slide agglutination or Ouchterlony double diffusion test and pathogenicity.
- If other pseudomonads are present in the investigated sample *P. glumae* can be distinguished as it produces crystals of calcium oxalate in potato-peptone-glucose agar (PPGA) medium containing 0.1% $CaCl_2$. This work has recently been published by Matsuda, Koiso, Iwasaki & Sato (1988) and appears to be a useful criterion.

Fig. 18.4. Rice plants showing symptoms on leaves 5 d after inoculation with *P. glumae*

Control

The bacterium can be eradicated from seed by heat therapy at 65°C for 6 d (Zeigler & Alvarez, 1988).

Control has also been obtained with applications of a new soil fungicide, methasulfocarb [S-(4-methylsulfonyloxyphenyl)N-methyl] thiocarbamate, Kayabest, NK-191 10% dust applications. Better control was obtained when air temperature was suitable for rice seedlings during nursery period or when inoculum concentration was low. The soil application procedure followed was the same used as in the control of seedling blight of rice caused by different pathogens (Ohmori & Watanabe, 1986).

Chapter 19

Bacterial Sheath Rot

The disease has been reported from Australia, China, Hungary and Japan (CMI Distribution Maps of Plant Diseases No. 508, ed. 1, 1975; Fig. 19.1), and more recently from South America (Zeigler *et al.*, 1987).

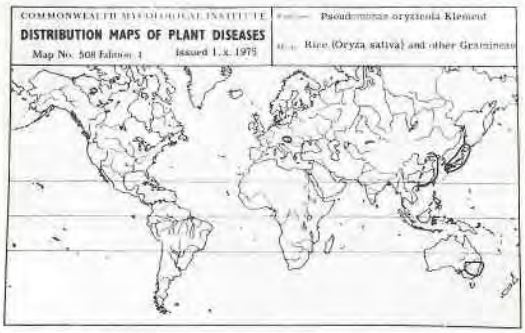

Fig. 19.1. Distribution map of bacterial sheath rot published by the CAB International Mycological Institute, Kew, U.K. in 1975

In Hungary very serious losses are incurred in some years by the so called "brusone" disease of rice where both the bacterium, *Pseudomonas syringae* pv. *syringae* and the blast fungus *Pyricularia oryzae*, are involved.

In 1953 the disease was particularly widespread in the rice fields between the Danube and the Tisza where as much as 40% of the plants were attacked. *P. syringae* pv. *syringae* was isolated from several hundred plants including young seed.

Symptoms

The pathogen attacks leaves, sheaths and stems of rice plants. It is characterized by brown or black lesions on sheaths, nodes and stems. Sheath lesions elongate, turn brown to reddish and the resulting necrosis leads to drying of entire plants. Grain discoloration (Fig. 19.2), seed infection and sterility occurs in extreme cases.

Fig. 19.2. Grain and sheath rot symptoms of rice caused by *Pseudomonas syringae* pv. *syringae*
(Photo: Courtesy Dr Robert S. Zeigler, CIAT, Colombia)

Pathogen

Pseudomonas syringae pv. *syringae* van Hall
(syn. *Pseudomonas oryzicola* Klement)

The bacterium is Gram-negative, motile

with 1-5 polar flagella, singly or in pairs, non-spore-forming, obligately aerobic, 2.0-3.5×0.8-1.0 μm.

Colonies on nutrient agar are small, circular, entire, whitish with a translucent margin which becomes serrate or undulate in older colonies. Green diffusible fluorescent pigment is produced on King's medium B. It liquifies gelatin, and does not reduce nitrate (see Table 1 in Chapter 20). For further information see CMI Descriptions of Pathogenic Fungi and Bacteria No. 236, 1970a. This pathovar shows characteristics listed for the species *P. syringae* (Bradbury, 1986). From the results of LOPAT determinative tests, strains of *P. oryzicola* should be classified in Group Ia, which contains a large number of distinct pathogens (Luketina & Young, 1979). It is distinguishable from *X. campestris* pv. *oryzae* and *X. campestris* pv. *oryzicola* by its whitish colour, production of fluorescent pigment and symptoms produced on the host.

Seed infection and plant infection

As mentioned earlier Klement (1955) isolated *P. syringae* pv. *syringae* from young seed but he did not provide any information on the transmission of the disease from seed to plant. However, in plants inoculated at the heading stage symptoms appeared in the panicle within 36 h. The seeds changed colour to greyish-green, olive, then brown. From 60 to 90% of the grains turned brown.

The reason why this disease is included in this book is because of its serious nature as reported from Hungary and its limited distribution in a few countries of the world.

Seed health testing

No testing method is available for the detection of *P. syringae* pv. *syringae*. The pathogen can be separated from other pseudomonads by the results of the LOPAT determinative test and pathogenicity test. The bacterium produces a fluorescent pigment on King's medium B, forming levan type colonies on nutrient agar containing 5% sucrose. Colonies are oxidase negative, do not produce soft rot in potato slices, and they are arginine dihydrolase negative inducing hypersensitive reaction in tobacco leaves.

Chapter 20

Isolation Techniques and Identification of Pathogenic Bacteria

20.1 **Isolation from infected parts of plants**

- Select leaf and stem areas where maximum number of bacteria are suspected. Cut small areas of the affected tissues on a glass slide including healthy looking neighbouring parts, and mount, in a drop of water. Put on a cover slip and examine under compound microscope at 40-100 magnification for streaming of bacteria (Fig. 20.1). Only if bacterial ooze is observed proceed with further steps of isolation.

Fig. 20.1. Bacterial ooze streaming from excised plant tissue

- Remove the cover slip and put the infected tissue in a few drops of water, tease apart the tissue and leave it for 10-15 min to obtain more bacterial cells in the water. Sometimes it is even preferable for making isolations to collect a number of small infected pieces from 4-5 seedlings.
- Using a bacteriological loop streak the water containing bacteria a number of times on to a suitable medium in a crosswise manner as shown in Fig. 20.2. Before streaking make sure that there is a film of water in the loop. The process of streaking is done in order to get single separate colonies.

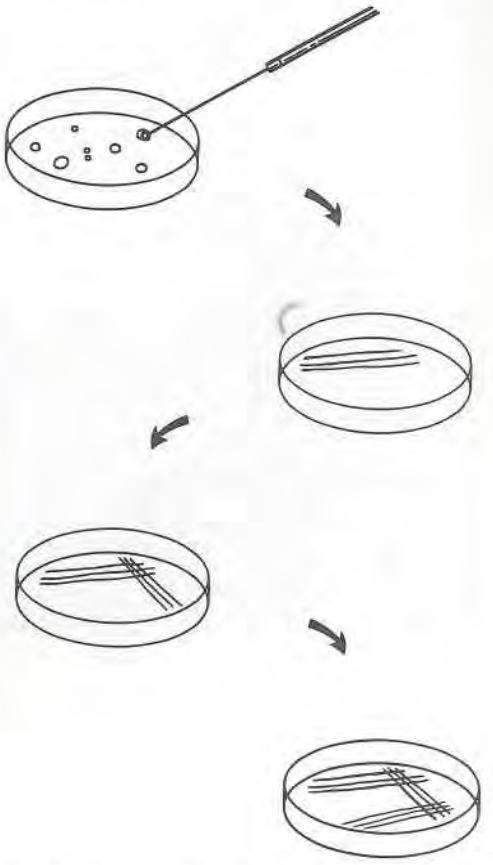

Fig. 20.2. Streaking of bacteria on agar media using bacteriological loop

- Media, generally used at the Institute of Seed Pathology for isolating rice bacterial pathogens, are:

Nutrient agar	*Pseudomonas* spp.
King's medium B	*Pseudomonas* spp.
Growth factor medium	*Xanthomonas* spp.

(For the recipes of the media and their preparation see section 20.6).

- Incubate the streaked plates in an inverted position at 25-30°C.
- Start observing the plates after 1-2 d for pseudomonads and up to 4 d for xanthomonads.
- The colonies of pseudomonads on nutrient agar are white, whitish-grey, circular, smooth, glistening and raised (Fig. 20.3). *Pseudomonas glumae* often produces a non-fluorescent yellowish green to brown diffusible pigment in nutrient agar medium. On King's medium B, colonies are whitish-grey, raised with or without the production of a diffusible yellowish-green pigment which shows fluorescence under ultraviolet light (Fig. 20.4).

Fig. 20.3. Suspected colonies of *Pseudomonas avenae* (arrow) on nutrient agar medium

- The colonies of xanthomonads on all the media are convex to domed, smooth, mucoid, creamy to yellow (Fig. 20.5).
- Suspected colonies should be transferred for purification. Select single colonies and transfer them to a suitable plating medium by streaking in order to obtain separate colonies (Fig. 20.2).

Fig. 20.4. Fluorescent colonies of pseudomonads on King's medium B.
Photo taken under UV lamp

Fig. 20.5. Suspected colonies of *Xanthomonas campestris* pv. *oryzae* (arrows) on growth factor medium

- Identification has to be confirmed after purification by conducting either biochemical, serological, pathogenicity and/or hypersensitive reaction tests.

20.2. **Biochemical tests**

20.2.1. *Gram reaction*

This reaction test is essential for differentiating bacteria into two broad groups: Gram-positive and Gram-negative. The non-staining Gram reaction test, better known as *Potassium Hydroxide Solubility Test*, is as follows:

- On a glass slide mix a loopful of bacteria from a well grown colony in a drop of 3% KOH aqueous solution. Mixing should not exceed 10 s. A toothpick can also be used for picking bacteria from a colony as well as for mixing.
- Raise the loop or the toothpick a few centimeters from the glass slide. If strands of viscid material are seen as shown in Fig. 20.6, the bacterium in question is Gram-negative. Gram-positive bacteria do not produce such strands even on repeated strokes of the loop/tooth pick.

Fig. 20.6. A Gram-negative bacterium giving the mucoid thread in the KOH test

20.2.2. *Pigmentation*

Plant pathogenic bacteria produce a variety of pigments some of which manifest themselves only on special media. *Pseudomonas* species produce several kinds of pigments which are of taxonomic and diagnostic value. One group of diffusible pigments consists of the fluorescence types which are produced by the so-called fluorescent pseudomonads (Fig. 20.4). The green diffusible fluorescent pigments are best produced in media of low iron content. The most widely used medium for this purpose is King's medium B (see media).

- The fluorescence can readily be seen under ultraviolet light.
- Yellow carotenoid pigments are produced by *Xanthomonas* spp. as seen in Fig. 20.5. *Erwinia herbicola* is also known to produce similar pigmentation. When the suspected colonies are purified and used further in pathogenicity tests only the pathogenic xanthomonads will produce symptoms in the inoculated plants.

20.2.3. *Arginine dihydrolase* (Lelliot & Stead, 1987)

The Arginine dihydrolase complex system permits certain pseudomonads to grow under anaerobic conditions. Ammonia is evolved which brings about the change in pH, indicating a positive reaction.

Fig. 20.7. Arginine dihydrolase test. Left, positive; Right, negative

- Inoculate by stabbing with a fresh culture test tubes containing 3 ml of Thornley's medium (see media).
- Cover with sterile mineral oil or molten Vaseline (molten white petroleum jelly).
- Incubate for 3 d at 27°C.
- A change of colour to red (alkaline) is a positive reaction (Fig. 20.7).

20.2.4. *Levan (poly-fructose) formation*

Pseudomonas syringae produces white, domed, shining, mucoid, levan type colonies on a 5% sucrose nutrient agar (see media). Isolated colonies are usually 3-5 mm after 3 d at 27°C.

Fig. 20.8. Colonies on nutrient agar containing 5% sucrose. Observe the levan colonies type (top), and the non-levan colonies (bottom)

Levan, formed by levan sucrase, is produced by most pseudomonads which utilize sucrose. The reaction is useful in the identification of pseudomonads (Fig. 20.8).

Inoculate plates in order to obtain separate colonies when testing pure cultures. Serial dilutions from infected samples or seed extracts are similarly used for the inoculation of the plates.

20.2.5. *Kovac's Oxidase Test* (Kovac's, 1956; Hildebrand & Schroth, 1972)

The test is of particular differential value within the genus *Pseudomonas*. It can be used to distinguish the complex of species related to *P. syringae*, which are oxidase negative from other pseudomonads which are oxidase positive.

- Place a Whatman filter paper No. 1 in a Petri dish and add 3-4 drops of freshly prepared 1% aqueous solution of tetramethylparaphenylene-diamine dihydrochloride in the centre of the paper. Change of the reagent to a purple colour within 10 s of application of the culture should be regarded as a positive test result (Fig. 20.9).

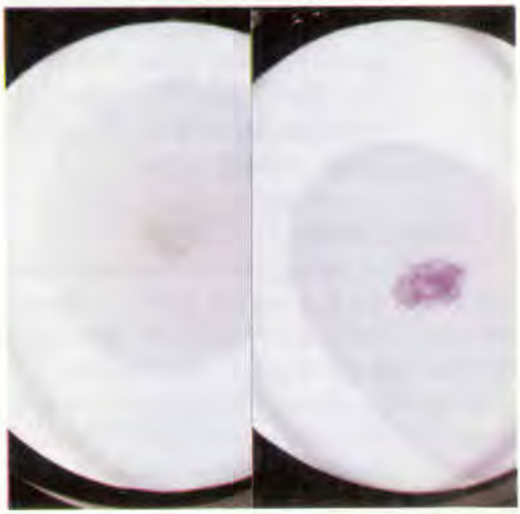

Fig. 20.9. Oxidase test. Left, negative; Right, positive

Table 1. Key tests which differentiate *Pseudomonas fuscovaginae, P. avenae, P. glumae* and *P. syringae* pv. *syringae* isolates
(Robert S. Zeigler & Elizabeth Alvarez, 1988, in press)

Characteristics	Fluorescent		Non-fluorescent	
	P. fuscovaginae	*P. syringae*	*P. avenae*	*P. glumae*
Lipase	+	–	–	–
Gelatin liquefaction	+	+	–	d
Arginine dihydrolase	+	–	–	–
Oxidase	+	–	+	–
Utilization of:				
Sucrose	–	+	–	–
Inositol	–	+	–	+
Arginine	+	–	–	+
H_2S production	+	–	–	–
NO_3 – reduction	–	–	+	+
Levan formation from sucrose	–	+	–	–
Growth at 37°C	+	–	+	+
41°C	–	–	+	+

Symbols: + = positive reaction; – = negative reaction; d = variable. Representing reaction of 30 isolates of *P. avenae*, 11 isolates of *P. syringae*, 6 isolates of *P. glumae* and 100 isolates of *P. fuscovaginae*.

Notice: Since Tetramethyl-paraphenylene-diamine dihydrochloride is a rather expensive reagent, prepare the solution only in the required amount to be used. The reagent can also be made weeky or fortnightly and stored in a stoppered dark glass bottle at 4°C. Care should be taken to avoid contact of the powder or solution with the skin.

20.3 **Serological tests**

Serological testing has become an important tool in the diagnosis of plant pathogenic bacteria. Three tests which have been used in testing rice pathogens are described here.

In the tube and slide agglutination tests a positive reaction is given by agglutination of bacterial cells due to multiple bridging between specific antibodies and antigens of the cell walls and flagella. Agglutination can be seen by the naked eye but weak agglutination is best seen under low magnification of a stereomicroscope. In the Ouchterlony double diffusion test the soluble antibodies and/or homologous antigens diffuse independently through a gel, usually agar, and when the reactants meet, a precipitin band is produced.

20.3.1. *Agglutination test*

Since antisera are expensive and sometimes the use of undiluted antisera in the agglutination tests does not give satisfactory results, dilution of antisera has become a normal practice.

Before agglutination tests are conducted in a seed health testing laboratory, the optimal dilution point of the antiserum where definite agglutination occurs must be established. This is done by preparing serial dilutions of the antiserum in 0.85% NaCl and testing these dilutions in test tubes by adding an equal volume of the bacterial suspension (10^9 cfu/ml) prepared in 0.85%

NaCl or 0.01 M buffer solution. The test tubes should be shaken a couple of times gently in half-clockwise and half-anticlockwise directions and later incubated at 37°C for 2 h. Record carefully the last dilution where agglutination is observed (titre). The "working dilution" of the antiserum is two steps lower than the titre of the antiserum (Fig. 20.10). In this figure the titre of the antiserum is shown at 1:2560. The working dilution should therefore be 1:640. The working dilution of a given antiserum is the one which should be used in routine seed health tests. When the test is per-

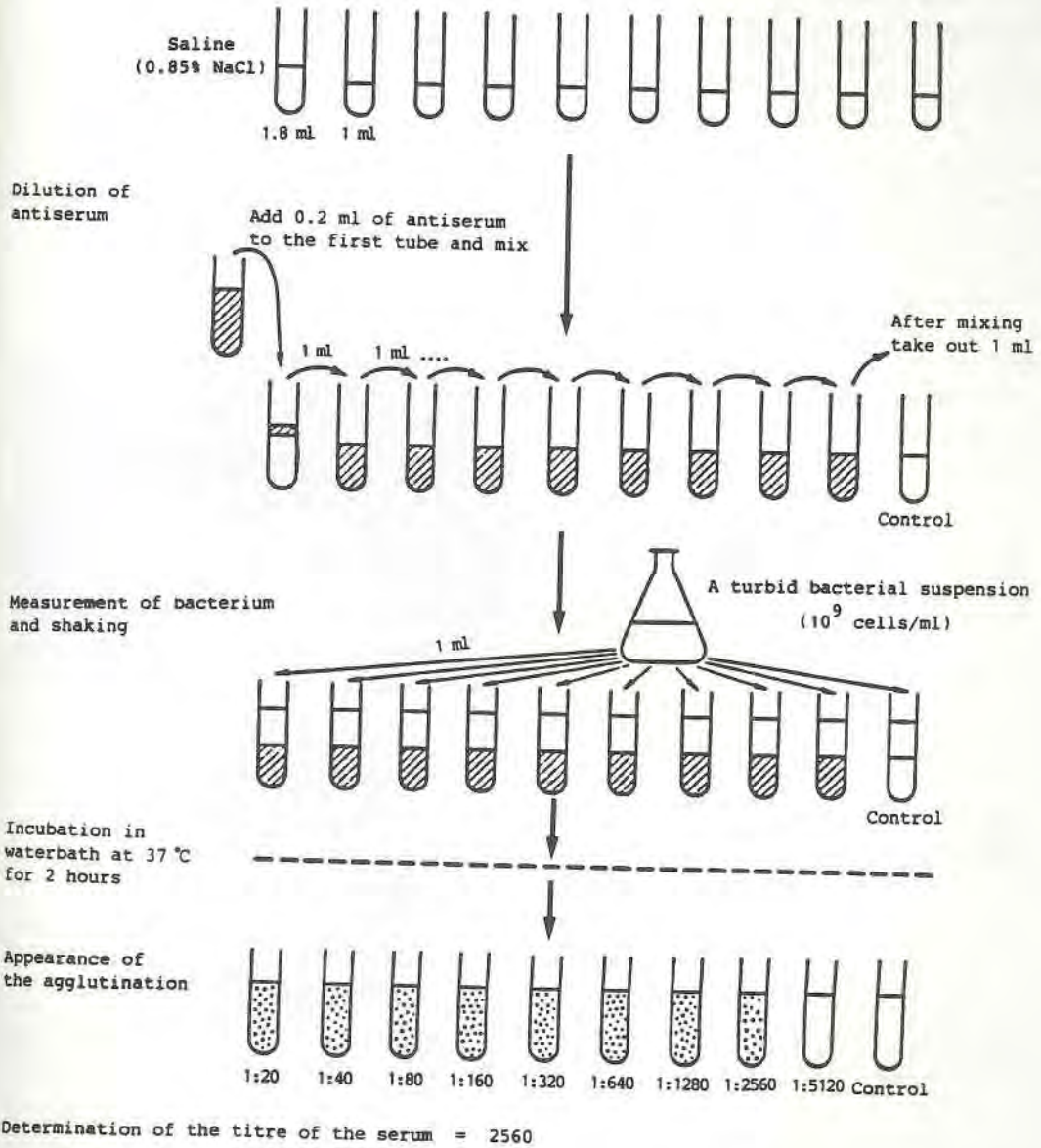

Fig. 20.10. Procedure for establishing the titre and the working dilution of an antiserum for conducting agglutination tests (modified after Kiraly, Klement, Solymosy & Vörös, 1970)

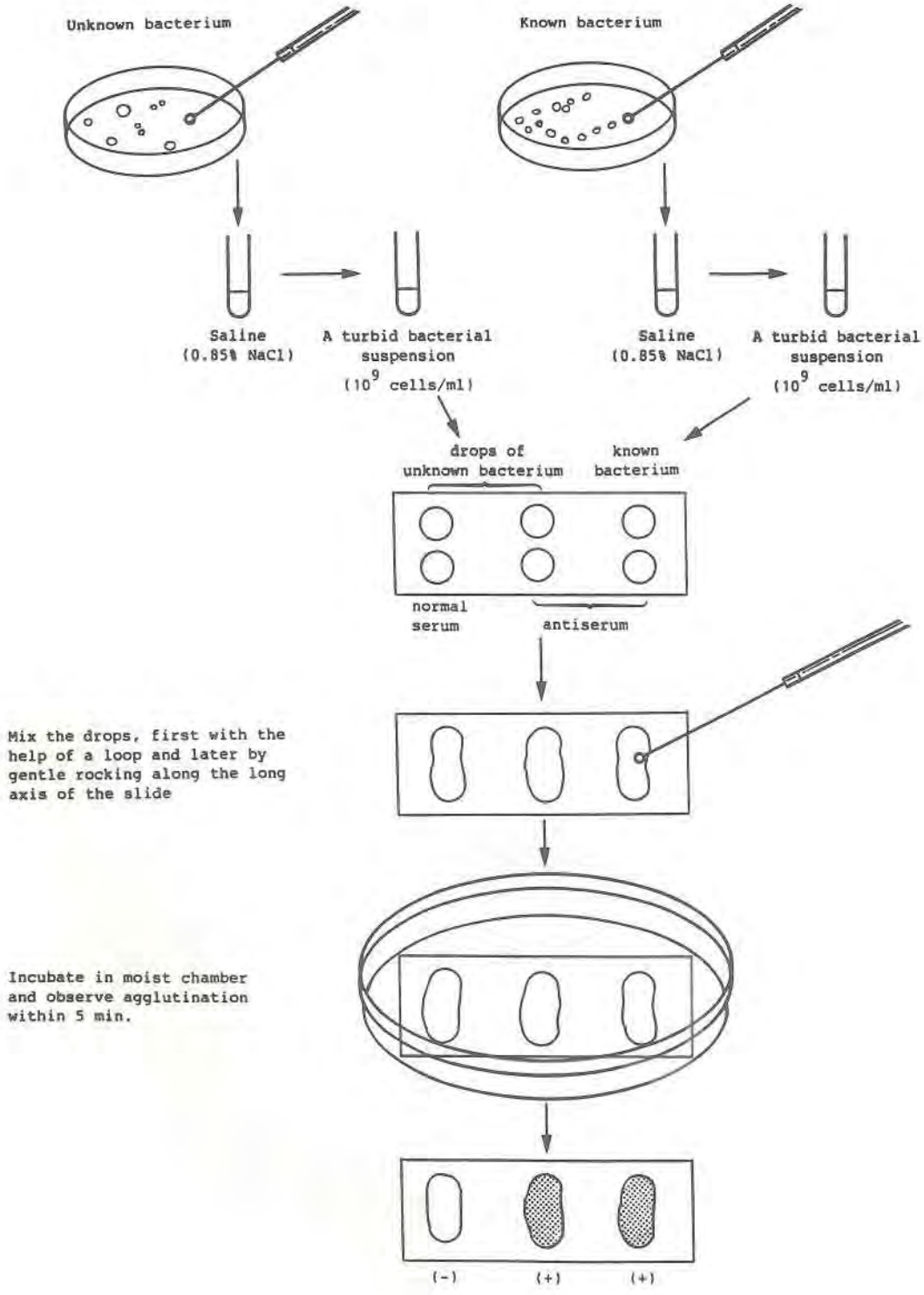

Fig. 20.11. Slide agglutination test

formed in test tubes, it is called Tube agglutination test and Slide agglutination when performed on glass slides (Fig. 20.11).

20.3.2. *Ouchterlony double diffusion test*

- Cut six peripheral wells equidistant from a central well (Fig. 20.12), preferably with a template or gel punch on a water agar plate (see media). The cutting of wells should be done just before performing the test. For Petri dishes of 9 cm diam (15 ml agar plate) all wells should be of 5 mm diam, peripheral wells 4 mm apart and approximately the same distance from the central well. A small drop of molten agar can be added to the base of each well to seal the well when glass Petri dishes are used.

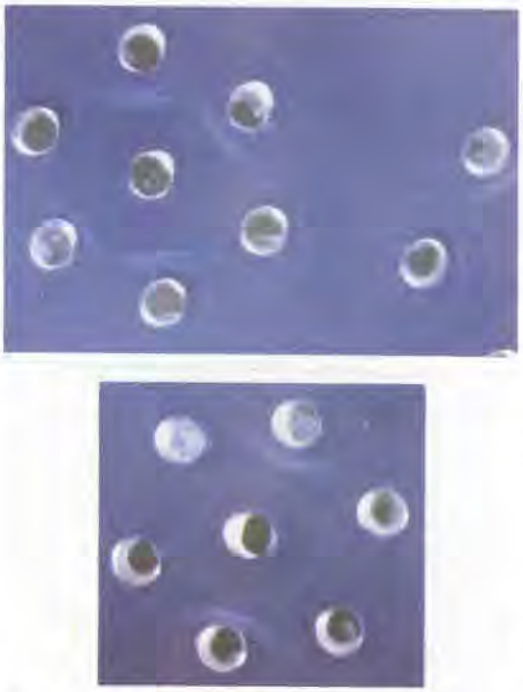

Fig. 20.12. Ouchterlony double diffusion test on water agar. Central wells filled with antiserum of the bacterium. Peripheral wells contain suspensions of the tested bacterial isolates. Observe formation of precipitin bands

- Fill the central well with undiluted antiserum and two opposite peripheral wells with a known control (homologous) antigen. The remaining four wells can be filled with suspensions of the unknown bacterial isolates. Bacterial suspensions are prepared by resuspending bacterial growth from an agar medium in 0.85% NaCl or 0.01 mol phosphate buffer saline, pH 7.2 (see media) to give a turbid bacterial suspension (10^9 cfu/ml).
- Cover the dishes and incubate in a moist chamber for 24 h.
- Examine for one or more white bands between the central well and the peripheral reference wells. If the same band pattern is found for the test wells and there is complete fusion of lines, homologous antigen is indicated. Spurs may be seen between adjacent antigen wells (Fig. 20.13). This indicates that the antigens are not identical, but related. Crossing of lines and no fusion indicates no serological relationship between the antigens.

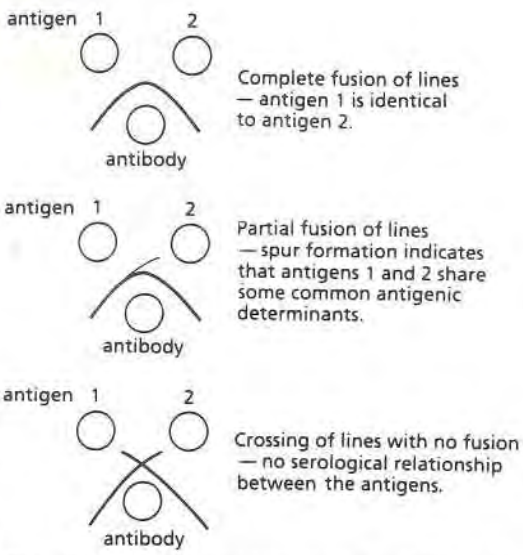

Fig. 20.13. Basic precipitation patterns in double immunodiffusion. (After Lelliot & Stead, 1987)

20.4. **Pathogenicity**

The aim of this test is to determine whether or not an isolated bacterium can cause symptoms in the host from which it was isolated. For many plant diseases this test is useful for confirming or negating an initial presumptive diagnosis.

Pseudomonas syringae pv. *syringae*, *P. glumae*, *P. fuscovaginae* and *P. avenae* are stem inoculated in the following manner:

- Bacterial suspensions from 24-48 h cultures in concentration of 10^6-10^7 cfu/ml, usually in amounts of 0.1 ml, are injected into the plant with a fine hypodermic needle, taking care not to pierce right through the stem. Known susceptible host plants are used for the inoculations.

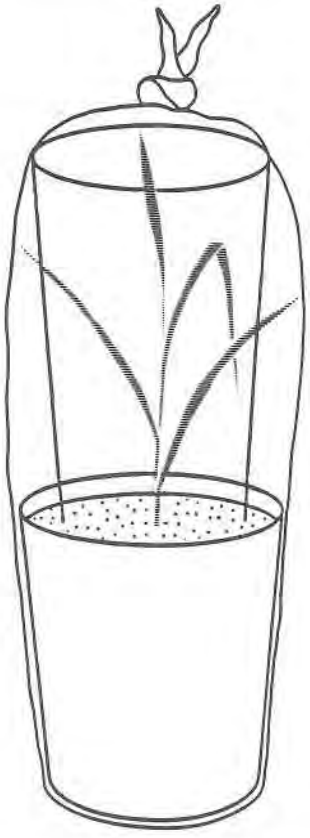

Fig. 20.14. Young host plant incubated in a polythene bag supported by a wire frame to prevent the plant touching the polythene

- Cover the inoculated plants with polythene bags for 2-4 d (Fig. 20.14) and incubate at appropriate temperature (25-30°C).
- Observe after 3 d and up to several weeks for water soaking, discoloration, wilting, streaks, stripes, necrosis and/or exudate production.

20.5. **Hypersensitive reaction**

Most bacterial plant pathogens can induce a hypersensitive response when injected into the tissues of a non-susceptible host plant (Fig. 20.15). This reaction results in rapid collapse of the infiltrated areas and water soaking followed by a dry, light brown necrosis. The reaction is an extremely useful presumptive diagnostic technique.

A variety of plants may be used, but for many bacteria tobacco is the preferred plant since it is easy to cultivate and maintain.

Tobacco hypersensitive test (HR)

- Prepare an aqueous suspension from a 24-28 h culture (10^8-10^9 cfu/ml).
- Inject the mesophyll of a large leaf blade with the suspension by inserting the needle of a hypodermic syringe into the cavity which runs along the side of the lateral veins or directly into a leaf vein. Use a narrow gauge needle of approximately 0.4 mm diam. The diagonal of the needle aperture should be adjacent and parallel to the surface of the tissue. Inject sufficient inoculum to flood the intercellular spaces (apparent by water soaking of the tissue).
- Inject a separate area of the lamina with sterile distilled water as a control and with a culture of the pathogen from the same group as the culture being tested and known to cause a typical hypersensitive reaction as a positive control.
- Mark the areas by wounding the leaf in a specific manner, e.g. by pin pricks or by using adhesive labels.

Fig. 20.15. Tobacco hypersensitive reaction test. Above: leaf areas 10 h after injection. Below: necrosis and collapse of the infected leaf areas within 24 h

- Inject more than one leaf and one sector of a leaf for each suspension and arrange controls and test injections on opposite sides of the main vein.
- Incubate plants in a well-shaded and ventilated glasshouse at less than 30°C or even at room temperature (22-25°C). If growth cabinets are available, incubate at 25°C and 85% relative humidity with a diurnal daylight regime of 16 h. Rapid collapse and watersoaking of inoculated tissues within 24 h, or at most 48 h followed by a dry, light-brown localized necrosis within 3 d indicates a positive hypersensitive reaction. Yellowing or browning without collapse or no visible reaction is considered negative.

20.6.2. *Preparation of media*

Both solid and liquid media are used for the cultivation of bacterial pathogens *in vitro*. Liquid media usually have the same composition as their solid counterparts except that agar is omitted. Commercial powder preparations are available on the market.

Most of the media used routinely can be prepared easily in the following way:

- The ingredients, in amounts as indicated in the formula or recipe, are added to a flask that should hold 1-1½ or 2 times the final volume of medium.
- Measure the required volume of water (de-mineralized or distilled water) and add it to the ingredients, little by little with constant stirring to wet the ingredients thoroughly and to avoid formation of lumps.
- Cover the flask with aluminium foil or plug the neck with cotton.
- Heat the flask to dissolve the ingredients (a microwave-oven can be used, but make sure that the flask is not too tightly closed). If agar is a component of the medium, heat mixture to dissolve the agar.

- Sometimes checking of pH is required. The reaction of the medium can be determined by pH indicator papers, or by a pH meter. The reaction is adjusted to the desired level by slowly adding drops of 1N NaOH or HCl to the hot solution.
- The medium while still hot is dispensed to appropriate containers for sterilization. If the medium is to be used for propagation of a bacterial pathogen, it is usually dispensed into test tubes (9 ml in ordinary, laboratory test tubes of 16 cm).
- Cap the flasks with aluminium foil or cotton. If medicine flasks are used, they should not be filled beyond the shoulder of the bottle. Caps should fit loosely during sterilization and tightened prior to removing the bottles from the sterilizer.
- The medium should be sterilized the same day that it is prepared. Sterilization is done in a steam autoclave or in a domestic pressure cooker. Tubes are loosely arranged in wire baskets or other containers. Sterilization is completed in 20 min at a temperature of 121°C. After the required time has elapsed, the pressure should be allowed to fall gradually to zero before the medium is removed from the sterilizer.
- Medium for slant cultures and plate cultures: tubes containing an agar medium are arranged in a slanted position to cool so that when the agar has solidified a sloping surface will extend from the bottom of the tube.

  If the medium is to be added to Petri dishes, it should be allowed to cool to about 50°C. Enough agar is added to give a depth of medium of about 5 mm.

20.6 **Media**

20.6.1 *Recipes*

Nutrient agar

Peptone	5.0 g
Beef extract	3.0 g
Agar	20.0 g
Distilled water	1000 ml

Nutrient broth

Peptone	5.0 g
Beef extract	3.0 g
Distilled water	1000 ml

Sucrose nutrient agar

Peptone	5.0 g
Beef extract	3.0 g
Sucrose	50.0 g
Distilled water	1000 ml

King's medium B

Proteose peptone	20.0 g
Glycerol	15 ml
K_2HPO_4	1.5 g
$MgSO_4.7H_2O$	1.5 g
Agar	20.0 g
Distilled water	1000 ml

Adjust pH to 7.2 before autoclaving the medium.

Water agar + NaN_3

Agar	20.0 g
NaN_3	0.2 g
NaCl	8.5 g
Distilled water	1000 ml

pH is adjusted to 7.0–8.0.

Arginine medium (Thornley's medium 2A) (Elliot & Stead, 1987)

Peptone	1.0 g
NaCl	5.0 g
K_2HPO_4	0.3 g
Phenol red	0.01 g
L-Arginine HCl	10.0 g
Agar	3.0 g
Distilled water	1000 ml

Mix, and dissolve by heating (pH is adjusted to 7.2 if necessary). Dispense 3 ml volumes in test tubes and sterilize by autoclaving for 20 min.

Selective medium for Pseudomonas glumae S-PG (Tshushima *et al.*, 1986)

KH_2PO_4	1.3 g
Na_2HPO_4	1.2 g
$(NH_4)_2SO_4$	5.0 g
$MgSO_4.7H_2O$	0.25 g
$Na_2MoO_4.2H_2O$	0.024 g
EDTA-Fe	0.010 g
D-sorbitol	10.0 g
Cetrimide	0.010 g
Phenol red	0.020 g
L-cystine	10.0 µg

(0.1 ml of an aqueous solution containing 0.01 g in 100 ml. heat to dissolve)

Methyl violet	1	mg
(1 ml of an aqueous solution of 0.01 g in 100 ml)		
Agar	20.0	g
Distilled water	1000	ml

Autoclave and add the antibiotic solutions which are filtersterilized (Millipore filter 0.4 μm pores diam) as follows:

Ampicillin sodium	10	mg
(add 1 ml of a solution containing 10 mg/ml in 75% ethanol)		
Pheneticillin potassium	50	mg
(add 5 ml of an aqueous solution containing 10 mg/ml)		

Growth factor medium (GF)

KH_2PO_4	0.4	g
$MgSO_4.7H_2O$	0.05	g
NaCl	0.1	g
$NH_4H_2PO_4$	0.5	g
$FeCl_3$	0.01	g
Yeast extract	3.0	g
Glucose	1.0	g
Agar	20.0	g
Distilled water	1000	ml

Dissolve by heating and autoclave.

Buffers

Phosphate-buffered saline (0.01 mol/l pH 7.2), PBS pH 7.2

NaCl	8.0	g
$Na_2HPO_4.12H_2O$	2.7	g
$NaH_2PO_4.\ 2H_2O$	0.4	g
NaN_3	0.2	g
Distilled water	1	litre

Chapter 21

White Tip

Widely distributed. It has been reported from more than 40 countries, including most of the rice growing areas of Africa, Latin America, Asia, Eastern Europe and Pacific Countries (Prot, 1989).

Loss estimates show 10-35% reduction in yield in Japan (Yoshii & Yamamoto, 1950); 40-50% in U.S.A. (Atkins & Todd, 1959); and 29-46% in Taiwan (Hung, 1959). According to Ou (1985) yield losses vary with country, year, cultivar and management. In recent years, yield losses due to white tip appear to be strongly reduced in the rice growing areas. These have been attributed to the successful use of insecticides such as carbofuran which is also a nematicide.

Symptoms

Infected plants develop chlorotic or white leaf tips (Fig. 21.1). Plants are stunted, lack vigour and produce small panicles. Affected panicles show high sterility and distorted glumes and kernels. Flag leaves may be twisted, causing incomplete panicle emergence. Not all infected plants show symptoms.

Fig. 21.1. Symptoms of white tip disease in rice plants
(Photo: Courtesy Dr M. Agyew-Sampong, WARDA, Liberia)

Causal organism

Aphelenchoides besseyi Christie
(syns. *Aphelenchoides oryzae* Yokoo; *Asteroaphelenchoides besseyi* (Christie) Drozdovski)

The nematode has a slender body varying between 0.44-0.84 mm in length by 14-22 μm wide. In females, the excretory pore is usually near the anterior edge of the nerve ring; spermatheca is elongate, oval and packed with sperm; the ovary is relatively short, the post-vulval uterine sac narrow, and the terminus bears a mucro of diverse shape with 3-4 pointed processes. In males, the proximal end of the spicule lacks a dorsal process (EPPO, 1979). For more details see Ou (1985).

Location in the seed

The quiescent nematodes are located in the space between the husk and caryopsis of dry seeds (Yoshii & Yamamoto, 1950; Todd & Atkins, 1958; Fukano, 1962; Huang & Huang, 1972). Rao (1972) further demonstrated that the nematode remains coiled up inside the palea and on the surface of the lodicules which look dried up and

papery as the seed matures. On average five or six nematodes are present in each seed (Thorne, 1961).

The nematode is known to survive on the infected seed for varying lengths of time, 8 months (Cralley, 1949), 3 years (Yoshii & Yamamoto, 1950), 23 or 24 months (Todd & Atkins, 1958), and as long as 8 years (Zem & Momteiro, 1977).

Seed infestation and crop performance

Fukano (1962) estimated that the critical inoculum density for susceptible paddy rice was approximately 30 viable nematodes per 100 seeds.

Detection of the nematode in the seed

The detection method adopted by Huang (1983) in Brazil is as follows:

- Peel the husks manually in five replicates of 100 seeds in a small quantity of water in a watch glass and leave for 24 h at 25±3°C.
- Wash the material on a standard sieve, pore aperture of 45 μm. The resultant material on the sieve will have nematodes and debris of husks and caryopsis. Transfer them to a Baermann funnel, leave the material overnight, collect the nematodes and then observe the filtrate under a stereoscopic microscope.
- Count the number of nematodes extracted from each replicate of 100 seeds. If needed, confirm the identity of the nematode at higher magnifications of a compound microscope.

  The use of Baermann funnel can be avoided. Extraction of *Aphelenchoides besseyi* can be made in small sieves placed in a Petri dish as is done routinely at the Seed Health Unit of the International Rice Research Institute in the Philippines (Evelyn B. Gergon, pers.comm., 1988).
- Count 500 seeds and pour them on a sieve placed in a Petri dish (Fig. 21.2). Fill the dish with water until the seeds are completely immersed. Leave the set-up for 72 h.
- Remove the seeds by lifting the sieve from the Petri dish and transfer the water into a beaker. The dish should be rinsed into the same beaker with a stream of water from a wash bottle.

Fig. 21.2. A working sample of 500 rice seeds ready for extraction of *Aphelenchoides besseyi*

Let the water stand for about an hour which allows the nematodes to settle down and concentrate at the bottom of the beaker.

- Remove water gently from the top with the help of a pipette leaving behind about 15-20 ml in the beaker (Fig. 21.3). Transfer this water into an examination dish which in turn is examined under the stereoscopic microscope with transmitted light (diascopic light). The nematodes are seen better in this type of light than in the incident type (episcopic).
- Count the number of nematodes extracted.

Control

Seed treatment by both hot water and chemicals has been found to be very effective in controlling the disease. Treat the seed with hot water, 15 min at 52-57°C.

Gomi & Kogure (1956) reported Folidal to be more effective than hot water treatment. Complete control was achieved by soaking the seed for 24 h in 1:600 solution of rhodanate-acetic ester-200 containing 20% active ingredient (Tanaka, 1959); or in 1:200-1:400 emulsion (20%) of ethyl thiocyanoacetate (Fukano, 1962).

In recent years systemic pesticides have been used to control the disease. Vuong & Rodriguez (1972) reported effective control by soaking seeds in Sassen, Dimecron (phosphamidon) or Thiabenzol (75% thiabendazol). Uebayashi, Amano & Nakanishi (1971) recommended soaking of seeds in baycid, diazinon, sumithion or cartap.

Lee, Han & Park (1976) obtained a 19% yield increase by applying ethoprop and carbofuran after transplanting and as soon as symptoms appeared in the field.

Cralley (1957) found that the rice sown in water was much less infected by *Aphelenchoides besseyi* than when drilled and flooded later.

Fig. 21.3. Removal of top water with a pipette from the beaker. The remaining 15-20 ml water will be poured into the dish for final examination under stereoscopic microscope

Quarantine

Aphelenchoides besseyi has been included in the import regulations of Africa, south of the Sahara (Neergaard, 1970). In India, Nath & Sethi (1970) intercepted the nematode in 1968 on rice seed imported from the Philippines and since then it has been repeatedly intercepted in rice germplasm at the National Bureau of Plant Genetic Resources, New Delhi.

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Appendix

List of pesticides appearing in the literature with their ISO names

(Sources: Agricultural Chemicals and Pesticides, A hand book of the toxic effects. Edited by Edwa[illegible] J. Fairchild, Castle House Publications Ltd., Kent, England, 1978, pp. 229 and original research pa[illegible] where the trade names have been mentioned)

Standard Common names (e.g. ISO or equivalent)	Trade names	Chemical names
	Agrimycin	Streptomycin
	Aureofungin	Polyene-heptaene
Jenthion	Baycid	Phosphorothioic acid, o, o-dimethyl-, o- (4-methylthio)-m-tolyl) ester
Benomyl	Benlate	1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester
Jentin chloride	Brestanol 45 WP	Triphenyltin chloride
Captan	Orthocode	4-cyclohexene-1, 2-dicarboximide, N-(trichloromethyl)thio-
Carbendazim	Bavistin	2-benzimidazolecarbamic acid, methyl ester
Carbofuran	Furadan	Carbamic acid, methyl-, 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl ester
Carboxin	Vitavax	1,4-oxathiin-3-carboxamide, 5, 6-dihydro-2-methyl-N-phenyl-
Cartap	Padan, Cadan	Carbamic acid,thio-s, s'-(2-(dimethylamino)trimethylene) ester, hydrochloride
Ceresan M		Mercury, ethyl(p-toluenesulfonanilidato)-
Copperoxy-chloride	Fytolan	Copper chloride mixed with copper oxide
Diazinon	Basudin	Phosphorothioic acid, o, o-diethyl o-(2-isopropyl-6-methyl-4-pyrimidinyl) ester
Phosphamidon	Dimecron	Phosphoric acid, dimethyl ester, ester with 2-chloro-N, N-diethyl-3-hydroxycrotonamide
Mancozeb	Dithane M-45	Manganese ethylene bis di thiocarbamate plus zinc ion (85 arae 4,52,76)

Standard Common names (e.g. ISO or equivalent)	Trade names	Chemical names
Ethoprop	Mocap	Phosphorodithioic acid, o-ethyl s, s-dipropyl ester
Parathion*	Folidol	o, o-diethyl o-4-nitrophenyl phosphorothioate
Isoprothiolane	Fuji-one	di-isopropyl 1, 3 dithiolan-2-glidenemalonate
	Granosan L*	
Guazatine	Panoctine	9-aza-1, 17-heptadecanediamine, N, N'-diamidino-, hydrogen sulfate
Edifenphos	Hinosan	Phosphorodithioic acid, o-ethyl-s, s-diphenyl ester
thiram + thiophanate methyl	Homai	dimethyl 4, 4'- (o-phenylene) bis (3-thioallophanate) + tetramethylthiuram disulphide
	HPMTS 80EC	2-hydroxypropyl-methane-thiosulphonate
	Kanamycin*	
Kasugamycin	Kasumin	3H-imidazo(4,5-b)pyridine, 7-chloro-3-beta-D-ribofuranosyl-
Iprobenfos	Kitazin P, IBP	o,o-bis(1-methylethyl)s-(phenylmethyl)phosphorothioate
Methasulfocarb	Kayabest NK-191	[s-(4-methylsulfonyloxyphenyl) N-methyl] thiocarbamate
Probenazole	Oryzamate	3-allyloxybenzol [1,2] thiazole -1, 2-dioxide
Phthalide 30 WP		Phenolphthalein
	Proraz EC*	
PCNB	Quintozene	Benzene, Pentachloronitro-
Futhalide	Rabacide	4,5,6,7-tetrachlorophthalide
	Rhodan	Thiocyanic acid, p-aminophenyl ester
	Sankel*	Nickel dimethyldithiocarbamate
	Sassen*	ethylthiocyano acetate
Fenitrothion	Sumithion	Phosphorothioic acid,o, o-dimethyl o-(4-nitro-m-tolyl) ester
	Terrcoat	1,2,4-thiadiazole,5-ethoxy-3-(trichloro-methyl)
Tetracycline		2-naphthacenecarboxamide, 4-(dimethylamino)- 1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5, 6, 10, 12, 12a-hexahydroxy-6-methyl-1, 11-dioxo-

Standard Common names (e.g. ISO or equivalent)	Trade names	Chemical names
Thiabendazole	Thiabenzole, Tecto-60	benzimidazole, 2-(4-thiazolyl)-
Thiram	Arasan	Bis(dimethylthiocarbamoyl) disulfide
Thiophanate	Topsin M	Allophanic acid, 4,4'-o-phenylenebis(3-thio) diethyl ester
	Tricyclazole	s-triazolo(3,4-b)benzothiazole, 5-methyl-

* Information incomplete