A Manual of Rice Seed Health Testing

Edited by T.W. Mew and J.K. Misra

IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

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The International Rice Research Institute (IRRI) was established in 1960 by the Ford and Rockefeller Foundations with the help and approval of the Government of the Philippines. Today IRRI is one of 18 nonprofit international research centers supported by the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is sponsored by thc Food and Agriculture Organization of the United Nations (FAO), the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). Its membership comprises donor countries, international and regional organizations, and private foundations.

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Foreword

Intensive collaboration characterizes the international exchange of rice germplasm. Efforts to increase rice yields demand that more germplasm be exchanged, more often. In 1992, IRRI dispatched 94,836 seed lots to rice scientists world wide and received 6,270 seed lots from outside the Philippines. This exchange of rice germplasm can be expected to increase exponentially as national breeding programs intensify their work and rice scientists strive to increase rice yields through breeding resistance to pests and tolerance for abiotic stresses into new varieties, to feed the growing number of rice consumers of the future.

But any exchange of crop materials involves the inherent risk of also exchanging pests and diseases. Certification of seed health involves three basic issues:

- Identifying the organisms that have quarantine importance.
- Determining the optimum sample size of a seed lot to test for those organisms.
- Using standard procedures to carry out the tests.

IRRI established its Seed Health Unit in 1983, in cooperation with the Plant Quarantine Service of the Bureau of Plant Industry of the Philippines. Staff members of the unit work to ensure that incoming seed lots consigned to IRRI meet or exceed Philippine quarantine requirements and that seed lots IRRI sends out meet or exceed the quarantine requirements of the countries to which they are consigned. Many methods have been developed, tested, and modified to meet the need for safe and rapid exchange of rice germplasm and breeding materials.

The Seed Health Unit also offers a short course on seed health maintenance and testing procedures to help improve the speed and safety of rice seed exchange worldwide. First offered in 1990, the course provides comprehensive, hands-on experience in all aspects of rice seed health testing.

This manual is based on the content of the short course. Tests for seedborne fungi are those used by IRRI's Seed Health Unit. Tests for bacteria are based on procedures used by IRRI's Plant Pathology Division. Diagnostic tests for viruses are not included because no tropical viruses are known to be borne by rice seeds. Standard procedures accepted by the International Seed Testing Association are described, in particular those on sampling (ISTA 1985).

Dr. J.K. Misra, visiting scientist at IRRI 1988-90, compiled the first draft. Staff of the Seed Health Unit; the Agronomy, Plant Physiology and Agroecology; Entomology; and Plant Pathology Divisions; and of the Genetic Resources Center and Biometrics Unit provided additional detail. Dr. T.W. Mew integrated the many contributions.

The manual was edited by Jill Gale de Villa, assisted by Teresita Rola, with graphic design by Ramiro Cabrera, Communication and Publications Services.

Klaus Lampe
Director General

Preface

In the last few decades, the international exchange of plant germplasm (seed) has increased tremendously. Global cooperation to boost food production and combat hunger has revolutionized agricultural production and, at the same time, has increased the risk of introducing exotic pests and pathogens. Consequently, the quarantine officials' job has become increasingly complex and challenging.

Quarantine personnel deal with a wide range of outgoing or incoming plant and other materials, their pathogens, and other undesired objects accompanying them. It has become difficult, if not impossible, for the limited number of people working at quarantine stations to know intimately the bizarre group of organisms associated with the various crops that frequently pass through them.

Thus, specialists dealing with specific crops and pathogens should publish all relevant information. The publications should be geared to help the users, be they scientists or tradesmen, allow safer, uninterrupted movement of germplasm. Yet, this type of publication is unavailable for many crops which are exported.

Rice is exchanged widely for both scientific and commercial purposes. Shortly after its founding, the International Rice Research Institute (IRRI) started exchanging seed for scientific purposes. In recent years, these exchanges have occurred with increasing frequency. Between 1985 and 1990, IRRI dealt with 936,547 seed lots (IRRI's Seed Health Unit data). Many other organizations are engaged in seed exchange for scientific purposes. There is enormous movement of seed for commercial purposes.

This manual compiles important information relating to common seedborne rice diseases and rice seed contaminants, details of the causal organisms, and methods of detecting their presence.

The manual has three major divisions. Part I discusses the importance of seeds, their health, and the risks involved in seed exchanges; reviews the historical development of quarantine systems; and describes rice plant morphology and ecosystems. Part II describes laboratory equipment needed for assessing rice seed health; samples and sampling methods; dry seed inspection; detection, isolation, and identification methods for fungi, bacteria, and nematodes; viruses and mycoplasmalike organisms (briefly mentioned); and methods for field inspection and seed treatment. Only methods pertinent to assessing seed health for quarantine are included. Part III describes the pathogens and the diseases they cause.

Appendix 1 contains sample recording sheets. Lists of media, stains, and reagents are given in Appendix 2 and some recipes for preparing them. Appendix 3 is a glossary of terms used in this manual. References cited in the text are listed before the appendices.

We hope this manual, and our approach to seed health, will help the readers assess rice seed health properly. However, no work can claim to be complete. There is always room for correction and/or amplification. We intend to update and improve this manual in the future, and thus ask that you send us suggestions and comments that will make future editions more complete and more useful.

Part 1 Introduction

CHAPTER 1

Rice Seed Health and Quarantine

Rice germplasm, its definition, and the importance of seed health in rice seed exchange

CHAPTER 2

The Rice Plant and its Environment

Morphology of the rice plant, definitions and descriptions of the rice plant's environments

CHAPTER 1

Rice seed health and quarantine

J.K. Misra, T.W. Mew, and S.D. Merca

What is seed?

The term seed connotes both immature and mature fertilized ovules, and rice seed generally refers to the whole fruit (caryopsis). However, for purposes of this manual, seed will refer specifically to mature ovules that contain an embryo, and rice seed will refer only to the rice grain.

Seed history

Seed, the germ of life, has received worldwide attention due to global agricultural cooperation and the increasing need to develop good, high-yielding food plants. Good seeds are essential to good crop production. They are today's treasure and the hope for feeding future generations.

Our nomadic ancestors gathered seeds. Neolithic farmers planted and cultivated food crops. They selectively collected seeds of plants that suited their environments. They brought seeds when they moved. Thus, they helped spread crop plants to different parts of the world.

Agriculture and animal husbandry diffused from centers in the Middle East and Asia to other parts of the world. Beginning in the 17th century, people brought cereal crops to the New World. Europeans returned home with maize acquired in Mexico. Numerous American crops were moved through Europe to Asia, while Asiatic and European crops were introduced into the Americas.

Farmers and enthusiastic plant lovers initiated plant and seed selection. During the late 19th and early 20th centuries, scientists followed suit.

Seed exchange

Since its inception, IRRI has been conscious of the need for a rice genebank large enough to permit crop improvement. As of 1990, IRRI had collected and conserved about 82,000 varieties of rice. This huge collection was achieved through the efforts of IRRI scientists with the collaboration of and donations by many national centers.

Samples are cared for by the International Rice Germplasm Center (IRGC). IRGC sends out as many seed samples as it receives. IRGC multiplies incoming samples and conserves the germplasm to meet an ever-increasing demand from researchers the world over.

Other IRRI divisions and programs, such as Plant Breeding, Genetics, and Biochemistry; Plant Pathology; Entomology; Agronomy, Plant Physiology, and Agroecology; and the International Network for Genetic Evaluation of Rice (INGER, formerly IRTP), also disseminate germplasm. The Plant Breeding, Genetics, and Biochemistry Division multiplies improved rice germplasm in various stages of development and disseminates them among researchers and farmers. The divisions sent 1,411,261 seed packets of breeding materials to 87 countries from 1983 through 1990. Seed movement to collaborative countries was boosted further by the introduction of shuttle breeding projects for better germplasm.

Since its inception in 1975, INGER has undertaken a vigorous, far-reaching seed exchange program to evaluate different germplasms under varying rice-growing environments around the world. Through this program, IRRI has worked with more than 800 rice scientists from 75 countries in Asia, Latin America, Africa, North America, Europe, and Oceania (Seshu 1985). Seventy-five percent of INGER's outgoing nursery varieties are tested in Asia. Thus, INGER has proved an effective link through seed exchanges among regional, national, and international scientists. INGER's activities will increase in the future (Chang et al 1988).

The importance of seed health

Seed health refers primarily to the presence or absence of disease-causing organisms such as fungi, nematodes, bacteria, viruses, and insects. Physiological conditions such as trace element deficiency may also affect health (ISTA 1985).

Seed health is crucial to crop production. Seed is an important exchange material for farming, seed production, and research at national, regional, and international levels. At the international level, seed exchanges help create new varieties of crops and materials for research. With the exchanges comes the danger of introducing pests and pathogens. These hazards may accompany, adhere to, or be inside the exchange material.

Safeguards are needed to stop the spread of pests and pathogens while allowing safe, uninterrupted seed movement. The safeguards include rules, regulations, import permits, phytosanitary certificates, inspections, treatments, isolation, passage through quarantine greenhouses, seed health testing, and other measures which have been adopted at national and interna-

levels (Kahn 1988). Quarantine officers enforce rules and regulations and implement safeguard procedures. Different quarantine procedures are applicd to seeds used for commnercial purposes and to those used for scientific ends. Commercial sced is stringently regulated. Seed for scientific use is handled more leniently, in reccognition of benefit considerations.

National, local, and state governments have enacted quarantine rules and regulations governing the movement of plant and other materials within their jurisdictions. The International Plant Protection Convention of 1951 binds almost all seed-exporting and -importing countries to the convention's rules, regulations, and policies. Signatory nations are bound by the convention's decisions, which have the force of a legal treaty and are administered by the Food and Agriculture Organization of the United Nations.

In recent years, regional and global cooperation in agricultural research has gained momentum. Consequently, many organizations (including the Inter-African Phytosanitary Commission, 1961; the European and Mediterranean Plant Protection Organization, 1965; the South Pacific Commission; the Caribbean Plant Protection Commission; and the Association of Southeast Asian Nations [ASEAN] Plant Quarantine Center and Training Institute [PLANTI], Kuala Lumpur, 1981) met and set up biological standards for national rules and regulations pertaining to the safer intercontinental and interregional movement of plant germplasm and other commodities. Asian genebanks (for instance, the Japanese genebank in Tsukuba, IRRI's genebank, the Chinese genebank, and the Indian National Bureau of Plant Genetic Resources) are cooperating commendably with many nations by exchanging germplasm. Each bank sets its own requirements for seed health during exchanges.

Quarantine

Pathogens can enter a new environment, country, or region through hosts, packings, other inert materials, insect vectors, birds, and air currents. Airborne pathogens can be stopped only by natural barriers such as oceans and high mountain ranges. Quarantine officers can intercept other pathogens and exclude or eradicate them.

Exclusion measures include compulsory quarantine, inspection (compulsory or voluntary), and certification of planting stock or germplasm as seed.

The importance of these measures became apparent when several devastating pathogens entered Europe and America through exchanged plant material. The pathogens included those that cause powdery mildew disease of grapes, citrus canker, potato wart, wheat flag smut, and Dutch elm disease.

Some pathogens endemic to parts of North America had spread through nursery stocks to other regions before the USA established an internal quarantine system. Pathogens of minor consequence in their native environments, such as chestnut blight, may be destructive in a new environment.

Quarantine systems and regulations developed after 1870. Individual American states initiated exclusion of insect pests through quarantine regulations. Early quarantine attention focused on propagative materials such as nursery stocks, but ignored edible fruits, vegetables, and seeds. The national government did not restrict plant movement until 1912, when Congress passed the Federal Plant Quarantine Act. Australia and the European nations had imposed restrictions earlier. Some pests were controlled through exclusion.

Quarantine systems control some diseases by eradicating the pathogens. Incoming plant materials are treated using physical means (precooling or heat treatment) and/or chemical methods (biocidal treatment) to climinate unwanted pathogens.

The importance of adequate quarantine methods to detect and stop disease cannot be overstated. Seed being moved internationally and interregionally should be healthy and pathogen-free to avert disease outbreaks.

Methods used to detect and identify pathogens and pests of rice seed are described in Part II of this manual. The pests and diseases are described in Part III. ■

CHAPTER 2

The rice plant and its environment

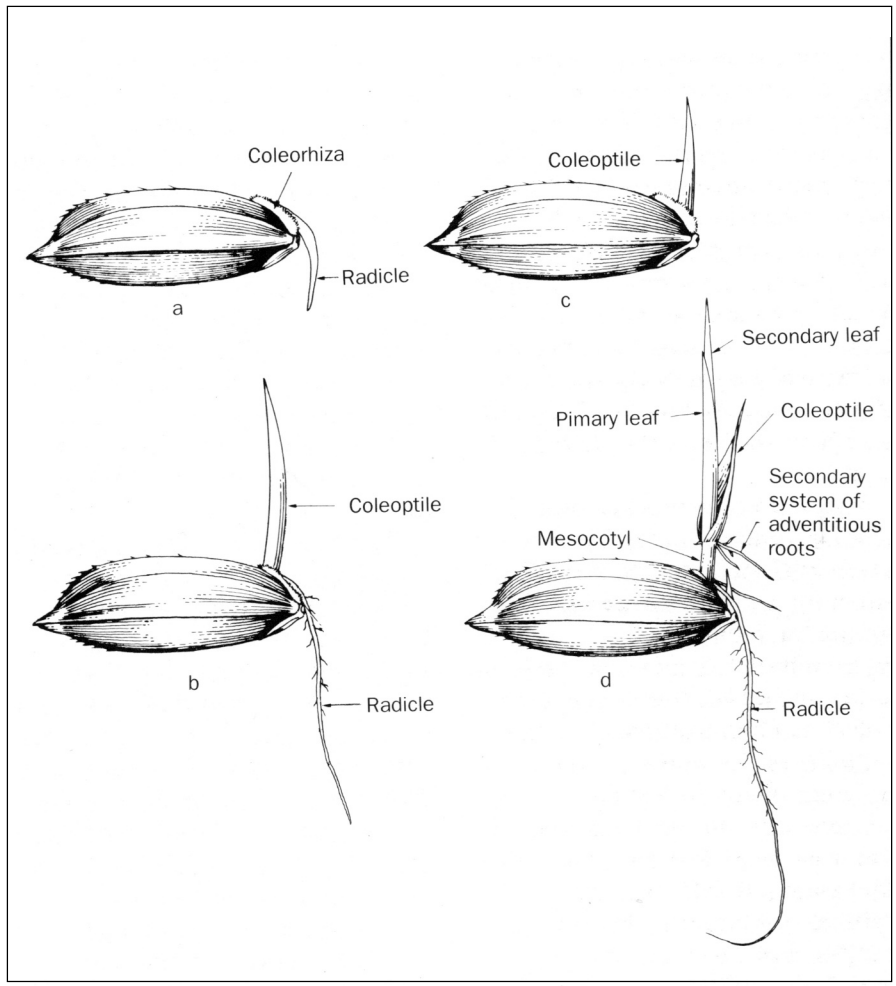
J.K. Misra, G.C. Loresto, and T.W. Mew

Oryza sativa L., the most commonly cultivated rice species, belongs to subfamily Pooideae of the grass family Poaceae. The genus *Oryza* is divided into several sections. *O. sativa* comes under section Sativae and its series *Sativa. O. sativa* is a diploid plant (2n=24). *O. glaberrima* Steud. (2n=24), anothcr *Oryza* species, is cultivated in West Africa. The two species have minor morphological differences, but terms discussed here apply to both. Both are annual grasses adapted to habitats ranging from aquatic to dry land.

Seedling morphology

Grains of nondormant varieties can germinate immediately after ripening. During germination, the coleorhiza emerges with the radicle first (Fig. 2.1a,b) in an aerated ervironment; the coleoptile protrudes first (Fig. 2.1 c) in a submerged medium. The seedling soon develops two or more secondary seminal roots. As growth progresses, a secondary system of adventitious roots (Fig. 2.1d) originates from the first node and replaces the seminal roots.

The cylindrical coleoptile encloses the primary leaf. The mesocotyl between the root and coleoptile grows to push out the coleoptile above the ground. The primary leaf, emerging out of thc coleoptile, is green and cylindrical, with no blade. The second leaf differentiates into sheath, blade, ligule, and auricles.



2.1 *Oryza sativa* germination.
a, b. Coleorhiza emerges, radicle first in an aerated environment.
c, d. Coleoptile protrudes first in a submerged environment.

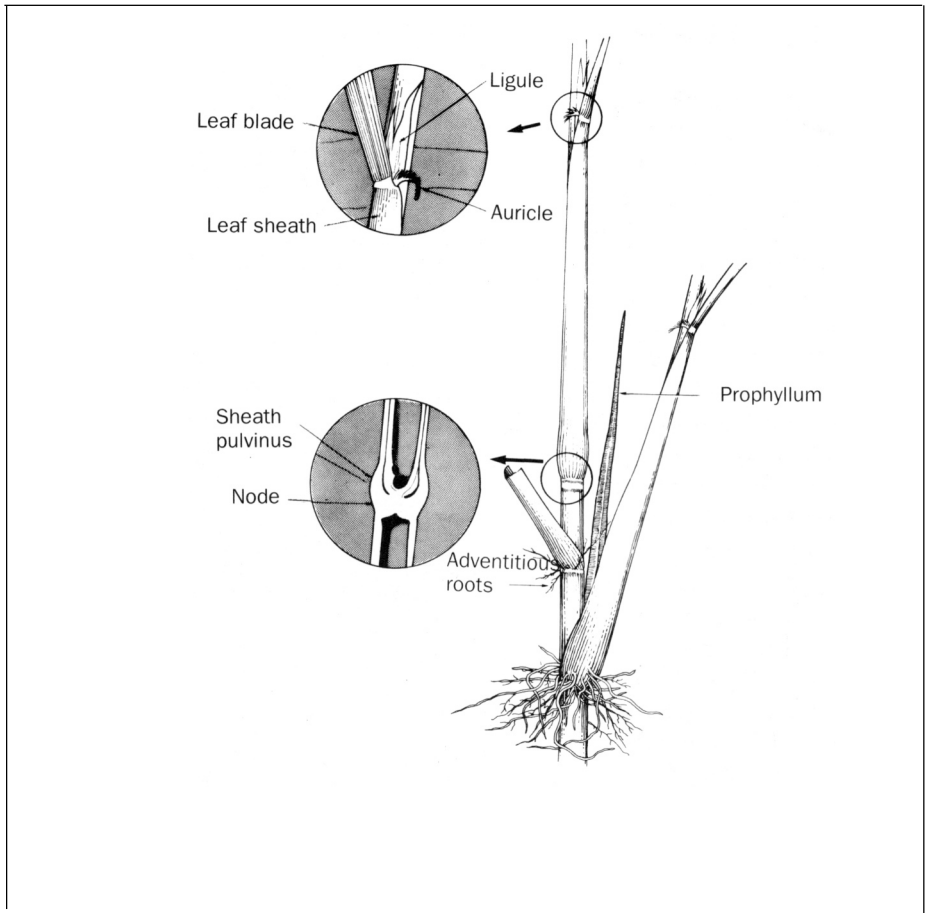
Morphology of the mature plant

Mature plants of different varieties vary in height from 1 to 5 m, depending on heredity and environment. Plants 1-2 m tall are common for varieties grown in some ecosystems. The mature plant (Fig. 2.2, 2.3) is discussed below. (For further reading, see Chang and Bardenas 1965, Vergara and Chang 1976, and Vergara 1991.)

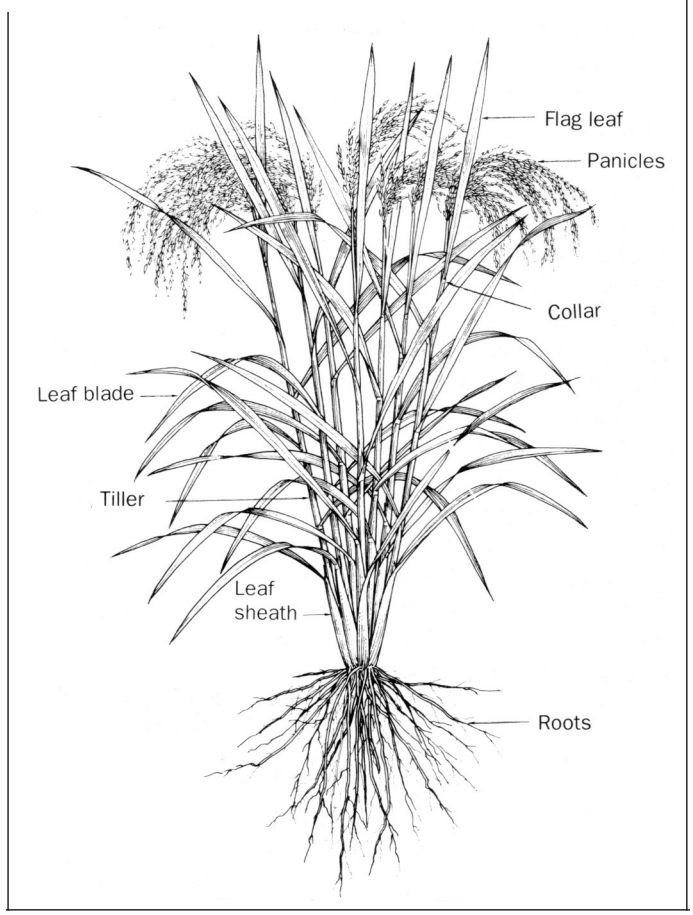
■ ***Root.*** Seminal roots disappear quickly and are replaced by secondary and adventitious roots which develop at nodes both below and above the ground. These roots emerge in a whorl at the nodes above the ground and can be seen at maturity.

■ ***Culm.*** The rice plant's culm (stem) is composed of several nodes and internodes. Leaves arise at the nodes, with a bud in the axil. The bud gives rise to a tiller. The internodes are hollow or fistular, with smooth outer surfaces, and varying lengths. Lower internodes are shorter and compressed near the base, and are thick and solid. Tillers arise alternately from the culm. Primary tillers arise from the lowermost nodes. In turn, they give rise to secondary and tertiary tillers.

■ ***Leaves.*** The leaves are sessile and are borne on the nodes. Each leaf consists of a blade and a sheath. The blades are flat and vary among varieties in size, shape, color, angle of insertion, and pubescence. The leaf sheath envelopes the culm above the node. The swelling at the base of the leaf sheath is called the sheath pulvinus and is often confused with the node. The leaf associated with thc panicle is called the flag leaf and differs in shape from others. Veins on the leaf are parallel and have a prominent midrib. At the base of the blade, on either side, are paired small appendages called auricles. Thcy may fall off in older leaves. The place where the sheath and blade join is called the collar or junctura. Oh its inner junction is a membra-



2.2 Parts of a primary and a secondary tiller.



2.3 A mature rice plant.

nous structure called the ligule, which may be glabrous or ciliate. Its size, color, and shape vary among varieties.

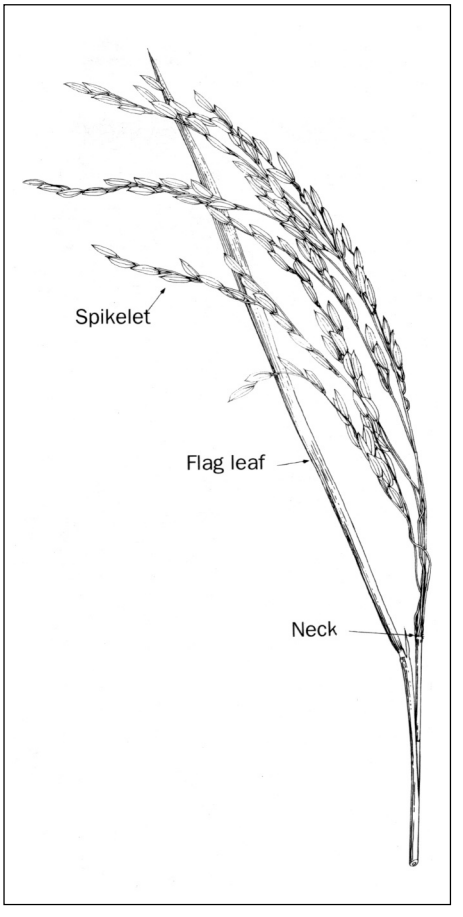
The number of leaves in the main culm is always greater than the number of tillers. The number decreases from the primary to the tertiary tillers. The prophyllum is at the base of the main tiller.

■ ***Floral organs.*** The rice plant terminates in an inflorescence (blossom, flower) called a panicle. Each unit of the inflorescence is a spikelet.

—The ***panicle*** (Fig. 2.4) grows from the uppermost internode. The upper extremity of the uppermost internode, where primary branches of the panicle arise, is called the panicle base or neck. The axis starting from the panicle base to its end is the primary panicle axis. It is differentiated into nodes and internodes. The nodes are solid; the internodes are fistular. In the axils of branches on the panicle are swollen portions called panicle pulvini. Each node gives rise to primary branches, which in turn bear secondary branches. The secondary branches bear the spikelets.

—Varieties vary greatly in panicle length, shape, insertion angle of primary branches, and weight and density.

—The ***spikelets*** (Fig. 2.5) are pedicelate. They have a short axis called the rachilla. The flower is borne in the axil of two types of bracts. The first pair of bracts are sterile, and are known as sterile lemmas (glumes, empty glumes, or outer glumes). These may be of unequal size, and are generally smaller than the fertile glumes. The upper pair of bracts is called the fertile (flowering) glumes and consists of lemma and palea. The glumes (lemma and palea) and the floral organs constitute the floret.



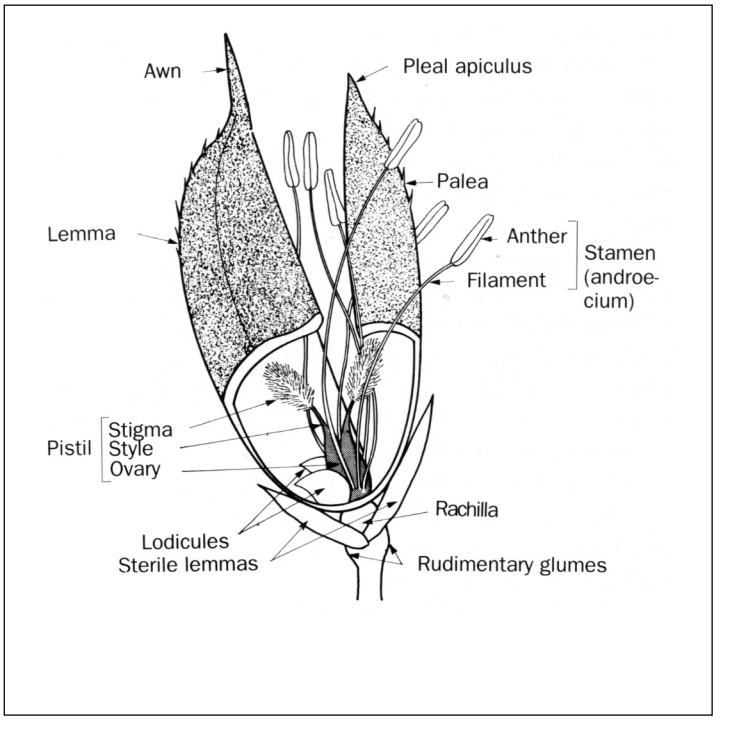
2.4 The panicle.

The lemma is a five-nervcd bract partly enveloping the smaller three-nerved bract palea. The middle nerve or keel may be hairy or smooth. These bracts extend into smaller structures called apiculi. The apiculus at the lemma is known as the lemmal apiculus while that at the palea is the paleal apiculus. The lemma may also bear an awn, which is the extension of its keel. The surface of the bracts may be smooth or hairy.

—The ***flower*** (Fig. 2.5) is bisexual, complete, hypogynous, and zygomorphic.

—The ***perianth*** (Fig. 2.5) is modified and greatly reduced into two succulent structures called lodicules, which are adnate to the palea. At the time of flowering, the lodicules help push apart the lemma and palea at the base to expose the stamens and the pistil.

—The ***androecium*** (male reproductive organ, Fig. 2.5) has six stamens in two whorls. Anthers are



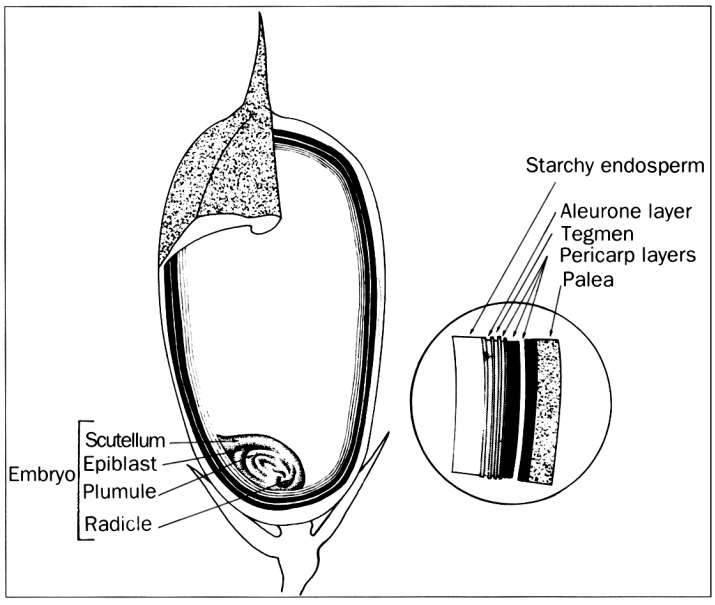
2.5 The spikelet and flower.

two-celled, besifixed, deeply sagittate, laterally dehiscent, with long, slender filaments.

—The ***gynoecium*** (female reproductive organ, Fig. 2.5) is monocarpellary, superior, ovoid, unilocular with one sessile ovule, and marginal placentation. There are two short styles. Stigmata are bifid, light yellow to amaranthus purple, and feathery (plumose).

—The ***fruit*** is the caryopsis (pericarp fused with testa). Most are indehiscent.

—The ripened ***seed*** (Fig. 2.6) has 90% starch and 10% protein in the endosperm. Radicle and plumule are well-developed in the embryo. The seed coat varies in color from light brown to red, to purple. Usually the seed (mature fruit plus lemma, palea, rachilla, sterile lemmas, and awn) is used to grow a crop. Brown rice (grain) can be used as seed, but it is more susceptible to microbial attack due to the absence of the protective hull (lemma, palea, etc.)



2.6 The seed.

Classification of seeds

Rice seed is usually categorized as follows:

■ ***Nucleus seed***—genetically pure seed available from the original plant breeder and used for breeder's seed production

■ ***Breeder's seed***—seed directly produced or controlled by the original plant breeder

■ ***Foundation seed***—seed directly produced from the breeder's seed by technically qualified staff

Certified seed—progeny of foundation or certified seeds.

Rice ecosystems

Rice-growing environments vary significantly in different localities within and between countries. Consequently, controversies have arisen regarding acceptable definitions and classification systems. Scientists and specialists from different disciplines and parts of the rice-growing world formed an international committee to determine terminology for rice-growing ecosystems. Khush (1984) described the results. Garrity (1984) reviewed existing taxonomic treatments. The ecosystem terminology briefly described below is used in subsequent chapters.

Terminologies and classifications of the rice ecosystems are based on water regime, drainage, temperature, soil type, and geomorphology. Rice-growing environments are grouped in to five main categories.

■ The ***irrigated environment*** has sufficient water available during the entire growing season. Rainfall supplements the water need. The irrigated environment contributes almost three-fourths of the total world rice production. It has been divided into three subcategories.

—The ***irrigated environment with favorable temperature*** has an 18-38 °C air temperature during the rice-growing season. This subcategory constitutes the greater portion of the irrigated ecosystem.

—The ***irrigated, low-temperuture, tropical zone environment*** has air temperatures below 18 °C during the seedling stage. This affects plant growth. Cold-tolerant and blast-resistant varieties are best suited to this ecosystem.

—The ***irrigated, low-temperature, temperate zone environment*** has air temperatures below 18 °C during the seedling and flowering stages. Cold-tolerant japonica varieties are best suited to this environment.

m The rainfed lowland environment is the most variable rice ecosystem. It comprises one-fourth of the world rice area which is dependent on amount and duration of rainfall, depth and period of standing water, frequency and time of flooding, and soil type and topography. It is divided into five subcategories.

—The ***rainfed, shallow, favorable environment*** receives adequate water from rainfall and experiences no serious drought or flooding.

—The ***rainfed, shallow, drought-prone environment*** may experience drought or moisture stress at any of the rice plant's growth stages.

—The ***rainfed, shallow, drought- and submergence-prone environment*** experiences both submergence and prolonged periods of drought.

—The ***rainfed, shallow, submergence-prone environment*** experiences prolonged rainfall, flooding, and submergence for as many as 10 d/yr.

–The ***rainfed, medium-deep, waterlogged environment*** experiences waterlogging, with water depths ranging from 25 to 50 cm in bunded or unbunded fields.

■ The ***deepwater environment*** has unbunded fields with 0.5-3 m of standing water. It has two subcategories.

–The ***deepwater environment*** has 50-100 cm of water.

–The ***very deepwater environment*** has water depth exceeding 100 cm.

■ The ***upland environment*** has bunded or unbunded rainfed fields with no surface or rhizosphere water accumulation. Hydromorphic ricelands in West Africa are examples of this ecosystem. Varieties suited for this environment should have deep root systems, be able to recover from drought, withstand aluminum toxicity and iron deficiency, and be resistant to blast. It has four subcategories.

–The ***favorable upland environment*** with long growing season has a long rainy season and highly fertile soil with good water-holding capacity.

–The ***favorable upland environment with short growing season*** has a short rainy period and favorable growing conditions, but drought is a major constraint.

–The ***unfavorable upland environment with long growing season*** has a long rainy season but unfavorable soil or topographic factors.

–The ***unfavorable upland environment with short growing season*** has a short rainy season and poor soil conditions.

■ ***Tidal wetlands*** are near the seacoasts and inland estuaries and are influenced by tides. This heterogeneous environment has four subdivisions.

–Tidal wetlands with perennially fresh water are away from the sea and do not have saline water.

–Tidal wetlands with seasonally or perennially saline water are found near seacoasts and at estuary mouths.

–Tidal wetlands with acid sulfate soils occur near coasts and inland estuaries, are affected by salt water, and are not planted to rice.

–Tidal wetlands with peat soils exist near seacoasts and estuaries. Not currently in use for rice production, these areas could be utilized for growing rice. ■

Part 2 Procedures for seed health evaluations

CHAPTER 3

Equipment
General procedures and equipment needed

CHAPTER 4

Samples and Sampling
Proper sampling methods for seed lots

CHAPTER 5

Dry Seed Inspection
Initial detection of disease symptoms in seeds and of weeds and insect contaminants

CHAPTER 6

Fungi
Detection methods

CHAPTER 7

Bacteria
Detection methods

CHAPTER 8

Nematodes
Detection methods

CHAPTER 9

Viruses and Mycoplasmalike Organisms
A brief note

CHAPTER 10

Field Inspection
Methods

CHAPTER 11

Seed Treatment
Methods for eradicating pests and pathogens

CHAPTER 3

Equipment

J.K. Misra, T.W. Mew, and S.D. Merca

Steps followed for the phytosanitary certification of rice seeds are shown in Figure 3.1.

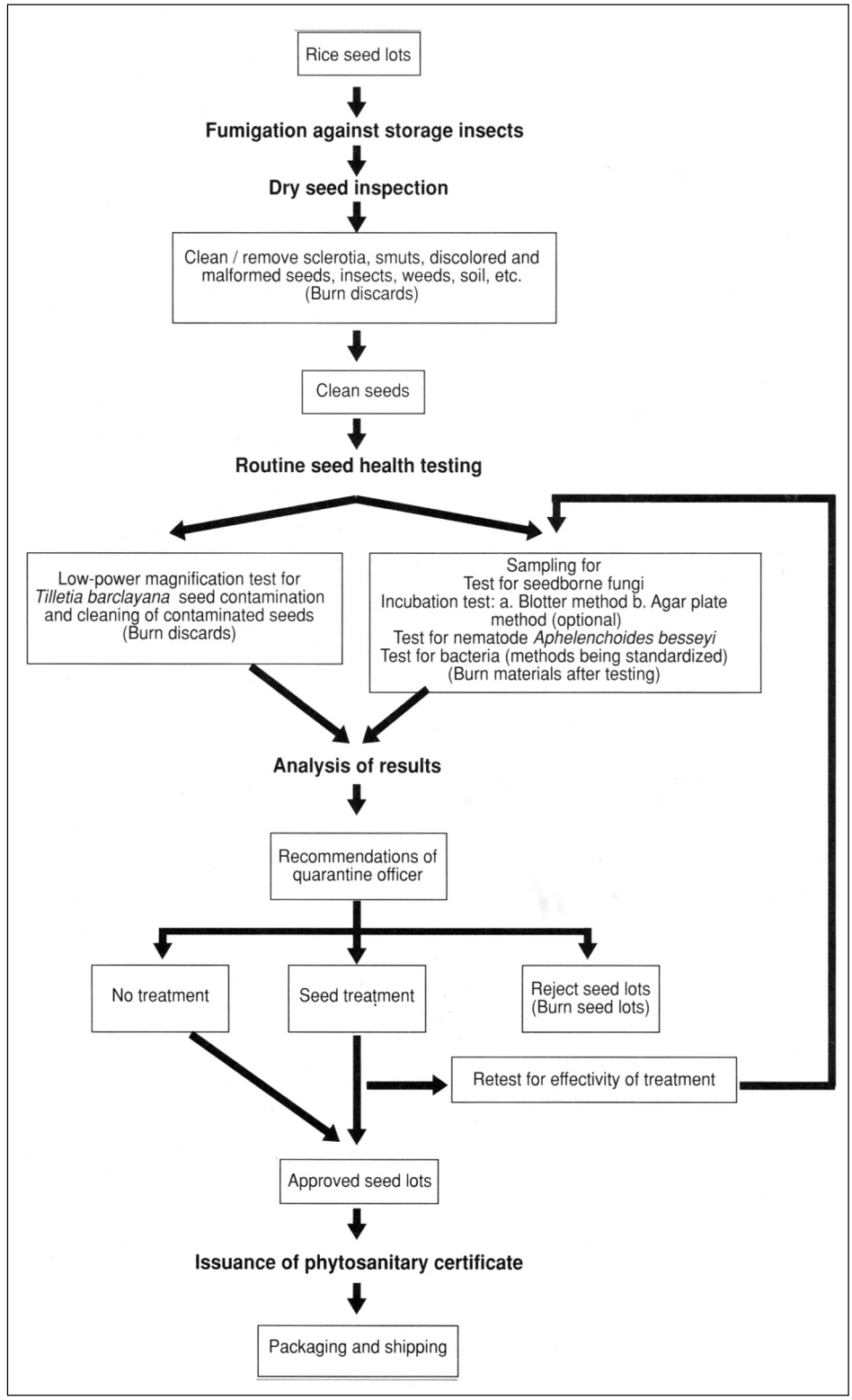
Seed testing laboratories use seed sampling equipment to draw and divide official seed samples (Table 3.1).

Standard plant pathology laboratory equipment and facilities suitable for many techniques used in rice seed health testing are listed in Tables 3.1 and 3.2 and briefly explained below. The equipment used for sampling is described in the next chapter.

A hand or magnifying lens (Fig. 3.2) may detect plant debris, sclerotia, smuts, malformations, and indications of infection such as dried bacterial ooze, resting mycelia on seed surfaces, and fruiting bodies.

A stereobinocular microscope (Fig. 3.3) can readily detect contamination of the seed surface with teleospores of *Tilletia barclayana.*

Additional equipment is needed for the washing test. An automatic shaker standardizes shaking, a low-speed centrifuge concentrates suspension, a compound microscope helps identify fungal spores, and a haemocytometer determines the spore load. Stereobinocular and compound microscopes are used to detect and identify nematodes in suspension after sedimentation.



3.1 Steps toward the phytosanitary certification of rice seeds.

The blotter test, an indirect method of major importance, requires near-ultra violet (NUV) light incubation chambers equipped with timers to regulate NUV for 12 h, followed by 12 h darkness, daily. The incubation chamber temperature for the standard blotter test is 20±2 °C. Some NUV chambers are constructed inside a walk-in room that is air-conditioned to maintain the recommended temperature. The NUV light source should be a 320-400 nm lamp, preferably Philips TL 40W/08, GE F 40 BL, or equivalent.

A freezing blotter may be used to kill the germinating embryo or seedling when germination is so vigorous that seedling growth elevates the petri dish cover and the blotter becomes dry. Freezing is accomplished on the second or third day of incubation by placing the seeded plates in a freezer for 24 h and then returning them to NUV incubation. Plastic petri dishes are recommended as they allow better NUV light penetration than do glass dishes.

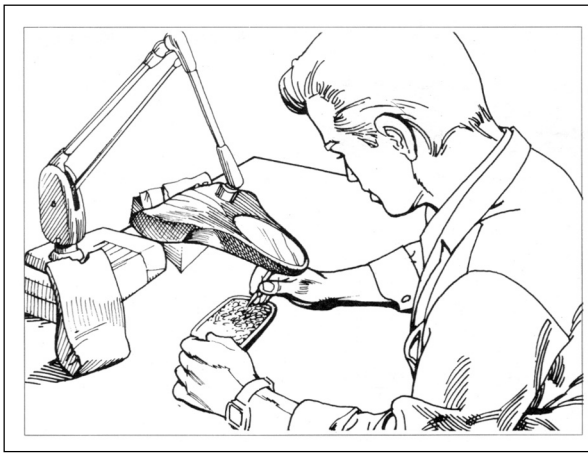
Agar for fungi test should be incubated at 28±1 °C in NUV light/darkness, and that for bacteria at 28 °C. This can be done in standard laboratory-top incubators.

For the growing-on test, container covers for the medium (substrate) and test seeds should be transparent to admit light and to facilitate visual examination without removing the covers. Transparent plastic bags supported by sticks or ready-made hard transparent covers used in germination tests are available.

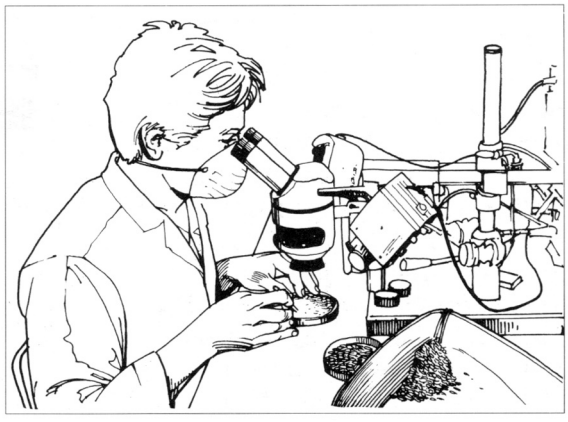
Table 3.1. Laboratory equipment, instruments, and glassware used for seed health testing.[a]

- Seed sampling
  - Stick or sleeve trier (Nobbe trier. Fig. 4.1)
  - Conical divider (Boerner type, Fig. 4.3)
  - Soil divider (Riffle type, Fig. 4.4)
  - Centrifugal divider (Gamet type. Fig. 4.5)
  - Magnifying lens (Fig. 3.3)
- Seed health testing
  - Essential
    - Autoclave
    - Sterilizing oven
    - Hot air
    - Refrigerator/freezer
    - Clean bench or laminar flow hood
    - Centrifuge (up to 20,000 rpm)
    - Centrifuge (table top model - 3,000-5,000 rpm)
    - Automatic shaker or rotary shaker (adjustable speed)
    - Distilling apparatus
    - Incubation chambers (25-32 °C)
    - NUV incubator (21 °C) with automatic time switch for lamps
    - Lighted incubator (25-32 °C)
    - Stereobinocular microscope - magnification up to 50X
    - Compound microscope - magnification up to 1000X
    - Mineral light lamp-UV
    - Hand lens
    - Haemocytometer
    - Tally counter
    - Mortar and pestle
    - Electric mixer
    - Vortex mixer
    - Hot water bath
    - pH meter
    - Alcohol lamp
  - Optional
    - Growth chamber
    - Hot plate
    - Filtration apparatus
    - Gas burner
    - Electrostatic air cleaner
    - Phase-contrast microscope
  - Glassware and other laboratory supplies
    - Petri dishes - glass, plastic
    - Test tubes - assorted sizes
    - Beakers - assorted sizes
    - Syringes - assorted sizes
    - Erlenmeyer flasks - assorted sizes
    - Atomizer or sprayer
    - Baermann funnel set-up (Fig. 8.1)
    - Funnels - glass, plastic
    - Graduated cylinders - 50, 100, 500, 1000 ml
    - Pipettes - assorted sizes
    - Germination trays with transparent covers
    - Glass rods, L-shaped
    - Microscope slides/cover glasses
    - Filter paper
    - Blotters, round, 8.5 cm diameter
    - Wire loop (transfer loop)
    - Inoculating needle
    - Plastic bags - different sizes
    - Mineral oil or paraffin
    - Marking pens for glass
    - Indelible pencils
    - Paper towels
    - Working box containing dissecting needles, forceps, scissors, scalpels, transfer needles, etc.

[a] Sources: IRRI Seed Health Unit and Langerak et al (1988).



3.2 Dry seed inspection using a magnifying lens.



3.3 Dry seed inspection using a stereobinocular microscope.

Data from the tests should be recorded on appropriate recording sheets and signed by the responsible analyst. The names of fungi should be recorded according to the state they are found during testing. If conidia are found, the conidial epithet is recorded; if the perfect stage is detected, the perfect stage epithet is recorded; if both are present, both epithets are recorded. For uniformity in recording, the currently accepted epithet should be used. (See Appendix 1 for sample recording sheets for dry seed inspection, washing test, blotter and agar plate tests, and for *Aphelenchoides besseyi.).* ■

Table 3.2. Methods and equipment used in rice seed health testing.

Method	Preparation procedures	Detection/identificatlon tools			
		Hand lens	Stereobinocular microscope	Compound microscope	Others
Direct method					
For sclerotia, smut, malformed and moldy seeds		+	+	+	
Tilletia barclayana contamination			+	+	
Washing test	Shake by hand or use automatic shaker; then centrifuge at low speed			+	Haemocytometer Tally counter
Aphelenchoides besseyi sedimentation test	Use modified Baermann funnel		+	+	Tally counter
Indirect method					
Blotter test	In NUV incubation 21 °C, with timer 12 h NUV light/ 12 h darkness		+	+	Freezer for deep freezing method
Agar test for fungi and bacteria	Seed surface sterilization in table top incubators (28 °C)		+[a]	+[a]	Laminar flow cabinet
Test tube agar growing-on test	In growth chamber (28 °C) 12 h light/ day or on laboratory table top near window	+[b]			
Inoculation test	In growth chamber (28 °C) 12 h light or in glasshouse	+			Sterile sand/ soil, light
Other methods					
Serological	As required				

[a]To verify spores. [b]To magnify developing lesions.

CHAPTER 4

Samples and sampling

J. Mojica and K.A. Gomez

Proper sampling is vital to assessing seed quality for planting value, seed health, and seedling vigor. Seed analysts, field inspectors, and quarantine personnel often have to collect samples or deal with samples provided to them. Thus, they need to be aware of the types of samples, of sampling procedures, and of the reasons for placing great emphasis on sampling.

General concept of sampling

Definition and importance of sampling

Sampling is a procedure of selecting a fraction of the total population, about which information is needed, to represent that population. It is used when information regarding one or more characteristics of the population is needed but measuring all individuals in that population is not possible (Snedecor and Cochran 1967).

A sample is a subcollection of objects (or organisms) selected from a population of interest, usually chosen so as to make inferences about one or more attributes of the population based on observation made on the sample. It must be a representative of the population if it leads to correct inferences about the population (Steel and Torrie 1960). If all the sampling units (say, plants, panicles, or seeds) in the population are alike, only one sample is needed to give all information about the population. However, sampling units differ from one another. Also, sampling units comprising a particular sample may differ from sample to sample and this inherent variability from different samples could give rise to different results. The differences among samples is called *sampling variation.* Despite sampling variation, appropriate conclusions about the population should be reached. Thus, the process of sampling must be guided by statistical techniques such as the use of efficient sampling designs and appropriate choices of estimation procedures (Snedecor and Cochran 1967).

A good sampling technique should have the following features: a) minimum sampling variation signifying a high level of precision of the estimate, b) unbiasedness implying accuracy of the estimate or the closeness of the expected values and the true value of the estimate, and c) feasibility and cost effectiveness (Cochran 1977).

Components of a sampling technique

A sampling technique consists of the following (Steel and Torrie 1960), Snedecor and Cochran 1967, Ostle and Mensing 1975, Cochran 1977, Gomez and Gomez 1984):

1. Definition of the target population and choice of sampling unit

The sampling unit is the unit on which actual measurement is to be made. A good sampling unit must be easy to identify, easy to measure, and fairly uniform.

Examples

Target population	*Sampling unit*
Seeds in a seed lot	1 seed
Plants in a plot	1 hill
Disease severity in a plant	1 leaf
Rice yield in a plot	5-m^2 area

2. Sample size or the number of sampling units taken from tha population

Sample size is governed by the size of the variability among sampling units and the desired degree of precision of thc estimate. Sampling variation, the variability among sampling units, decreases as samplc size increases.

3. Sampling design or the method (of selecting the sample

To obtain a representative sample of the population, the principle of randomness should be applied. Some commonly used sampling designs applying this principle are:

■ *Simple random sampling.* Each unit in the population is given an equal chance of being selected into the sample.

Example. Let the population be a collection of seeds placed in a bag (or a seed lot). Each seed in the bag has an equal chance of being chosen if a sample of size 100 is drawn randomly from the bag.

■ *Multistage random sampling.* This is characterized by a series of sampling stages which involves several types of sampling units. The selection of the sample is done separately and independently at each stage of sampling.

Example. In a two-stage random sampling, let the population be the collection of all ricefields in a town. The attribute of interest is the average number of nematodes per hectare. The primary sampling unit is a field and the secondary sampling unit is a 200-ml soil sample. A sample of *n* fields is first selected and from each of these selectcd fields, a sample of *m* soil samples are taken.

■ *Stratified random sampling.* The population of N units is divided into strata of $N_1, N_2, \ldots\ldots N_k$ units and from each stratum N_i, sample size n_i is taken. Stratification is done to bring about a gain in precision in the estimates of attributes of a heterogeneous population. The heterogeneous population is divided into strata, each of which is internally homogeneous. The estimates of samples of each homogeneous stratum when combined give a more precise estimate of the population value.

Example. A field where discase intensities vary from one part of the field to another. The whole field is divided into k sub-areas, each sub-area representing a different level of disease intensity. All plants of each sub-area will be harvested separately. From the bulk seeds of each sub-area, *n* sample seeds will be taken for seed testing.

Sampling from binomial population (Steel and Torrie 1960, Snedecor and Cochran 1967)

There are sampling situations which allow only two possible outcomes. An example is the number of individuals showing the presence or absence of a qualitative characteristic. In assessing seed quality, it could be the number of seeds infected or not, diseased or not, discolored or not, viable or not, and so on. An estimate of a population proportion of a particular trait is often required. The parameter to be estimated by a confidence interval is the proportion of, say, infected seeds in the population. The parameter is generally denoted by p and its estimate, the observed

Table 4.1. Estimated sampling variation (x 10^{-4}) at varying sample sizes for different proportions of infected seeds.

Sample size (n)	True percentage of infected seeds in population				
	50	40	30	20	10
10	250.0	240.0	210.0	160.0	90.0
25	100.0	96.0	84.0	64.0	36.0
50	50.0	48.0	42.0	32.0	18.0
100	25.0	24.0	21.0	16.0	9.0
200	12.5	12.0	10.5	8.0	4.5
300	8.3	8.0	7.0	5.3	3.0
400	6.2	6.0	5.2	4.0	2.2
500	5.0	4.8	4.2	3.2	1.8

Table 4.2. Probability of not detecting infected seeds for different sample sizes and different true proportions of infected seeds.

Sample size (n)	True percentage of infected seeds[a]			
	1	2	5	10
10	0.90	0.82	0.60	0.35
25	0.78	0.60	0.28	0.07
50	0.61	0.36	0.08	0.01
100	0.37	0.13	0.01	0.00
200	0.13	0.02	0.00	0.00
300	0.05	0.00	0.00	0.00
400	0.02	0.00	0.00	0.00
500	0.01	0.00	0.00	0.00
1000	0.00	0.00	0.00	0.00

[a] 0.00 is given in table if actual probability is less than 0.005.

Table 4.3. The values of 2SD for varying values of sample size and true proportion of infected seeds.[a]

Sample size (n)	True percentage of infected seeds in population								
	10	15	20	25	30	35	40	45	50
10	0.19	0.23	0.25	0.27	0.29	0.30	0.31	0.31	0.32
25	0.12	0.14	0.16	0.17	0.18	0.19	0.20	0.20	0.20
50	0.08	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14
100	0.06	0.07	0.08	0.09	0.09	0.10	0.10	0.10	0.10
200	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07
300	0.03	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.05
400	0.03	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05
500	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04
1000	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03

[a] Applicable when estimating the proportion p of infected seeds.

proportion, by $\hat{p}$. Any estimate of the proportion of infected seeds can vary from the true proportion due to sampling variation. The estimated sampling variation based on the formula $\hat{p}\ (1-\hat{p})/n$ at varying sample size (n) and proportion of infected seeds is computed and shown in Table 4.1. As sample size increases, sampling variation decreases. As the true proportion of infected seeds approaches 50%, sampling variation increases. When the true proportion of infected seeds is relatively low (i.e., <10%), the chance of detecting any infected seeds increases with increasing sample size (Table 4.2).

Some researchers report the estimated proportion $\hat{p}$ with a ± SD or ±2SD following it, where SD stands for standard deviation which is the square root of the sampling variation. The limits $\hat{p} - 2SD$ and $\hat{p} + 2SD$ are meaningful in the sense that approximately 95% of the time, the true proportion of infected seeds will fall within these two limits. Since in practice, the standard deviation is unknown, it is estimated from the sample data via the formula

$$SD = \sqrt{\hat{p}\,(1-\hat{p})/n}$$

where $\hat{p}$ is the proportion of infected seeds in the sample. The estimated values of 2SD for varying sample sizes and true proportion of infected seeds are shown in Table 4.3.

Application of sampling in seed testing

Appropriate sampling techniques in seed testing have not yet been developed. Studies have to be undertaken in order to prescribe the appropriate size of sampling unit, the number of samples to be taken, and the sampling design to be used in seed testing. Preliminary studies are being done but results so far obtained are not yet enough to recommend the appropriate sampling procedures to be employed in seed testing. Further studies still have to be done.

Meanwhile, the International Seed Testing Association (ISTA) has come up with some guidelines on the sampling procedures used in international seed testing. Excerpts of these procedures from the ISTA Rule Book (1985) are presented below, and the appropriate rule numbers are stated in parenthesis.

Definitions (Rule 2.2)

- ***Lot***—a specified quantity of seeds, physically identifiable, in respect of which an International Analysis Certificate may be issued.
- ***Primary sample***—a small portion taken from one point in the lot.
- ***Composite sample***—formed by combining and mixing all the primary samples taken from the lot.
- ***Submitted sample***—a sample submitted to the testing station. It must be of at least the size specified in Rule 2.6.3 and may comprise either the whole or a subsample of the composite sample.
- ***Working sample***—subsample taken from the submitted sample in the laboratory, on which one of the quality tests in these Rules is made.
- ***Subsample***—the portion of a sample obtained by reducing the sample using one of the sampling methods prescribed in Rules 2.6.6 and 2.7.2.

Objective (Rule 2.1.a)

The quantity of seed tested in the laboratory is minute compared with the size of the seed lot which it is intended to represent. To obtain uniform and accurate results in seed testing, it is essential that the primary, composite, and submitted samples be taken and prepared with care and in accordance with the methods prescribed in the rules of sampling. No matter how accurate the laboratory work is done, the results can only show the quality of the sample submitted for analysis; consequently, every effort must be exerted to ensure that the samples sent to the seed testing station accurately represent the composition of the seed lot in question, Likewise, in reducing the sample in the laboratory, every effort must be made to obtain a working sample that is representative of the sample submitted.

Procedures for sampling the lot (Rule 2.6)

Sampling shall be carried out only by persons trained and experienced in seed sampling (Rule 2.6.1).

1. Sample intensity (Rule 2.6.2)

a) For seed lots in bags (or other containers of similar capacity that are uniform in size), the following sampling intensity shall be regarded as the minimum requirement:

Up to 5 containers: Sample each container and always take at least five primary samples.

6-30 containers: Sample five containers or at least one in every three containers, whichever is the greater.

31-400 containers: Sample 10 containers or at least one in every five containers, whichever is the greater.

401 or more containers: Sample 80 containers or at least one in every seven containers, whichever is the greater.

b) Sampling intensity for small containers (Rule 2.6.2.a)

If the seed is in small containers such as tins, cartons, or packets as used in the retail trade, the following procedure is recommended:

A 100 kg weight of seed is taken as the basic unit and the small containers are combined to form sampling units not exceeding this weight, e.g., 20 containers of 5 kg, 33 containers of 3 kg, or 100 containers of 1 kg. For sampling purposes, each unit is regarded as one 'container' and the sampling intensity above is applied.

2. Instruments and methods (Rule 2.6.4.a.)

a) The sleeve-type trier (grain probe) (Fig. 4.1) is the instrument most commonly employed for sampling large lots. It consists of a hollow brass tube inside a closely fitting outer shell or sleeve with a

solid pointed end. The tube and sleeve have open slots in their walls. When the slots are alined, seeds drop into the cavity of the tube. To close the slots, turn the tube. Tube length and diameter depend on the size of the container and kind of the seed to be sampled.

The trier can be used horizontally for vertically. A vertical trier should be partitioned to assure even sampling through the layers.

Insert the closed trier diagonally into the bag or container. Alter insertion, agitate it gently, and open and close it several times to ensure filling. Finally, close carefully before drawing out the sample.

b) The Nobbe trier (Fig. 4.2a) is suitable for sampling seed in bags but not in bulk. It is constructed in varying dimensions depending on the kinds of seeds to be sampled. The Nobbe trier is a pointed tube, long enough to reach the center of the bag, with an oval hole near the pointed end, The instrument should be approximately 500 mm long, including the handle (about 100 mm long) and point (about 60 mm), leaving about 340 mm to penetrate the bag. This should reach the center of all types of bags. For cereals, the internal diameter of the tube should be about 14 mm. For clovers and similar seeds, 10 mm is sufficient.

Insert the trier gently inlo the bag (Fig. 4.2b), pointing upward at an angle of about 30° to the horizontal, with the hole facing downward, until it reaches the center of the bag. Rotate the trier 180°, bringing the hole on top. Withdraw the trier with decreasing speed so the quantity of seed obtained from successive locations increases progressively from the center to the side of the bag. If, howerver, the trier is long enough to penetrate to the opposite side of the bag, withdraw it at a relatively constant speed. During withdrawal, the trier should be gently agitated to maintain an even flow of seed. The more polished the inner surface of the trier, the more freely the seed will flow.

To sample the bottoms of standing bags, raise them off the floor and place them on top of other bags. The holes that the trier made in the bags may be closed by pushing the weave back together.

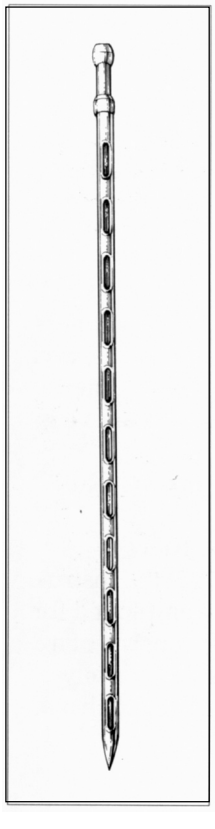
c) Sampling by hand is difficult, and should not be used for chaffy seeds like rice.

3. Taking primary samples (Rule 2.6.4)

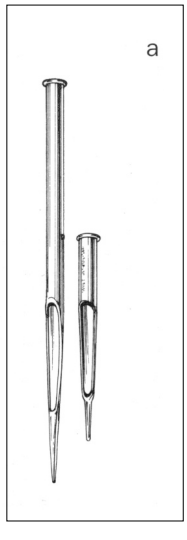
Primary samples of approxirnately equal size shall be taken from each container sampled. When the lot is in containers (including bags), the containers to be sampled shall be selected at random throughout the lot and primary samples drawn from top, middle, or bottom portions of the container but not necessarily from more than one position in any container. When the seed is in bulk or in large containers, the primary samples shall be drawn from random positions or depths.

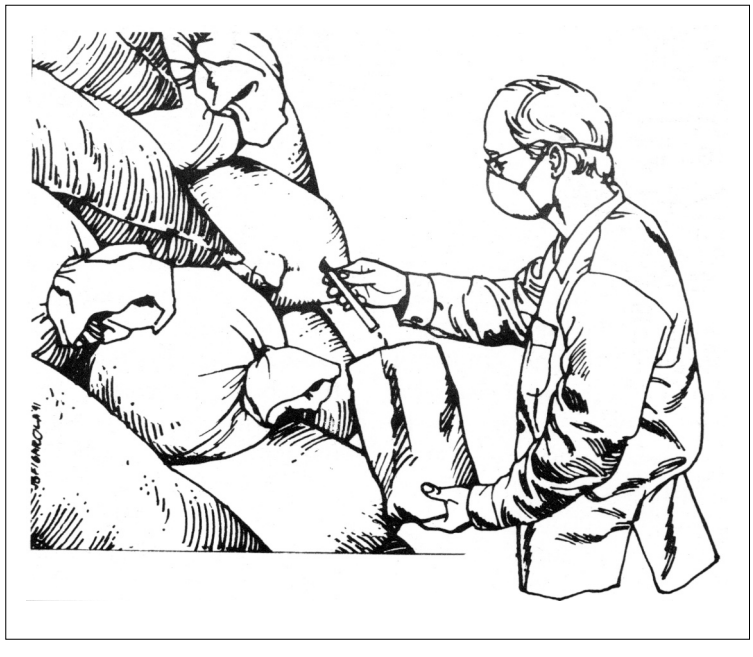
4. Obtaining the composite sample (Rule 2.6.5)

If the primary samples appear uniform, they shall be combined to form the composite sample.



4.1 A sleeve-type trier (grain probe).





4.2 a. Stick triers (Nobbe trier).
b. Inserting a stick trier in a sack of rice to draw seeds.

5. Obtaining the submitted sample (Rule 2.6.6)
The submitted sample shall be obtained by reducing the composite to an appropriate size by one of the methods referred to in Rule 2.7.2., using larger equipment if necessary.

If it is difficult to mix and reduce the sample properly under warehouse conditions, the entire composite sample shall be forwarded to the seed testing station for reduction.

If the composite sample is of appropriate size, it may be regarded as the submitted sample without reduction.

Additional samples requested not later than at the time of sampling, by the owner of the lot, if granted, shall be prepared in the same way as the submitted sample and marked 'Duplicate'.

6. Weight of submitted sample (Rule 2.6.3.)
For rice, thc submitted sample should be 400 g. If less, it should be indicated that 'The sample submitted weighs only ....g and is not in accordance with the International Rules for Seed Testing.'

Procedures in the laboratory

1. Obtaining the working sample (Rule 2.7.2.)
The submitted sample received by the seed testing station generally needs to be reduced to a working sample equal to or greater than the size prescribed for each test.

The submitted sample shall first be thoroughly mixed. The working sample shall then be obtained either by repeated halving or by abstracting and subsequently combining small random portions.

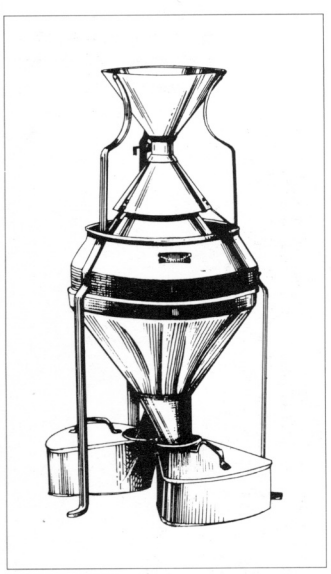
For routine seed health testing, 400 seeds are taken as working sample (Rule 7.4.3.a.3.).

2. Instruments and methods (Rule 2.7.2.a)

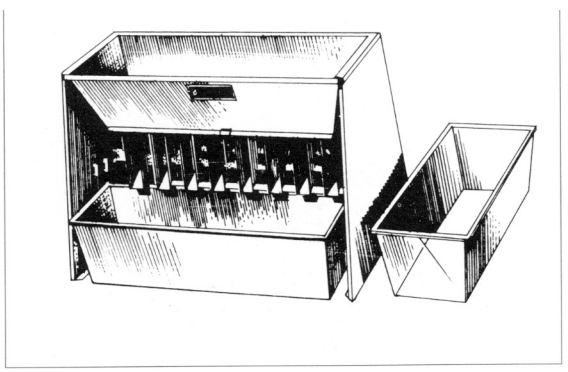
a) The mechanical divider method is suitable for all kinds of seeds except extremely chaffy types. The apparatus, a conical (Fig. 4.3), soil (Fig. 4.4), or centrifugal (Fig. 4.5) divider, splits samples into two approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the two parts and passing the whole sample through a second time, and, if necessary, a third time. The sample is reduced by passing the seed through repeatedly and removing one half on each occasion. This process of successive halving is continued until a sample of approximately, but not less than, the required size is obtained.

b) The modified halving method (Fig. 4.6). Thc apparatus comprises a tray into which fits a grid of equal-sized cubical cells, open at the top and every alternate one having no bottom. After preliminary mixing, the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. The submitted sample is successively halved until a working sample of approximately, but not less than, the required size is obtained.

c) The random cup method, the spoon method, and the hand halving method are not applicable to rice (ISTA 1985). ■

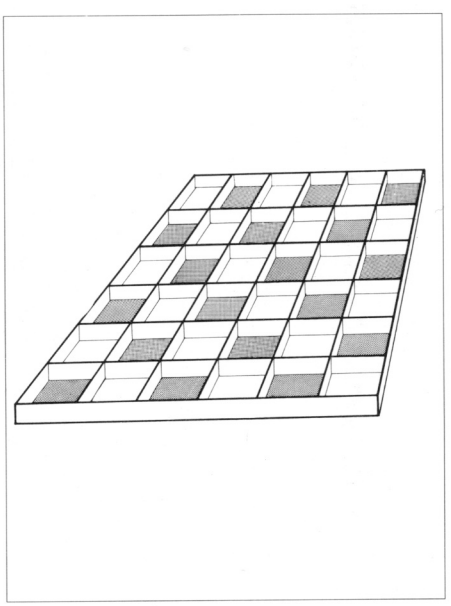


4.3 A conical divider (Boerner type).



4.4 A soil divider (Riffle type).

4.5 A centrifugal divider (Gamet type).



4.6 Sampling tray for the modified halving method.

CHAPTER 5

Dry seed inspection

J.K. Misra, C.C. Huelma, and T.W. Mew

Dry seed inspection detects smut balls (false smut); smutted seeds (kernel smut); diseased, discolored, or deformed seeds; germinated seeds; insect-damaged seeds; seeds of rice varieties other than that specified; weed seed contaminants; insects; mites; other plant parts; and inert materials such as soil.

The whole submitted sample should be visually inspected with or without a stereobinocular microscope or a magnifying lens (Fig. 3.2, 3.3).

Weeds

Most weed species have been, and still are, inadvertently disseminated between and within continents and countries as contaminants in traded seed (Horne 1953, Delouche 1988). Weeds of Philippine origin have been spread widely through exportation of seed of high-yielding rice varieties. In California, all the introduced species of rice weeds (except *Echinochloa crus-galli* (L.) P. Beauv. var. *crus-galli*) were brought into the state in rice seed (Fuller and Barbe 1983).

Weeds that mature at the same time as rice are harvested and threshed with the rice. Rice seed that is contaminated with weed seeds may introduce new species to a field or add to an existing weed population and is a source of weed perpetuation. In the southern United States, many weeds are spread almost entirely in rice seed (Smith et al 1977). Once planted, contaminated seed can result in weed management problems for many years (Rao and Moody 1990).

Admixture of weed seeds in marketable grain detracts from its quality and appearance, thereby lowering its grade and price. In Thailand, the price of rice drops by about one-half when the grain is contaminated with weed seeds (Suwunnamek 1986).

Important weed pests are listed in Chapter 12.

Insects

Insects are among the most important pests affecting seed health in the field, in transit, or in storage. Many stored grain pests are distributed worldwide.

Insect pests spread mainly through trade between countries and continents. They may be transported in packaging, cabins, etc. The maize weevil, lesser grain borer, Indian meal moth, flour moth, and other pests entered the USSR from the USA with imported maize stalks, French beans, and cacao beans from 1921 to 1923. Some insects acclimatized and spread further. The rice stem borer was introduced to Iran in 1977 with rice-straw wrapping on citrus cuttings imported from Japan (M.D. Pathak, 1988, pers. commun.).

"Some dangerous pests of stored grains like the khapra beetle, broad-nosed grain weevil, cowpea beetle, and flour-spotted bean weevil... were introduced into the USSR several times with imported cereals and legumes, but they were localized at insignificant points, thanks to timely quarantine measures" (Zakladnoi and Ratanova 1987).

Although not all newly introduced pests have damaged their new environments, some have wreaked great havoc.

Important insect pests are listed in Chapter 13. ■

CHAPTER 6

Fungi

J.K. Misra, S.D. Merca, and T.W. Mew

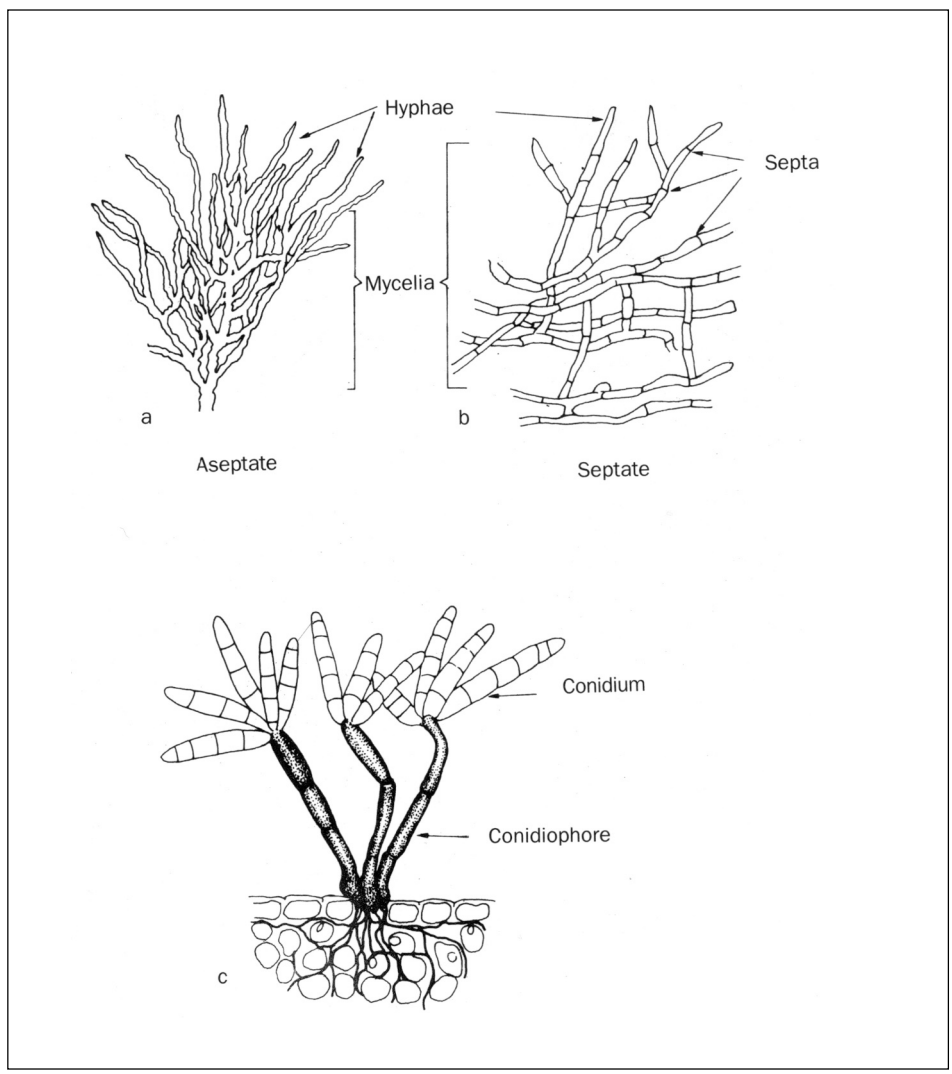
Fungi are a unique, bewildering, and fascinating group of organisms. "Biologists use the term fungus (pl. fungi; L. fungus = mushroom from Gr. sphongos = sponge) to include eukaryotic, spore-bearing, achlorophyllous organisms that generally reproduce sexually and asexually, and with usually filamentous, branched somatic structures that are typically surrounded by cell walls containing chitin or cellulose, or both of these substances, together with many other complex organic molecules" (Alexopoulos and Mims 1979).

Fungi are ubiquitous and play significant roles in our lives. They attack and destroy a variety of organic materials; cause fermentation; are used in the production of organic acids, vitamins, and medicines; and cause diseases. As saprophytes, they help recycle a variety of complex organic materials. As pathogens, they cause a number of destructive diseases. In rice, fungi are known to cause 55 diseases, 43 of which are seedborne or seed-transmittable (Ou 1985; Richardson 1979, 1981; Neergaard 1979). Tables 6.1 through 6.3 give the incidence of detection of seedborne fungal pathogens at IRRI's Seed Health Unit.

The thalloid body of fungi (Fig. 6.1a,b) lacks chlorophyll, the green plant pigment, and in most cases (except for the lower fungi and yeast), consists of branched, septate or aseptate, multinucleate microscopic threads or filaments called hyphae. A mass of hyphae forming the thallus is called a mycelium. At certain stages during the life cycles of higher fungi, mycelia form compact or loose tissues. Most fungi grow optimally between 20 and 30 °C. A few are thermophilic (high ternperature-loving) or psychrophilic (low temperature-loving). Light, while not necessary for growth, is required for spore production.

Fungi reproduce asexually by spores of various shapes, sizes, and colors. Conidial production (sporulation) occurs through specialized hyphae usually referred to as conidiophores or sporophores (Fig. 6.1c). The spores exhibit a variety of colors and shapes that help in species identification.

Sexual reproduction in fungi is described in Alexopoulos and Mims (1979), Webster (1970), and Moore-Landecker (1972).



6.1 The thalloid body of fungi showing aseptate (a) and septate hyphae (b), and conidiophores and conidia (c).

Detection

Blotter and agar plate methods are standard tests. The washing test reveals identifiable spores or mycelia adhering to or growing on the seed surface. Thc growing-on test is a supplementary test to detect readily transmittable seedborne pathogens.

Blotter method

General: This standard test is a simple and inexpensive way to detect seedborne fungi that respond to sporulation. During routine seed health testing at IRRI, this procedure satisfactorily detects important seedborne fungal pathogens. Data in Tables 6.1-6.3, indicate the varying frequencies of detection among some common rice pathogens from 1987 to 1990 at the Seed Health Unit.

Procedure:
1. Use 9.5-cm petri plates made of Pyrex glass or clear plastic to allow NUV light to penetrate. The plates should contain 2-3 layers of good-quality white or colored blotter papers moistened with distilled water (Fig. 6.2a).
2. Place seeds rom the working sample, (with or without pretreatment) equidistant on the petri plates at 25 seeds/plate (Fig. 6.2b).
3. Incubate seeds at 22 °C under a 12-h light and 12-h dark cycle with NUV light for 6-8 d (Fig. 6.2c). (See Chapter 3 for light source specification and other details.)

Results: Express results as a percentage of the number of total seeds.

Table 6.1 Percent incidence of seedborne pathogens on untreated outgoing seed lots received for certification by the IRRI Seed Health Unit, 1987-90.[a]

Pathogen	1987			1988		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	91.4	1-92	17.8	97.9	1-92	22.2
Curvularia spp.	88.1	1-69	6.8	93.1	1-79	12.6
Sarocladium oryzae	71.8	1-70	6.0	78.1	1-63	6.5
Microdochium oryzae	31.8	1-17	3.0	36.9	1-49	2.6
Fusarium moniliforme	11.3	1-5	1.1	12.0	1-13	1.3
Drechslera oryzae	19.3	1-16	1.4	23.1	1-28	1.9
Pyricularia oryzae	0.2	1	1.0	0.4	1	1.0
Tilletia barclayana	3.2	1-60	6.3	9.3	1-98	12.0
Aphelenchoides besseyi[b]	3.3	1-134	15.3	6.8	1-107	9.8

Pathogen	1989			1990		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	99.2	1-81	23.5	95.4	1-98	40.6
Curvularia spp.	92.2	1-71	8.1	88.5	1-79	7.9
Sarocladium oryzae	71.8	1-70	4.3	68.5	1-66	4.5
Microdochium oryzae	41.3	1-18	2.2	61.2	1-24	3.3
Fusarium moniliforme	14.4	1-19	1.5	32.5	1-17	1.6
Drechslera oryzae	25.1	1-25	1.4	18.5	1-14	1.7
Pyricularia oryzae	0.1	1	1.0	0.5	1	1.0
Tilletia barclayana	11.9	1-84	12.1	4.8	1-57	4.4
Aphelenchoides besseyi[b]	7.6	1-712	13.9	6.4	1-73	6.5

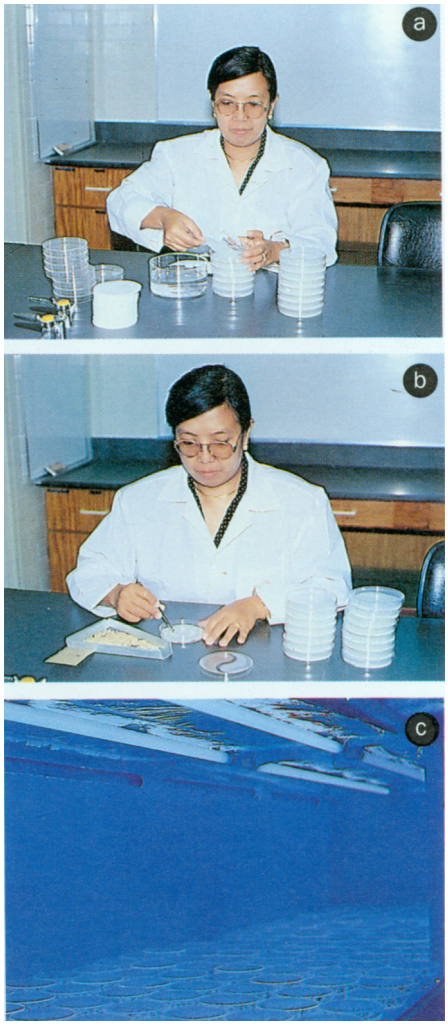
[a]Based on 200 seeds per seed lot. No. of seed lots examined: 1325 (1987), 1600 (1988), 1703 (1989), 2373 (1990). [b]Actual nematode count.

Table 6.2 Percent incidence of seedborne pathogens on untreated incoming seed lots received for postentry clearance by the IRRI Seed Health Unit, 1987-90.[a]

Pathogen	1987			1988		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	54.0	1-69	9.8	92.7	1-95	27.8
Curvularia spp.	78.7	1-783	12.5	91.0	1-75	11.3
Sarocladium oryzae	26.1	1-40	4.2	31.8	1-40	4.2
Microdochiurn oryzae	22.1	1-29	6.2	13.0	1-9	1.4
Fusarium moniliforme	10.7	1-4	1.3	12.3	1-13	1.5
Drechslera oryzae	46.3	1-41	7.9	73.7	1-88	6.9
Pyricularia oryzae	7.0	1-39	8.4	0.9	1-2	1.3
Tilletia barclayana	15.8	1-93	14.3	19.2	1-84	14.9
Aphelenchoides besseyi[b]	19.9	1-544	69.0	7.3	1-9	2.1

Pathogen	1989			1990		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	91.2	1-93	21.5	78.9	1-88	28.1
Curvularia spp.	83.2	1-59	12.3	88.9	1-80	7.2
Sarocladium oryzae	54.3	1-81	6.2	39.5	1-24	3.3
Microdochium oryzae	29.5	1-28	3.2	45.2	1-68	4.4
Fusarium moniliforme	25.2	1-25	2.2	28.0	1-10	1.7
Drechslera oryzae	81.0	1-72	9.8	64.0	1-52	6.7
Pyricularia oryzae	2.2	1-17	2.7	0.8	1-2	1.5
Tilletia barclayana	17.1	1-99	12.2	14.9	1-88	20.3
Aphelenchoides besseyi[b]	15.8	1-204	11.3	23.4	1-58	7.6

[a]Based on 200 seeds per seed lot. No. of seed lots examined: 272 (1987), 422 (1988), 457 (1989), 261 (1990). [b]Actual nematode count.



6.2 The blotter method.
a. Moistening blotters. b. Placing 25 seeds on blotters.
c. Incubating seeds.

Table 6.3 percent incidence of different seedborne pathogens on treated incoming seed lots received for postentry clearance by the IRRI Seed Health Unit, 1987-1990. [a]

Pathogen	1987			1988		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	33.3	1-44	10.5	17.5	1-40	8.0
Curvularia spp.	44.4	1-15	3.1	24.9	1-41	5.4
Sarocladium oryzae	10.2	1-27	6.3	4.6	1-11	2.1
Microdochium oryzae	9.3	1-14	5.0	0.9	1-3	2.0
Fusarium moniliforme	1.8	1	1.0	1.4	1	1.0
Drechslera oryzae	36.0	1-62	8.4	18.9	1-44	6.2
Pyricularia oryzae	0.0	0		0.0	0	
Tilletia barclayana	46.2	1-52	10.6	37.3	1-75	11.4
Aphelenchoides besseyi [b]	44.0	1-797	67.0	25.8	1-344	26.5

Pathogen	1989			1990		
	Affected seed lots	Detection levels	Mean value	Affected seed lots	Detection levels	Mean value
Alternaria padwickii	4.1	1-49	11.4	24.2	1-21	3.1
Curvularia spp.	30.3	1-10	1.6	44.7	1-66	10.8
Sarocladium oryzae	7.4	1-4	1.6	0.8	1	1.0
Microdochium oryzae	1.6	1-3	2.0	2.3	1	1.0
Fusarium moniliforme	0.0	0		1.5	1	1.0
Drechslera oryzae	14.8	1-12	1.9	35.6	1-31	2.5
Pyricularia oryzae	12.3	1-17	5.5	0.0	0	
Tilletia barclayana	98.4	1-89	24.4	41.7	1-83	18.0
Aphelenchoides besseyi [b]	27.0	1-84	15.8	31.1	1-54	10.9

[a] Based on 200 seeds per seed lot. No. of seed lots examined: 225 (1987), 217 (1988), 122 (1989), 132 (1990).
[b] Actual nematode count.

Agar plate method

General: Detects and identifies seedborne fungi through colony characteristics which they exhibit when grown on nutrient agar. Although data are not statistically conclusive, our experience indicates that common pathogens (including *Alternaria padwickii, Bipolaris oryzae, Sarocladium oryzae,* and *Microdochium oryzae*) are detected more easily using natural agar media than using synthetic ones. (See Appendix 2 for recipes.) We have worked with water agar, potato dextrose agar, potato sucrose agar, Czapek-Dox agar, guicol agar, polished rice agar, rice hull decoction agar, rice straw decoction agar, rice bran agar, and malt extract agar. Germination inhibitors such as 2,4 dichlorophenoxyacetic acid (an herbicide) can be used but IRRI achieved satisfactory results without the inhibitor. Plant debris and other inert matter can be similarly plated.

Procedure:
1. Take 400 seeds as working sample. Pretreat seeds with 1% sodium hypochlorite for 10 min.
2. Drain off excess liquid. Place seeds (10 seeds per agar plate) (Fig. 6.3) on either malt extract agar or potato dextrose agar in 9.5-cm petri dishes.
3. Incubate at 22 °C for 5-8 d, either under alternate cycles of NUV light and darkness, or in darkness.

Results: Examine plates for characteristic pathogen colonies, beginning on the third day and continuing through the eighth day of incubation. Also examine seeds under a stereobinocular microscope. View spores and other fungal structures under a compound microscope to distinguish the fungal forms. Express results as a percentage of seeds infected.

Washing test

General: Detects identifiable spores on seed surfaces.

Procedure: 1. In a convenient vessel (beaker or flask), place the working sample and add water, with or without a wetting agent, or alcohol. Shake vigorously to remove organisms adhering to the seed surface.

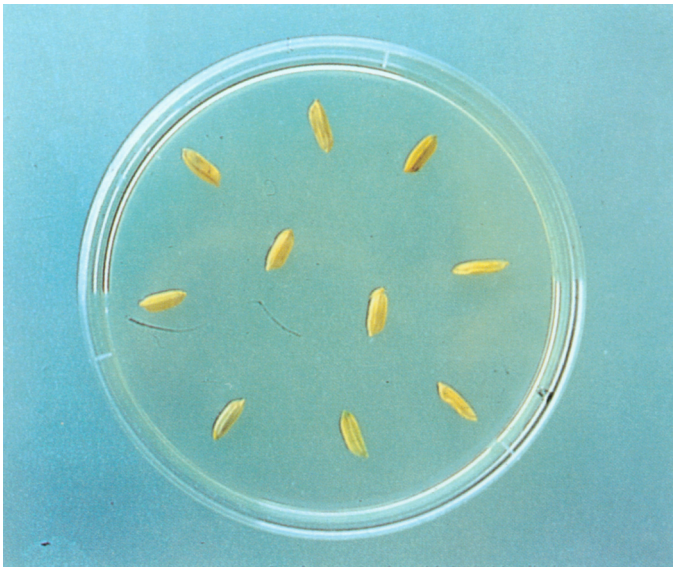
2. Transfer washings into centrifuge tubes and centrifuge for about 5 min at low speed (3000-5000 rpm).

3. Decant excess liquid from each centrifuge tube and examine extracted material under a compound microscope for fungal spores, hyphae, and nematodes.

4. Stain with lactophenol blue to color spores and hyphae and thus detect fungi more easily.

Results: Use a haemocytometer to count the number of spores.

6.3 Agar plate showing ten evenly spaced rice seeds.



Growing-on test

General: Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable.

Procedure: Sow seeds on a suitable medium (sterilized soil, sand, or water agar) under optimal conditions for germination in order to detect symptoms of infection that are not normally detected by other methods.

Isolation

Seedborne fungi can be conveniently isolated using standard mycological techniques.

Procedure: 1. Pick up actively growing mycelia or spores with a sterile needle under aseptic conditions.

2. Inoculate mycelia or spores on petri plates or slants containing the appropriate agar medium.

3. Incubate at 28 °C.

Identification

Identify fungi after viewing them under a stereobinocular microscope at different magnifications.

Procedure: 1. Prepare slide mounts of spores or other bodies in a drop of water or lactophenol blue and examine under a compound microscope for shape, size, and color.

2. Compare findings with those on pp. 75-89 and with literature available on fungal taxonomy (Booth 1977; Ellis 1971, 1976; Ou 1985; Raper and Fennell 1977; Raper and Thom 1984; Sutton 1980). ■

CHAPTER 7

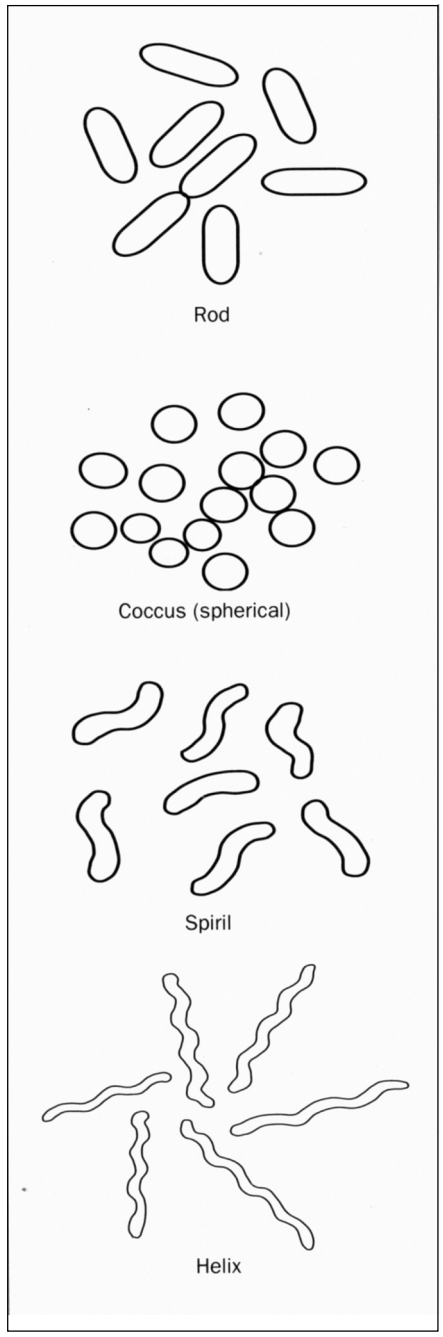
Bacteria

B. Cottyn, M.T. Cerez, and T.W. Mew

Bacteria (from bacterium, Greek, a small staff) are free-living unicellular organisms widely distributed in nature. They are prokaryotes—organisms with a single chromosome which is not enclosed in a nucleus. They possess a rigid multilayered cell wall that gives them a defined spherical-, rod-, helical-, or spiral-like shape (Fig. 7.1). Often the cells remain together in groups or clusters; for instance, it is very common for cocci or rods to occur in long chains. Bacteria reproduce asexually by binary fission. A bacterium growing on an agar surface forms a colony. Therefore, colony formation allows one to determine the number of bacteria in a culture. For instance, if 100 cells are plated, 100 colonies will appear. Bacteria can be grown in liquid or on solid growth media.

Bacterial rice pathogens

About 10 rice diseases are known to be caused by bacteria. The causal organisms are classified into three genera: *Erwinia, Pseudomonas,* and *Xanthomonas.* This manual discusses in detail only bacterial rice pathogens that occur commonly, are important for seed health, and are reported or suspected to be seedborne. Although a standardized, precise, and quick method of detecting, isolating, and characterizing bacterial rice pathogens has not yet been developed, some traditional methods described here can serve seed health testing purposes. Photographs of the species which concern this manual are given in Chapter 15.



7.1 Various Shapes of bacteria.

Detection tests are used to determine the presence of certain bacteria. If presence is indicated, the bacteria are then isolated. In fact, detection tests may serve as additional confirmation for identification tests, or as a first screening of unknown isolates to detect possible pathogens based on revealed symptoms or characteristics.

Erwinia species isolated from rice are commonly considered to be saprophytes although they may also act as opportunistic pathogens. Only two *Erwinia* spp. have been reported as rice pathogens: *E. herbicola* and *E. chrysanthemi, E. herbicola* was reported in Japan as the causal organism for palea browning, hence affecting rice grain quality, *E. chrysanthemi* causes foot rot which affects both the leaf sheath (causing sheath browning as in sheath rot) and the culm, and the root crown, resulting in severe decay at the foot of the rice plant. The disease has been reported in Japan (Goto 1979), Bangladesh, Korea, India, and the Philippines. However, this manual does not discuss the genus *Erwinia* further, since these pathogens have not been confirmed as seedborne. *E. chrysanthemi* is thought to be disseminated in irrigation water. The disseminating medium for *E. herbicola* remains a matter of controversy. It may or may not be seedborne.

This chapter will cover extraction and isolation, purification, identification, and detection of seedborne pathogenic *Pseudomonas* and *Xanthomonas* species. Recipes for all media, stains, and buffer preparations are supplied in Appendix 2.

Extraction and isolation

It is essential to be able to isolate one microorganism from all others and to maintain it in culture in a pure state. Only after a pure culture has been isolated can one proceed to study the organism's characteristics. (For schematic diagram of these processes, see Figure 7.2)

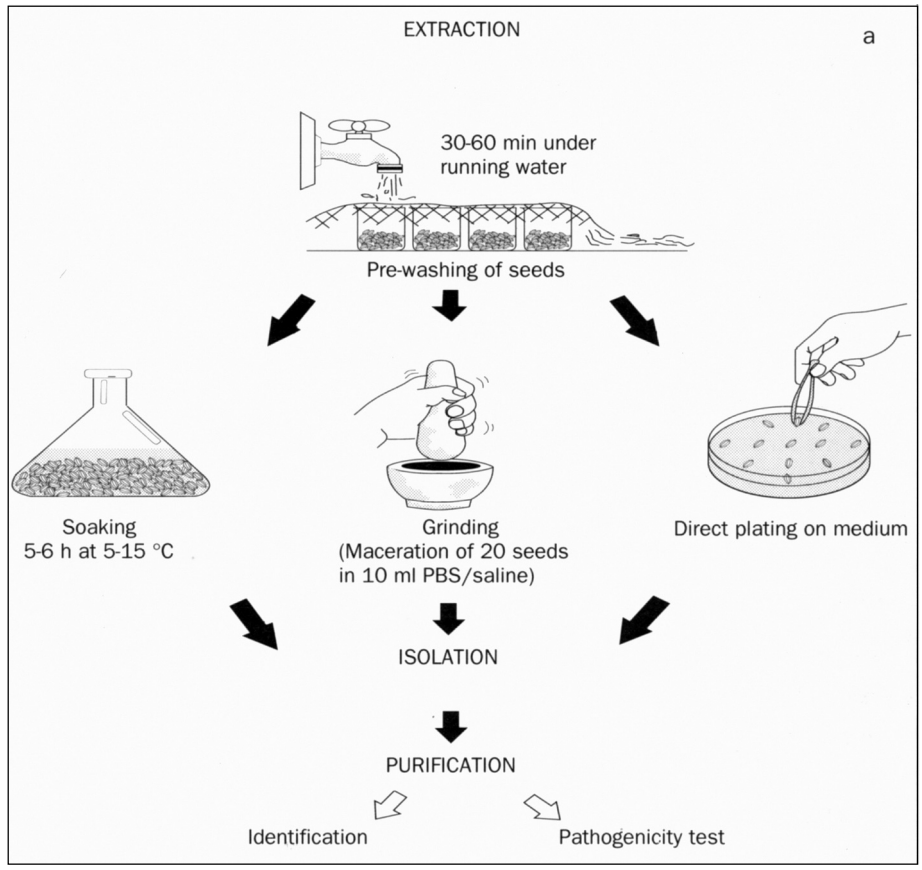
Pseudomonads

There are two main groups of phytopathogenic pseudomonads: the fluorescent group which produces a fluorescent pigment and the nonfluorescent group which does not.

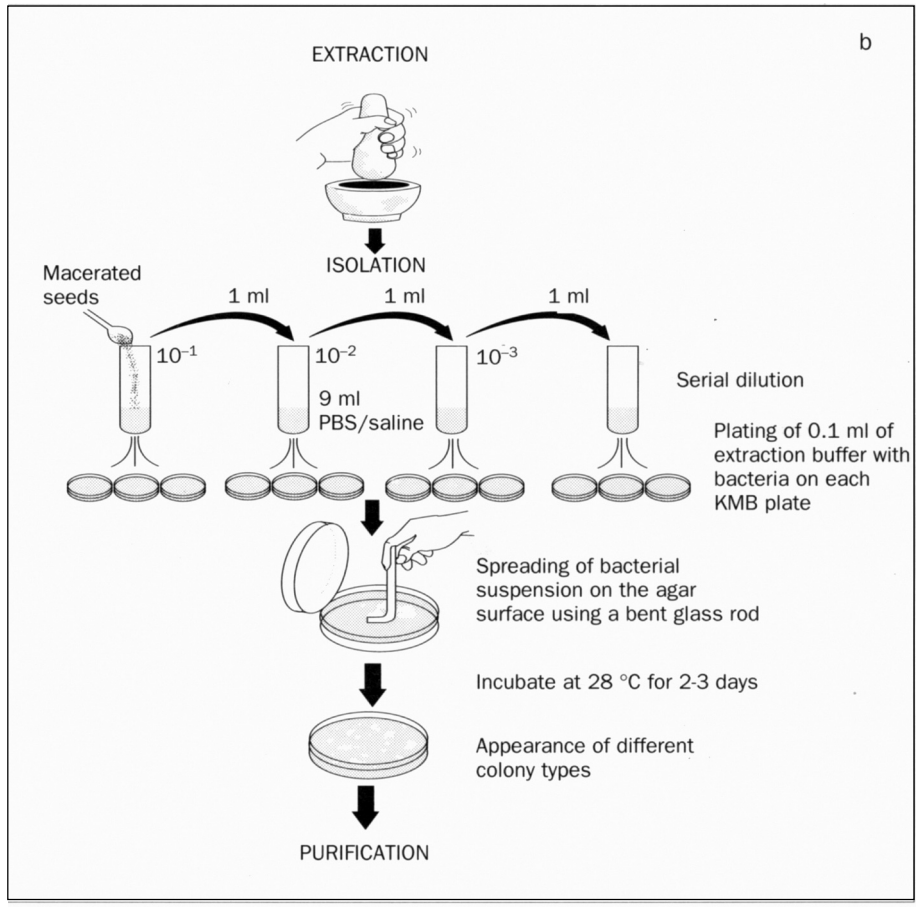
The most widely used medium for fluorescent pigment production is King's medium B (King et al 1954). Various other media and procedures for isolating *Pseudomonas* species can be found in the *Laboratory guide for identification of plant pathogenic bacteria* (Schaad 1990).

Seeds must often be prewashed to reduce debris and surface microflora, such as fungi, which otherwise will overgrow and interfere with the isolation of bacteria. To prewash, place beakers containing the different seed samples under running water for 30-60 min.

After prewashing, prepare sample extracts either by macerating the seeds or by soaking them for several hours at low temperature (5-15 °C) in phosphate buffer. The soaking method is usually used for large seed lots; for small-scale seed tests, grinding the seeds or plating them directly on medium is best. A sterile phosphate-buffered saline is the most commonly used extraction medium for both methods. Addition of detergents such as Tween 20



7.2a. Extraction of bacterial culture.

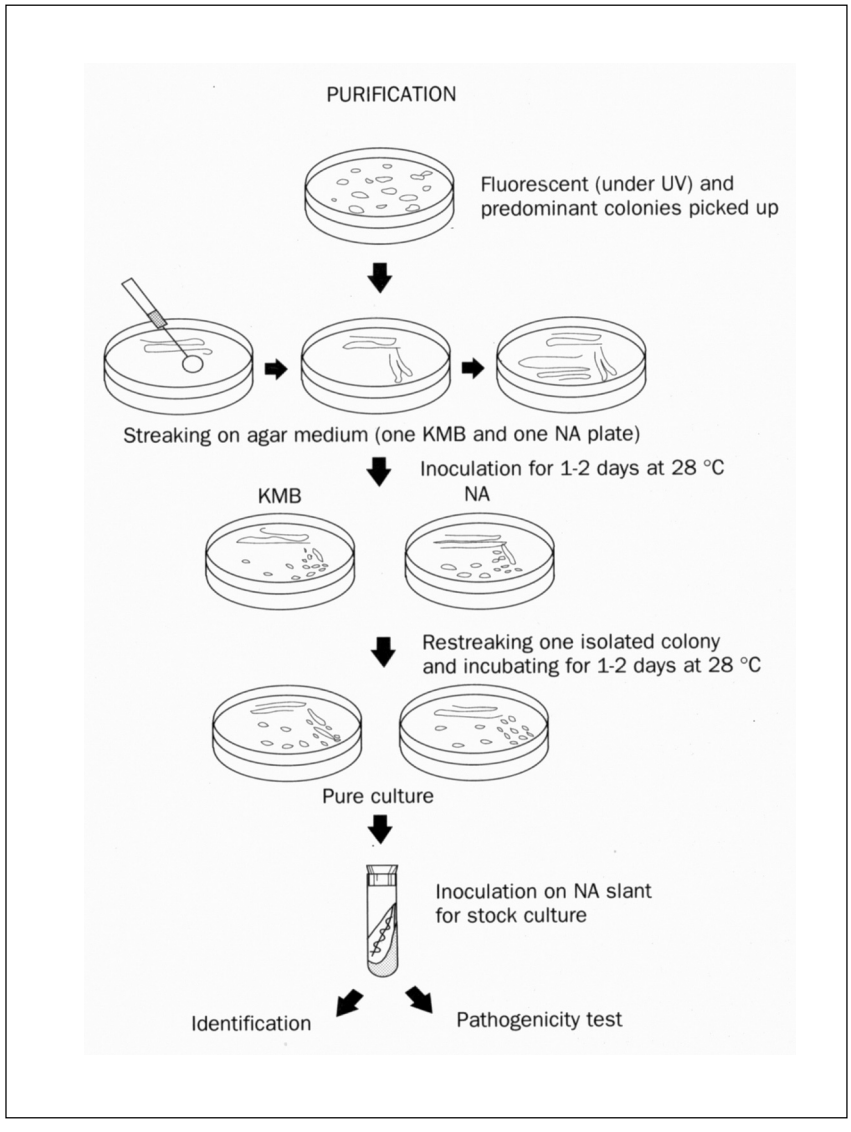


7.2b. Isolation of bacterial culture.

(0.001 %) to the extraction buffer can enhance the recovery of bacteria from the seeds, while addition of cycloheximide (0.01%) to the extraction buffer decreases fungal interference.

For the isolation step, 0.1 ml of undiluted extraction buffer and 10^{-1}, 10^{-2}, and 10^{-3} dilutions of the buffer are transferred onto 3 plates each of King's medium B. The 10^{-3} dilution is important because some seed lots can be contaminated with saprophytic or antagonistic bacteria which, when present in large numbers, might inhibit growth of some pathogenic pseudomonads. Bacterial suspension is spread on the surface of the agar, and the plates are then incubated.

Table 7.1 presents a grid of the morphological and biochemical characteristics that differentiate pathogenic pseudomonads.



7.2c. Purification of bacterial culture.

Table 7.1 Morphological and biochemical characteristics that differentiate pathogenic pseudomonads.

	P. syringae pv. *syringae*	*P. fuscovaginae*	*P. avenae*	*P. glumae*
Size	2.0-3.5 × 0.8-1.0 µm	0.5-0.8 × 2.0-3.5 µm	0.4-0.8 × 1.8-4.4 µm	0.5-0.7 × 1.5-2.5 µm
Fluorescence	+	+	–	–
Oxidase	–	+	+[a_b]	–
Oxidation-fermentation (O-F) test	0	0	0	0
Nitrate reduction	–	–	+	+[a_c]
2-ketogluconate production	–	–	–	–
ADH	–	+	–	–
Starch hydrolysis	–	+[e] +/–[f]	+/–	+/–[d] –
Growth on				
Inositol	+	–	–	+
Trehalose		+	–	

[a]Zeigler and Alvarez 1989. [b]Bradbury 1986, Bergey's manual 1986. [c]Bergey's manual 1986. [d]Bradbury 1986. [e]Miyajima et al 1983. [f]Rott and Notteghem 1989.

GRINDING METHOD (FOR SMALL-SCALE SEED TESTING)

General: The following is one procedure for small-scale seed testing using the grinding method.

Procedure:
1. Thoroughly crush 20 seeds in 10 ml sterile phosphate buffer using either a sterile pestle and mortar or an electric mixer.
2. Leave suspension resting at room temperature for 2 h (25-28 °C). This allows bacteria to multiply to a more detectable level. (Pathogenic bacteria might represent only 1% of the total microflora of the seed.)
3. Vortex the suspension for 5 min and transfer 0.1 ml of undiluted and 10^{-1}, 10^{-2}, and 10^{-3} dilutions onto 3 plates each of King's medium B. Plate by using an L-shaped glass rod while spinning the plate, so that the liquid is evenly spread on the agar surface.
4. Incubate plates at 28 °C far 2-3 d.
5. Examine plates under UV light for fluorescent *Pseudomonas.* Pick up and restreak all fluorescent and predominant nonfluorescent colonies on both nutrient agar and King's medium B for purification.

Table 7.2. Morphological and biochemical characteristics that differentiate *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola.*

	X. oryzae pv. *oryzae*	*X. oryzae* pv. *oryzicola*
Size	0.5-0.8 X 1.3-2.2 µm	0.4-0.6 X 1.0-2.5 µm
Oxidase test	–	–
Oxidation-fermentation (O-F) test	0	0
Nitrate reduction	–	–
2-ketogluconate production	–	–
Starch hydrolysis[a]	±	+
Carbon source utilization		
Trehalose	+	+
Inositol	–	–
Alanine	–	+
0.001% $CuNO_3$	+	–

[a] Bradbury (1986) registered Xoo as – and Xcola as +. Swings et al (1990) registered Xoo as +, + = positive, – = negative, 0 = oxidative.

Xanthomonads

Most species of the genus *Xanthomonas* are plant pathogens with a vast variety of plant hosts. Bacterial blight and bacterial leaf streak are two major rice diseases caused by *Xanthomonas* spp. Seed transmission of *X. oryzae* pv. *oryzicola* (Xcola) was confirmed by Goto et al (1988). Whether *X. oryzae* pv. *oryzae* (Xoo) is seed-transmitted remains a controversial issue despite intensive studies in both temperate and tropical countries (Mew 1989, Coto et al 1988).

Both pathogens are very closely related in many morphological, cultural, physiological, and biochemical characters. However, differentiation is possible by

- the symptoms they cause on rice,
- a few phenotypic features,
- polyacrylamide gel electrophoresis protein fingerprints,
- fatty acid profiles, and
- pathovar-specific monoclonal antibodies (Swings et al 1990).

Also, Xoo grows on media containing 0.001% $CuNO_3$ but not on media containing alanine as a sole carbon source, while Xcola grows on alanine but not on 0.001% $CuNO_3$ (Vera Cruz et al 1984).

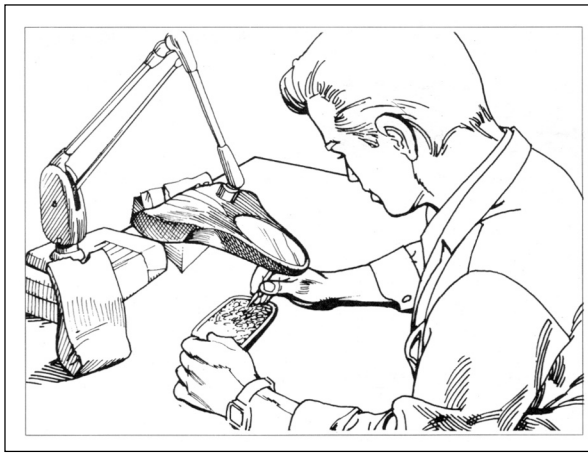
Table 7.2 presents, in grid form, the morphological and biochemical characteristics that differentiate Xoo from Xcola.

DIRECT ISOLATION FROM SEEDS COLLECTED FROM NATURALLY INFECTED FIELDS

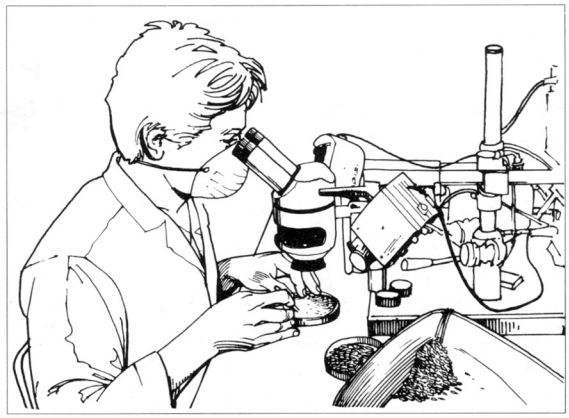
General: Infected seeds are ground into a fine powder and used for isolation. This is the same method described for the isolation of *Pseudomonas,* with a few modifications to make the method more suitable for isolation of *Xanthomonas.*

Procedure:
1. Use peptone sucrose agar (PSA) instead of King's medium B as the isolation medium.
2. Incubate plates at 28 °C for 3-5 d.
3. Resultant yellow colonies which appear after 48 h and which are similar to Xoo and Xcola (see Figs. 15.5a-15.6a, pp. 92-93) are picked up and restreaked on PSA plates.

Note: Direct isolation of Xoo and Xcola from seed is quite difficult because these bacteria grow slowly on laboratory isolation media. This brings about the significant problem of major overgrowth by a wide variety of fast-growing contaminants. Moreover, many of these contaminants, such



3.2 Dry seed inspection using a magnifying lens.



3.3 Dry seed inspection using a stereobinocular microscope.

Data from the tests should be recorded on appropriate recording sheets and signed by the responsible analyst. The names of fungi should be recorded according to the state they are found during testing. If conidia are found, the conidial epithet is recorded; if the perfect stage is detected, the perfect stage epithet is recorded; if both are present, both epithets are recorded. For uniformity in recording, the currently accepted epithet should be used. (See Appendix 1 for sample recording sheets for dry seed inspection, washing test, blotter and agar plate tests, and for *Aphelenchoides besseyi.).* ■

Table 3.2. Methods and equipment used in rice seed health testing.

Method	Preparation procedures	Detection/identificatlon tools			
		Hand lens	Stereobinocular microscope	Compound microscope	Others
Direct method					
For sclerotia, smut, malformed and moldy seeds		+	+	+	
Tilletia barclayana contamination			+	+	
Washing test	Shake by hand or use automatic shaker; then centrifuge at low speed			+	Haemocytometer Tally counter
Aphelenchoides besseyi sedimentation test	Use modified Baermann funnel		+	+	Tally counter
Indirect method					
Blotter test	In NUV incubation 21 °C, with timer 12 h NUV light/ 12 h darkness		+	+	Freezer for deep freezing method
Agar test for fungi and bacteria	Seed surface sterilization in table top incubators (28 °C)		+[a]	+[a]	Laminar flow cabinet
Test tube agar growing-on test	In growth chamber (28 °C) 12 h light/ day or on laboratory table top near window	+[b]			
Inoculation test	In growth chamber (28 °C) 12 h light or in glasshouse	+			Sterile sand/ soil, light
Other methods					
Serological	As required				

[a]To verify spores. [b]To magnify developing lesions.

flagellum. Cells occur singly, in pairs, or sometimes in chains.

Erwinia are Gram-negative straight rods, 0.5-1 µm × 1-3 µm, and possess 4-6 peritrichous flagella. Most cells occur singly. Endospores are not present in these genera.

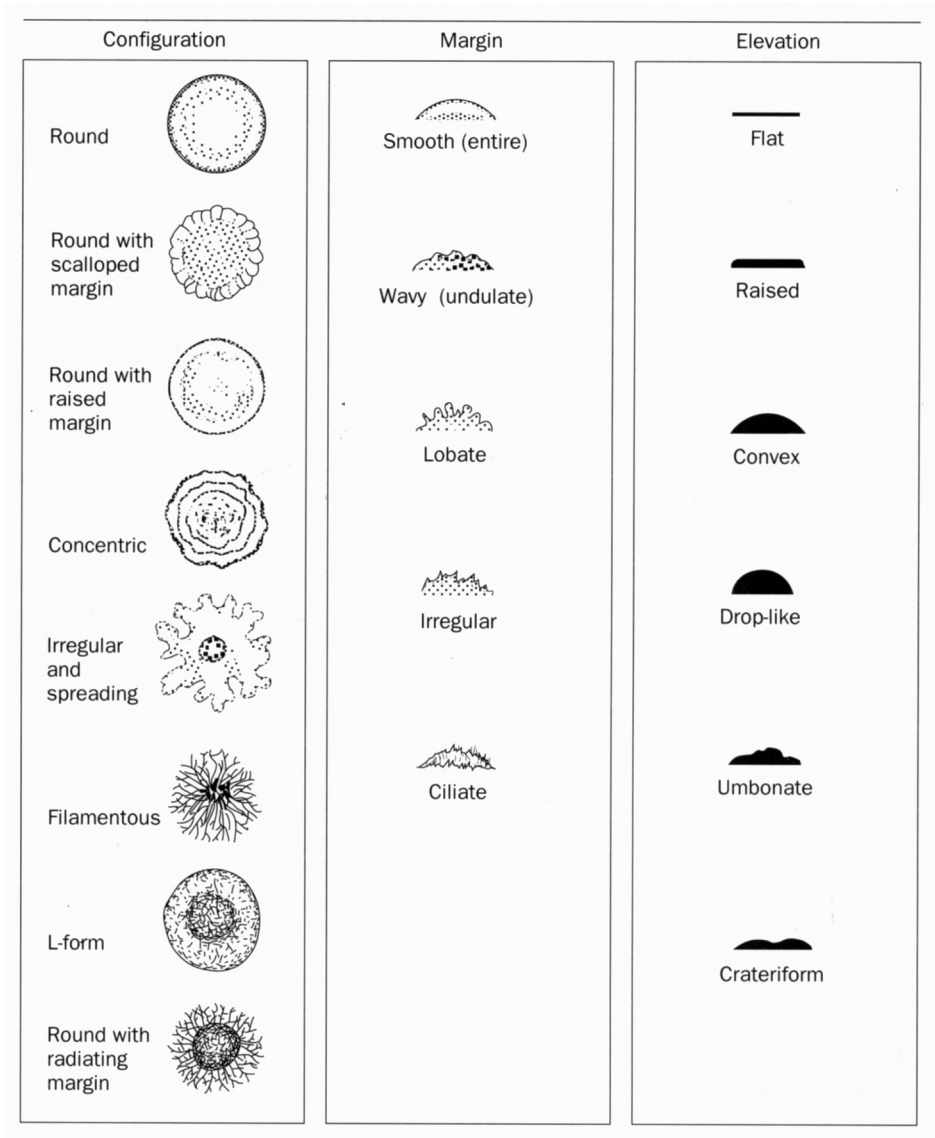
MOTILITY

Bacteria are motile if they possess flagella. Two types of flagellar arrangements exist: polar and peritrichous. A bacterium with a single flagellum or a group of flagulla at one or both poles of the cell is called polarly flagellated. Organisms with this flagellar arrangement move rapidly with a tumbling motion. Peritrichously flagellated bacteria have flagella attached around the surface of the cell. Such bacteria move more slowly, usually in a straight line, and rotate along the long axis. Motility is observed using a wet mount and preferably with a phase-contrast microscope. Only freshly grown cultures (grown overnight or for 1 d) should be examined since older bacterial cultures may be only weakly motile.

Procedure:
1. Place 1-2 drops of sterile physiological water (0.85% NaCl) on a clean glass slide.
2. Take a few bacteria by carefully dipping a sterile wire loop or sterile toothpick in a freshly grown colony and streaking them in the water on the glass slide.
3. Gently place a cover slip and observe motility under a 100X oil immersion objective.

COLONY CHARACTER

The form, elevation, and margin of bacterial colonies may help identify the type of bacterium (Fig. 7.3). In most cases, these properties lack diagnostic value unless the bacterium reveals a highly charactcristic colony type under certain conditions—e.g., on certain selective media.



7.3 Morphology of bacterial colonies.

General colony appearance of the described *Pseudomonas* species on nutrient agar is as follows (see also Figs. 15.la., 15.2a, 15.3a, and 15.4a.

■ *P. avenae* colonies are round, smooth, raised, chalk white, and glistening. Old colonies are sticky and adhere to the agar.

■ *P. fuscovaginae* colonies are round, smooth, raised, white to light brown, glistening, translucent, and 3-5 mm in diameter.

■ *P. glumae* colonies are round, smooth, raised, grayish white, and viscid.

■ *P. syringae* pv. syringae colonies are small, round, smooth, raised, whitish with a translucent margin which becomes undulate in older colonies.

Colony morphology of the described *Xanthomonas oryzae* pathovars on nutrient agar is as follows (see also Figs. 15.5a and l5.6a).

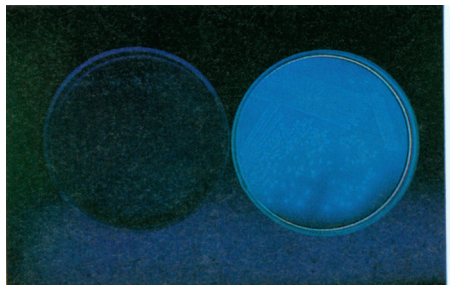
■ Xoo colonies are round, smooth, convex, butyrous, whitish yellow to straw yellow later, and opaque against transmitted light. The colonies appear as small dots on the 3rd or 4th d and reach 1-2 mm diameter on the 5th to 7th d—they grow slowly.

■ Xcola colonies arc round, smooth, convex, viscid, whitish to pale yellow later with maturity. The colonies reach 1 mm diameter in 3 d—they grow much more rapidly than Xoo.

PIGMENTATION

The two main groups of phytopathogcnic pseudomonads are the fluorescent group and the nonfluorescent group.

Some *Pseudomonas* species produce diffusible yellow-green pigments that are sometimes mistaken for fluorescent pigments. These can be distinguished by examining the cultures on solid media with ultraviolet light of short wavelength (254-360 nm) under which only the fluorescent pigments will fluoresce. The most widely used medium for fluorescent pigment production is King's medium B.



7.4 Fluorescence under UV light. L = without fluorescent pigments, R = with fluorescent pigments.

■ *P. fuscovaginae* and *P. syringae* pv. *syringae* belong to the fluorcscent group and hence produce a fluorescent pigment on King's medium B (Fig. 7.4).

■ *P. avenae* and *P. glumae* belong to the nonfluorescent group. (*P. glumae* occasionally produces a diffusible yellow-greenish, nonfluorescent pigment.)

Yellow pigments are found in both Xoo and Xcola (as well as in *Erwinia herbicola* which is frequently isolated from rice). The yellow pigments from *Xanthomonas* have been given the common name *xanthomonadins* and are insoluble in water.

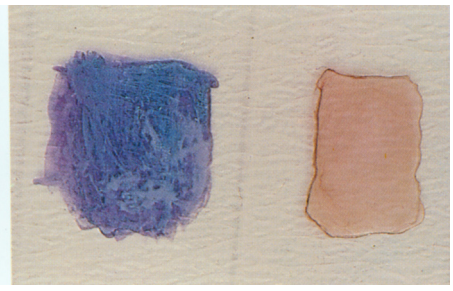
Biochemical characteristics

GRAM STAINING

General: This stain is essential for differentiating bacteria in two broad groups: Gram-positive and Gram-negative. Gram-staining characteristics are related to structural and chemical properties of the cell wall. These characteristics are basic to the initial Identification of plant pathogenic bacteria.

Procedure:
1. On a clean slide, thinly spread a bacterial film (a few bacteria spread in a drop of sterile distilled water on the glass slide). Dry in air for a few minutes. Then lightly flame the underside of the slide to fix the bacteria to it.
2. Flood the smear with crystal violet solution for 1 min.
3. Wash in tap water a few seconds. Drain off excess water.
4. Flood the smear with iodine solution for 1 min.
5. Wash in tap water a few seconds.
6. Decolorize (about 30 s) with solvent (e.g., acetone-alcohol decolorizer) until the solvent flows colorless from the slide.
7. Rinse in tap water for about 2 s.
8. Counterstain for 3 min with safranine solution.
9. Wash briefly in tap water. Dry in the air and examine under the microscope.

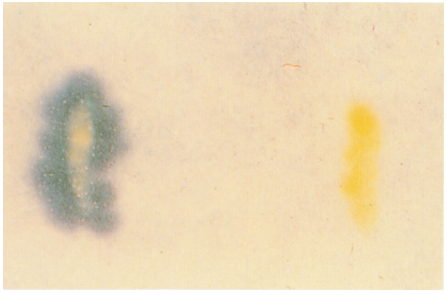
Results: Gram-positive bacteria appear purple to blue-black. Gram-negative bacteria appear red (Fig. 7.5).



7.5 Gram staining. L = color of Gram-positive reaction, R = color of Gram-negative reaction.

OXIDASE TEST

General: This test determines the presence of cytochrome c (oxidase enzymes of the respiratory chain) and is positive only for bacteria containing cytochrome c as a respiratory enzyme. Oxidase-positive organisms



7.6 Oxidase test reaction. L = color of an oxidase positive reaction, R = oxidase negative reaction: no color around the streak of yellow pigmented bacteria.

are usually either aerobes or facultative anaerobes.

Obligate anaerobic organisms lack oxidase activity since they are unable to live in the presence of atmospheric oxygen and do not possess a cytochrome oxidase system.

Procedure: 1. Place a piece of Whatman No. 1 filter paper in a petri dish.

2. To the paper, add some freshly prepared Kovacs' oxidase reagent (1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride).

3. With a platinum wire inoculating needle, smear a colony onto the reagent-impregnated paper in a line 2-3 cm long.

Results: A purple color (positive) occurs within 5-10 s if the organism is oxidase-positive—an anaerobe or a facultative anaerobe (Fig. 7.6). *P. avenae* may be positive or negative, *P. fuscovaginae* are oxidase-positive, and *P. syringae* pv. *syringae* and *P. glumae* are negative.

Oxidation-fermentation (O-F)—The Two Tubes Test

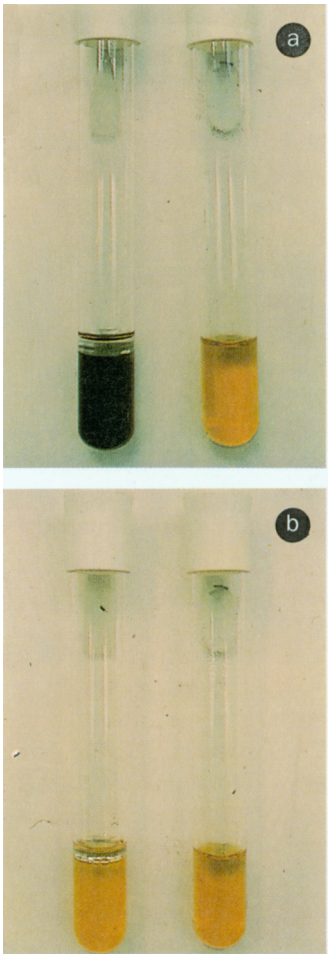
General: The Hugh and Leifson O-F basal medium is used to differentiate fermentative from oxidative metabolism of carbohydrates. Glucose is commonly employed in the O-F basal medium; however, there are times when an organism being tested is unable to metabolize glucose, yet can utilize other carbohydrates.

Fermentation is an anaerobic process and bacteria that ferment carbohydrates are usually facultative anaerobes—they can grow and reproduce under either aerobic or anaerobic conditions.

Oxidation of glucose is an aerobic process and bacteria that oxidize carbohydrates are usually strictly aerobic.

Procedure: 1. For each organism tested, inoculate a pair of O-F tubes with a small loop containing cells from a young culture.

2. Set up control sets—one inoculated set with no carbohydrate added and one uninoculated set with carbohydrate. Include a positive control by inoculating a well-known fermentative organism such as *Escherichia coli.*



7.7 Oxidation-fermentation test. a. Results of oxidation. b. Results of fermentation.

3. To detect fermentation, cover the medium in one tube with sterile mineral oil to a depth of 1-2 cm to exclude all oxygen.

4. Incubate at 28 °C for 48 h or longer.

Results: Observe for production of acid. If present, acid will cause a yellow discoloration in the medium because of pH change.

Oxidative organisms, such as *Pseudomonas* and *Xanthomonas,* produce acid reactions (resulting from the breakdown of glucose) in the uncovered medium only.

Lack of acid production in both tubes indicates that the organism is either unable to catabolize the glucose or the medium is unsuitable for growth of the organism.

Yellow color in the uncovered tube alone indicates oxidation (Fig. 7.7a); yellow color in both tubes indicates fermentation (Fig. 7.7b), and no color change in both tubes or a blue color (denoting alkaline reaction) indicates that neither oxidation nor fermentation took place.

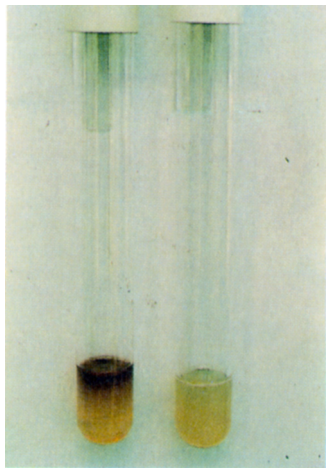
Note: Of the organisms under discussion, *Erwinia* is the only fermentative one; both *Pseudomonas* and *Xanthomonas* are oxidizers.

NITRATE REDUCTION TEST

General: This test determines the ability of an organism to reduce nitrate to nitrite, which in turn can be further reduced to free nitrogen gas. The process is an anaerobic respiration whereby an organism is able to derive its oxygen from nitrate. The reduction of nitrate is denoted by the development of color, as a colored compound is formed by the reaction of nitrite with the two added reagents (sulfanilic acid and dimethyl-α-naphthylamine).

Procedure A:

1. Prepare potassium nitrate agar (semisolid) and place 5 ml portions in each tube (one tube per isolate).
2. Autoclave tubes containing the medium and cool before use or refrigerate for storage.
3. Stab-inoculate tubes using a wire loop with an 18-24-h-old pure colony grown from a nutrient agar plate.



7.8 Nitrate reduction. L = red color shows nitrate reduction, R = no color change means no nitrate has been reduced.

4. Set up control tubes.

   Negative control: Incubate uninoculated control tube and test with reagents in conjunction with inoculated tubes to determine if the initial medium is nitrite-free.

   Positive control: Inoculate one tube with a known nitrate positive organism (e.g., *Escherichia colt*) and test to determine if the medium and reagents are functioning properly.
5. Incubate the tubes at 28 °C for 48 h.
6. Add nitrate reagents to an incubated nitrate agar tube: 5 drops of reagent A (0.6% dimethyl-a -naphthylamine) together with 5 drops of reagent B (0.8% sulfanilic acid) both dissolved in 30% 5 N acetic acid.

Results A:

1. If a pink to deep red color develops within 1·2 min, the test is positive (Fig. 7.8). The organism reduced nitrate to nitrite. The test is completed. *P. avenae* reduces nitrate; *P. glumae* may or may not reduce nitrate; *P. syringae, P. fuscovaginae,* and Xoo and Xcola do not reduce nitrate.
2. If no color develops, no nitrite is present in the medium. The result is negative, or the nitrate has not been reduced, or the nitrite has been further reduced to free nitrogen. Continue to procedure B to test for presence of unreduced nitrate.

Procedure 8: Zinc reduction. To the test tubes which did not develop color, add a pinch (approximately 20 mg) of nitrate-nitrite-free zinc dust.

Results B:

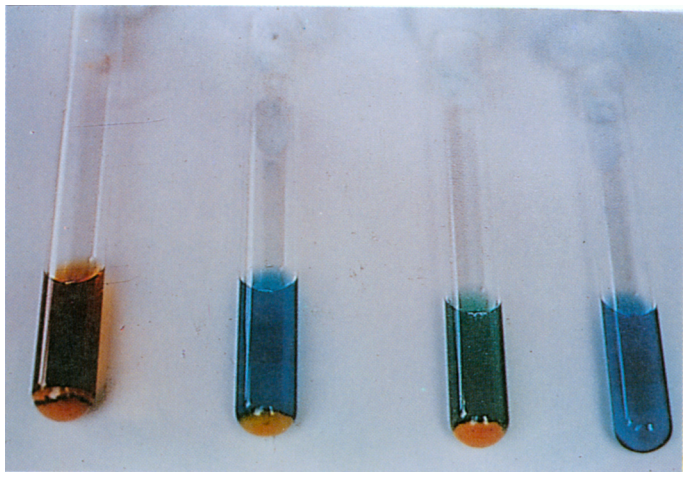
1. Positive if no color develops—nitrate is absent, and the organism reduced nitrate to nitrite and then further reduced nitrite to free nitrogen or other end products.
2. Negative if deep red color occurs within 5-10 min– the zinc reduced the nitrate, which was still present in the test tube, to nitrite. The organism did not reduce the nitrate.

PRODUCTION OF 2-KETOGLUCONATE

General: The ability of an organism to oxidize gluconic acid (potassium gluconate) as its sole carbon source can easily be determined by the formation of the reducing compound 2-ketogluconate. Since 2-ketogluconate is a reducing agent, it can be detected by adding cupric ions, which are present as copper sulfate in Benedict's reagent. The originally blue cupric ions change to a yellow or orange-red precipitate of cuprous oxide when reduced.

Procedure:

1. Prepare gluconate peptone broth and dispense 2.0 ml per tube (one tube per isolate).



7.9 Production of 2-ketogluconate. The yellow precipitates in the three tubes on the left show a positive reaction. The clear blue solution with no precipitate in the tube on the right indicates a negative reaction.

2. Include an uninoculated tube in the test as a negative control.
3. Autoclave the tubes together with the broth and let them cool before use.
4. Inoculate the broth heavily from an 18-24 h pure culture grown on nutrient agar.
5. Incubate the inoculated tubes at 28 °C for 48 h.
6. Add 1.0 ml of Benedict's reagent directly to the incubated gluconate tube. Mix the solution well and place the tube in a boiling water bath for 10 min.

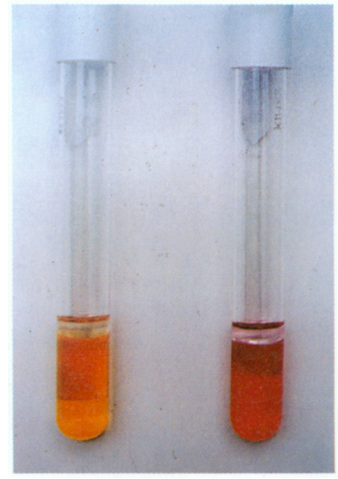
Results:
1. Positive—a yellow to orange-red precipitate indicates the presence of 2-ketogluconate as reducing substance (Fig. 7.9).
2. Negative—if there is no change, there is no precipitate. There is no reducing substance (2-ketogluconate) produced.
3. The uninoculated control tube should show negative results.

ARGININE DEHYDROLASE TEST

General: This test measures the ability of an organism to catabolize arginine. The arginine dehydrolase enzyme system couples arginine degradation to adenosine triphosphate (ATP) generation, thus permitting the organism to grow under anaerobic conditions. The bacterium to be studied is cultivated under anaerobic conditions by overlaying the surface of the medium with either paraffin or mineral oil. L-arginine breakdown involves: two enzymes: arginine desmidase which degrades arginine to citrulline + NH_3, and citrulline ureidase which converts citrulline to ornithine + CO_2 + NH_3. The process can be monitored by including a pH indicator (phenol red) since the pH of the medium rises (to alkalinity) as NH_3 is produced.

Procedure:
1. Prepare the arginine medium and dispense 3 ml volumes in test tubes (one tube per isolate).

   Include a control tube without arginine for each organism to be tested and an uninoculated tube with arginine.
2. Sterilize the tubes by autoclaving for 15 min and cool before use, or refrigerate for storage.
3. Stab-inoculate into the medium a freshly grown test organism (a culture grown for 18-24 h on nutrient broth).
4. Overlay all tubes with 2 ml of either sterile paraffin or sterile mineral oil to create an anaerobic condition.
5. Incubate the tubes for 3 d at 28 °C.



7.10 Arginine dehydrolase test. L = positive, R = negative for production of NH_3.

Results:
1. Positive (alkaline) if the medium turns red, due to production of NH_3. Only *P. fuscovaginae* produces NH_3 (Fig. 7.10).
2. Negative if there is no color change, indicating no degradation of arginine (Fig. 7.10).
3. The inoculated control tube without arginine and the uninoculated one with arginine should remain yellow (the initial color).

STARCH HYDROLYSIS TEST

General: The starch hydrolysis test assesses an organism's ability to hydrolyze starch by enzymatic activity. Starch is a homopolysaccharide composed of many a-D-glucose units. The basic structure of starch is a mixture of two polyglucose molecules:

linear amylose (10-20%) and branched amylopectin (80-90%).

Starch hydrolysis occurs by enzymatic action of a-amylase (also called endoamylase). This digestive enzyme attacks the interior of polysaccharide chains. Amylose is split completely into maltose and glucose units, while complete breakdown of amylopectin requires the additional presence of another enzyme, glucosidase. Partially digested starch molecules are called dextrins. The more the starch is hydrolyzed, the smaller the dextrins become.

Starch hydrolysis can be followed using an iodine reagent. As the enzymatic reaction proceeds (i.e., the large polysaccharides are split into smaller units), the color produced by iodine gradually changes from blue to purple to red-brown (partial hydrolysis) to no color (complete hydrolysis).

Procedure:
1. To assure that the reagents are effective, they should be tested on known starch-hydrolyzing bacteria (e.g., *Bacillus subtilis*) and nonstarch-hydrolyzing bacteria (e.g., *E. coli*).
2. Prepare starch medium as described in Appendix 2.
3. Inoculate the starch agar plates by streaking with an 18-24 h pure culture from nutrient agar.
4. Incubate the inoculated plates at 28 °C for 5 d.
5. Flood the incubated plates directly with the reagent (aqueous Lugol's iodine) and interpret the reaction immediately.

Results: *P. syringae* does not hydrolyze starch; Xcola does; *P. avenae, P. fuscovaginae, P. glumae,* and Xoo may or may not hydrolyze starch.

1. Positive if the medium is purple-blue with slight yellow or colorless zones (hydrolysis zones) around or under the bacterial growth. In this case, starch is completely hydrolyzed by the bacteria.
2. Negative if the medium and the area around the bacterial growth are purple blue. This indicates that starch is still present, and no hydrolysis occurred. The bacteria lack the ability to hydrolyze starch.
3. Partial hydrolysis has occurred if reddish-brown zones occur around growth, indicating the presence of dextrins. The bacteria are not able to complete starch hydrolysis.

CARBON SOURCE UTILIZATION

General: This procedure tests the ability of an organism to utilize certain sugars (e.g., trehalose and inositol, Tables 7.1 and 7.2 as a sole source of carbon. Utilization is demonstrated by the presence of growth.

The medium for the carbon source utilization tests comprises the inorganic minerals for growth and the sugar to be tested.

Procedure:
1. Prepare the Ayers et al mineral salts medium and sterilize by autoclaving at 121 °C for 15 min.
2. The carbon source (sugar) to be tested is filter-sterilized and added at 0.5% (wt/vol) final concentration to the autoclaved and cooled (45 °C) Ayers et al mineral salts medium. Prepare control plates without sugar.
3. Pour the medium in petri dishes (one plate per isolate) and let them solidify in a sterile flow-bench.
4. Streak freshly grown bacteria onto the medium and incubate at 28 °C for 3, 7, and 14 d.

Results: Record presence or absence of growth as an indication of the organism's ability to utilize the tested sugar. Compare growth in plates containing no added sugar.

P. syringae and *P. glumae* grow on inositol and not on trehalose; *P. fuscovaginae* grows only on trehalose; *P. avenae* does not grow on either.

Both Xoo and Xcola grow on trehalose but not on inositol; however, Xoo will grow on $CuNO_3$ but not on alanine, while Xcola will grow on alanine but not on $CuNO_3$.

Detection

Pathogenicity test

Pathogenicity tests have not been standardized. There are numerous applicable inoculation methods, including:

- wound inoculation by blade, scissors, or syringe;
- spraying the plants with a bacterial suspension;
- vacuum infiltration of bacteria into plant tissue; and
- wound inoculation by rubbing plant parts with a bacterial suspension.

All of these techniques have value, depending on the nature of the disease and purpose of the experiment.

Some principles stated in the *Laboratory guide for identification of plant pathogenic bacteria* (Schaad 1990), when making pathogenicity tests are quoted below:

1. Grow pathogen-free plants under conditions most favorable for their growth and which most closely approximate the conditions for disease development in the field.

2. Select an inoculation technique which most closely simulates the nature method of inoculation or infection..

3. Use relatively low dosages of bacteria in inoculation (10^3 to 10^5 colony-forming units(cfu)/ml) when spraying or infiltrating plants.

4. Symptoms obtained from plant inoculation should closely resemble those that occur in the field.

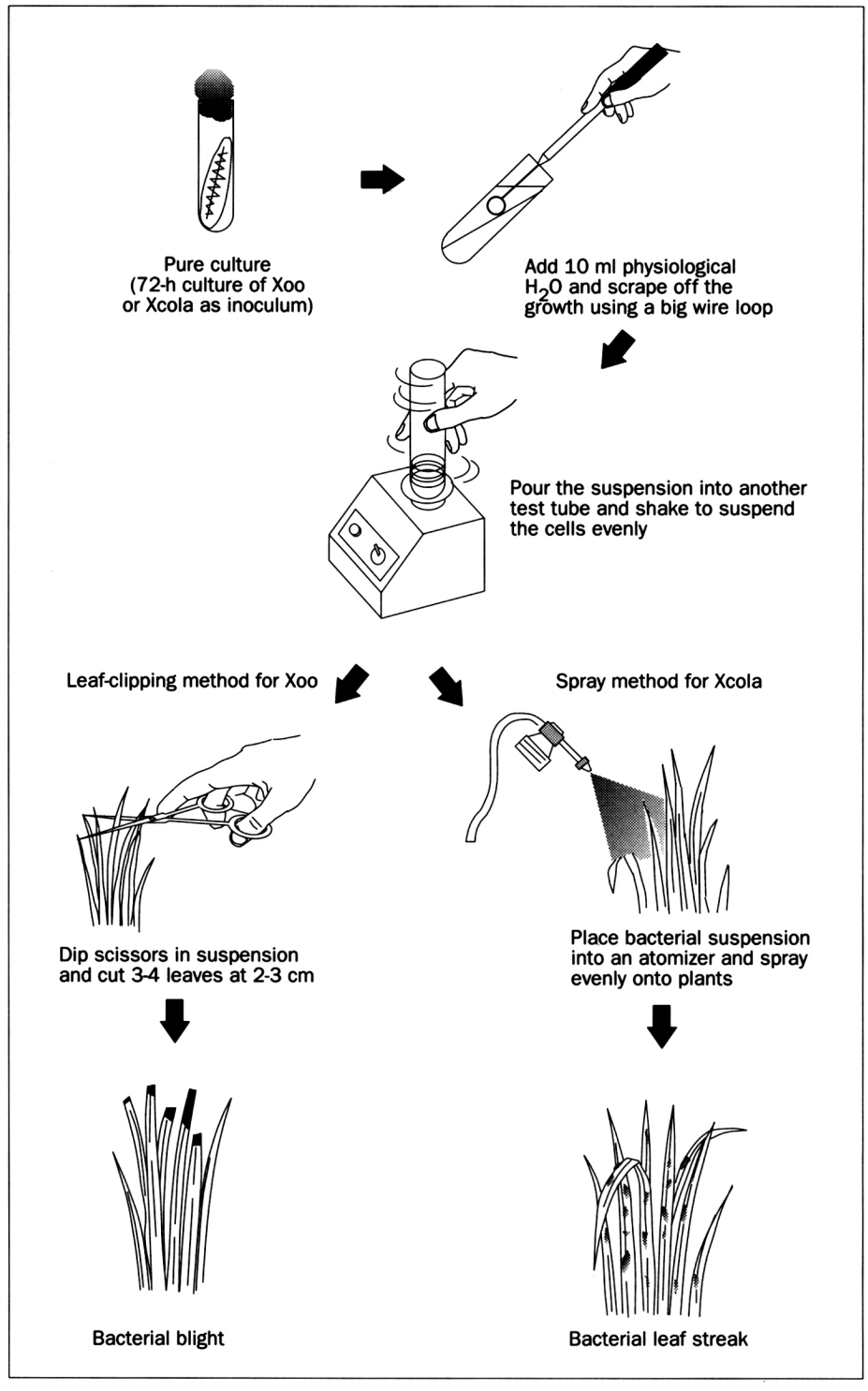
PSEUDOMONAS PATHOGENICITY TEST

General: The following is one method used at IRRI for detecting pathogenic *Pseudomonas* associated with rice seed. After isolating and purifying suspected bacteria from diseased seed samples, a pathogenicity test on seedlings is performed in the greenhouse.

Procedure: 1. *Plant preparation.* To clean the seeds, wash in 70% ethanol for 30 s and rinse three times in sterile distilled water. To germinate, place seeds on moistened filter paper in petri dishes and put in a growth chamber. After 1 wk, transplant seedlings to soil in plastic trays and put in a greenhouse.

2. *Preparation of inocula.* Inoculate the nutrient broth with seed isolates collected from nutrient agar slants and grow overnight. These nutrient broth cultures will be used to inoculate the seedlings.

3. *Inoculation by injection.* Inoculate 3- to 4-wk-old seedlings by injecting 0.3 ml bacterial suspension into the sheath. Inoculate 3 seedlings per isolate. Inoculate controls by injecting nutrient broth only.



7.11 Pathogenicity test for Xoo and Xcola on mature plants in the greenhouse.

Results: Disease symptoms are recorded 1 wk after inoculation. Carefully observe and note typical symptoms and the nature of the lesions. Due to the nature of the disease symptoms, presumptive evidence concerning the causal organism can sometimes be made. Nevertheless, the organism should be reisolated from any resulting lesions, subcultured, and identified (Koch's postulate).

Notes: Environmental conditions (temperature, relative humidity, presence of other interfering organisms, etc.) can have an important impact on disease development.

Symptoms caused by the *Pseudomonas* spp. discussed in this manual may be very similar, thus preventing reliable diagnosis by symptomatology alone. Nevertheless, pathogenicity testing is a useful tool to study disease symptoms and to detect the possible causal organism. Once an organism is suspected to be pathogenic, further characterization and identification must be made.

XANTHOMONAS PATHOGENICITY TEST (FOR MATURE PLANTS IN A GREENHOUSE)

General: A pathogenicity test is one of the most reliable methods for confirming bacterial identity from infected seeds or diseased seedlings. Some rice varieties are resistant to bacterial blight and bacterial leaf streak, thus it is important to use a susceptible variety when doing pathogenicity tests for these diseases.

Xoo infects the vascular tissues of leaves; thus all methods of artificial inoculation used for bacterial blight, such as clipping and pin-prick methods, are based on wounding the leaves and simultaneously introducing the bacteria into the vascular system. Both the pin-prick and clipping methods are effective but the latter is faster (Fig. 7.11).

Natural Xcola infection is believed to occur through the stomata of the rice leaves. Therefore, the spraying method has been proven effective for bacterial leaf streak inoculation. Spraying is convenient and gives more reliable results than methods that wound the leaves (Fig. 7.11).

Procedure A: *Preparation of inoculum*

1. Grow a pure bacterial culture in a slant of modified Wakimoto's medium for 72 h.
2. Pour 10 ml sterile physiological water (0.85% NaCl) into the culture slant and scrape the bacterial mass off with a sterile wire loop.
3. Suspend the cells evenly in a mixer. The resulting suspension gives approximately 10^{8}-10^{9} cfu/ml.

Procedure B: *Inoculation of plants*

1. *Inoculation for bacterial blight* (Xoo): Using the leaf-clipping method, inoculate susceptible varieties (e.g., IR24 or IR8) when they are 35-40 d old. Cut only fully expanded leaves, 2-3 cm from the tip, with a pair of sterile scissors dipped in bacterial suspension. To assure inoculation, clip only 3-4 leaves before dipping the scissors again in the inoculum. Label plants with date and isolate code, and keep in a greenhouse until scoring date.
2. *Inoculation for bacterial leaf streak* (Xcola): Place bacterial suspension, together with a drop of sticker (Tween 20), into an atomizer and spray evenly onto IR24 or IR50 plants. After spraying, plants should ap pear moistened, but the suspension should not be dripping from them. Label plants as above and keep in a greenhouse.

Procedure C: *Scoring. Xoo:* In practice, disease symptoms are recorded 2 wk after inoculation in case of screening for varietal resistance. Since this procedure aims to determine the identity of the organism, 1 wk after inoculation may be enough time to observe typical bacterial blight symptoms—wavy, elongated, water-soaked lesions.

Xcola: Bacterial leaf streak lesions appear about 10 d after inoculation (Fig. 15.6b,c, and d).

If no characteristic symptoms are produced, the isolates may be considered as saprophytes.

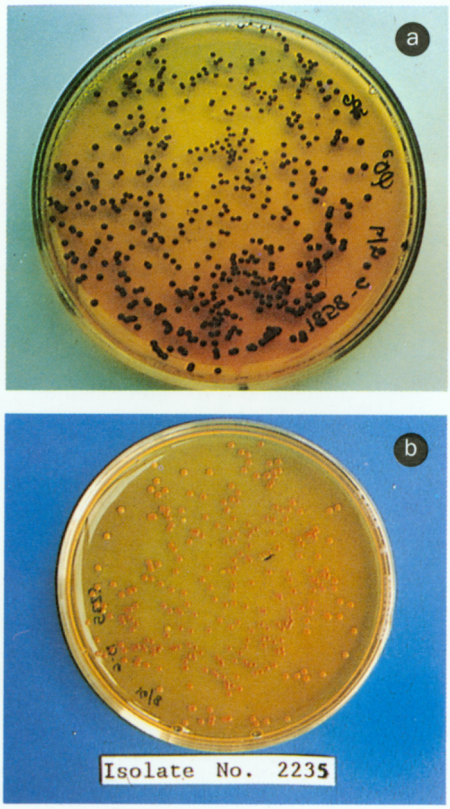
Reisolation and confirmation of virulence from the lesions are necessary to confirm identity of the pathogen.

Semiselective media

PSEUDOMONAS GLUMAE — TYPICAL COLONY FORMATION

General: A selective medium (S-PG) was developed by Tsushima et al (1986) and was used by Mogi (1988) to detect *P. glumae* in rice seed. On the S-PG medium, *P. glumae* forms two types of colonies (Fig. 7.12), depending on the isolate:

- Type A (Fig. 7.12a) colonies are round, smooth, convex, and reddish brown.
- Type B (Fig. 7.12b) colonies are round, smooth, convex, and opalescent purple.



7.12 a. *P. glumae* type A colony on S-PG medium. b. *P. glumae* type B colony on S-PG medium.

Other pseudomonads (fluorescent and nonfluorescent) may grow on this medium, but only *P. avenae* is reported to produce colonies similar to type B.

Procedure:
1. Prepare the S-PG medium.
2. Make serial dilutions of 10^{-2}, 10^{-4}, and 10^{-6} in sterile physiological water (0.85% NaCl in distilled water) from a culture grown for 12 h in nutrient broth.
3. Pipette 0.1 ml of the serial dilutions on 2 plates of S-PG medium. Spread the bacteria out over the medium with an L-shaped glass rod while rotating the plate.
4. Incubate the plates at 28 °C for 3 d.
5. Check the plates for colony formation typical of *P. glumae*.

Notes: At IRRI, *Xanthomonas* did not grow on S-PG, but all the nonfluorescent pseudomonads tested grew well on S-PG and showed type B colonies. Although their identity is not yet confirmed, the colonies might be *P. avenae* (found to produce colonies on S-PG medium similar to type B of *P. glumae*). Type A colonies are more conclusively characteristic.

Pseudomonas glumae—calcium oxalate crystal formation

Matsuda et al (1988) observed that when *P. glumae* was grown on potato peptone glucose agar (PPGA), a selected medium for *P. glumae* supplemented with 0.1% $CaCl_2$, characteristic crystals were produced in the colony. This appears to be useful for rapid detection of *P. glumae*. However, at IRRI we were unable to observe any crystals.

Pseudomonas fuscovaginae—Miyajima's selective medium

General: Miyajima (1989) developed a selective medium to detect *P. fuscovaginae*. After 4-5 d, *P. fuscovaginae* grown on this medium produces round, smooth, raised, translucent, beige or cream colonies, some of which have green pigments in the center (Fig. 7.13). The green pigments fade after 8 d or more.

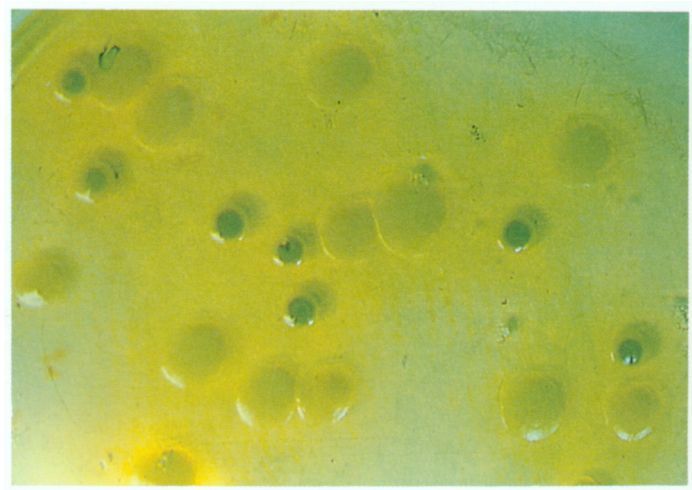
Procedure:
1. Prepare Miyajima's medium.
2. Make serial dilutions of 10^{-2}, 10^{-4}, and 10^{-6} in sterile physiological water (0.85% NaCl in distilled water) from a culture of *P. fuscovaginae* grown for 12 h in nutrient broth.
3. Pipette 0.1 ml of the serial dilutions on the medium. Spread the bacteria over the medium with an L-shaped glass rod while rotating the plate.
4. Incubate the plates at 28 °C for 5 d.

Results: Check the plates for colonies which show the green pigmented spot in the center, typical of *P. fuscovaginae* (Fig. 15.2a).

Notes: Suspected *P. fuscovaginae* strains grown on this medium showed two different colony types in one plate.

Type 1: round, smooth, raised, translucent, light brown colonies with a green spot in the center.

Type 2: round, smooth, raised, opaque, cream-colored colonies without green pigments, and somewhat larger diameter.



7.13 *Pseudomonas fuscovaginae* on Miyajima's medium showing beige or cream-colored colonies. Note some colonies with green pigment at the center.

A MODIFIED BLOTTER TEST TO DETECT SEEDBORNE *P. AVENAE* (SHAKYA AND CHUNG 1983)

General: Bacterial stripe caused by *P. avenae* has been shown to result from naturally infected seeds. Shakya and Chung observed that symptom development increased when nitrogen was added. They developed a method for detecting *P. avenae* in which rice seeds are plated on filter paper moistened with 230 ppm nitrogen (urea) solution.

The test is described for 100 seeds (4 replicates of 25 seeds each).

Procedure:
1. Plate 25 seeds in petri dishes (9 cm diam) on 3 layers of filter paper moistened with 230 ppm of urea solution.
2. Incubate plates at 27-30 °C and 12-h daylight cycles.
3. After 3 d, remove lids so that seedling growth is not hindered. Flood seeds again with urea solution. Keep plates in a high-humidity tent (e.g., in a polyethylene bag) to prevent seeds from drying out.
4. Open polyethylene bag periodically to allow air circulation.
5. During the first week, add nitrogen solution 2-3 times.
6. Starting from the second week, add only sterile water to keep filter papers well-moistened.
7. Record symptoms after 12-14 d of incubation.

Results: Distinct brown stripes on the coleoptile, leaf sheath, and leaf blade are characteristic symptoms produced by *P. avenae* (Fig. 15.1b and c).

Phage techniques to detect Xoo

Fang et al (1982) stated that the existence of a Xoo species-specific phage in rice seeds was related to disease occurrence. Hence, isolation of the Xoo bacteriophage from infected materials indirectly indicates the presence of the pathogen.

Although phage techniques are an indirect method of detecting Xoo, thcy have proven quite sensitive and can detect as few as 10^2 cfu / ml of a pure Xoo culture (Katznelson and Sutton 1951). However, it is difficult to detect Xoo populations below 10^4 cfu/ml from samples which have high concentrations of saprophytic microorganisms (Goto 1971). Also it was reported by IRRI that the phages seem to survive much longer than do bacterial cells, particularly at higher temperatures (IRRI 1969). Phage techniques are also used to assay the disinfecting effect of various seed treatments for controlling the bacterial blight discase.

For an overview of the presently identified phage strains in different regions, refer to *Rice diseases* by Ou (1985).

Phage techniques can be applied in two ways to detect the presence of Xoo:

- by demonstrating the presence of the bacteriophage of Xoo in diseased leaves, infected seeds, or in ricefield water; or
- by using a Xoo-specific phage to identify a suspected isolate as Xoo.

PHAGE ISOLATION FROM NATURALLY INFECTED SEEDS

General: This indirect method detects the pathogen by demonstrating the presence of its specific bacteriophage. With naturally infected seeds, one must first know the indicator bacterium (i.e., the corresponding Xoo strain sensitive to most of the Xoo-specific phages) to be added in the assay.

Procedure (see Fig. 7.14):
1. Macerate 100 seeds in 10 ml of sterile peptone sucrose broth (PSB).
2. Centrifuge this seed suspension at 10,000 rpm for 10 min. Keep the supernatant.
3. Add 1 ml of a full-grown broth culture of indicator bacteria to 1 ml of undiluted and to 1 ml of appropriate dilutions of the supernatant.
4. Add 3-4 ml autoclaved peptone sucrose agar (PSA) medium, cooled to 40 °C, to the sample. In a vortex mixer, shake samples carefully to achieve a homogeneous distribution. Pour samples into petri dishes and let them solidify.
5. Incubate the plates at 28 °C.
6. Check the plates the next day for plaque formation.

Results: If the sample contains phages specific to the Xoo indicator strain, there will be lysis as shown by plaque formation (Fig. 7.15). Hence, if the phage is present in the sample, so is the pathogen.

Notes:
1. It is advisable to confirm this indirect method of detecting Xoo by testing the specificity of the phages isolated from the infected seeds against Xoo, Xcola, *Erwinia herbicola* (a common contaminant in isolation of Xoo), and other yellow colonies Isolated from the seeds.
2. At IRRI, phages are most commonly isolated from ricefield water.

A1. Macerate 100 seeds in 10 ml of sterile PSB.

A2. Centrifuge at 10,000 rpm for 10 min.

A3. Separate supernatant and pellet.

A4. Remove 0.1 ml of supernatant and use it to determine the phage count in the original sample (steps B1-5)

A5. Mix the remaining supernatant with the pellet, and add a known amount of species-specific phages (approximately 10^2 phages).

A6. Immediately after adding the phages, centrifuge the sample again at 10,000 rpm for 10 min.

A7. Separate supernatant and pellet.

A8. Remove 0.1 ml of supernatant and use it to determine the phage count in the original sample plus the number of phages added in step A5 (steps C1-5).

A9. In a vortex mixer, mix the remaining supernatant and pellet.

A10. Incubate for 10 h at 28 °C. This allows the phages to infect Xoo bacteria present in the seed sample, resulting in multiplication of the phages.

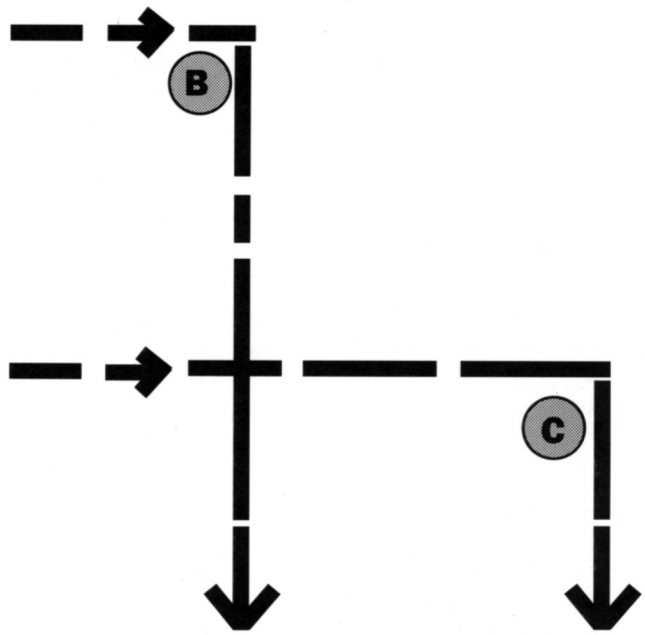
A11. Centrifuge at 10,000 rpm for 10 min.

A12. Separate the supernatant and prepare serial dilutions up to 10^6.

A13. To 0.1 ml samples of each dilution, add 0.5 ml indicator bacteria that has been fully grown in PSB and 4 ml molten PSA that has been cooled to 10 °C.

A14. Shake carefully and pour each into a petri dish. Incubate overnight at 28 °C.

A15. Count the plaques. A significant increase indicates the presence of Xoo.



B1. While doing step A12, use the 0.1 ml of supernatant taken at step A4 and make dilutions up to 10^{-3}.

B2. Proceed as in step A13.

B3. Proceed as in step A14.

B4. Proceed as in step A15.

B5. Count plaques to estimate the number of phages in the original sample.

C1. While doing step A12, use the 0.1 ml of supernatant taken at step A8 and make dilutions up to 10^{-3}.

C2. Proceed as in step A13.

C3. Proceed as in step A14.

C4. Proceed as in step A15.

C5. Count plaques to ascertain the total number of phages which include those added in step A5, and the initial number of phages in the original sample.

7.14 Phage multiplication procedure.

PHAGE MULTIPLICATION

General: In the phage multiplication technique, a known amount of species-specific phage is added to the homogenized seed suspension for incubation at 28 °C for 10 h. A significant increase in phage number, determined by plaque count, indicates the presence of the bacterial cells in the seed sample.

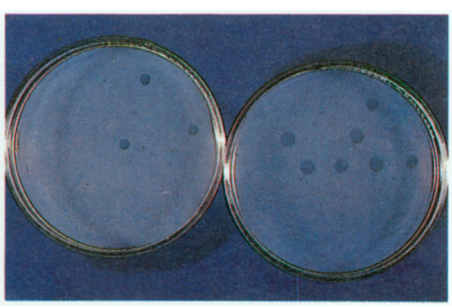
Indeed, if the traced bacterium is present, the added specific phages will adsorb to and infect the bacterial cells, resulting in lysis of the bacteria through which a large amount of new phages are released. These, in turn, can infect other bacterial cells, and so on. The result is vast multiplication of phages, reflected through the number of lysed bacteria shown by clear zones on the medium (plaques).

Procedure: see Figure 7.14.

Results: A significant increase in phage number (plaque count at 10 h vs plaque count at 0 h) indicates the presence of Xoo in the seed sample.

Note: This method can provide an estimation of the amount of bacterial blight pathogens present in the seed sample, as long as the bacterial population is above the phage multiplication threshold. To estimate disease levels, also consider the average time the phage needs for one infection cycle and the average burst size (i.e., the average yield of virus particles per infected host cell). For further information, see *Bacteriophages* (Adams 1959).

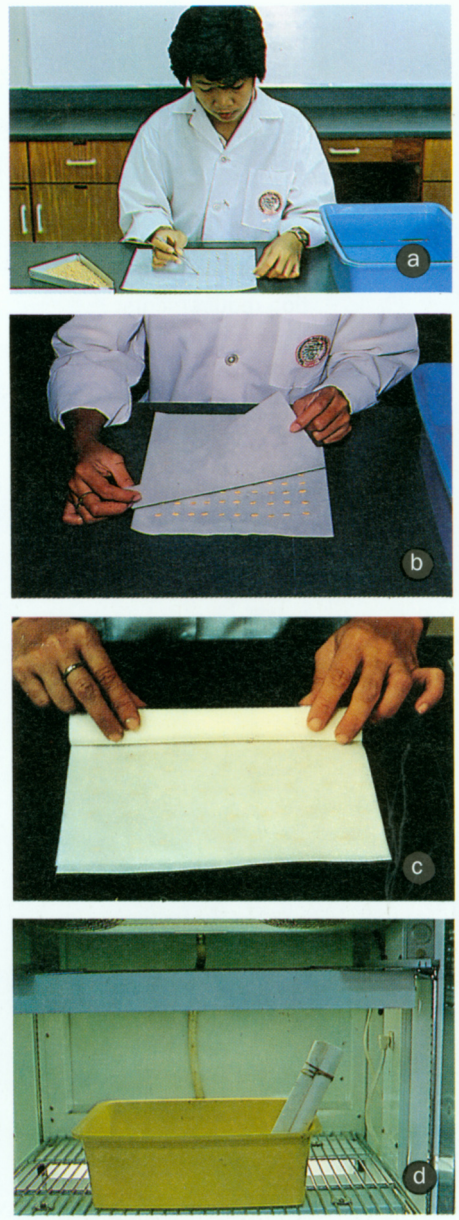
7.15 Plaque formation (clear zones result from lysis of the bacteria).



Roll towel method to detect Xanthomonas oryzae *pv.* oryzae *from rice seed (Singh and Rao 1977)*

General: This test is similar to the growing-on test, but between wet paper towels. It is used to detect Xoo infection of seeds by examining the resulting seedlings. The method can be a routine procedure for seed health testing of suspected seed lots.

Procedure:
1. Soak two 45- x 28-cm paper towels in tap water.
2. Place 100 seeds, equally spaced in 10 replicates of 10 seeds on one of the wet paper towels (Fig. 7.16a). Use the other wet paper towel to cover the seeds (Fig. 7.16b).
3. Roll the towels (Fig. 7.16c) and close the ends with rubber bands. The rolled towels may be placed in a plastic bag to maintain humidity.
4. Place towels in an upright or inclined position in a plastic tray (Fig. 7.16d). Incubate the whole setup for 5-9 d at 28-30 °C under a 12-h light regime.
5. After 5-9 d, remove the rubber bands, unroll the towels, and examine the seedlings carefully for bacterial blight symptoms. Small pieces of coleoptile, leaf sheath, and leaf which show symptoms of water soaking (brown or yellow discoloration) must be examined microscopically for bacterial ooze.
6. Crush the pieces showing bacterial ooze in a little sterile physiological water (0.85% NaCl) in a petri dish.



7.16 Paper towel method. a. Plating 100 seeds on wet paper towel. b. Covering the seeds. c. Rolling the towels. d. Incubation position.

7. Streak the bacteria onto PSA medium by dipping a wire loop in the suspension. Incubate the plates for 72-96 h at 28 °C.

Results: Examine the PSA plates for typical Xoo colonies. Pick up possible candidates and proceed with the purification requirements.

Using pure colonies, perform the pathogenicity test to confirm the identity of the isolates. ■

CHAPTER 8

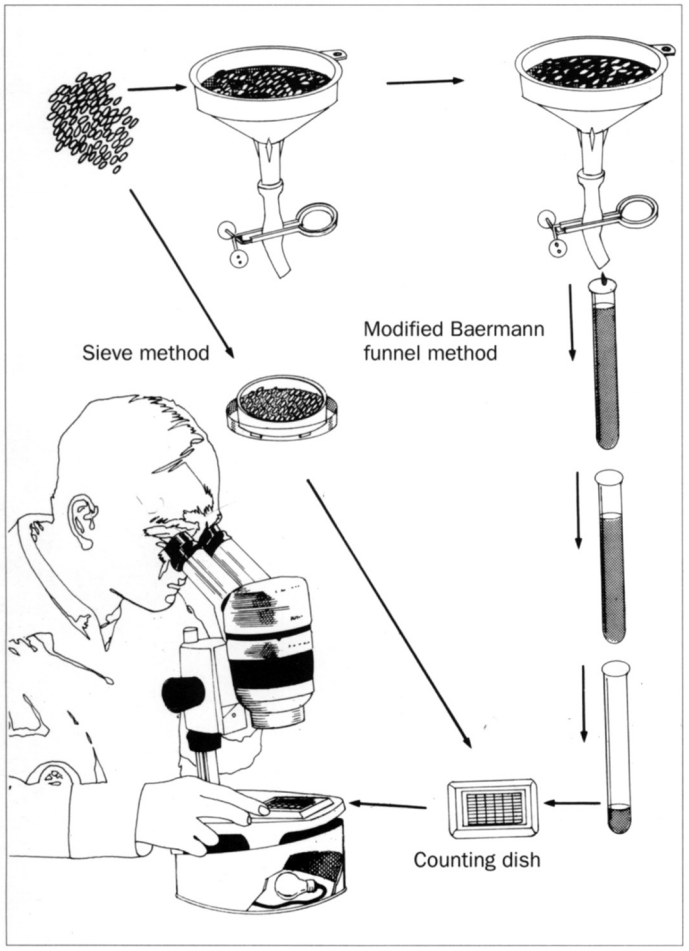
Nematodes

J-C. Prot and E.B. Gergon

Nematodes—unsegmented roundworms—feed on microorganisms, plants, or animals. They live in water or in a film of water in the soil.

Plant parasitic nematodes are microscopic and transparent. They live in the rhizosphere, inside roots, or in aerial plant tissues. They are disseminated through irrigation and flood water, wind, animals, human activities, tools, and plant materials. Plant parasitic nematodes feed and reproduce on root and aerial plant tissues. Nematodes damage the plants by feeding and by producing toxic metabolites or secretions.

Although more than 100 specics of plant parasitic nematodes occur in rice ecosystems, only a few have been proven pathogenic to rice. The pathogenic species of economic importance are *Aphelenchoides besseyi, Criconemella onoensis, Ditylenchus angustus, Heterodera* spp., *Hirschmanniella* spp., *Hoplolaimus indicus, Meloidogyne* spp., *Paralongidorus australis,* and *Pratylenchus* spp. Only *A. besseyi* is considered as it is the only nematode known to be borne by rice seed, and is, therefore, of quarantine importance.



8.1 Modified Baermann funnel and sieve methods.

Detection

Methods available for detecting *A. besseyi* include the modified Baermann funnel method, the sieve method, and the manual dehulling method. Nematodes may also be detected during the washing test for fungi.

For routine seed health testing (RSHT), the modified Baermann funnel or sieve method as described by Gergon and Mew (1991) is less tedious and more practical than the manual dehulling method. Manual dehulling should be employed for quantitative analysis.

Nematodes may be detected from seeds that have been used for RSHT and from 100-500 seeds soaked in water for 2 d.

Modified Baermann funnel method (Fig. 8.1)

Procedure:
1. Place seeds over a 10-cm diam, 40- × 40- mesh steel wire dish fitted in a funnel (12 cm diam) and filled with 250 ml tap water. Let the setup stand for at least 48 h.
2. After incubation, draw approximately 20 ml of the water into a test tube through rubber tubing attached to the funnel. Allow the collected water to stand for 1 h. Pipette out excess water, leaving 10-15 ml in the tube.
3. Examine water rernaining in the tube for nematodes. Count nematodes under a stereobinocular microscope.

Sieve method (Fig. 8.1)

Procedure:
1. The setup consists of a sieve and a plastic dish. The sieve, made from nylon cloth mesh, is stretched over a polyvinyl chloride (PVC) drain pipe rim (15 x 15 mm) that has 3 legs. Place sieve in a plastic dish.
2. Place seeds over the mesh. Fill dish with enough water to submerge seeds. Leave at 25±2 °C for at least 2 d.
3. After 48 h, gently remove the sieve from the dish and collect the water in a beaker. Wash the dish 2 to 3 times to ensure that all nematodes are transferred to the beaker.
4. Allow beaker to stand for 1 h or so to allow nematodes to settle down. Pipette out excess water. Count nematodes on a De Griss counting dish under a stereobinocular microscope. This method is efficient for seeds with or without hulls and can detect nematodes even after 24 h.

Manual dehulling method

Procedure:
1. Soak seeds in water for 24 h.
2. Using a scalpel and needle, dehull seeds while they are soaking.
3. Transfer contents (kernels, hulls, and water) either to a Baermann funnel or a sieve. Recover and count nematodes after 48-72 h. When dehulled grains are used, more nematodes are recovered through the sieve method than the Baermann funnel method.

   Nematode count increases as seeds soak longer, but seeds putrify after about 72 h.

Identification

Parasitic plant nematodes have stylets, which are not present in nonparasitic nematodes. To see the stylets, view the nematode under a stereobinocular microscope with the light source coming from below.

A. *besseyi* is described on p, 65. ■

CHAPTER 9

Viruses and mycoplasmalike organisms

T.W. Mew and S.D. Merca

Twenty-one rice viruses and mycoplasmalike organisms have been reported. The majority (17) are vector-transmitted. The rest are mechanically transmitted (mosaic viruses), soil-transmitted (necrosis mosaic viruses), and two are confirmed as seed-transmitted (wrinkled stunt virus and witches' broom virus).

Ou (1985) reported that wrinkled stunt and witches' broom viruses were first observed in 1976 and 1978, respectively, in a breeding plot in Surinam, South America. He noted they were seedborne. Dr. F. Klas of the Agricultural Experiment Station in Paramaribo, Surinam, also confirmed that wrinkled stunt virus is seedborne (Ou 1985).

The two seedborne diseases appear confined to Surinam. Seed health testing for these two diseases has not been established. ■

CHAPTER 10

Field inspection

J.K. Misra, T.W. Mew, and S.D. Merca

Field inspections—observations of standing crops—are done at various stages of plant growth. Field inspections are carried out by agencies responsible for certifying or producing certified seeds, by seed technologists, and by quarantine personnel.

Officers who inspect fields for plant quarantine, commonly termed *crop health inspection,* may benefit from employing some methods used during crop inspection for other purposes. These methods, as well as the more traditional quarantine procedures, are presented below. For seed certification, field inspectors must check

- crop land requirements;
- the source of the crop's seed;
- sowing dates, pattern, spacing, plant ratio, and proper rouging;
- purity of the cultivar (e.g., no admixture with other cultivars);
- harvesting methods (to prevent admixtures);
- postharvest operations such as cleaning, drying, sampling, tagging, labeling, and sealing; and
- other special requirements the crop may have.

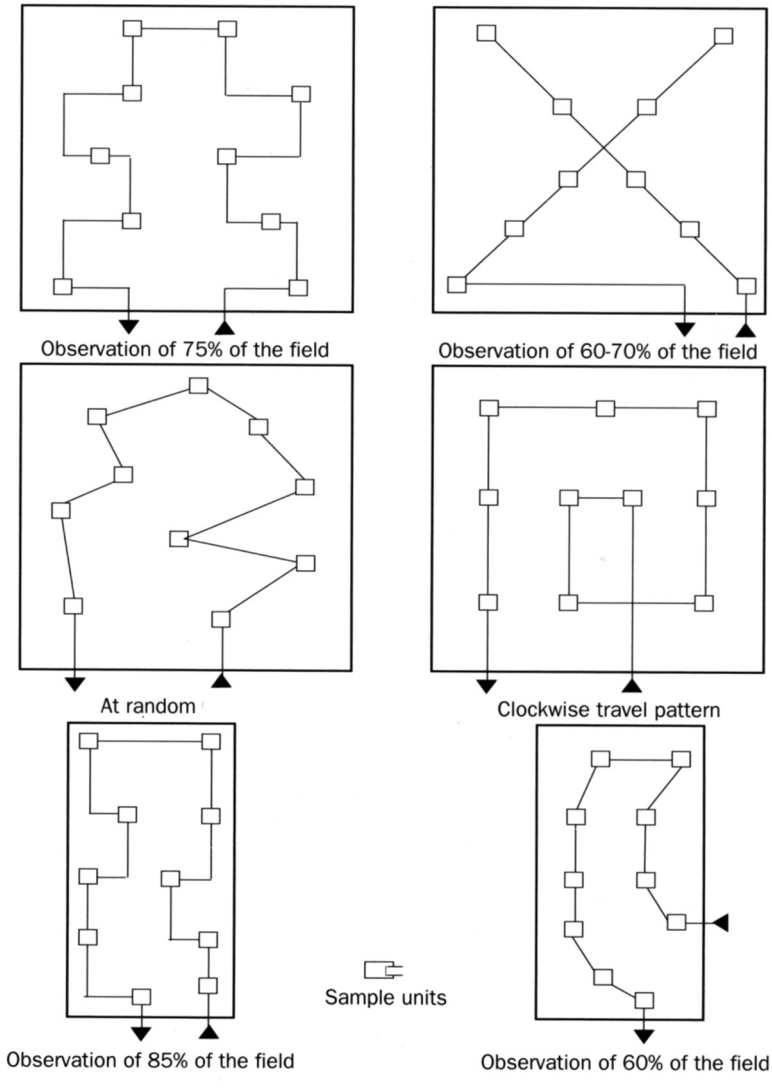
Quarantine officers inspect fields as pre- or postentry checking for export and import control to ensure that no new pathogen is introduced and to estimate the incidence and severity of existing diseases. Field inspections are conducted by experienced plant pathologists and field inspectors knowledgeable about crop diseases in the field. Inspections start at the seedling stage and are repeated through maturity to harvesting.

General principles

Some general procedures normally followed by seed or crop inspectors are helpful to plant quarantine. The helpful suggestions include:

- Inspect so observations can be made. from the tops of the plants.
- Have the sun at your back or side. Do not face the sun, as this decreases visibility.
- Inspect the field so as to cover the maximum possible area. Svensson et al (1975) prescribed schematic patterns of walking that are useful for inspecting large fields (Fig. 10.1).

10.1 Schematic patterns for walking a field (after Svensson et al 1975).



Plant growth stages, number of field inspections, and counts per inspection

Crop health inspections must he timed with specific plant growth stages. Schedule observations to allow verification of the incidence of various diseases when they appear at specific plant parts (such as the leaf, leaf sheaths, neck, panicle, and spikelets). For production of foundation and certified rice seed, a minimum of two inspections are required from flowering to harvest stages (Svensson et al 1975). Make 5 counts per inspection if the field is not larger than 2 ha. Increase counts by one for each additional 2 ha or part thereof. Inspect 1000 plants or heads per count (Anonymous 1972).

Field count methods

For field counts and seed certification, more plots and number of plants or heads are counted than for quarantine inspections. Procedures are devised to randomly check the specified number of heads or plants at different field sites. For seed certification, the methods outlined by Agarwal (1980) are useful when inspecting large areas and many plants.

For quarantine purposes, the fields or number of plants to be observed are small, and the inspector can check every plant. For example, in germplasm exchange, a small number of seeds are multiplied in glasshouses or in open quarantined fields, and all are examined very carefully.

Table 10.1. Growth stages of the rice plant and their respective codes.

Code	Stage
1	Germination
2	Seedling
3	Tillering
4	Stem elongation
5	Booting
6	Heading
7	Milk stage
8	Dough stage
9	Mature grain

Table 10.2. Disease, stage of inspection, and scale of symptoms.

Disease	Growth stage at which inspection is made[a]	Scale (degree of severity)
Fungi		Scale (for blast nursery) (Note: scale 1-4 is qualitative, 5-9 is quantitaitve).
Leaf blast (Bl)	2-3	
Pathogen: Magnaporthe grisea (*Pyricularia oryzae* – imperfect or anamorph stage)		0 No lesions 1 Small brown pinpoint-size specks, or larger brown specks without sporulating center 2 Small roundish to slightly elongated, necrotic gray spots, approximately 1-2 mm in diam, with distinct brown margin; lesions mostly found on the lower leaves 3 Lesion type as in scale 2, but a significant number of lesions are on the upper leaves 4 Typical susceptible blast lesions as in types 5, 7, and 9 below, 3 mm or longer, infecting less than 2% of the leaf area. Lesion type 3 mm or longer is the qualitative type for susceptible blast lesions. Increasing amount of these lesions on leaves will classify reaction into scales 5-9. 5 Typical blast lesions infect 2.10% of the leaf area 6 Typical blast lesions infect 11-25% of the leaf area 7 Typical blast lesions infect 26-50% of the leaf area 8 Typical blast lesions Infect 51.75% of the leaf area; many dead leaves 9 More than 75% leaf area affected
	2-3 (field or greenhouse)	Scale (predominant lesion type) 0 No lesions 1 Small brown pinpoint-size specks, or larger brown specks without sporulating center 3 Small, roundish to slightly elongated necrotic sporulating spots, approximately 1-2 mm diarn with a distinct brown margin or yellow halo

Table 10.2. continued

Disease	Growth stage at which inspection is made[a]	Scale (degree of severity)
Fungi		Scale (for blast nursery) (Note: scale 1-4 is qualitative, 5-9 is quantitaitve).
Note: Lesion types 5,7, and 9 are considered typical susceptible lesions.		5 Narrow or slightly elliptical lesions, 1-2 mm long with brown margin 7 Broad, spindle-shaped lesions with yellow, brown, or purple margin 9 Rapidly coalescing small, whitish, grayish, or bluish lesions without distinct margins
Panicle blast (PB) Pathogen: *P. oryzae*	8 (20-25 d after heading)	Scale (based on symptoms) 0 No visible lesion or lesions on only a few pedicels 1 Lesions on several pedicels or secondary branches 3 Lesions on a few primary branches or the middle part of panicle axis 5 Lesion partially around the panicle base (node) or the uppermost internode or the lower part of panicle axis near the base 7 Lesion completely around panicle base or uppermost internode or panicle axis near the base with more than 30% of filled grain 9 Lesion completely around panicle base or uppermost internode or panicle axis near base with less than 30% of filled grains
	8-9	Scale (incidence of severely infected panicles)
Note: For the mass evaluation of PB incidence, count only the number of panicles with lesions completely around node, neck, or lower part of panicle axis (symptom type 7-9).		0 No incidence 1 Less than 5% 2 5-10% 5 11-25% 7 26-50% 9 More than 50%
Brown spot (BS) Pathogen: Cochliobolus miyabeanus (*Bipolaris oryzae, Drechslera oryzae*)	2 and 5-9	Scale (affected leaf area) 0 No incidence 1 Less than 1% 2 1-3% 3 4-5% 4 6-10% 5 11-15% 6 16-25% 7 26-50% 8 51-75% 9 76-100%
Note: This scale may also be used to assess eyespot disease caused by *D. gigantea.*		
Narrow brown leaf spot (NBLS) Pathogen: *Sphaerulina oryzina* (*Cercospora janseana*)	3-9	Scale (affected leaf area) 0 No incidence 1 Less than 1% 3 1-5% 5 6-25% 7 26-50% 9 51-100%

Table 10.2. continued

Disease	Growth stage at which inspection is made[a]	Scale (degree of severity)
Bacteria		
Bacterial leaf streak (BLS) Pathogen: *Xanthomonas oryzae* pv. *oryzicola* Note: This scale may also be used to assess leaf smut caused by *Entyloma oryzae.*	3-9	Scale (affected leaf area) 0 No incidence 1 Less than 1% 3 1.5% 5 6-25% 7 26-50% 9 51-100%
Leaf scald (LSc) Pathogen: *Monographelia albescens (Gelachia oryzae Microdochium oryzae)*	5-8	Scale (affected leaf area) 0 No incidence 1 Less than 1% (apical lesions) 3 1-5% (apical lesions) 5 6-25% (apical and some marginal lesions) 7 26-50% (apical and marginal lesions) 9 51-100% (apical and marginal lesions)
Bacterial blight (BB) Pathogen: *Xanthomonas oryzae* pv. *oryzae*	3-4 (kresek, greenhouse evaluation of leaf blight)	Scale (for greenhouse test, lesion area) 1 0-3% 2 4-6% 3 7-12% 4 13-25% 5 26-50% 6 51-75% 7 76-87% 8 88-94% 9 95-100%
	5-8 (leaf blight)	Scale (for field test, lesion area) 1 1-5% 3 6-12% 5 13-25% 7 26-50% 9 51-100%
Viruses		
Note: Scale (1-9) based on symptom severity in greenhouse test to evaluate varieties for symptomatic resistance (tolerance) and resistance to virus infection. Individual plants are scored at 3 wk after inoculation. Severity = $\frac{\text{Sum of scores}}{\text{Total no. of plants observed}}$	Score on each plant and compute average score.	
Rice virus and viruslike diseases		Scale (infected plants) 0 No infection 1 1-10% 3 11-30% 5 31-50% 7 51-70% 9 71-100%

Table 10.2. continued

Disease	Growth stage at which inspection is made[a]	Scale (degree of severity)
Rice tungro (RTV)	2 (greenhouse)	Scale (based on symptom severity 3 wk after inoculation)
	3-5 (field)	1 No symptoms 3 1-10% plant height reduction, no distinct leaf discoloration 5 11-30% height reduction; no distinct leaf discoloration 7 31-50% height reduction and/or yellow to orange leaf discoloration 9 More than 50% height reduction; and yellow to orange leaf discoloration
Rice ragged stunt (RSV)	2-3 (greenhouse)	Scale (based on symptom severity 5 wk after inoculation)
	4-6 (field)	1 No symptoms 3 0-10% plant height reduction; no short, ragged, or curled leaves
See note in RTV.		5 0-10% height reduction; youngest normal leaves, 1-2 older leaves abnormal 7 10-30% height reduction; with youngest normal leaves; may have 3-4 abnormal older leaves 9 More than 30% height reduction; or with abnormal leaves including the youngest
Grassy stunt (GSV) Note: Scale being developed.	2-3 (greenhouse) 3-6 (field)	General scale can be used in the meantime 0 No infection 1 1-10% 3 11-30% 5 31-70% 7 51-70% 9 71-100%
Sheath blight (ShB) Pathogen: *Thanatephorus cucumeris* (*Rhizocotonia solani*)	7-8	Scale (based on relative lesion height) 0 No infection observed 1 Lesions limited to lower 20% of the plant height 3 20-30% 5 31-45% 7 46-65% 9 More than 65%
Sheath rot (ShR) Pathogen: *Sarocladium oryzae*	7-9	Scale (incidence of severely affected tillers) 0 No incidence 1 Less than 1% 3 1-5% 5 6-25% 7 26-50% 9 51-100%

Field inspection at IRRI

Rice scientists at IRRI, in consultation with scientists from 26 countries, have developed a standard evaluation system for rice (SES) for its varietal improvement. This system has been used since 1975 by scientists involved in genetic evaluation. The relevant information (IRTP 1988, available from IRRI) is summarized in Tables 10.1 and 10.2 and Figures 10.2 and 10.3.

At IRRI, where thousands of seed lots are exchanged yearly, strenuous efforts are exerted to exclude unwanted organisms and objects. The Seed Health Unit at IRRI, in collaboration with the Bureau of Plant Industry (Quarantine Services) of the Philippines, conducts phytosanitary certification. Procedures include crop health inspections for both incoming (postentry quarantine) and outgoing (pre-export quarantine) seed lots. Some importing countries require certification of disease incidence on the mother plants. As an

Table 10.2. continued

Disease	Growth stage at which inspection is made[a]	Scale (degree of severity)
Grain discoloration (Gd) Pathogen: Several spp. of fungus and bacteria	8-9	Scale (grains with severely discolored glumes) 0 No incidence 1 Less than 1% 3 1-5% 5 6-25% 7 26-50% 9 51-100%
Note: Severity of grain discoloration can be estimated by counting grains with more than 25% of glume surface affected.		
False smut (FSm) Pathogen: *Ustilaginoidea virens*	9	Scale (infected florets) 0 No incidence 1 Less than 1% 3 1-5% 5 6-25% 7 26-50% 9 51-100%
Kernel smut (KSm) Pathogen: *Tilletia barclayana*	9	
Udbatta disease (UDb) Pathogen: *Balansia oryzae-sativae* (*Ephelis oryzae*)		Scale (infected panicles or tillers) 0 No incidence 1 Less than 1% 5 1-25% 9 26-100%
Bakanae disease (Bak) Pathogen: *Gibberella fujikuroi* (*Fusarium moniliforme*)	3-6	Scale (infected tillers) 0 No incidence 1 Less than 1% 5 1-25% 9 26-100%
Stem rot (SR) Pathogen: *Magnaporthe salvinii* (*Nakataea sigmoidea, Helminthosporium sigmoidea* var. *irregulare* *Sclerotium oryzae* (anamorph)	7-9	Scale (stems with lesions and sclerotia) 0 No incidence 1 Less than 1% 3 1-5% 5 6-25% 7 26-50% 9 51-100%

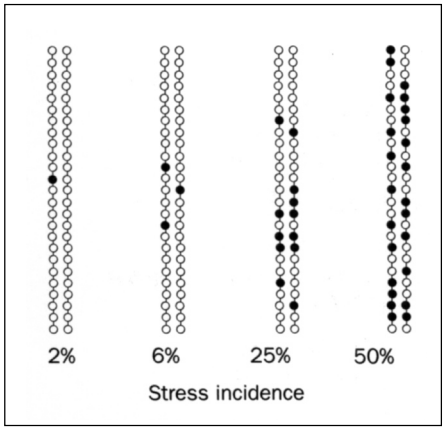
[a] See Table 10.1 for growth stage codes.

additional precaution, the multiplication plots of the International Rice Germplasm Center (IRGC), INGER, and the Plant Breeding, Genetics, and Biochemistry Division are inspected regularly at specified growth stages. Data are recorded as outlined in SES. The Philippine Plant Quarantine Service's certifying officer uses the data as basis for issuing a phytosanitary certificate. A summary of crop health data taken in 1990 is presented in Table 10.3. ■

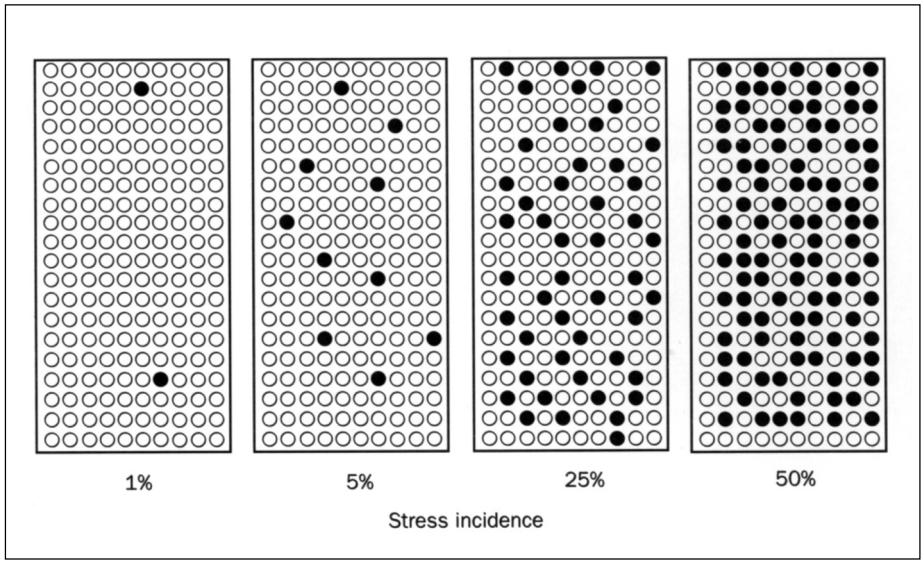
Table 10.3. Percentage of entries affected by rice diseases among IRRI's incoming[a] and outgoing seed multiplication lots, 1990 dry and wet seasons.

Disease	Incoming[b]		Outgoing[c]	
	Dry season	Wet season	Dry season	Wet season
Entries without diseases	86.39	0.38	77.50	4.60
Rice tungro	1.44	0	2.27	3.99
Grassy stunt	0	0	0	0.02
Ragged stunt	0	0	0.01	0
Sheath rot	7.85	18.01	12.14	14.29
Sheath blight	0	0.76	0.08	1.97
Stem rot	0	0	0	0
Narrow brown leaf spot	0.72	0	0.28	3.08
Leaf scald	0.06	1.14	0	59.26
White leaf streak	0	0	0	0.01
Blast	0.28	0.38	0.14	0.76
Brown spot	0	0	0.01	0
Bakanae	1.55	0	1.22	0
Bacterial leaf streak	0.11	76.24	6.90	68.89
Bacterial blight	0	1.14	0.07	1.18
Leaf smut	0	0	0	1.98
False smut	0.06	0	0.04	0.28
Bacterial stripe[d]	1.33	0	0.01	0.01
Sclerotium seedling blight	0.33	0	0.02	0

[a]Diseases observed on plants originating from incoming seeds were not introduced. [b]Based on a total of 1,808 entries (dry season) and 261 entries (wet season). [c]Based on a total ot 14,148 entries (dry season) and 10,381 entries (wet season). [d]Bacterial stripe and sclerotium seedling blight observed at the seedbed stage.



10.2 Schematic drawing showing percentage of plants in a 2-row field plot with 4 levels of stress incidence.



10.3 Schematic drawing showing percentage of plants in a field plot with 4 levels of stress incidence.

CHAPTER 11

Seed treatment

J.K. Misra. T.W. Mew, and C.C. Huelma

Seed treatment is physical or chemical seed therapy. Seed treatment dates back at least to the Roman empire, when wine and crushed cypress leaves were used to treat seeds for better harvest.

Today, seed treatment is important in pest management. In western countries and Japan, where mechanized farming is practiced, seed treatment has become part of normal farm procedures. Seed treatment revolutionized production (Nakamura 1986, Bowling 1986) by controlling many pests and pathogens and by repelling birds. As new technologies and chemicals become available, the dimensions of seed treatment change significantly.

Seeds are treated

- in the early stages of their germination and establishment to protect them from pests and pathogens,
- to promote better seedling stands,
- to minimize yield loss,
- to maintain and improve seed quality, and
- to avoid introduction and spread of harmful organisms.

Advantages of seed treatment

Among the many advantages of seed treatment are that it

- protects seeds and seedlings from pests and pathogens in the early stages of germination and establishment,
- is easier than plant treatment and can be done indoors with or without machinery,
- is unaffected by weather conditions,
- requires less chemicals than does plant treatment,
- pollutes the environment less than does plant treatment,
- does not result in much development of resistance in insect pests (although fungi are developing resistance to fungicides), and
- uses biocides that affect only targeted organisms and not the other beneficial entities in the soil.

Limitations of seed treatment

Advantages of seed treatment outweigh the limitations which include

- lack of effective broad-spectrum systemic fungicides for internally seedborne fungi and for fungal resting structures;
- no protection beyond the early growth or seedling stages;
- no potent biocides that protect the seeds from rodents, nematodes, insects, slugs, and birds; and
- chemicals do not continue to adhere to the seed surface, do not maintain the desired level during germination, and do not protect germinating seeds and seedlings from seedborne and soilborne pests and pathogens.

Methods

Seeds are treated by chemical, physical, or both methods. Aspects of general seed treatment are presented by Jeffs (1986), Neergaard (1979), and Anselme (1988). Information about rice seed treatment for quarantine purposes is presented here.

Physical seed treatment is any method that does not employ chemicals. Normally, heat treatment is accomplished using hot water, dry heat, or steam.

Heat treatment kills seedborne pests and pathogens without injury to most germplasm. Heat therapy may be injurious to and should be used cautiously with old or damaged seeds and varieties which have low heat tolerance. Japonica varieties are generally more heat-sensitive than indica varieties.

Hot water treatment at 52-57 °C for 15 min eradicates seedborne *Aphelenchoides besseyi.*

Heat therapy is often given in conjunction with chemical treatment.

Biocidal chemicals (fungicides, bactericides, insecticides, nematicides) are applied as liquids or powders. Depending on the type of seed and the nature of the chemical, biocides may be applied as dust treatments, fumigants, wet treatments (steep, sprinkle, quick wet, and slurry), oil fungicide treatments, and by pelleting. Since no broad-spectrum fungicide exists, mixtures of fungicides are commonly used.

In Japan, the following chemicals are used to control blast, bakanae, and brown spot rice diseases (Nakamura 1986):

- thiram 20% and benomyl 20% (benlate T);
- thiram 30% and thiophanate-methyl 50% (Homai);
- thiram 10% and thiophanate-methyl 10% (Homai coat);
- captan 30% and kasugamycin 3% (kasumin C); and
- copper sulfate, basic 29%, and oxine-copper 20% (oxybordeau).

Chemicals used for seed treatment in the USA are

- captan,
- thiram,
- maneb,
- chloroneb,
- difolatan (*cis*--N-((l,l,2,2-tetrachloroethyl) thio)4-cyclohexene-1,2, dicarboximide),
- Vitavax (2,3-dihydro-6-methyl-5-phenyl carbamoyl-1,4-oxathiin),

■ terracoat (5-ethoxy-3-trichloromethy1-1,2,4-thiadiazole), and

■ pentachloronitrobenzene.

The most widely used fungicides for rice seed treatment are Bedate T and Homai. These also partly control *A. besseyi.*

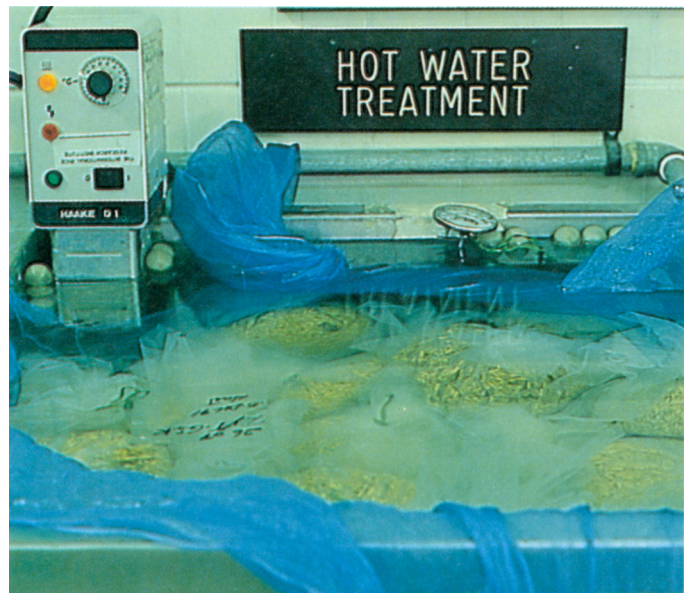
In Guyana, seeds are commonly treated with fungicides to control seedling diseases (Kennard 1965).

The rice water weevil (*Lissorhoptrus oryzophylus)* is an important insect pest that is not seedborne. It is controlled in the USA by treating seeds with organophosphate or carbamate insecticide. In Japan, seedlings are treated with cartap or propoxur granules (100 g/box) during transplanting. This effectively controls the larvae. Cartap (Padan), MEP (Sumithion), and MPP (Baycid) are common nematicides used to treat seeds for *A. besseyi.*

To treat bacterial grain rot (caused by *Pseudomonas glumae*), seeds in Japan are disinfected with kasumin, kasumin C, hot water, and dry heat.

Seed treatment for quarantine

No single treatment can eliminate all the important seedborne or seed-transmitted pests and pathogens. Therefore, a combination of therapies (thermo- and chemotherapy) is needed to ensure that seeds do not harbor pests or pathogens. (See Table 11.1 for recommended treatments.) Almost all importing and exporting countries meet international quarantine obligations. Some nations require exporting countries to apply prescribed treatments to seed lots before shipment. Treatments required by importing nations arc listed in Table 11.2.



11.1 Hot water treatment of rice seeds at 52-57 °C for 15 min.

11.2a. Slurry treatment. Small plastic bottle for 50-g seeds and bigger plastic bottle for 100-g seeds. b. Slurry treatment. Big plastic bottle for 1-kilo seeds placed on a roll mill and rotated for 510 min.

Seed treatment at IRRI

IRRI's Seed Health Unit treats outgoing seed lots to meet the phytosanitary requirement of various countries. Hot water treatment (52-57 °C for 15 min, after presoaking for 3 h in cold water; Fig. 11.1) followed by slurry treatment with 0.3% benlate and 0.3% Dithane M-45 by seed weight (Fig. 11.2) is applied to all shipments intended for Southeast Asian nations. ASEAN PLANTI (1981) prescribed hot water treatment at 52-57 °C for 15 min followed by dressing seeds with 1 g benlate/ 100 ml of seed.

At IRRI, all outgoing and incoming seed lots are fumigated with 1.2 g phosphine/m^3 for 72 h, NAT, NAP (Fig. 11.3).

Future biocides

Seed treatment has revolutionized crop production and minimized chances of spreading exotic pests and pathogens. In the future, biocides are needed that will do the following:

- act against the target organisms without having undesirable side effects;
- not affect germinating seeds and seedlings;
- be compatible with all other chemicals used in rice production (for example, fertilizers and herbicides);
- be environmentally safe; and
- not have deleterious effects on nontarget organisms and lives (Bowling 1986).

11.3 Fumigation using atmospheric-type fumigation chamber and phosphine.

Precautions

Seed treatment (physical or chemical) requires utmost care. Improperly applied, treatment can damage or kill the seeds. Application modes and doses should be correct. Most biocidal chemicals are health hazards if not properly handled.

Treated seeds should not be eaten and should be adequately labeled to prevent inadvertent consumption. Some biocidal chemical formulations color treated seeds, providing visible warning to anyone who handles them. Coloring also helps evaluate the uniformity and thoroughness of chemical application. ■

Part 3 Pests and pathogens

CHAPTER 12

Weed Seed Contaminants

CHAPTER 13

Insect Pests

CHAPTER 14

Fungal Pathogens

CHAPTER 15

Bacterial Pathogens

CHAPTER 16

Nematode Pest

CHAPTER 17

Organisms Associated with Grain Discoloration

CHAPTER 12

Weed seed contaminants

K. Moody and R. Lubigan

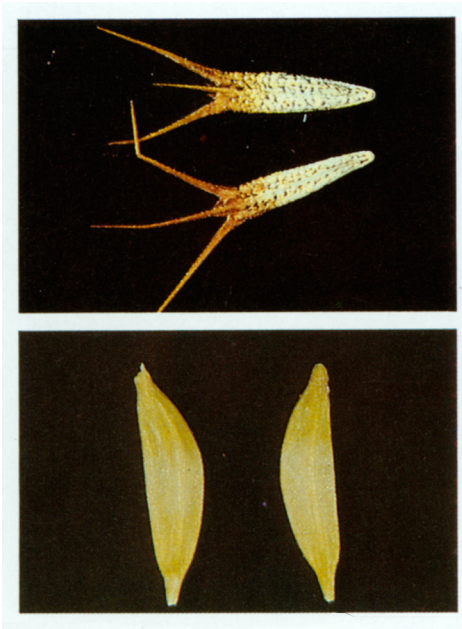
Most weed species have been, and still are, inadvertently disseminated between and within continents and countries as contaminants in traded seed (Horne 1953, Delouche 1988). Weeds of Philippine origin have been spread widely through export of high-yielding rice varieties. Weeds are also being disseminated in the continuing rice trade within South America; between South and North America; among Africa, North America, and Asia; and among most other combinations of trading partners (Delouche 1988). In California, with the exception of *Echinochloa crus-galli* (L.) P. Beauv. var *crus-galli,* all the introduced species of rice weeds have been brought into the state in rice seed (Fuller and Barbe 1983).

Rice seed contaminated with weed seeds may introduce new species to a field or add to an existing weed population and is a source of weed perpetuation in crop fields. In the southern USA, many weeds are spread almost entirely in rice seed (Smith et al 1977).

If there are 1,000 weed seeds per kilogram of rice seed, 100,000 weed seeds will be planted per hectare ($10/m^2$) if the seeding rate is 100 kg/ha. Planting contaminated seed will be costly to a weed management program for many years (Rao and Moody 1990).

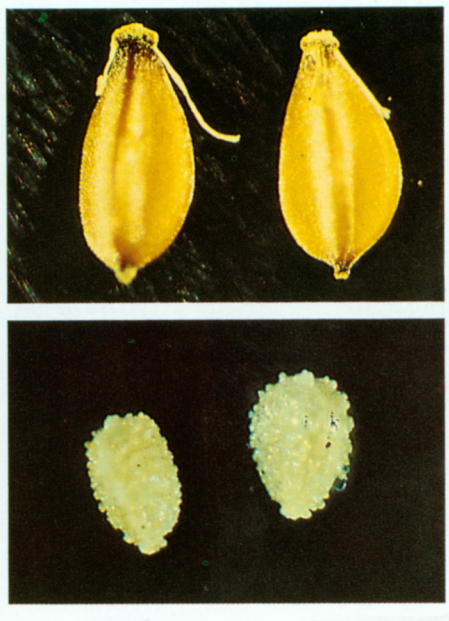
In marketable rice grain, mixtures of weed seeds detract from its appearance and lower its grade and price. In Thailand, the price of rice drops by about one-half when the grain is contaminated with weed seeds (Suwunnamek 1986).

Standardized procedures are available for the types of inspection and testing needed to prevent weed seed contamination of crop seed. The



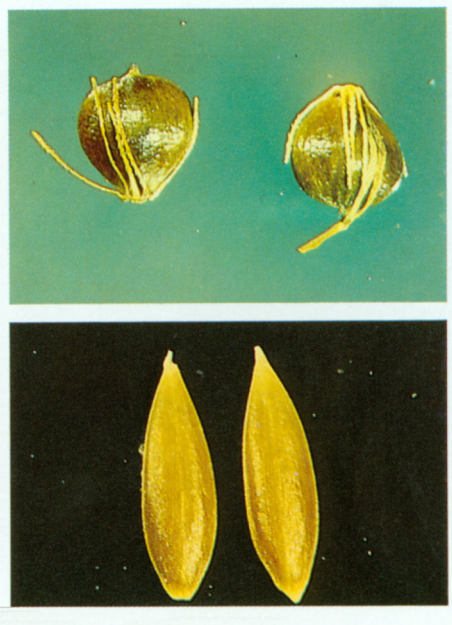
12.1 Seeds of *Synedrella nodiflora* (courtesy of K. Moody and R. Lubigan).

12.2 Seeds of *Cyperus compactus.*



12.3 Seeds of *Cyperus iria* (courtesy of K. Moody and R. Lubigan).

12.4 Seeds of *Fimbristylis miliacea* (courtesy of K. Moody and R. Lubigan).



12.5 Seeds of *Scirpus juncoides* (courtesy of K. Moody and R. Lubigan).

12.6 Seeds of *Digitaria ciliaris* (courtesy of K. Moody and R. Lubigan).

relevant tests for determining the incidence of weed seed contamination are purity analysis and determination of other species by number.

To obtain a composite sample, see Chapter 4.

Purity analysis

One of the objectives of this test is to determine the identity of the various species of seeds and inert particles in thc sample.

The working sample is separated into three component parts: pure seeds, other seeds (identified by their Latin names), and inert matter. The percentage of each part is determined by weight, calculated to one decimal place. Components of less than 0.05% are reported as trace.

Determination of other species by number

The objective of this test is to determine the number of seeds of other species. This test is used mainly to determine the presence of noxious or undesirable seeds (e.g., weed seeds).

The working sample (minimum 500 g for rice) is examined either for seeds of all other species (complete test) or of certain designated species (limited test). The number of seeds found of each species sought is counted.

The result is expressed as the number of seeds belonging to each designated species or category found in the actual quantity examined. In addition, the number per unit weight (e.g., per kg) may be calculated.

The actual weight of seed examined, its Latin name, and the number of seeds of each species sought and found shall be reported on the Analysis Certificate. The certificate should be endorsed as *a complete test,* or *a limited test,* or *a reduced test* (if the whole working sample was not examined).

Weed seeds encountered during dry seed inspection are listed and briefly described in Table 12.1 and are shown in Figures 12.1-12.12. ■

12.7 Seeds of *Echinochloa colona* (courtesy of K. Moody and R. Lubigan).

12.8 Seeds of *Echinochloa crus-galli* ssp. *hispidula* (courtesy of K. Moody and R. Lubigan).

12.9 Seeds of *Ischaemum rugosum* (courtesy of K. Moody and R. Lubigan).

12.10 Seeds of *Panicum repens* (courtesy of K. Moody and R. Lubigan).

12.11 Seeds of *Paspalum distichum* (courtesy of K. Moody and R. Lubigan).

12.12 Seeds of *Rottboellia cochinchinensis* (courtesy of K. Moody and R. Lubigan).

Table 12.1. Weed seed Contaminants of rice encountered during dry seed inspection of incoming seeds.

Family and species	Seed description	Range of detection in incoming seeds[a]	Sample of country in which detected[a]	Figure number
Asteraceae				
Synedrella nodiflora (L.) Gaertn.	Achenes black, achenes of the tubular flowers compressed, oblong, 4.5 x 1.1 mm, coarsely strigose, the apex 2-awned, 2-3.5 mm long, strigose			12.1
Caryophyllacea				
Stellaria media (L.) Vill.	Seed circular in outline with marginal notch, 1-1.3 mm in diameter, reddish brown, surface covered with curved rows of tubercles	0-.1%	Nepal	
Cyperaceae				
Cyperus compactus Retz.	Seed trigonous, oblong to linear, slightly convex on one side, apiculate rostrate, brown, 1-2x0.5 mm	0-.33%	Egypt	12.2
Cyperus iria L.	Seeds (achenes) brown to red brown, egg shaped, 1-1.5 mm long and 0.1-0.5 mm wide with large base and short pointed minute tip	.01-.1%	Bangladesh, Madagascar, China	12.3
Fimbristylis miliaceae (L.) Vahl	Seed (achene), inverted egg-like, tip blunt, pale ivory to brown, finely reticulate, 0.6-1 mm long by 0.75 mm wide	0-.04%	Bangladesh Bhutan, Nepal Nigeria, Vietnarn	12.4
Scirpus juncoides Roxb.	Seed (achene) pale brown to black, three sided, almost smooth, with minute tubercles, up to 6 barbed bristles originating from the base, seed about 2 mm long and 1.5-1.75 mm wide	.1-.12%	Bangladesh Bhutan, China, India. Madagascar, Malaysia, Nepal	12.5
Scirpus sp.		.1-.12%	Bangladesh, Bhutan, China (Taiwan), India, Madagascar, Malaysia, Nepal	
Poaceae				
Digitaria ciliaris (Retz.) Koel.	Lower glume minute but distinct, deltoid. 0-5-2 mm long, upper glume about 2/3 the length of spikelet, narrow, with 1-mm-long marginal hairs and 3 smooth nerves; lemma broadly lanceolate, as long as the spikelet with 5-7 smooth nerves: palea lanceolate, leathery (coriaceous), pale green to yellow brown, as long as lemma; yellow-white caryopsis tightly enclosed by the lemma and palea, generally elliptical and dorsally compressed with a punctiform hilum: embryo 0.6-0.8 mm by			12.6

Table 12.1 continued

Family and species	Seed description	Range of detection in incoming seeds[a]	Sample of country in which detected[a]	Figure number
	2 mm about half the length of the caryopsis.			
Digitaria sp.		0-.10%	Madagascar	
Echinochloa colona (L.) Link	Seed (spikelet) pubescent green tinged with purple, seeds white to yellow, egg-shaped (ovate) 1.3 mm long and 1 mm wide	.32-.40%	Bangladesh, Bhutan, China Egypt, India Iran, Madagascar, Nepal, Pakistan, Sri Lanka, Vietnam, South Africa	12.7
Echinochloa crus-galli (L.) Beauv. ssp. *hispidula* (Retz.) Honda	Seed (spikelet) glumes hairy, long awn present, smooth, yellow green to yellow brown, ovate, convex, about 1.5 mm long by 1 mm wide			12.8
Ischaemum rugosum Salisb.	Glumes transversely ribbed, nerves many and winged above, seeds light brown, biconvex. measuring 2 mm long by 1 mm wide, smooth or very finely reticulated	.05-.30%	Bhutan Nepal	12.9
Panicum repens	Spikelets oblong-ovate. acute or slightly acuminate, 3 mm long			12.10
Panicum sp.		0-.10%,	Madagascar	
Paspalum distichum L. [= *P. Paspalodes* (Michx.) Scribn.]	Fruit nearly as long as the spikelet, 1.2 mm wide. smooth, usually without hairs (glabrous). but some may have a few bristly hairs at the apex, first glume very small or absent, second glume as long as the spikelet anti 3- to 5-nerved, lemmas 3- to 5 nerved and shiny, with prominent but often off-center midnerve. lemmas of fertile florets finely roughened by longitudinal rows of minute tubercles, producing faintly striate effect, glumes and sterile lemma often finely pubescent, with soft hairs on the upper portion and along their sides, spikelels flattened, never strongly arched, with a broad base on the fertile floret and short, pointed apex			12.11
Paspalum sp.		0-.10%	Egypt Madagascar	
Rottboelia cochinchinensis (Lour.) W. D. Clayton	Spikelets 4-5 mm long. the stalkless one yellowish, smooth and hard, sunk into the thick internode; stalked spikelets green and leafy, with thick flattened stalk fused with the internode	.04-.40%	Bangladesh Madagascar Pakistan	12.12

[a]Based on observations at SHU, IRRI.

CHAPTER 13

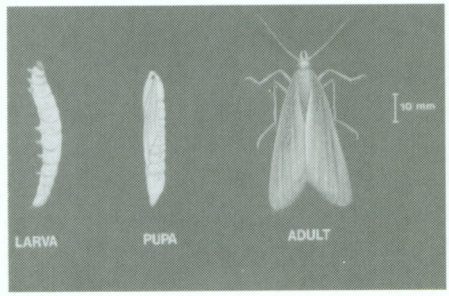
Insect pests

J.A. Litsinger and A.T. Barrion

Several dozen insect pests feed on rice seeds in storage. Although the rice plant's tough husk is naturally resistant to most insect pests, several species can penetrate the seed. These are called primary pests. Primary pests are of greatest interest to quarantine. Most species, however, are secondary pests that cannot enter or feed in whole grains. Nevertheless, poor storage and handling practices can create quarantine problems where secondary pests have been introduced from one country to another in infested rice straw (e.g., stem borer and rice water weevil). No field pests can be transferred in whole grains.

The pests discussed here are pests of stored grain. This group of pests can present minimal quarantine risk if the following procedures are followed:

- Ship only whole seeds, eliminating broken grain and detritus from seed lots,
- Do not dry seed in the sun as excessive heat creates openings between the lemma and palea, allowing secondary pests to enter the whole seed.
- Do not stack seed as this creates pressure that opens the lemma and palea.
- Fumigate seeds to kill all insects that may be inside.



Rice moth

Scientific name: *Corcyra cephalonica* (Stainton)
Common name: rice moth
Quarantine status: secondary pest
Stage of entrance or attachment to seed: larva, egg

The adult's body is 12-15 mm long and uniformly gray-brown. Diagnostic features are the upper side of the forewing without spots but with moderately darkened veins; and straight labial palps that are short and inconspicuous in the male but long and prominent in the female. The moth lives for 1 wk.

Each female lays 100-300 eggs on grain and in crevices. Larvae develop in silken chambers that are attached to milled rice. Pupal cocoons are dense, white, tough, and occur in clumps. Larvae are dirty white; the first abdominal segment bears a pigmented ring enclosing a membranous area around the base of the seta; abdominal spiracles have the posterior rim prominently thicker than the anterior part. The life cycle of the rice moth is 5-7 wk.

The moth commonly attacks rough rice and is rarely found in flour mills.

Rusty red grain beetle

Scientific name: *Cryptolestes ferrugineus* (Stephens)
Common name: rusty red grain beetle
Quarantine status: primary pest
Stage of entrance or attachment to seed: larva

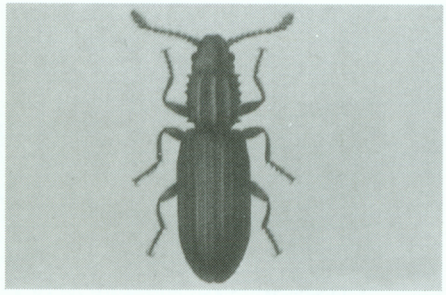
The adult's body is 1.5-2 mm long and light reddish brown. The rusty red grain beetle can be confused with *Cryptolestes pusillus* Schönherr (see figure on p. 70) or *C. turcicus* Grouvelle. Its diagnostic features are an extremely flat and rectangular body;
V-shaped, threadlike antennae; and a thorax with lateral ridges strongly to moderately contracted posteriorly. The beetle lives 6-9 mo.

Females lay an average of 423 eggs in cracks and crevices of the grain, or loosely in farinaceous material, in intergranular spaces, and in grain dust. Many larvae are found outside the grain kernel. Larvae spin cocoons and prefer the embryo to the endosperm. They complete their development exclusively on some species of seedborne fungi.

Larvae are white to straw-colored with flat slender bodies; the posterior end of the abdomen has two dark slender horns. The life cycle lasts 4-9 wk.

Cryptolestes pusillus Schonherr

The rusty red grain beetle thrives in hot moist grain. Larvae are cold-hardy, tolerant of low relative humidity, and have a climatic plasticity index of 570. The adult is a strong flier.



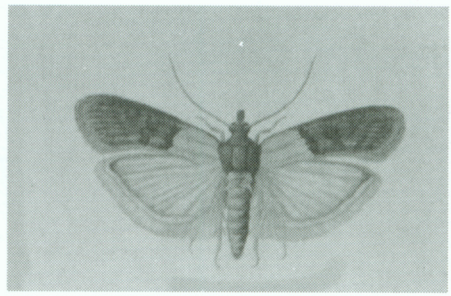
Saw-toothed grain beetle

Scientific name: *Oryzaephilus surinamensis* (Linnaeus)
Common name: saw-toothed grain beetle
Quarantine status: primary pest
Stage of entrance or attachment to seed: none

The adult's body is 2.5-3.5 mm long and dark brown. Although the saw-toothed beetle can be confused with the merchant grain beetle (*O. mercator*), *O. surinamensis* has smaller eyes and longer rounded checks behind the eyes. Diagnostic features include a flat body and the prothorax bears six prominent teeth along each side and three longitudinal ridges on the dorsal side. The adult usually lives 6-10 mo, but may survive as long as 3 yr.

Each adult female lays 50-400 eggs loose among grains. Larvae feed on the external parts of broken grains. They have long white bodies, brown heads, and three pairs of distinct legs behind the head; and the abdomen tapers posteriorly. The larvae form a protective chamber by sticking small food particles together. The life cycle is 4-5 wk.

The saw-toothed grain beetle can tolerate high moisture content in grain. Undamaged seeds or kernels are immune to attack, unless they are moist and soft. The beetle is cold-hardy, tolerant of low relative humidity, and has a climate plasticity index of 500. Scarring and roughing of the food or grain surface indicates feeding damage of this beetle. The adult is very active but rarely flies.



Indian meal moth

Scientific name: *Plodia interpunctella* (Hubner)
Common name: Indian meal moth or dried fruit moth
Quarantine status: primary pest
Stage of entrance or attachment to seed: larva, egg

The adult's body is 16 mm long and reddish brown. It may be confused with the rice moth *Corcyra cephalonica.* Diagnostic features are the basal half of the forewing is pale yellow to whitish gray with occasional dark spots; the rest of the body is reddish brown with a coppery luster and pale to dark transverse, irregular, leaden gray stripes. At rest, the wings are folded closely together like a roof along the bodyline and antennae lie flat on the wings. Mutant forms are common—e.g., forms without normal color patterns or with uniformly dark forewings. The adult lives 14 d.

Each female lays 40-400 eggs on grain. Larvae feed on both external and internal parts of the rice. They prefer the embryo. Larval color depends on the food consumed. Larvae spin silk to bind grains together and then spin a web over the grains.

Larvae have dirty white legs (3 pairs on the thorax and 4 on the abdomen) along a hairy body. Each side of the head has 5-6 eye spots. The front of the head extends slightly more than two-thirds into a vertical triangle. The life cycle is 3-8 wk.

This cold-hardy species has a climatic plasticity index of 330. It feeds not only on cereals but also on nuts, dried fruits, and other dry foods inside groceries and households.

It prefers the germ, bran, and endosperm (in that order), and can overwinter in unheated granaries in temperate countries.

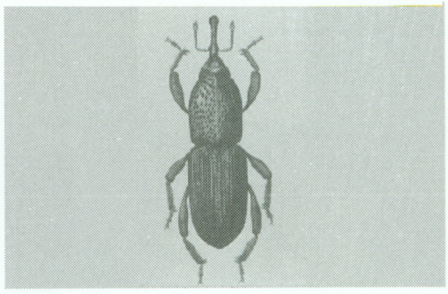
Lesser grain borer

Scientific name: *Rhizopertha dominica* Fabricus
Common name: lesser grain borer or Capucine beetle
Quarantine status: primary pest
Stage of entrance to seed: larva

The adult's body is 2.5-3 mm long and dark brown. Diagnostic features include a cylindrical body with rows of round punctures on the hard wing cover; deflexed head, hidden by a prothorax which has a coarsely tuberculate anterior margin; and antennae with large and distinctly separated 3-segmented club. It lives 1 yr.

Each female lays 300-600 eggs loose among the grains. Larvae feed on inner and outer parts of the grain. They prefer the endosperm and can tolerate grain moisture as low as 8%. The larvae are grublike and have front legs. The head is withdrawn into the curved body. The life cycle is 4-5 wk.

The adult is a strong flier. The presence of flour dust inside a warehouse indicates heavy infestation by the lesser grain borer. The insect is voracious but does not attack leguminous and sunflower seeds.



Granary weevil

Scientific name: *Sitophilus granarius* (Linnaeus)
Common name: granary weevil
Quarantine status: primary pest; unable to fly and thus can only infest grains in storage
Stage of entrance or attachment to seed: egg, larva, pupa, adult

The body of the adult is 2.5-4.7 mm long and shiny reddish brown to black brown with no spots on the wings. It may be confused with the rice and maize weevils. The granary weevil's diagnostic features include sparse oblong-oval or elongated punctures on the thorax, and a head telescoped into a distinct snoutlike structure. The adult lives 3-8 mo.

The adult cannot fly because of its poorly developed hindwings.

Each female lays 50-400 eggs singly in a hole chewed in a rice seed. Each larva hollows out its seed. The larva is grublike, white, legless, has a brown head, and the lower side of its body is straight. The life cycle is 4-8 wk. If physically disturbed, larvae do not successfully complete development.

The granary weevil is a cold-hardy species with a climatic plasticity index value of 173. The adult feeds on whole stored cereals and leaves small holes on damaged seeds. When disturbed, the adult feigns death, as does *S. oryzae.* The granary weevil does not develop on millet, oil seeds, and pulses except chickpeas.

Rice weevil

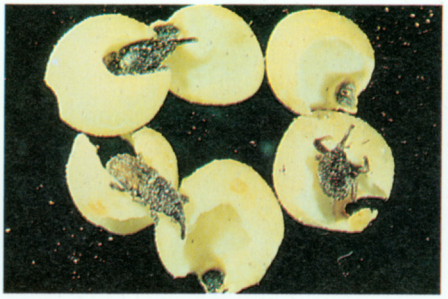
Scientific name: *Sitophilus oryzae* (Linnaeus)
Common name: rice weevil
Quarantine status: primary pest; can infest rice in the field
Stage of entrance or attachment to seed: egg, larva, pupa, or adult

The adult rice weevil is a 2.5-4-mm long reddish brown to brown-black beetle, and can be confused with the maize weevil. Its diagnostic features are densely rounded or irregularly shaped punctures on the prothorax; a long narrow snout with eight-segmented, elbowed, or club-shaped antennae on the head; and hard front wings with four reddish brown spots. The adult lives 7-8 mo.

Each female lays 300-500 eggs singly in holes chewed in seeds. The single larva hollows out cach seed. The larvae are grublike, white, legless, have brown heads, and the lower side of the body is straight. The life cycle is 4-7 wk.

The newly formed adult chews its way out of the grain, leaving a round hole. The adult rarely flies, prefers smaller grains, and its feeding causes irregularly shaped holes. When disturbed, the adult feigns death by drawing its legs close to its body, falling, and remaining motionless for several minutes.

Grains with closed lemma and palea are resistant to entry by this weevil.



Maize weevil

Scientlfic name: *Sitophilus zeamais* Motschulsky
Common name: maize weevil
Quarantine status: primary pest; can infest grain in the field
Stage of entrance or attachment to seed: egg, larva, pupa, adult

The adult has a 3-5-mm long, brown-black to reddish-brown body and may be confused with the rice weevil. Its diagnostic features are densely rounded or irregularly shaped punctures on the prothorax, a long narrow snout on the head, clubbed or elbow-shaped antennae with eight segments, and hard front wings with four reddish-brown spots. The adult lives 4-5 mo.

The maize weevil is larger than the rice weevil, although both have hindwings. The adult is a strong flier, and prefers larger grains. *Sitophilus oryzae* and *S. zeamais* can only be differentiated by the internal character of the male aedeagus. The aedeagus of *S. zeamais* is flat and has two distinct impressions. That of *S. oryzae* has an even, convex upper surface. Females of the two species appear very similar.

Each female lays 100-150 eggs singly in holes chewed in seeds. The larva hollows out each seed. The larva is grublike, white, legless, has a brown head, and the lower side of its body is straight. The life cycle is 5-7 wk.

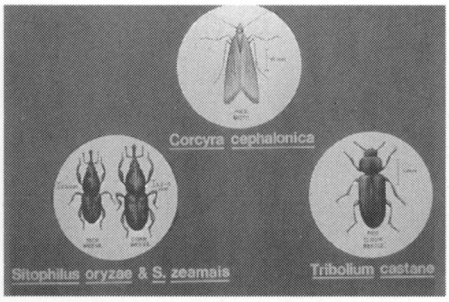
Angoumois grain moth

Scientific name: *Sitotroga cerealella* (Olivier)
Common name: Angoumois grain moth (named after the French province which experienced an outbreak of the moth in 1760)
Quarantine status: primary pest; can infest grain in fields and storage
Stage of entrance or attachment to seed: larva, pupa

The moth's body is 5-10 mm long and yellow-brown with several black dots on the forewing. It may be confused with the white-shouldered house moth *Endrosis sarcitrella* Linnaeus. The Angoumois grain moth's diagnostic features include pointed wing tips; a black spot beyond the center of the upper side of the front wing; very elongated, sharply pointed, and needlelike apex of the hindwing; fringed wings; and curved labial palp. Adults live 10-30 d.

Each female lays 30-150 cggs singly or in clumps on the surface of grain. Each larva hollows out the inside of one grain. Larvae are yellow-white with dark heads. Their life cycle is 5-6 wk or as long as 12-13 wk when exposed to low temperatures.

After emerging from the grain, the adult leaves a *trap door.* Adults prefer whole grains to milled rice. The infested grain has a small round opening in it. The Angoumois grain moth has a climatic plasticity index of 550. The moth dies at temperatures below 10 °C; eggs and larvae die when temperatures exceed 60 °C.



Red flour beetle or bran bug

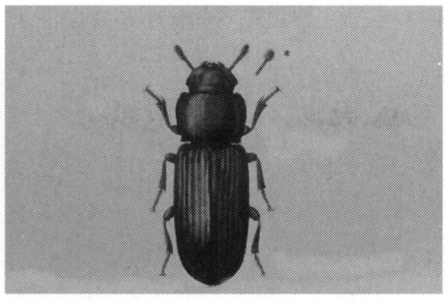
Scientific name: *Tribolium castaneum* (Herbst)
Common name: red flour beetle or bran bug
Quarantine status: primary pest
Stage of entrance or attachment to seed: adult, larva, and egg

The body of the adult is 3-5 mm long and shiny red brown to blackish brown. It may be confused with the confused flour beetle. The red flour beetle's diagnostic features are a relatively flat body with a broader-than-long pronotum, and parallel-sided abdomen; 11-segmented antennae with a 3-segmented pronounced club; a head lacking a ridge above the eyes; and eyes separated ventrally by a space less than two times the diameter of the eye. The adult lives 335-540 d.

Each female lays 300-500 eggs on the outside of grains, attaching the eggs with sticky glue.

Larvae feed both outside and inside the grain. The larva has a prominent head, three pairs of distinct legs, forked abdomen tip, and an elongated and cylindrical white body tinged with yellow. The life cycle of the red flour beetle lasts 4-6 wk but may decrease to 20 d under optimum conditions.

The length of the life cycle depends on the availability of food. The pest is omnivorous and cannibalistic. It is more cold-susceptible than the cold-hardy *Cryptolestes*. The adult is a strong flier.



Confused flour beetle

Scientific name: *Tribolium confusurn* **(Jacquelin du Val)**
Common name: confused flour beetle or bran bug
Quarantine status: primary pest
Stage of entrance or attachment to seed: adult, larva, and egg

The adult beetle is 3-4 mm long and shiny reddish brown. It may be confused with the red flour beetle. Diagnostic features include a flat, oval-shaped body; 11-segmented antennae gradually thickening toward the apex, forming a poorly differentiated 5-segmented club; a head with a ridge above the eyes; and eyes separated ventrally by a space three times the diameter of the eye. The adult lives 1 yr.

Females lay 450-500 eggs on grain. Larvae feed externally on the grains. Larvae have prominent heads, the pairs of distinct legs, forked abdomen tips, and are pale yellow. The life cycle is 4-6 wk.

The confused flour beetle prefers to feed on the grain embryo but can complete development exclusively on seedborne fungi. It is more cold-hardy than the red flour beetle and is a weak flier. Both adults and larvae are omnivorous and cannibalistic. ■

CHAPTER 14

Fungal pathogens

J.K. Misra, S.D. Merca, and T.W. Mew

Fungi are the most numerous of the seedborne rice pathogens. Their epidemic potential varies between species, races, ecosystems, and with their immediate adaptation to their environments. Species of concern to quarantine are *Alternaria padwickii, Bipolaris oryzae, Cercospora janseana, Curvularia lunata, Ephelis oryzae Fusarium moniliforme, Microdochium oryzae, Nakataea sigmoidea, Pyricularia oryzae, Rhizoctonia solani, Sarocladium oryzae, Tilletia barclayana,* and *Ustilaginoidea virens.*

Alternaria padwickii

Pathogen: *Alternaria padwickii* (Ganguly) Ellis (Ellis 1971)
Other acceptable names: *Trichoconis padwickii, Trichoconiella padwickii*
(Etymology: from Latin *alteres,* a kind of dumbbell and Padwick, a scientist)

Disease: stackburn

Detection level: frequently detected (1-100% of incoming seed lots), with low epidemic potential

Where detected: infected seeds and plant parts

How detected: blotter or agar plate methods

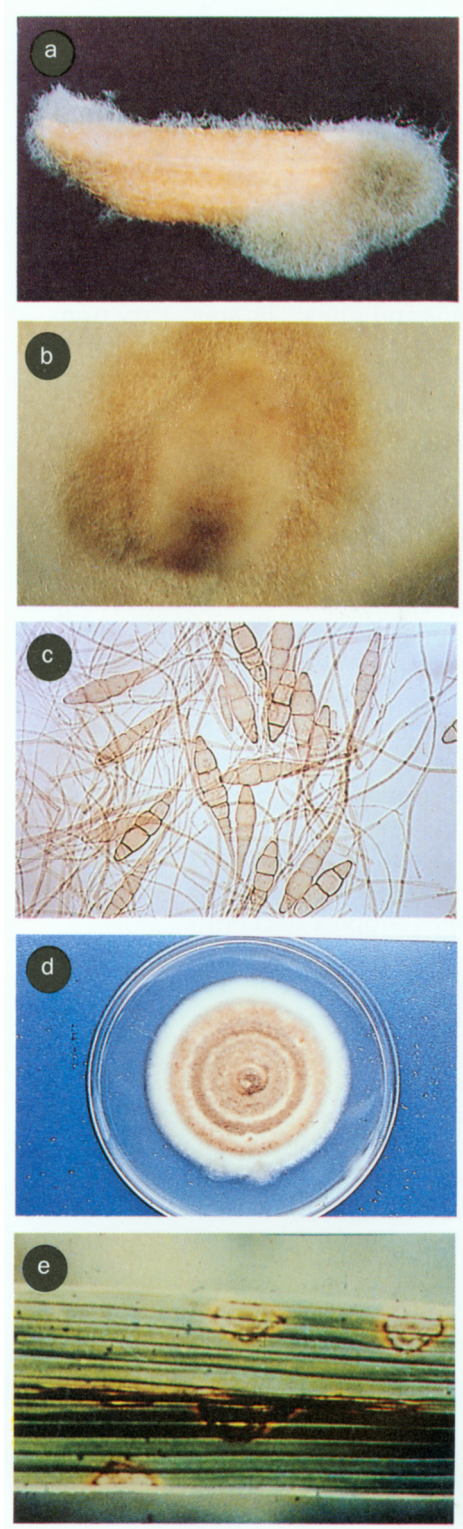
Appearance: see Figure 14.1.

Under a stereobinocular microscope, restricted to profuse mycelial growth with conidia can be seen over the seed on the blotter after 6-8 d incubation (Fig. 14.1a). The profusely growing mycelia are grayish brown, a characteristic of this fungus. Sometimes, pinkish to brownish areas are seen over the blotter around the seed (Fig. 14.1b). Aerial hyphae with straw-colored to dark brown conidia with long terminal appendages are easily discernible at 25X. Figure 14.1c shows a slide mount of conidia with long apical appendages.

A colony on potato dextrose agar is light salmon to dark grayish-brown and attains 4.1 cm in diameter after 5 d incubation at 25 °C (Fig. 14.1d). The reverse is greenish black with salmon edge. Mycelia are effuse, thin, well-developed, copiously branched, hyaline while young and become salmon to dark brown at maturity, 3-6 nm thick, and septate at almost regular intervals of 20-25 nm. Conidiophores are 100-175 × 3-6 nm, swollen apically, and minutely echinulate at the tip. Conidia are fusiform, nondeciduous, measure 103-173 × 9-20 nm (the broadest cell measuring 9-20 nm), have three to five (commonly four) transverse septa, are constricted at the septa, hyaline, and turn from straw-colored to grayish-brown at maturity, with a long terminal appendage. The appendage is half or more of the length of the conidium. One or more septa are seen in the body of the appendage (Fig. 14.1c).

THE DISEASE—STACKBURN

Stackburn is widely spread. It occurs in China, several Southeast Asian countries, Egypt, Nigeria, Madagascar, Surinam, and the USSR.



14.1a. *Alternaria padwickii* mycelial growth and conidia on seed. b. Pink to brownish coloration rendered by *A. padwickii* (courtesy of S. Merca). c. Conidia of *A. padwickii.* d. A. *padwickii* colony on potato dextrose agar. e. Stackburn lesion on leaves.

Symptoms

Stackburn causes lesions on leaves. Significant seed infection and discoloration result in poor germination and rotting of seeds, roots, and coleoptile. *A. padwickii* infects the endosperm and reduces the rice quality. Although stackburn is rarely seen in the Philippines, a high percentage of seed infection is revealed by the blotter test.

Where severe infections occur, symptoms are visible on seedlings, on leaves of adult plants, and on grains as discoloration. The fungus causes typical dark brown spots on the leaves (Fig. 14.1e). The spots are oval to circular, with distinct margins and rings. The spots vary from 1 to 5 mm in diameter. The center of the spot is pale brown. Later it turns white and develops minute black dots, the sclerotia. Similar spots may appear on seedling roots where they cause root tissue to rot. In severe infections, seedlings wilt and finally die. Infected grains have pale brown to whitish spots with a dark brown border and black dots in the center. Similar symptoms arise from various other organisms.

The fungus can penetrate deep into the glumes, causing the kernel to shrivel and become brittle.

Disease development

Both upland and lowland ecosystems support stackburn.

The disease cycle has not been determined yet. *A. padwickii* is thought to survive in the soil and on old rice straw and cause infection in the next season. Infected seeds may be the source of primary inoculum. The stackburn pathogen infects wild grass in ricefields. The wild grasses may be a source of inoculum (Padwick 1950).

Little is known about the influence of environmental factors on stackburn. Sreeramulu and Vittal (1966) found conidia in the air over ricefields in greater numbers in the late morning than at other times of the day. This indicates the role of temperature in spreading the disease.

Control

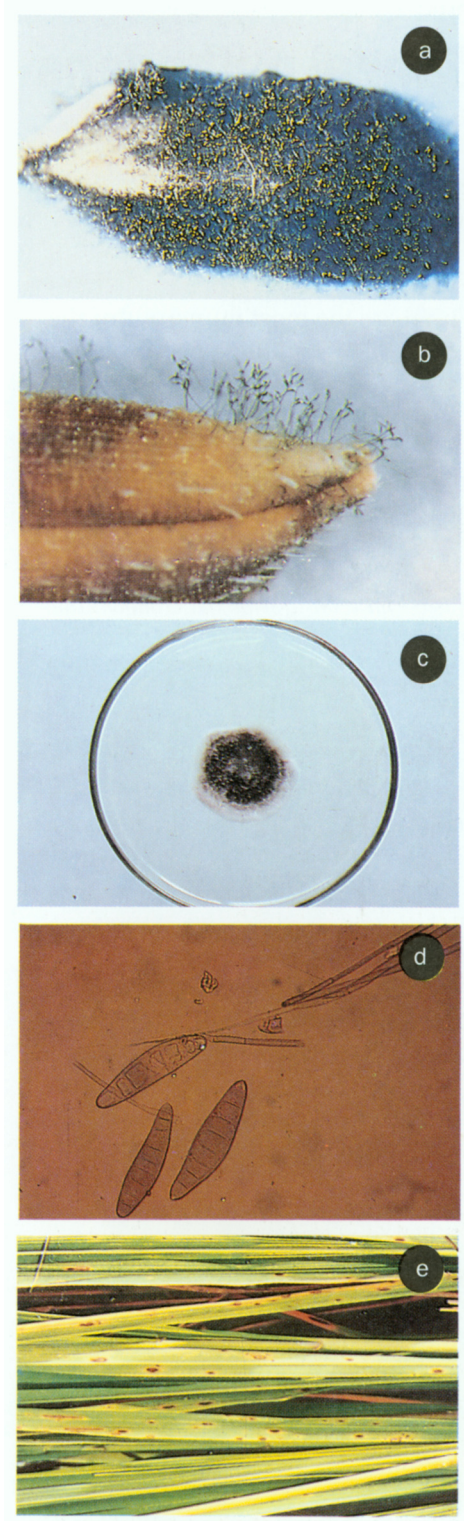
Seed treatment with Dithane M-45 (0.3% by seed weight) provides satisfactory control (Vir et al 1971). Other fungicides and hot water treatment at 50-54 °C for 15 min are also suggested. Burning stubble and rice straw reduces inoculum potential.

Bipolaris oryzae

Pathogen: *Bipolaris oryzae* (Breda de Haan) Shoemaker
Other acceptable names: *Drechslera oryzae* and *Helminthosporium oryzae*
Teleomorph: *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler ex Dastur
(Etymology: from *bipolaris,* bipolar, referring to the bipolar germination of the spores)
Disease: brown spot
Detection level: frequently detected (1-65% of seeds tested), with low epidemic potential
Where detected: infected seeds and plant parts
How detected: blotter or agar plate methods, washing test
Appearance: see Figure 14.2.

Infected seeds incubated on blotters appear dark brown to black, with profuse growth of mycelia visible to the unaided eye (Fig. 14.2a). Under a stereobinocular microscope, at 12-25X magnification, erect dark conidiophores appear scattered or in groups over the seeds. Conidia on the conidiophores are curved both apically and laterally (Fig. 14.2b).

After 5 d incubation at 30 °C , a colony on potato dextrose agar measures 8.1 cm in diameter (Fig. 14.2c). It is effuse, dark brown to black, with blackish reverse. Hyphae are branched, darker, and measure 8-15 nm in diameter. Conidiophores occur in small groups, seldom singly, and are flexuous, geniculate, light to dark brown, long, and thick. Conidia are numerous, curved, naviculate, light brown, smooth, pseudoseptate with 6-14 septa, 63-153 × 14-22 nm, with minute hilum (Fig. 14.2d).



14.2a. Habit character of *B. oryzae* on severely infected seeds. b. Habit character of *B. oryzae* on lightly infected seeds. c. *B. oryzae* colony on PDA. d. *E. oryrae* conidia showing minute hilum at the base of the conidia (courtesy of S. Merca). e. Brown spot symptoms on leaves (courtesy of S. Merca).

THE DISEASE—BROWN SPOT

Sir John Woodhead reported in 1945 that brown spot was the principal cause of the 1942 Bengal famine (cited in Ou 1985). Vidhyasekaran and Ramadoss (1973) reported that severe infections can reduce yield by 20-40%. Abnormal soil conditions increase damage.

Symptoms

Conspicuous brown spots appear on the leaves. Spots measure 2-1 × 0.5 cm, are oval and evenly distributed. They are brown with gray or whitish centers on maturity (Fig. 14.2e). In severe infections, spots fuse and leaves wither. Spots also develop on glumes. When conditions favor fungal development, a velvety growth can be seen over the seeds (Fig. 14.2a), and the fungus may enter the glumes and leave blackish spots on the endosperm.

Brown spot symptoms may appear on the leaf coleoptile (Fig. 14.2e), leaf sheaths, and panicle branches. Blackish lesions may be seen on young roots.

Disease development

Both lowland and upland ecosystems support brown spot development.

Brown spot is seedborne. Seedling infection (seedling blight) arises from infected seeds. Secondary infection, which appears at the posttillering stage, occurs through windborne spores (conidia).

Stubble of the previous crop and collateral hosts, such as *Leersia hexandra, Echinochlaa colona, Pennisetum typhoides,* and *Setoria italica* may be sources of secondary inoculum.

Conidia germinate at 25-30 °C and are infectious at 90-100%) humidity.

Control

Seed treatment methods effectively control primary infection of seedlings.

Before sowing, treat seeds with hot water (53-54°C) for 10-12 min. This controls primary infection at the seedling stage. Presoaking the seed in cold water for 8 h increases effectivity of the treatment.

Griseofulvin, Nystatin, Aureofungin, and similar antibiotics have been found effective in India in preventing primary seedling infection.

Secondary airborne infections may be controlled by spraying Hinosan and Dithane M-45.

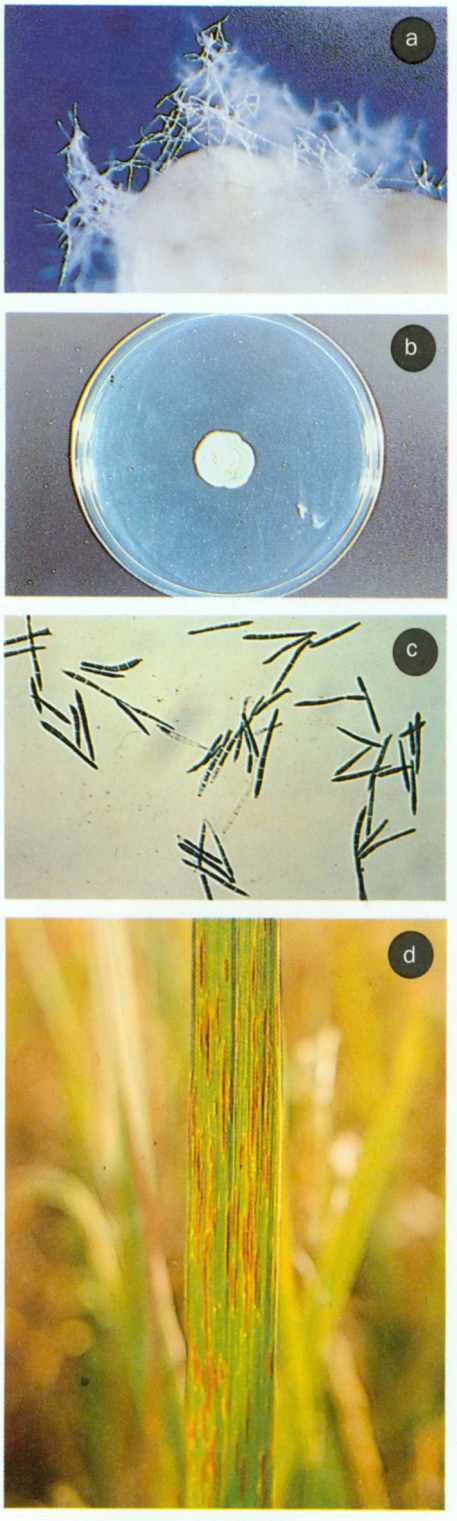
Proper agronomic practices such as crop rotation, field sanitation, balanced application of fertilizers, proper water management, and soil amendments can help control brown spot.

Cercospora janseana

Pathogen: *Cercospora janseana* (Racib.) O. Const.
Teleomorph: *Sphaerulina olyzina* Hara (Etymology: from cercos, worm; spora, spore)
Disease: narrow brown leaf spot
Detection level: infrequently detected (1.15% of seeds tested), with low epidemic potential
Where detected: infected seeds and leaf blades
How detected: blotter or agar test methods; washing test
Appearance: see Figure 14.3,

Under a stereobinocular microscope, dark color or almost black, erect, solitary or grouped conidiophores bearing long, hyaline conidia can be seen (Fig. 143).

After 5 d incubation at 25 °C, a colony on potato dextrose agar attains 2.9 cm diam (Fig. 14.3b). Growth is compact, restricted, cream with hyaline margin, and reverse black. Hyphae are branched and septate. Conidiophores occur singly or in groups, arc olivaceous-brown to almost black, simple to sometimes branched, straight or flexuous, with or without geniculations, and vary in length. Conidia are single, often greatly variable in size and shape, mostly cylindrical, hyaline, sometimes subhyaline, smooth, and three or more septate, and measure 25-48 × 4-6 nm depending on whether they are from the host or culture medium (Fig. 14.3c).



14.3a. Habit character of *Cercospora janseana* on sterile glumes. b. *C. janseana* colony on PDA. c. Conidia of *C. janseana* stained with lactophenol blue. d. Lesions caused by *C. janseana.*

The teleomorph, *Sphaerulina oryzina,* was described by Hara (1918). It is not commonly seen on seeds during rice seed health testing.

THE DISEASE—NARROW BROWN LEAF SPOT

Constantinescu (1982) renamed the fungus *Cercospora janseana* (Racib.) O. Const.

Narrow brown leaf spot occurs in almost all rice-growing countries in Asia, Latin America, Africa, and in the USA, Australia, and Papua New Guinea.

Symptoms

Narrow brown elongated spots or lesions measuring 2-12 × 1-2 mm appear on the leaves (Fig. 14.3d), leaf sheaths, pedicels, and glumes. In resistant varieties, lesions may be narrower, shorter, and darker than those on susceptible varieties (Ou 1985). Spots appear just prior to flowering stage.

Infection causes severe damage in susceptible varieties by reducing the green surface area of the leaves, killing them and the sheath.

Disease development

Estrada and Ou (1978) reported that 30 d or more are required for symptoms to develop after artificial inoculation. This may account for the late appearance of the disease in the field although young and old leaves are equally susceptible.

Both upland and lowland environments support disease development.

Control

The disease can be controlled by using resistant varieties and chemicals. lnformation is not available on the effectivity of seed treatment to control the disease.

Curvularia spp.

Pathogen: *Curvularia* Boedijn (Boedijn 1933)
(Etymology: from *curvus,* curved, referring to curved spores)
Disease: black kernel
Detection level: frequently detected (1-60% of seeds tested), with very low epidemic potential
Where detected: seeds and other plant parts; decaying plant parts
How detected: blotter or agar plate methods; washing test
Appearance: see Figure 14.4.

One of the most commonly encountered fungal genera during rice seed health testing, *Curvularia* spp. may infect up to 80% of seeds and cause grain discoloration. In severe infections, *Curvularia* may weaken seedlings and cause leaf spot (Ou 1985). The most common species infecting rice is *C. lunata;* however, *C.* affinis, *C. geniculata, C. oryzae,* and *C. pallescens* may also be involved in black kernel disease.

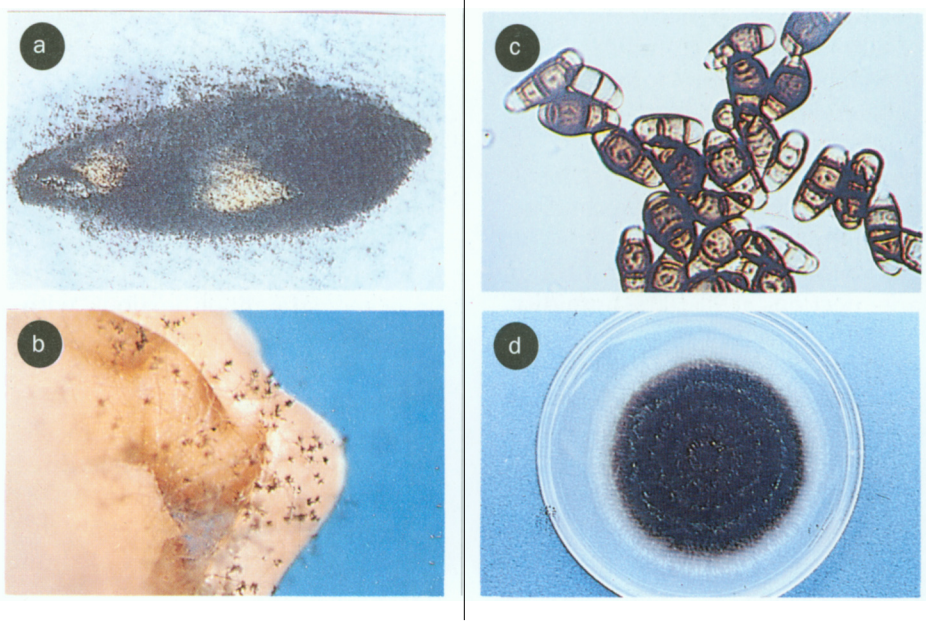
Identification of the species is not necessary during rice seed health testing. However, to identify the species, view mycelia, spores, and conidiophores under a compound microscope. Mount specimen in water or lactophenol cotton blue. Water is preferable as it does not interfere with ascertaining the color.

Under a stereobinocular microscope, infested seeds on a blotter show light to dark brown or somewhat blackish erect conidiophores (macronematous) scattered or (sometimes) grouped (Fig. 14.4a), with terminal or/and laterally borne light to dark brown conidia (Fig. 14.4b). Conidia are often curved but may show other shapes as well.

On infected seeds, dark brown to almost black, unbranched, and septate (apically) conidiophores are seen. They have boat-shaped, brownish conidia. Conidia are born terminally, spirally, or in whorls giving a clustered appearance. Viewed under a compound microscope in water or lactophenol cotton blue mount, the conidia appear triseptate. The third cell from the base is larger, shows prominent curvature, and the basal cell has a scar from its attachment with the conidiophores. Conidia measure 19-32 × 8-16 nm (Fig.14.4c).

Curvularia lunata (Walker) Boedijn colony on potato dextrose agar at 30 °C attains 7.2 cm diameter (Fig. 14.4d). The colony is dark brown to black with hyaline edge.

14.4a. Habit character of *Curvularia* showing dark mass of conidiophore and conidia. b. Flowerlike conidiophores of *Curvularia* spp. c. *C. lunata* conidia. Note scar on basal cell. d. *Curvularia* spp. colony on PDA.



Symptoms
Glumes are discolored, and, in severe infection, the rice kernel shows black discoloration.

Disease development
High humidity and tropical temperatures during crop growth provide a congenial environment for *Curvularia* growth and the development of black kernel.

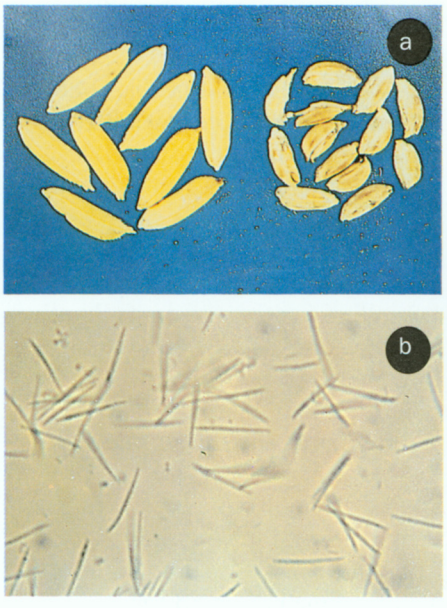
Control
Specific control measures are lacking for black kernel, but spraying with broad-spectrum fungicides can effectively arrest infestation over glumes and other plant parts. To treat seeds, slurry treatment with mancozeb has been found effective at 0.3% by seed weight.

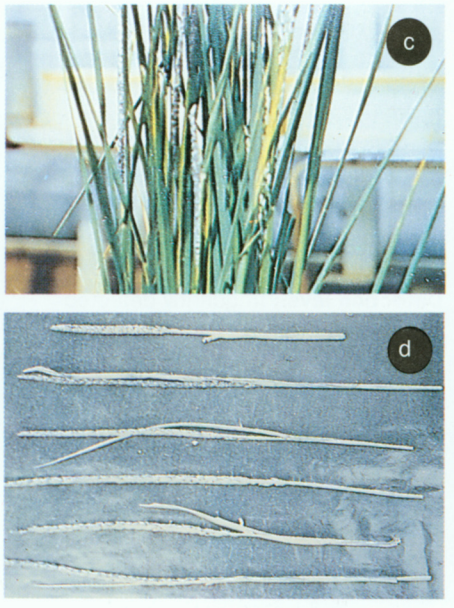
Ephelis oryzae

Pathogen: *Ephelis oryzae* (Sydow 1914)
Teleomorph: *Balansia oryzae sativae* (Hashioka 1971)
(Etymology: from *udbatti, agarbatti,* an incense stick)
Disease: udbatta
Detection level: infrequently detected, with low epidemic potential
Where detected: infected seeds
How detected: dry seed inspection, blot ter test, washing test
Appearance: see Figure 14.5.

Infected seeds appear discolored, abnormally small, deformed, and have whitish masses of conidia (Fig. 14.5a). Blackish perithecia can also be seen. Materials should be examined under a compound rnicroscope to ascertain the identity. The washing test can indicate presence of *E. oryzae* if typical needle-shaped conidia are visible under a compound microscope.

Conidia are hyaline, needlelike, and measure 13-35 × 1-2 nm (Fig. 14.5b). The perithecial stage





14.5a. Healthy seeds (L) and seeds infected with udbatta (R). b. *E. oryzae* conidia. c, d. Udbatta-infected panicles.

(Hashioka 1971) consists of perithecia embedded in stroma. Perithecia are ovate to pyriform and measure 125-200 × 85-100 nm. Asci are cylindrical, hyaline, eight-spored, and measure 92-100 × 6 nm. Ascospores are filiform, straight or curved, 12-27 × 1 nm (CAB 1979).

THE DISEASE—UDBATTA

In India, udbatta occurs in Bihar, Orissa, Maharashtra, and Karnataka states, on low hills where high soil temperature (28 °C) and high soil moisture prevail (Ou 1985); Udbatta has been reported in Southwest China (Tai and Siang 1948), Hongkong, New Caledonia, and West Africa. Udbatta is internally seedborne.

Symptoms
Infected panicles (Fig. 14.5c,d) emerge from their sheaths as grayish white cyclindrical rods with black dots (conidial acervuli). Plants are stunted. Before panicle emergence, conidia and white mycelia form narrow stripes over the flag leaf. Systemic infection appears in all tillers of an infected plant.

Disease development
Mohanty (1964) report that hilly upland areas are prone to udbatta.

Udbatta recurs through fungi surviving on diseased seeds. Other grass hosts may assist the spread. Udbatta may occur in cycles.

Mohanty (1976) noted that high soil temperature (28 °C) and high soil moisture favor the development of udbatta.

Control
Hot water treatment at 54 °C for 10 min effectively controls udbatta (Mohanty 1975).

Fusarium moniliforme

Pathogen: Anamorph: *Fusarium moniliforme* Sheldon
Teleomorph: *Gibberella fujikuroi* (Sawada) Ito (Ito and Kimura 1931). (Etymology: from Latin *mille,* necklace, collar)

Disease: bakanae and foot rot

Detection level: frequently detected (1-59% of seeds tested), with low epidemic potential

Where detected: infected seeds, panicles, lower regions of diseased plants

How detected: blotter or agar plate methods, washing test, growing-on test

Appearance: see Figure 14.6.

Under a stereobinocular microscope, infected seeds on a blotter show moderate to heavy growth of white, fluffy mycelia, often covering the entire seed (Fig. 14.6a). The blotter under the seed sometimes turns violet. Later growth appears powdery due to microconidia formation (Fig. 14.6b).

14.6a. *Fusarium moniliforme.* Note white, fluffy mycelia and microconidia covering the seed. b. Habit character of *F. moniliforme* showing mycelial and false heads (microconidia). c. *F. moniliforme* colony on potato dextrose agar (courtesy of S. Merca). d. *F. moniliforme* microconidia and macroconidia spores (courtesy of S. Merca). e. Bakanae symptoms—highly elongated tillers. f. Sporulation of *F. moniliforme* (microconidia) on stem of bakanae-infected tiller.

A colony on potato dextrose agar, after 5 d incubation at 20 °C, measures 4.7 cm in diameter (Fig. 14.6c), is pinkish, and floccose. At 25 °C, the color becomes reddish violet. Hyphae are branched and septate. Microconidia form in a chain or false head on laterally borne conidiophores. Conidia are 1-2 celled, fusiform to ovate, hyaline, and measure 5-12 × 1.5-2.5 nm. Macroconidia are borne on phialides, 3 to 7 septate, straight or slightly curved basal cell slightly or distinctly pedicellate, formed in sporodochia or pionnotes with color varying from buff, salmon orange, to carrot red, and measure 5-82 × 2.0-4.2 nm. (See Fig. 14.6d for microconidia and macroconidia.)

Perithecia are dark blue, spherical to ovate, measure 250-330 × 220-280 nm. Asci are cylindrical, 4-8 spores, measure 90-102 × 7-9 nm. Ascospores are septate and measure 15.0 × 5.2 nm (Wollenweber and Reinking 1935).

The disease—bakanae and foot rot

Bakanae is widely distributed in all rice-growing areas. Its name varies in different countries. In the Philippines it is called as *play lalake* (male rice); in China, *white stalk;* and in Guyana, *man rice.* In India, Thomas (1931, 1933) described it as *foot rot* disease.

Loss reports vary widely, 3.7-50% (Ito and Kimura 1931, Kinki-Chugoku Regional Agricultural Committee 1975, Pavgi and Singh 1964).

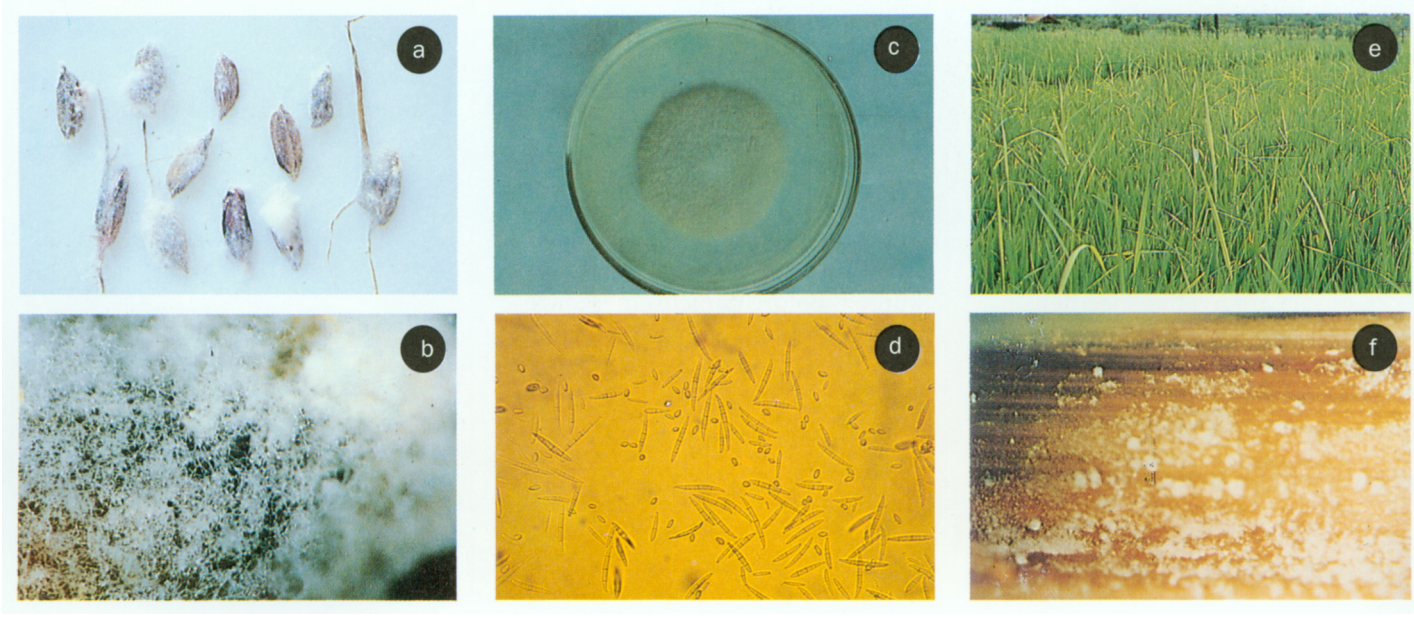
Symptoms

The most visible symptom is the bakanae tillers, which are highly elongated and can be seen from the distance in fields and seedbeds (Fig. 14.6e). Diseased plants appear abnormally elongated, thin, and yellow green compared with other plants. Diseased plants may be distributed irregularly in an infected field. In the seedbed, heavily infected seedlings with necrotic lesions on roots die before or after transplanting. White powdery growths of conidiophores can be seen over the lower regions of the diseased plants (Fig. 14.6f). Diseased plants bear few tillers and their leaves dry up quickly. The diseased plants survive but bear empty panicles. *Gibberella fujikuroi* may also produce perithecia under certain environmental conditions (Sun and Synder 1978).

Disease development

Upland and lowland ecosystems support bakanae development.

Fusarium moniliforme is usually seedborne but can also be soilborne (Seto 1933, Kanjanasoon 1965, Sun 1975). Ascospore release is facilitated



by low relative humidity. Released ascospores lodge over emerging panicles and contaminate seeds. The fungus survives in seeds for many months, depending on the storage conditions. Misra et al (1989) found the fungus associated with seed samples stored for 28 mo in the Philippines.

High moisture and temperature and the germination stage favor bakanae development in seedbeds and seedboxes.

Infection usually occurs during flowering and maturation stages. Infected plants produce numerous conidia and perithecia. Seedlings may be infected at an early growth stage. Infection then becomes systemic but does not reach the floral parts (Seto 1937). Ungerminated seeds sown in infested soil are more susceptible to infection than presoaked or germinated seeds (Kanjanasoon 1965).

Control

Seed treatments with benomyl and thiram-benomyl combinations are effective. Benomyl or benomyl-t at 1-2% of seed weight should be used for dry seed coating. Soaking seed with fungicide solution at 1:1000 for 1 h or 1:2000 for 5 h gives satisfactory results (Ou 1985).

Microdochium oryzae

Pathogen: Anamorph: *Microdochium oryzae* (Hashioka and Yokogi) Samuels and Hallett (Syns. *Gerlachia oryzae* [Hashioka and Yokogi], W. Gams; *Rhynchosporium oryzae* Hashioka and Yokogi) Teleomorph: *Monographella albescens* (Thumen), (Parkinson et al 1981) (Etymology: from Latin *micro,* small; Greek *docheion,* container, referring to the spore-bearing region)

Disease: leaf scald

Detection level: frequently detected (1-54% of seeds tested), with moderate epidemic potential

Where detected: infected seeds and leaf blades

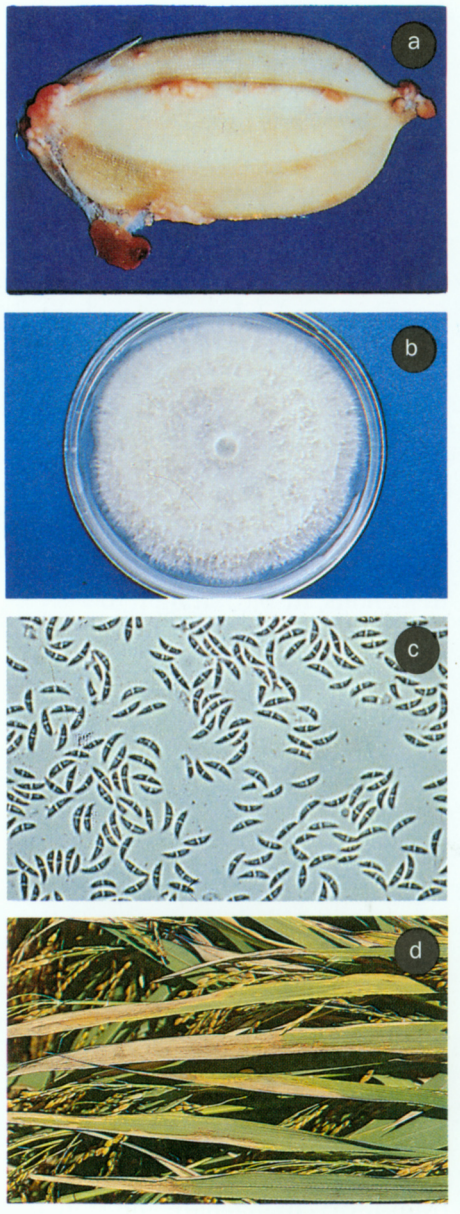
How detected: blotter or agar plate method

Appearance: see Figure 14.7.

Plainly visible with the naked eye or under a stereobinocular microscope, salmon- to orange-colored islands of the fungus' conidial mass (pionnotes) can be seen on the infested seed on a blotter (Fig. 14.7a). The fungus has anamorphic and teleomorphic stages. The anamorph is usually seen on seeds.

A colony on potato dextrose agar, after 5 d incubation at 25 °C, measures 8.4 cm in diameter (Fig. 14.7b). It is compact to slightly floccose, hyaline to salmon in color, and reverse salmon. Mycelia are hyaline and branched. Salmon-colored islands of conidia are seen in older colonies. Conidia are sickle or bow-shaped; 1-4 celled, commonly 2-celled; aseptate when young; hyaline when single but pinkish or salmon colored when in groups; measure 9-14 × 3.0-4.5 nm; and are borne on conidiophores which are not easily distinguishable from the conidium itself (Fig. 14.7c).

Parkinson et al (1981) describe the teleomorph: "perithecia scattered, brown, immersed, subepidermal, occupying almost the entire depth of the leaf, solitary or aggregated into small groups of 1 to 4 perithecia, globose to subglobose, sometimes with a more or less flattened base, 150-180



14.7a. *Microdochium oryzae.* Note orange pionnotes on seed. b. *M. oryzae* colony on potato dextrose agar. c. *M. oryzae* conidia stained with lactophenol blue. d. Leaf scald lesions caused by *M. oryzae* (courtesy of S. Merca).

× 90-120 nm, with a distinctly protruding apical papilla. The ostiole is lined on the inside by hyaline periphyses. The thin smooth wall of 9-18 nm thick is composed of two layers. The outer wall of 3-5 layers is made of light brown colored, angular, somewhat elongated pseudoparenchymatic cells up to 6 nm long and 2-3 nm wide. The inner rather thin wall is almost hyaline with thin-walled compressed cells which usually disappear as the asci mature. Asci cylindrical to cylindric-clavate, thin-walled, 8-spored, unitunicate, 40-85 × 8-12 nm with a distinct amyloid apical structure. Ascospores obliquely distichous to tristichous, fusoid, straight to slightly curved, hyaline, 3-5 mostly 3 septate, not or slightly constricted at the septum, 14-23 (30) × 3.5-4.5 (7.5) nm. Paraphyses filiform, hyaline."

THE DISEASE—LEAF SCALD

Leaf scald is common in rice-growing countries. It has been reported to cause considerable damage in Latin America and West Africa.

Symptoms

Symptoms appear on mature leaves as zonate lesions starting on leaf tips or edges (Fig. 14.7d). Lesions are parallel to oblong, with light brown halos. On mature leaves, lesions vary from 1 to 5 cm in length and from 0.5 to 1 cm in breadth. Coalescing lesions may blight the greater part of the leaf blade and extend to 25 cm long. Zonations become indistinct with age.

Kwon et al (1973) in Korea observed typical leaf symptoms, and reddish-brown, small spots on the leaves and long elliptical or rectangular, purplish-black necrotic spots on the leaf sheaths and panicle necks. Spots enlarged and became bright purplish-brown or light gray.

Microdochium oryzae can also cause coleoptile decay and root rot (De Gutierrez 1960).

IRRI has found salmon-red islands of *M. oryzae* conidia during rice seed health testing.

Disease development

Leaf scald develops in all rice ecosystems.

Infection initiates through the stomata (Naito et al 1975). *M. oryzae* has been isolated from dry plant litter, leaf tissue, and seeds (Boratynski 1979), which may be the source of primary inoculum. The weed *Echinochloa crus-galli* has been found infected by *M. oryzae* (Singh and Gupta 1980), and thus may be a potent source of primary and secondary inoculum.

Control

Seed treatment with carbendazim and thiram 12 h before sowing is effective. Spray treatment with thiophenyl-methyl significantly arrests disease incidence (Swain et al 1990). Benomyl and mancozeb slurry treatment, both at 0.3% by seed weight, effectively eradicates seed infection.

Nakataea sigmoidea

Pathogen: *Nakataea sigmoidea* (Cav.) Hara (Hara 1918)
(Etymology: after Nakata, a scientist; from Latin *sigma,* refers to the shape of the conidium)

Disease: stem rot

Detection level: infrequently detected (0·1%), with low epidemic potential

Where detected: blotter test; during dry seed inspection, sclerotia may be found with the seeds

How detected: infected seeds, panicle branches, sclerotia found in infected stems

Appearance: see Figure 14.8.

Under a stereobinocular microscope, infected seeds on a blotter show blackish, erect conidiophores with three-septate, slightly curved, fusiform conidia, borne singly on pointed sterigmata (Fig. 14.8a). Conidia measure 9.9-14.2 × 29-49 nm (Fig. l4.8b). *N. sigmoidea* is the conidial state of *Magnaporthe salvinii.*

14.8a. Habit character of *Nakataea sigmoidea.*
b. Conidia of *N. sigmoidea.*
c. Stem rot lesions on rice tillers (courtesy of S. Merca).
d. Sclerotia formed inside an infected leaf sheath.

THE DISEASE—STEM ROT

Stem rot was first reported in 1879. Since then, it has been reported in rice-growing countries in Europe, Africa, South America, and Asia.

Lesions develop on stems and cause decay of the leaf sheath and culm. The weakened tillers lodge, thus contributing to grain yield loss and to poor grain milling quality. Estimated losses due to stem rot range from 18 to 80% of yield.

Symptoms

The disease usually develops on older rice crops where sclerotia initiate a small, blackish irregular lesion on the leaf sheath near the waterline (Fig. 14.8c). The lesion advances and penetrates the inner leaf sheath. Here it causes the leaf sheath to partially or entirely rot, and the infection penetrates the culm. Brownish-black lesions may develop in one or two internodes causing the stem to collapse and lodge. Sclerotia are usually formed inside thc affected leaf sheath (Fig. 14.8d) and culm but some may be found outside the leaf sheath. The disease continues to develop as the crop matures. At maturity, examination of infected tillers and panicles reveals sclerotia in the culm; conidiophores and conidia on the leaf sheath; and sclerotia, conidia, and conidiophores on the panicle and spikelet.

Disease development

Sclerotia of *M. salvinii,* which serve as the primary source of inoculum, survive in the stubble and soil surface for 190 d or for 133 d buried in the soil (Park and Bertus 1932). Sclerotia stay in the upper 2-3 inches of soil and float to the surface of the water during land preparation. These sclerotia later come in contact with the rice leaf sheath and germinate to form appressoria or infection cushions and initiate lesions on leaf sheaths. Stem rot progresses to infect the inner leaf sheaths and culm.

Wounds from lodging or insects directly increase the disease incidence. Artificial lodging has caused the disease to spread. Kobari (1961) reported 2-3 times more stem rot on rice plants with stem borers than those free from stem borers.

Control

Burning rice stubble in the field minimizes inoculum levels in the field. Deep plowing using a moldboard reduces inoculum potential by burying a large percentage of sclerotia. Proper use of fertilizers, avoiding excess nitrogen availability, and increasing potassium tend to reduce the damage.

Although many fungicides are effective, chemical control has not been used against stem rot. Resistant and nonlodging varieties are the preferred disease controls.

Pyricularia oryzae

Pathogen: *Pyricularia oryzae* Cav. (Etymology: from *pirum,* pear shape, describing the spores)

Disease: blast

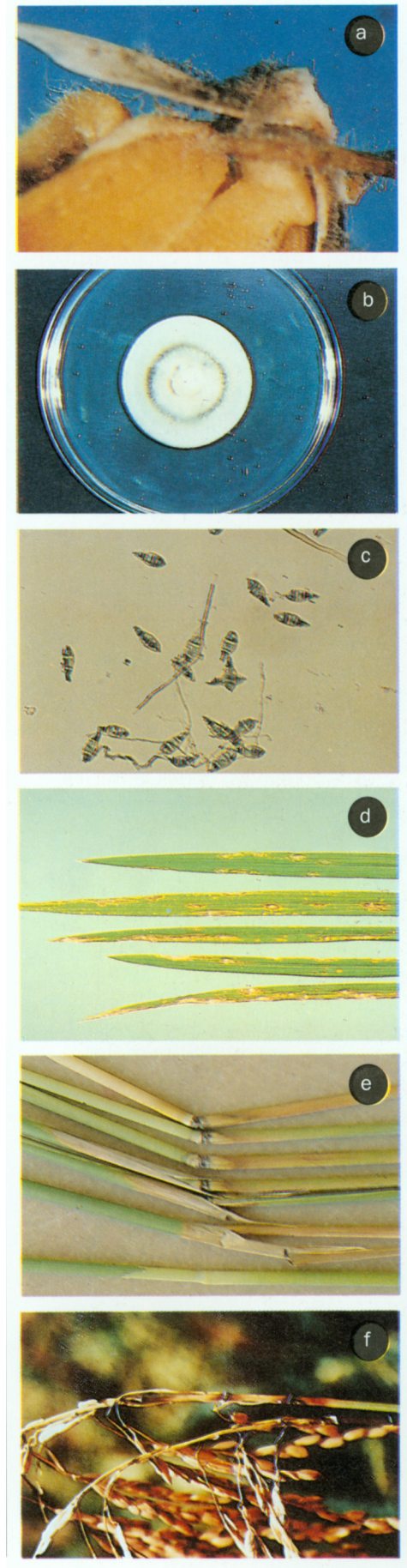
Detection level: infrequently detected (1.4% of seeds observed), with high epidemic potential

Where detected: infected seeds, panicles, nodes, leaves

How detected: blotter or agar plate methods, washing test

Appearance: see Figure 14.9.

Under a stereobinocular microscope, infected seeds on a blotter exhibit a fine, grayish growth of erect conidiophores bearing conidia mostly on their sterile glumes (Fig. 14.9a). *Cladosporium* appears similar, but it has bigger dark brown to almost black conidiophores and smaller conidia.



14.9a. Habit character of *P. oryzae* on the embryonal end of seed showing grayish colony growth on sterile glumes. b. *P. oryzae* colony on potato dextrose agar. c. *P. oryzae* conidia stained with lactophenol blue. d. Blast symptoms—spindle-shaped spots with brown or reddish brown margins, ashy centers, and pointed ends. e. Node blast. Leaf sheaths have been removed to show infected node. f. Blast symptoms—rotten neck infection at panicle base.

Colonies on potato dextrose agar at 25 °C measure 4.1 cm in diameter after 5 d (Fig. 14.9b). Mycelia are thin, effuse, almost black in the center with whitish margins; reverse blackish. Hyphae are hyaline and branched. Conidiophores are distinct from vegetative hyphae (macronematous), single or in groups, simple, and septate with distinct geniculations. Conidia are produced singly in sympodial fashion, pyriform to obclavate, usually biseptate, rarely 1-3 septate, almost hyaline to pale olive-colored, measuring 14-40 × 6-13 nm; with distinct protruding hilum (Fig. 14.9c).

THE DISEASE—BLAST

Blast is one of the most widely distributed rice diseases. Losses due to blast are difficult to estimate because other factors complicate the disease. However, losses are always significant.

Symptoms

Pyricularia oryzae produces spots or lesions on leaves, nodes, panicles, and grains, but rarely on leaf sheaths.

Leaf spots are spindle-shaped with brown or reddish-brown margins, ashy centers, and pointed ends (Fig. 14.9d). Fully developed lesions normally measure 1.0-1.5 cm in length and 0.3-0.5 cm in breadth. These characteristics vary with the spots' age, susceptibility of the cultivar, and environmental factors.

When nodes are infected, they become black and rotten (Fig. 14.9e). Infection of the panicle base causes *rotten neck* or *neck rot* (Fig. 14.9f) and causes the panicle to fall off. In severe infection, secondary rachillae and grains are also affected.

Disease development

Found in both upland and lowland environments, blast occurs most often in upland environments in the tropics. Water deficiency predisposes the crop to severe infection in all environments.

Low night temperatures favor blast infection and lesion development. Disease development is very much affected by the humidity and soil moisture. The upland ecosystem with high night humidity, where rice is grown in dry soil, presents a favorable environment for development of blast. Rice grown in moist and flooded conditions is moderately to highly resistant to the disease.

Airborne conidia, which may be present all year in the atmosphere, are the most potent source of infection. Conidia may be seedborne, or they may come from straw, stubble, or numerous alternate or collateral hosts (weeds). Environmental factors, such as temperature and relative humidity, play significant roles in conidia production and, thus in cycling the disease.

High relative humidity (more than 90%) favors conidial formation. A typical lesion can produce 2000-6000 conidia daily for at least 14 d under laboratory conditions (IRRI, unpubl. data).

Applying high amounts of nitrogenous fertilizers induces a heavy incidence of blast, irrespective of the supply of phosphorus or potassium.

Control

Blast may be controlled by

- planting resistant varieties,
- using chemicals, and
- using balanced agronomic practices.

Many blast-resistant rice varieties have been developed, but the pathogen adapts to them relatively quickly. Hence, constant efforts are needed to breed rice varieties with several resistant genes.

If planting time is suitably adjusted to avoid low night temperatures and high humidity, blast infection can be avoided.

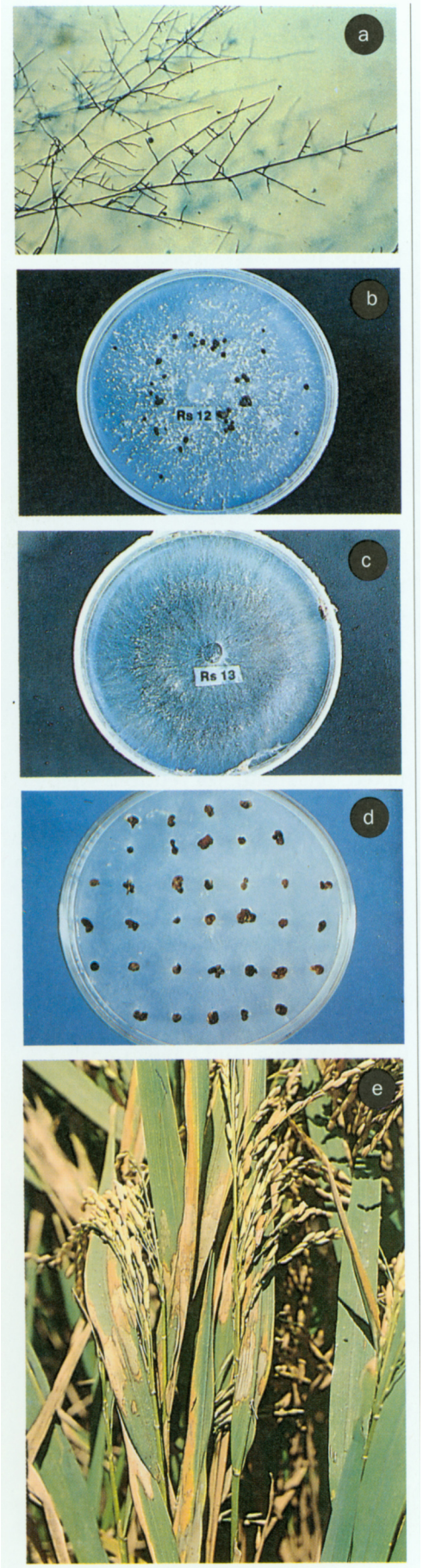
Seed is effectively treated with a benomyl slurry at 0.3% by seed weight, or by hot water at 52-57 °C for 15 min.

Rhizoctonia solani

Pathogen: Anamorph: *Rhizoctonia solani* Kuhn
Teleomorph: *Thanatephorus cucumeris* (Frank) Donk
(Etymology: from *rhiza,* root; *ketinein,* to kill)
Disease: sheath blight
Detection level: infrequently detected, with low epidemic potential
Where detected: infected seeds and other plant parts
How detected: sclerotial bodies seen during dry seed inspection; blotter test
Appearance: see Figure 14.10.

Under a stereobinocular microscope, infected seeds on a blotter show dark brownish spreading mycelia. Under a compound microscope, *Rhizoctonia's* characteristic branching pattern identifies the pathogen (Fig. 14.10a).

Rhizoctonia solani can be grown on potato dextrose agar or Czapek Dox agar media at 28-30 °C. Colonies are initially coloriess, then turn brown with age (Fig. 14.10b,c). Hyphae are hyaline when young but turn brown at maturity, measure 8-12 nm in diameter, and are septate. Three types of mycelia may be seen—straight runner hyphae; short, swollen, and branched or lobate hyphae which give rise to penetration pegs; and mycelia consisting of moniloid cells which take part in sclerotia formation. The hyphae can be seen in cultures, over the edge of the petri plate, or on the wall of the culture tube. On culture media, superficial sclerotia are produced. These are composed of thick-walled, dark, compact cells (Fig. 14.10d). Sclerotia may be irregular to somewhat spherical, and measure 4-5 mm or more in diameter. Basidia and basidiospores are formed under natural conditions and measure 10-15 × 7-9 nm and 8-11 × 5-6.5 nm, respectively; however, measurements between isolates vary. Sclerotial size and number depend on concentration and source of nitrogen in the medium.



THE DISEASE—SHEATH BLIGHT

Sheath blight, a widespread disease, occurs in almost all rice-growing regions. Higher tillerage of the modern varieties increases humidity around the plants, predisposing them to sheath blight infection.

Loss records vary depending on cultivars, plant growth stage, and environment. Yield losses range from 22 to 40% (Gangopadhyay and Chakrabarti 1982). In the tropics, total crop failure has been reported due to death of all the leaves in susceptible cultivars (Ou 1985).

Symptoms

Symptoms become apparent at tillering or flowering stage. Spots or lesions first develop near the water (in lowland fields) or soil (in upland fields). Spots initially appear on the leaf sheath. Spots may be oval or ellipsoidal, and measure 1-3 cm long. Lesions on the leaf blade are usually irregular and banded with green, brown, and orange coloration. Lesions are grayish white in the center with brown margins. At advanced stages, when the flag leaf is infected (Fig. 14.10e), panicle exsertion is affected. Some cultivars raised under conditions favoring disease development may later develop spots extending up to the leaf blades. Leaves with spots eventually die.

Sclerotia form on the spots. Sclerotia are usually large, 4-5 mm in diameter, white when young, turn brown or purplish brown at maturity, and fall off easily. Sclerotia can be seen between the leaf sheath and the culm.

14.10a. *Rhizoctonia solani* showing characteristic branching pattern on agar stained with lactophenol blue. b. Young *R. solani* colonies on agar. C. *R. solani* colonies turn brown with age. d. Sclerotia of *R. solani* germinating on agar medium. e. Sheath blight lesions affecting flag leaves and panicles (courtesy of S. Merca).

Disease development

Both upland and lowland ecosystems support the disease. Incidence is higher in drained ricefields than in upland fields.

Infection normally occurs through sclerotia which survive in the soil for a long time, depending on the temperature and moisture levels. In the tropics, infected straw, stubble, weeds, and sclerotia cause primary infection. Sclerotia cannot withstand dry soil for more than 21 mo. Surviving sclerotia come to the water surface during agronomic operations. They infect healthy plants upon contact. The number of sclerotia in the soil determines the intensity of primary infection. Mycelia enter host tissue through stomata or directly penetrate the cuticle. Mycelia proliferate throughout the host. Mycelia of younger lesions actively cause secondary infections.

High humidity and temperature (up to 40 °C) aid sheath blight spread and development.

Younger plants contract sheath blight at tillering and flowering stages. Upward development takes place after the heading stage under favorable conditions.

Close planting helps spread sheath blight by increasing humidity and temperature of the plants' microenvironment.

High doses of nitrogenous fertilizer cause susceptibility. Potassium induces some resistance.

Control

The disease can be controlled through

- cultural practices such as green manuring with *Seshania aculeata,* soil solarization, and deep plowing to bury infested plant residues into the soil (Dath 1979, Grinstein et al 1979, Papavizas and Lewis 1979);
- treating seed with thiram 75% ai and quintozene 23.2% at 1 g/kg of seed (Marcos 1975);
- applying organo-arsenic compounds, such as methylarsene sulphide and methylarsine bisdimethyl dithiocarbamate, effective at about 50 ppm with only two applications:

one application at the appearance of lesions and another at booting stage (Kozaka 1961);

- applying benomyl (Kannaiyan and Prasad 1976) or other fungicides such as Kitazin, Hinosan, and Dithane M-45;
- applying antibiotics such as Validamycin and Polyoxin (developed in Japan), and Chingfeng meisu and Jinggangmycin (developed in China); and
- using pentachlorophenol (PCP), a weedicide which inhibits sclerotial germination.

Sarocladium oryzae

Pathogen: *Sarocladium oryzae* (Sawada) (Gams and Hawksworth 1975) (Etymology: from Greek *saron,* broom; *klados,* branch)

Disease: sheath rot

Detection level: frequently detected (1-9% of seeds observed), with moder ate epidemic potential

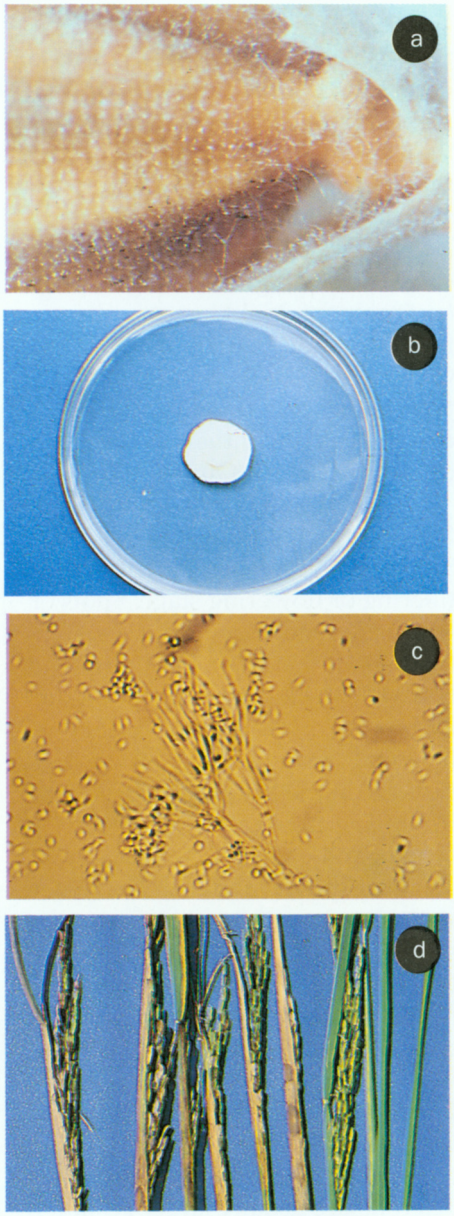
Where detected: infected seeds and leaf sheaths

How detected: blotter or agar plate methods

Appearance: see Figure 14.11.

Under a stereobinocular microscope, infected seeds on a blotter show short, erect, hyaline conidiophores with tiny, transparent, spherical conidial masses which resemble water droplets over their tips (Fig. 14.11a).

Colonies on potato dextrose agar are slow-growing, compact, and attain 1.9 cm diameter in 5 d at 30 °C (Fig. 14.11b). Aerial mycelia are sparse, orange, reverse darker orange. Hyphae are branched, septate, measure up to 2.5 nm in diameter. Gnarled, wider hyphae are also present. Conidiophores are irregularly branched. Branches arise in whorls. Phialides may develop on vegetative hyphae singly or on slender conidiophores in a fascicular manner. Conidia terminate in slimy masses, are hyaline, aseptate, cylindrical, smooth, and measure 3.5-7 × 0.8-1.5 nm (Fig. 14.11c).



14.11a. Habit character of *Sarocladium oryzae* on rice seed. b. *S. oryzae* colony on potato dextrose agar. c. *S. oryzae* phialides (conidiophores) and spores. d. Sheath rot disease on panicles.

Sarocladium attenuatum may be encountered during rice seed health testing and is indistinguishable from *S. oryzae* under the stereobinocular microscope. However, slide mounts of *S. attenuatum* show more regularly formed appressed verticillate conidiophores and longer conidia with truncated ends than *S. oryzae* (Gams and Hawksworth 1975).

A novice at seed health testing may confuse the growth of *Verticillium* spp. with that of *Sarocladium* spp. Careful examination under high magnification will show that *Verticillium alboatram* colonies are larger and colonies of *V. cinnabarinum* are smaller than those of *Sarocladium.*

The disease—sheath rot

Sheath rot occurs in all rice-growing countries. It causes significant yield loss during rainy season in both rainfed and upland ecosystems. Chen (1957) reported losses up to 85% in Taiwan. In India, Chakravorty and Biswas (1978) noted that sheath rot reduced yields of some varieties by 9.6-26.0%. Kang and Rattan (1983) reported losses up to 50% in Punjab.

Symptoms

Sheath rot appears on the uppermost leaf sheath which encloses the emerging panicle (Fig. 14.11d). Sheath rot lesions are irregular, 0.5-1.5 cm long, and have grayish centers and brownish margins. Sometimes the whole lesion appears gray-brown. In infected tillers, panicles emerge partially or not at all. Inside the sheath, a whitish powdery mass of fungus can be seen. Grains discolor brown. In the tropics, it is easily confused with sheath discoloration (caused by many *Pseudomonas* pathogens).

Disease development

Severe sheath rot infections occur most frequently in lowland environments and less frequently in the uplands.

Sarocladium oryzae is seedborne. The fungus enters through stomata or wounds. Secondary infections may be windborne, the fungus entering the host through injured tissue (Amin et al 1974, Chin 1974).

Plants are most vulnerable at tillering to panicle initiation stages. Infection at these stages becomes serious.

Severe infections occur in densely planted fields, especially where stem borers have infested the plants, or the plants are under stress.

Control

No effective control methods are currently available. However, seed treatment with fungicides such as Dithane M-45 and Benlate effectively eliminates seedborne inocula.

Tilletia barclayana

Pathogen *Tilletia barclayana* (Bref.) Sacc. and Syd. (Duran and Fischer 1961) (Etymology: after Tillet and Barclay, plant pathologists)

Disease: kernel smut

Detection level: frequently detected (1-100% of seeds observed), with low epidemic potential

Where detected: infected seeds

How detected: during dry seed inspection under a stereobinocular microscope; washing test; soaking in 0.2% solution of NaOH

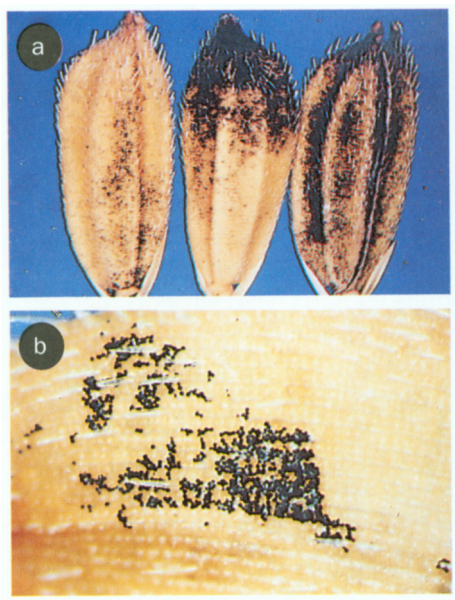
Appearance: see Figure 14.12.

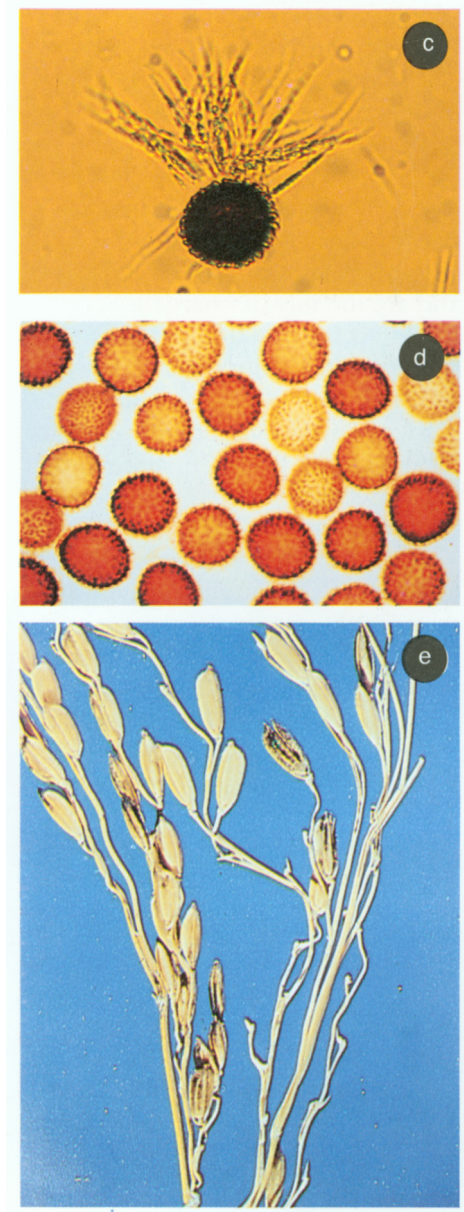
Under the stereobinocular microscope, infected seeds on a blotter show blackening of glumes. Dissected glumes show masses of spores on and in the kernel. In some instances, grains burst, revealing the spore masses. Dark, black, minute spots (singly or as masses of spores-teliospores) may be seen on the infested grain (Fig. 14.12a,b). Under a compound microscope, white, erect, primary sporidia may be seen issuing from spores on incubated seeds (Fig. 14.12b) and details of teleospores with primary sporidia (Fig 14.12c) may be recognized.

Takahashi (1896), who named the fungus *Tilletia horrida,* described it as follows: "spore masses pulverulent, black produced within the ovaries and remaining covered by the glumes. Spores globose, irregularly rounded, or sometimes broad-elliptical, the round ones 18.5-23.0 nm in diameter and the elongated 22.5-26.0 × 18.0-22.0 nm in size. Epispore deep olive brown, opaque, thickly covered with conspicuous spines (Fig. 14.12d). The spines hyaline or slightly colored, pointed at the apex, irregularly polygonal at the base, more or less curved, 2.5-4.0 nm in height, and 1.5-2.0 nm apart at their free ends. Sporidia filiform or needle-shaped, curved in various ways, 10 to 12 in number and 38-53 nm in length." Subsequently, the fungus has been studied by various workus. Padwick and Khan (1944) placed it with the genus *Neovossia* as *N. horrida* (Tak.) Padwick and Azmat Khan. Later on, Tullis and Johnson (1952) renamed it *N. barclayana.* Duran and Fischer (1961) returned it to the genus *Tilletia,* and renamed it *Tilletia barclayana* (Bref.) Sacc. and Sycl.

THE DISEASE—KERNEL SMUT

Takahashi and Anderson reported kernel smut in Japan and the USA in 1896 and 1899, respectively. It is now known in almost all rice-growing countries.





14.12a. *Tilletia barclayana* infected and -contaminated rice seeds. b. *T. barclayana* spores germinating. Note whitish growth of promycelia and primary sporidia. c. Germinating teliospore giving rise to promycelin and primary sporidia (courtesy of S. Merca). d. *T. barclayana* spores. e. Smutted seeds in panicles caused by *T. barclayana.*

Kernel smut is seed-transmitted. It may reduce grain quality if incidence is high. Most rice-importing countries exclude seeds infected with kernel smut or demand that seeds be properly treated before shipment.

Symptoms

The disease can be detected in the field only at panicle maturity. Minute carbon black pustules or dots can be seen on the glumes. In severe infections, rupturing glumes show beaklike outgrowths (Fig. 14.12e). When smut balls burst, spores lodge on healthy spikelets and leaves. The black spores can be seen in the field.

Disease development

Kernel smut develops more often and more severely in lowland than in upland ecosystems.

Chlamydospores persist for more than 3 yr. At germination, they produce many sporidia which in turn may produce secondary sporidia. Secondary sporidia are sickle-shaped and are forcibly discharged. They can infect healthy florets. Infected florets may be fully or partially smutted.

Heavy application of nitrogenous fertilizers and the use of late-maturing varieties increase the incidence of kernel smut.

High humidity and high temperature (25-30 °C) at anthesis favor disease development.

Control

Seed treatment with fungicides, hot water, or both is applied to infected seeds as a precautionary quarantine measure prior to shipment.

Ustilaginoidea virens

Pathogen: *Ustilaginoidea virens* (Cooke) Takahashi (Takahashi 1896) (Etymology: from Latin *ustilo,* to burn a little; *viren,* green)

Disease: false smut or green smut

Detection level: infrequently detected (0.1-7% of seed lots tested), with low epidemic potential

Where detected: infected seeds

How detected: dry seed inspection, washing test

Appearance: see Figure 14.13.

The fungus appears from within infected mature spikelets (Fig. 14.13a,b). The spore ball is initially yellowish to orange, later turns olive green to dark greenish black. Fleshy inside while young, thc ball becomes hard at maturity. The central mycelial mass is hard and composed of thin, hyaline hyphae. The outer sporiferous region is three-layered. The outermost layer is greenish black with powdery spores; the middle layer, orangish; the innermost, yellowish. Spores are small, smooth to warty, olivaceous, spherical, and measure 3-5 × 4-6 nm in diameter (Fig. 14.13c).

The teleomorph is not commonly encountered.

The disease—false smut or green smut

False smut or green smut was first reported in Tirunelveli, Tamil Nadu, India, in 1878, and later in many countries (Japan, the USA, Philippines, Myanmar, Sri Lanka, China, and others).

Because the disease attacks crops that are healthy, many farmers believe the appearance of false smut signals a bumper harvest. Observations by Rao (1964) support the farmers' belief.

14.23a. Panicle showing smutted seeds. b. Smutted seeds (L) and healthy seeds (R). c. Spores of *U. virens.*

Although not currently considered a serious disease, false smut is widely spread. Cultivation of some varieties in hybrid rice-growing areas with continuous cropping and high inputs may make false smut an endemic problem. Recently, false smut has become a serious problem both in hilly and foot hill regions of Uttar Pradesh (Pathak 1990; U.S. Singh, 1990, pers. commun).

Symptoms

Not more than four spikelets are usually affected per panicle. Affected spikelets are transformed into a large yellow-green velvety mass of spores about 1 cm in diameter or more. Initially contained in a membrane, the mass later bursts. The color of the ball darkens or changes to greenish black. If cut open, the ball appears white in the center. The center layer, consisting of mycelia, has three distinguishable parts. The innermost layer is yellowish with mycelia and developing spores. The middle layer is orange, with mycelia and spores. The outer layer is green and consists of mature spores. The surface of the ball is covered with powdery dark green spores.

Disease development

Both upland and lowland environments support false smut development.

Ascospore release from the perithecia coincides with anthesis of early varieties of rice. Ascospores are believed to be the source of primary infection. Ascospores lodge on the stigma and infect the spikelet. They convert the ovary into a smut ball which produces abundant conidia. Conidia are disseminated by wind and germinate within a few hours, serving as the source of secondary inoculum.

Reports regarding the stage at which infection occurs conflict. Infection has been reported at booting stage, at the early stage of flowering, at maturity, or just before heading.

Cloudy days with high relative humidity favor infection and disease development during the flowering stage.

Applying fertilizer at flowering stage increases false smut incidence and development. Where no fertilizer has been applied, disease incidence is low.

Control

Hashioka (1952) noted that spraying fungicides a few days before heading helps avert the disease. Copper oxychloride effectively arrests the disease without affecting yield (Kannaiyan and Rao 1976). ■

CHAPTER 15

Bacterial pathogens

B. Cottyn, M.T. Cerez, and T.W. Mew

Two bacterial genera are known to be of quarantine importance in rice seed health. However, there is no reliable technique for detecting *Xanthomonas* from rice seed; the detection of *Pseudomonas* may be difficult because identification up to species level is not easy.

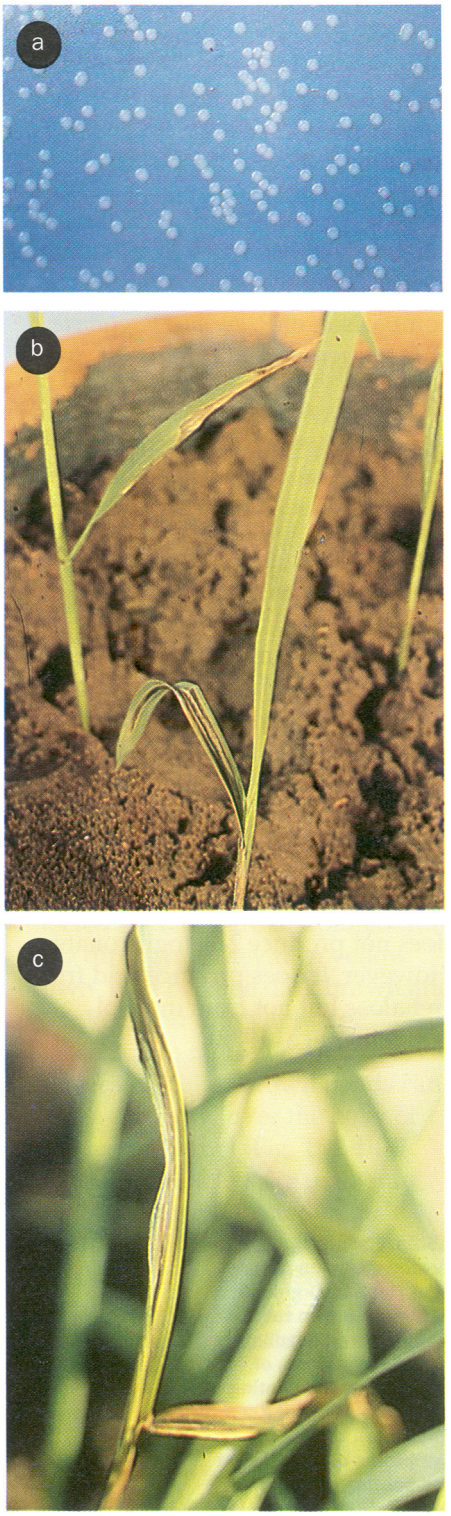
Pseudomonas avenae

Pathogen: *Pseudomonas avenae* Manns (Etymology: from Greek *pseudes,* false; *monas,* unit; *avenae* from *Avena* genus of plants)
Disease: bacterial stripe or bacterial brown stripe
Detection level: frequently detected, with low epidemic potential
Where detected: infected seeds, leaf sheath, leaf blade, and coleoptile
How detected: see Chapter 7.
Appearance and characteristics: see Figure 15.1.

See Chapter 7 and Figure 15.la for a comparison of *P. avenae, P. glumae, P. fuscovaginae,* and *P. syringae* pv. *syringae.*

THE DISEASE—BACTERIAL STRIPE OR BACTERIAL BROWN STRIPE

Bacterial stripe, a seedborne and seed-transmitted seedling disease, is known to occur widely in rice-growing countries (Shakya et al 1985), but it does not cause much damage to production.



15.la. *Pseudomonas avenae* on nutrient agar (blue background). b. Bacterial blight symptoms—longitudinal stripe. c. Bacterial blight symptoms —stripe has turned dark brown.

Symptoms

Symptoms seen at the base of the leaf sheath are water-soaked, dark green, longitudinal stripes. The stripes later turn dark brown (Fig. 15.1b,c). The coleoptile and leaf blade may also show symptoms. Mildly infected seedlings recover. Severely affected seedlings are stunted and eventually die.

Disease development

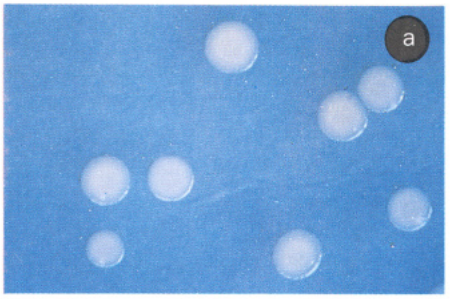
Upland and wetland ecosystem nurseries are affected with bacterial stripe.

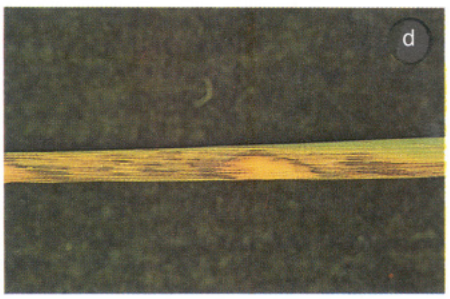
High humidity favors disease development. Seeds are the primary source of inoculum.

Bacterial stripe occurs only at seedling stage. How the pathogen reaches the rice seeds and where its presence has been recorded are unanswered questions (Mew 1992).

Control

Kasugamycin is effective against *P. avenae* and controls damage in nursery boxes (Yaoita and Fujimaki 1984). The pathogen can be eliminated from seeds by applying dry heat treatment at 65 °C for 6 d (Zeigler and Alvarez 1988).





Pseudomonas fuscovaginae

Pathogen: *Pseudomonas fuscovaginae* Miyajima, Tanii, and Akita (Etymology: from Greek *pseudes,* false; *monas,* unit; from Latin adj. *fuscus,* fuscous; from Latin fem. n.) *vagina,* sheath)

Disease: bacterial sheath brown rot

Detection level: uncertain, epidemic potential depends on temperature at panicle initiation

Where detected: infected seeds, rotted sheaths and seedlings

How detected: see Chapter 7.

Appearance and characteristics: see Figure 15.2.

See Chapter 7 and Figure 15.2a for a comparison of *P. avenue, P. glumae, P. fuscovaginae,* and *P. syringae* pv. *syringae.*

The disease—bacterial sheath brown rot

Bacterial sheath brown rot is now widely distributed in Latin America, Asia, the Burundi highlands of Central Africa, and Madagascar.

Precise information regarding the loss caused by the disease is lacking, but it is known that appreciable losses have occurred in tropical and temperate South America.

Pseudomonas fuscovaginae differs from other oxidase and arginine dehydrolase positive nonpathogenic fluorescent pseudomonads in that it does not produce 2-ketogluconate. However, it does produce acid from trehalose but not from inositol (Duveiller et al 1988).

15.2a. *Pseudomonas fuscovaginae* on nutrient agar (blue background). b. Sheath brown rot symptoms on seedling. *c.* Early symptoms of infection at the booting stage (courtesy of K. Miyajima). d. Late symptoms of infection at the booting stage (courtesy of K. Miyajima).

Symptoms

Sheath brown rot symptoms appearing at seedling and later stages include discoloration and rotting of sheath (Fig. 15.2b,c, and d). After transplanting, infected seedlings initially show yellow-brown discolorations on their lower leaf sheaths. Later the discolorations turn gray-brown to dark brown. Ultimately, rot results in death of the infected seedling. The infected flag leaf sheath becomes water-soaked and necrotic. Other sheaths also exhibit lesions. Spikelets of emerging panicles are discolored, sterile, or may be symptomless except for small brown spots. In acute infections, the sheaths turn completely grayish brown or dark brown and panicles shrivel and dry.

Disease development

Bacterial sheath brown rot is active in irrigated temperate regions and rainfed upland rice ecosystems.

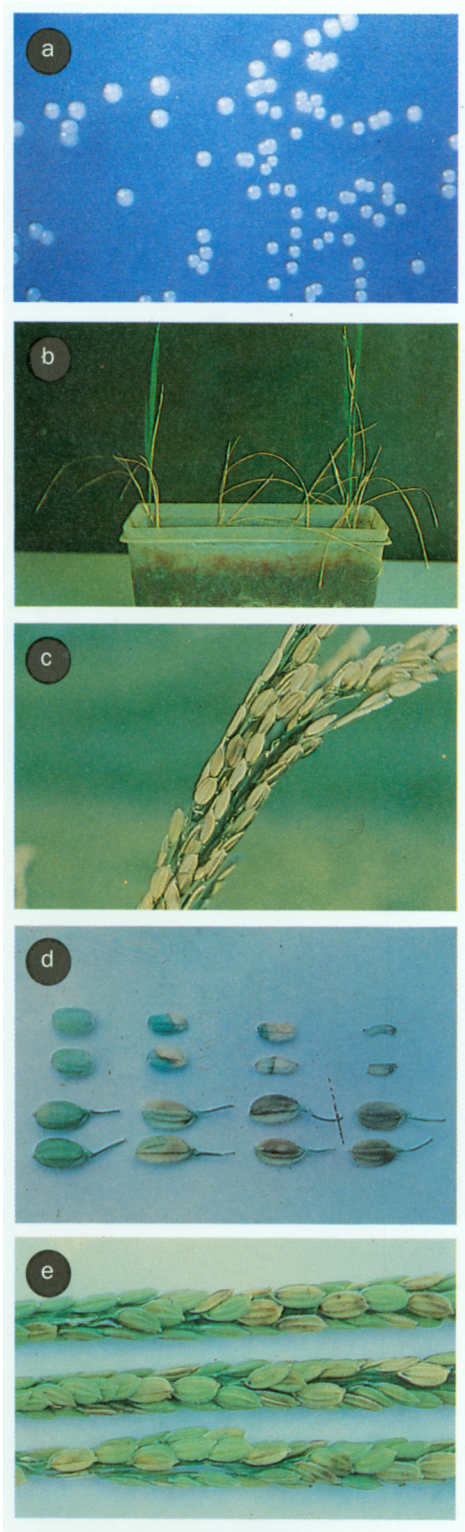
Cold temperature stress probably predisposes plants to severe attacks of bacterial sheath brown rot.

In temperate regions, *P. fuscovaginae* can survive in rice straw only if it is stored indoors. In the tropics, other host plants and infected seeds harbor the bacterium. These can be a source of primary inoculum.

The secondary infection or disease cycle in the field may arise through a bacterial population already proliferating on symptomless leaf blades and sheaths. The secondary occurrence is most infectious at booting stage.

Control

Dry heat treatment at 65 °C for 6 d eliminates *P. fuscovaginae* from seeds (Zeigler and Alvarez 1987). Streptomycin alone or combined with oxytetracyclin (15% + 1.5%) effectively arrests bacterial sheath brown rot. Kasugamycin is effective against *P. fuscovaginae* but does not eliminate it completely from the seed.



15.3a. *Pseudomonas glumae* on nutrient agar (blue background). b. Seedling rot *(P. glumae)* after inoculation. The two healthy seedlings at both sides are the controls. c. Grain rot d. Infected grains. Top = dehulled grain, bottom = whole grain (courtesy of C.C. Chien). e. Infected spikelets are unevenly distributed in the affected panicle (courtesy of C.C. Chien).

Pseudomonas glumae

Pathogen: *Pseudomonas glumae* Kurita and Tabei
(Etymology: from Greek *pseudes,* false; *monas,* unit; glumae refers to glume)

Disease: bacterial grain rot

Detection level: frequently detected, with moderately high epidemic potential

Where detected: infected seeds and rotten seedlings

How detected: see Chapter 7.

Appearance: see Figure 15.3.

See Chapter 7 and Figure 15.3a for a comparison of *P. avenae, P. glumae, P. fuscovaginae,* and *P. syringae,* pv. *syringae.*

THE DISEASE—BACTERIAL GRAIN ROT

The disease occurs widely (Kaku 1988, Zeigler and Alvarez 1988).

Infection causes spikelet sterility and poor or no grain ripening. There is no exact estimate of losses caused, but 900,000 ha of rice in North Kyushu Island (Japan) have been infected, indicating the intensity and severity the disease can assume (Mew et al 1989).

Symptoms

Bacterial grain rot symptoms appear on seedlings and spikelets. Seedlings raised in boxes turn brown and rot (Fig. 15.3b). The glumes (lemma) of infected spikelets are discolored. Initially dirty gray, they turn yellow brown, then become dark brown and shrunken, and ultimately dry (Fig. 15.3c). in mild infections, only the palea discolor. Brown bands can be seen distinctly over the belly of the infected kernel (Fig. 15.3d).

Infected spikelets are unevenly distributed in an affected panicle (Fig. 15.3e). In severe infections, 50% of spikelets may be affected. The bacterium is active only in spikelets but spreads intercellularly in the lemmal tissues.

Disease development

Irrigated tropical ecosystems support development of bacterial grain rot.

The disease prospers in high temperature and humidity.

Bacterial grain rot usually occurs at the milk stage. Infections occurring at heading stage are more damaging.

Control

Treating seeds with dry heat at 65 °C for 6 d helps eliminate *P. glumae* from seed (Zeigler and Alvarez 1988).

Pseudomonas syringae pv. *syringae*

Pathogen: *Pseudomonas syringae* pv. *syringae* van Hall. (Syn *P. oryzicola* Klement)
(Etymology: from Greek *pseudes,* false; *monas,* unit; *syringae* from Syringa, generic name of lilac)

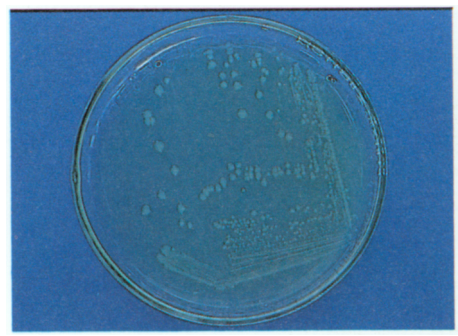
Disease: bacterial sheath rot

Detection level: frequently detected, with low epidemic potential

Where detected: infected seeds, rotting flag sheaths, stem, node

How detected: see Chapter 7. (No specific detection method.)

Appearance: see Figure 15.4.



15.4 *Pseudomonas syringae* pv. *syringae* on nutrient agar.

See Chapter 7 and Figure 15.4a for a comparison of *P. avenae, P. glumae, P. fuscovaginae,* and *P. syringae* pv. *syringae.*

THE DISEASE—BACTERIAL SHEATH ROT

Bacterial sheath rot was first reported by Klement (1955) in Hungary as a new bacterial rice disease affecting the panicle leaf sheath. Bacterial sheath rot occurs now in rice-growing continents and seems to have worldwide distribution. The causal bacterium may be present simultaneously with other *Pseudomonas* spp.

Symptoms

The infected panicle sheath develops brown lesions which later rot and cause the panicle to dry. The stem and nodes may develop blurred spots which turn brown or black (Ou 1985). Grains become discolored. Spikelets of infected panicles become sterile.

Disease development

Bacterial sheath rot primarily occurs in irrigated environments.

Wet and windy weather helps spread the disease (Klement 1955).

The disease may be seedborne and seed-transmitted, and may spread in a field through rain splash. Both hypotheses need confirmation.

Although the disease frequently occurs at the heading stage, it may develop earlier.

Control

Treating seeds with dry heat at 65 °C for 6 d helps eliminate *P. syringae* pv. *syringae* from seed (Zeigler and Alvarez 1988).

Xanthomonas oryzae pv. *oryzae*

Pathogen: *Xanthomonas oryzae* pv. oryzae (Ishiyama 1922, Dye 1978, Dye et al 1980)
(Etymology: from Greek *xanthus,* yellow; *monas,* unit; *oryza,* rice)

Disease: bacterial blight

Detection level: infrequently detected, with high epidemic potential

Where detected: seeds and other diseased plant parts

How detected: see Chapter 7. (Note that detection methods are not standardized for Xoo.)

Appearance: see Figure 15.5.

See Chapter 7 and Figure 15.5a for a comparison between *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola.*

THE DISEASE—BACTERIAL BLIGHT

Bacterial blight is one of the most destructive rice diseases in Asia. It occurs in China, Korea, India, Indonesia, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam. The disease also occurs in Northern Australia and Africa.

Symptoms

In the tropics, bacterial blight exhibits three distinct symptoms.

Leaf blight symptom is the most common manifestation seen in the farmer's field (Fig. 15.5b). Water-soaked stripes appear a few centimeters below the leaf tip, on the margin of the leaf blade (Fig. 15.5c). Stripes enlarge and turn yellow within a few days. They have wavy margins. One or both leaf margins may have lesions. As the disease progresses, lesions cover the whole leaf and the leaf turns white to gray with an overgrowth of saprophytic fungi.

15.5a. *Xanthomonas oryzae* pv. *oryzae* colonies on nutrient agar. b. Field symptoms of bacterial blight. c. Water-soaked stripes on leaves. d. Bacterial ooze.

In susceptible varieties, lesions may also appear over the leaf sheath, extending to its base. Lesions may occur on an injured part of a blade. Sometimes infected blades wilt and roll up while the other leaves are still green. Yellow stripes sometimes appear toward the inner side of the leaf blade, especially in resistant varieties. These later become necrotic.

Bacterial ooze—milky or pale amber-colored droplets—exudes from the surface of the young lesions in the early morning (Fig. 15.5d). The droplets dry up and impart a certain roughness to the leaf. The dry droplets fall off easily into the field water, where they may become a source of secondary inoculum.

In severe infections, grains are involved. Glumes show discolored spots surrounded by water-soaked margins. Spots can easily be seen while the grain is young and green; when the grain is mature, the spots become gray or yellowish white.

In temperate regions, the disease appears at the heading stage, rarely in the seedbed. On seedlings, small water-soaked spots develop on the margins of fully developed lower leaves. Later, the leaves yellow, dry rapidly, and wither.

Kresek symptoms were described in Indonesia almost four decades ago, as a separate bacterial rice disease. Leaves of the infected plant become grayish-green, fold up, and roll along the midrib about 1-2 wk after transplanting.

Pale yellow leaf symptom is seen in the youngest leaves of mature plants. These leaves become uniformly pale yellow or have yellow or greenish-yellow broad stripes on their blades. The mechanism of the appearance of this symptom is not well-understood. Bacteria have not been detected in the infected leaves but have been found in the stem and in the internodes of the infected leaves. Some workers consider this symptom to be a secondary effect.

Sometimes it is difficult to distinguish the symptoms of bacterial blight from symptoms of other diseases. Kresek symptoms may be confused with stern borer injuries. In order to confirm bacterial leaf blight infection, a few tests can be made.

Perform the pathogenicity test for *Xanthomonas* (see Chapter 7).

Disease development

Irrigated and rainfed lowland ecosystems support bacterial leaf blight development.

Heavy rains with strong winds facilitate disease development by causing wounds in plants. Dry weather helps bacterial exudates fall into irrigation water and spread the disease to neighboring fields. Moderately high temperature (25-30 °C) increases the disease incidence.

Where and how long Xoo survives in the environment to cause disease recurrence is not settled, as scientists of different rice-growing areas report varying findings. Other alternate hosts (weeds), diseased straw, rice stubble, and irrigation water (field or canal) may be potent inoculum carriers for the next season.

Xoo can enter into the host's body through numerous points such as stomata, wounds and other injuries to leaves, water pores (hydathodes) on the leaf blades, and cracks at the base of the leaf shield caused by emergence of new roots.

Xoo may be detected from the late seedbed stage to the tillering stage, or even later.

Excessive use of nitrogen (especially organic nitrogen) as a late topdressing, phosphate and potassium deficiency, and excess silicate and magnesium are importmt factors that predispose plants to bacterial leaf blight infection.

Control

The disease can be avoided effectively by using resistant varieties. However, the pathogen has different races in different areas and it is essential to know the pathogen's racial distribution to plan effective varietal deployment.

Xanthomonas oryzae pv. *oryzicola*

Pathogen: *Xanthomonas oryzae* pv. *oryzicola* (Fang, Ren, Chen, Chu, Faan & Wu) Dye (Dye 1978)
(Etymology: from Greek *xanthus,* yellow; *monas,* unit; *oryza,* rice)

Disease: bacterial leaf streak

Detection level: infrequently detected, high epidemic potential

Where detected: seeds and other plant parts

How detected: see Chapter *7.*

Appearance and characteristics: see Figure 15.6.

See Chapter 7 and Figure 15.6a for a comparison between *X. oryzae* pv. *oryzae* and *X. oryzicola.*

THE DISEASE—BACTERIAL LEAF STREAK

Bacterial leaf streak is widespread in tropical Asia.

Symptoms

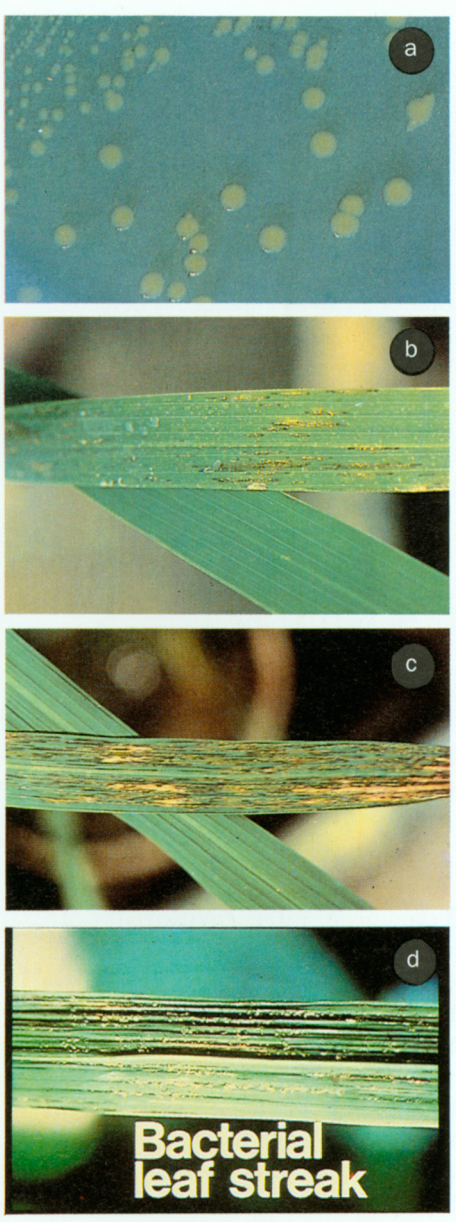
Translucent interveinal streaks of various length appear on the leaves (Fig. 15.6b,c). Yellowish droplets of bacterial ooze may be seen over the lesions under humid conditions (Fig. 15.6d). When dry, the ooze looks like small beads. Windborne beads then infect healthy leaves. Old lesions become light brown. A yellow halo may appear around the lesions in susceptible varieties.

At advanced stages of the disease, the leaves turn brown and die. Necrotic lesions become covered by saprophytic organisms. At this point, it is difficult to distinguish the symptoms of bacterial leaf streak from those of bacterial leaf blight.

Disease development

Bacterial leaf streak develops in both lowland and upland ecosystems.

High temperature (more than 30 °C) favors disease development. High humidity (more than 80%) favors initiation of infection and dissemination of the bacteria.



15.6a. *Xanthomonas oryzae* pv. *oryzicola* on nutrient agar. b, c. Translucent streaks on leaves (various lengths). d. Bacterial ooze.

Infected seeds and contaminated water are suspected sources that introduce the disease to new areas. Naturally infected wild rice (*Oryza perennis*) is suspected as a source of inoculum.

Xcola enters the host plant through the stomatal openings and wounds. Bacteria multiply in the parenchymatous tissue. The disease is spread mainly through rain splash, irrigation water; and leaf contact (within a field). Strong wind and rain facilitate the secondary spread of the disease.

Control

Bacterial leaf streak is best controlled by cultivating resistant varieties. Satisfactory control has been achieved by using disease-free seeds that are treated with fungicides and antibiotics. ■

CHAPTER 16

Nematode pest

J.-C. Prot and E.B. Gergon

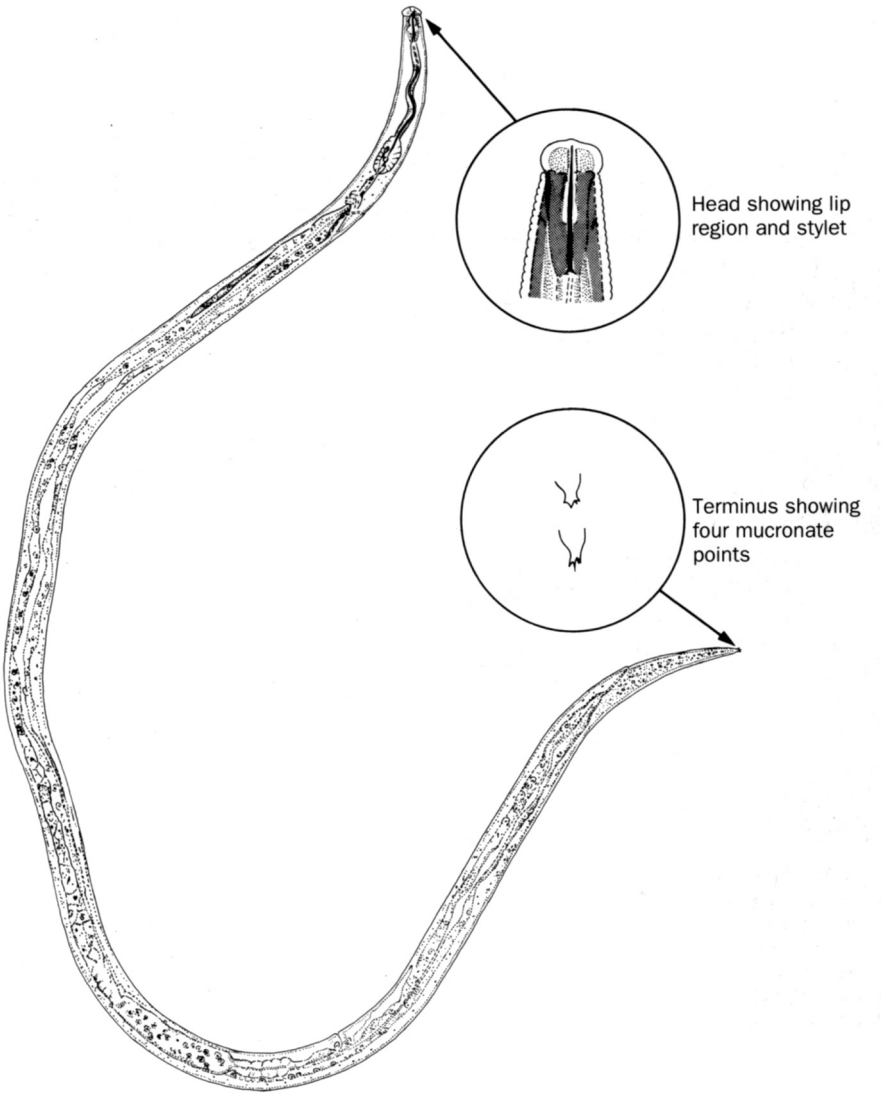
Only one nematode is of importancc to rice seed health testing.

Aphelenchoides besseyi

Pathogen: *Aphelenchoides besseyi* Christie
(Etymology: from *aphelen,* simple; *enchos,* spear; and after Besseyi, a scientist)
Disease: white tip
Detection level: up to 100 nematodes per seed from infected seed lots
Where detected: seeds
How detected: dehull grains or extract using small sieve or Baermann funnel techniques
Appearance: see Figure 16.1.

Aphelenchoides besseyi is an ectoparasite found on rice and other hosts. Its host range includes more than 35 genera of higher plants (Fortuner and Williams 1975). Rice is thc most important host. Other important hosts include wild rices, common weeds of ricefields, and food crops such as maize, onion, sweet potato, taro, and yam. It can also feed and reproduce on many saprophytic and pathogenic fungi, e.g., *Alternaria* spp., *Fusarium* spp., *Helminthosporium* spp., *Sclerospora* spp., and *Sclerotium oryzae.* It is bisexual and males and females occur in equal numbers. It usually reproduces by amphimixis, occasionally by parthenogenesis. Males are usually shorter than females.

Some characteristics that distinguish *A. besseyi* are a slender, slightly curved body, rounded lip, oval median esophagal bulb, lateral fields with four incisures, and a conoid tail with variably shaped mucro with 3-4 processes (Fig. 16.1).



16.1
***Aphelenchoides besseyi* highly magnified to show structures.**

For further details, see the Commonwealth Institute of Helminthology *Description of plant parasitic nematodes,* Set 1, No. 4 by Franklin and Siddiqi (1972).

THE DISEASE—WHITE TIP

The disease is known in most of the world's rice-growing areas. Yield loss depends on infestation level and other factors such as climate, variety, and agronomic practices (Bridge et al 1990).

Symptoms

Leaf tips first turn white, from the tip and extending downward 3-5 cm. Infected plants lose vigor and are stunted. The flag leaf twists, affecting panicle emergence. Small panicles are partially exserted, the uppermost part remains sterile and lacks spikelets (Fig. 16.2). In severely infected plants, panicles are affected. Infected grains are sometimes distorted. Yoshii and Yamamoto (1950) note that not all infected plants or tillers exhibit symptoms. Tolerant varieties are sometimes asymptomatic while harboring the pests.

In white tip caused by insect damage, leaves also show perforations.

16.2 White tip disease of rice caused by *A. besseyi.*

Disease development

Both upland and lowland environments support the disease.

The nematode survives in glumes from 8 to 36 mo, depending on storage conditions. *A. besseyi* feeds ectoparasitically on leaf apical meristems. It proliferates and enters the spikelets before anthesis and feeds on ovaries, stamens, and embryos. As the grain matures, nematodes stop multiplying and coil in the glume.

Infestation rarely occurs through the soil, commonly through infested seeds and diseased husks. The nematode is disseminated through irrigation water. Infestation and disease development are facilitated by flooded soil, high temperature, and nitrogenous manures.

Control

Seeds may be treated with nematicide or hot water (52-57 °C for 15 min).■

CHAPTER 17

Organisms causing grain discoloration and damage

J.K. Misra, S.D. Merca, and T.W. Mew

A number of weak parasites or saprophytes which have not yet been discussed in this manual in feet rice seeds at pre- and postharvest stages, causing syndromes referred to as grain discoloration. Grain discoloration may be on glumes, kernels, or both. The type and magnitude of discoloration vary with the place, environmental conditions, organisms involved, and several unknown factors. Not all organisms that cause grain discoloration have been studied or identified.

Disease development

Environmental factors such as continuous rain, humidity and temperature (27-35 °C) conducive to fungal and bacterial growth help increase thc incidence of grain discoloration. Strong winds, which cause plants to lodge against each other or to fall to the ground, contribute to grain discoloration.

Grain discoloration may also occur during storage where poor aeration, high humidity, and high temperature prevail. Under conditions favoring luxuriant growth during storage, some species of *Aspergillus, Fusarium,* and *Penicillium* produce toxins as well as cause discoloration.

Symptoms

Symptoms may be one spot of any color (ranging from pale yellowish, brown, gray to black dots), lesions to bleached areas over glumes, or combinations of many discernible patches, lesions, or spots. Spots on the glumes or kernels affect both physical and chemical qualities of the grains.

Fungi and bacteria associated with discolored grains affect germinability (Misra et al 1990, Ou 1985).

Table 17.1 lists some fungi which are associated with discolored seeds.

Bacteria are found associated with 28-32% of discolored seed (Misra et al 1990, Baldacci and Corbetta 1964). *Pseudomonas* spp. (fluorescent and

Table 17.1. Some fungi associated with discolored seeds.

Species	Frequency of detection (%)	Reference
Sarocladium oryzae	46	Misra et al (1990)
Curvularia spp.	41	
Trichoconiella padwickii	36	
Phoma spp.	21	
Fusarium moniliforme	10	
Fusarium sp.	8	
Verticillium cinnabarinum	7	
Nigrospora oryzae	6	
Fusarium semitectum	5	
Cochliobolus miyhabeanus	23.4	Baldacci and Picco (1948)
Alternaria sp.	13.4	
Epicoccum purpurascens	4.9	
Penicillium sp.	4.9	
Fusarium sp.	1.2	
Cephalosporium sp.	1.2	
Sterile spp.	19.7	
Fusarium	40	Teunisson (1954)
Penicillium spp.	25	
Aspergillus flavus	22	
Curvularia	60	Rao and Salam (1954)
Drechslera oryzae	9.1	Johnston (1958)
Alternaria padwickii	7.9	
Fusarium spp.	14.6	
Epicoccum sp.	30.2	Baldacci and Corbetta (1964)
Alternaria sp.	19	
Drechslera oryzae	9.2	
Penicillium sp., *Fusarium* sp., *Curvularia* sp. and *Pyricularia oryzae*	2.6-0.42	

nonfluorescent) and *Erwinia herbicola* are normally found associated with discolored seed. Nonfluorescent *Pseudomonas* strains cause discoloration and sterility in grains (Zeigler and Alvarez 1990). However, other bacteria present may cause this syndrome. More information is needed in this regard.

Detection

For details, see Chapters 6 and 7.

Fungi. Blotter and/or agar plate methods detect most of the fungi. Some slow-growing fungi are better seen on a blotter. Somewhat different flora appear when seeds are plated on agar after surface sterilization with 1% sodium hypochlorite for 10 min.

Control

Fungicidal spray reduces the incidence of grain discoloration in the field. Weekly sprayings of benomyl (50% WP) at 1.0 kg formulation/ha, applied from early booting to early ripening stages, help reduce grain discoloration. Mancozeb (80% WP) is also effective (Bandong et al 1983). Postharvest operations to keep seeds clean, dry (13-14% moisture), and free from rodents and insects during storage can help prevent grain discoloration and consequent damage to seeds. ■

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Appendices

Appendix 1. **Sample recording sheets.**

RECORDING SHEET FOR THE WASHING TEST

Accession no.
IQ /EQ

Botanical name

Sender/organization

Date of dispatch (in case of import)

Date of receipt

Phytosanitary certificate

Treated/untreated

Additional declaration

Field observation report on disease(s)

Weather conditions

Treatment given, if any

Dispatch date
To whom/address

Remarks

Date of analysis _____

Sample size tested: Weight of sample _____

Number of seeds _____

Name(s) of pathogen(s) investigated

Qualitative test: Pathogen present

Quantitative test: Number of spores detected

per gram of seed _____

per seed _____

Analyst

RECORDING SHEET FOR THE BLOTTER AND AGAR PLATE METHODS

Accession no.
IQ /EQ

Botanical name

Sender/organization

Date of dispatch (in case of import)

Date of receipt

Phytosanitary certificate

Treated/untreated

Additional declaration

Field observation report on disease(s)

Weather conditions

Treatment given, if any

Dispatch date
To whom/address

Method _____

Date of sowing _____ Recording date _____

Total no. of seeds tested _____ Number of seeds per replicate _____

Analyst number _____

Fungus	Replicate numbers										Percent
	1	2	3	4	5	6	7	8	9	10	

Remarks _____

_____
Analyst

RECORDING SHEET FOR *APHELENCHOIDES BESSEYI*

Accession no.
IQ /EQ

Botanical name

Sender/organizatiom

Date of dispatch (in case of import)

Phytosanitary certificate

Treated/untreated

Additional declaration

Field observation report on disease(s)

Weather conditions

Treatment given, if any

Dispatch date
To whom/address

Method

☐ Modified Baermann funnel
☐ Sieve method

Date of sowing

Date of recording

Number of seeds 500 Replicates : 2

(250/rep)

If less than 500 seeds:
Number of seeds used

Number of *A. besseyi*	Rep. 1	Rep. 2	Total *A. besseyi*

Saprophytic nematode noted

Remarks

Analyst

RECORDING SHEET FOR THE INSPECTION OF DRY SEED

Accession no.
IQ /EQ

OBSERVATIONS

Botanical name

Sender/organization

Date of dispatch (in case of import)

Date of receipt

Phtyosanitary certificate

Treated/untreated

Additional declaration

Field observation report on disease(s)

Weather conditions

Treatment given, if any

Dispatch date
To whom/address

Remarks

Date of inspection _____

Size of sample inspected _____ No. of sample inspected _____

PO

(A) VISUAL OBSERVATION
Inert matter
(empty seeds, pieces of plant parts such as stem, petioles, flower parts; pieces of broken or damaged seed units [half or less than half of the original size], nematode galls, fungus bodies such as ergot, sclerotia and smut balls, soil, sand, stones, and all other nonseed matter)

Seed abnormalities
(shriveled seeds, spotting/discoloration of various types, malformation, hypertrophies, cracks, holes, etc.)

(B) STEREOBINOCULAR MICROSCOPE OBSERVATION
Surface-borne pathogenic propagules
(smut and bunt spores, uredo- and teleutospores of rust fungi, oospores of downy mildews, sclerotia, and pycnidia of different fungi or/in the seed coat, spores of other fungi, etc.

Analyst

Appendix 2. **Media, stains, buffer and their preparations.**

I. Media preparation

Unless otherwise specified, all media are made up to 1 liter with distilled water and sterilized at 121 °C for 15 min (15 psi).

Filter-sterilized cycloheximide can be added to the molten media to give a final concentration of 0.01%. [Dissolve 1 g cycloheximide in 10 ml distilled water and filter sterilize (10% solution). Add 1 ml to 1 liter molten medium.]

Aqueous Lugol's iodine

Iodine powdered crystals (I_2)	5.0 g
Potassium iodide (KI)	10.0 g
Distilled water	100.0 ml

Method of preparation:
1) Stock solution. Dissolve KI in distilled water. Slowly add I_2 crystals and shake until dissolved. Keep the solution in a tightly closed brown bottle.
2) Working solution. Make up a 1:5 dilution of the stock solution with distilled water each time before using.

Arginine medium (*Thornley's Medium 2A*)

Phenol red	0.01 g
Dipotassium phosphate (K_2HPO_4)	0.3 g
Peptone	1.0 g
Agar	3.0 g
Sodium chloride (NaCl)	5.0 g
Arginine HCl	10.0 g
Distilled water	1.0 liter

In case it has an orange color, adjust pH to 6.8 with 1N HCl until the medium turns yellow.

Ayers et al mineral salts medium

Potassium chloride (KCl)	0.2 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Ammonium phosphate ($NH_4H_2PO_4$)	1.0 g
Agar	12.0 g
Distilled water	1.0 liter

Sugars to be tested: to the autoclaved basal medium (at 45 °C), add filter-sterilized solutions of the test carbohydrates for a 0.5% final concentration.

To demonstrate acid production:
—Incorporate 1 ml bromthymol blue (1.6% alcohol solution) in the basal medium.
—Adjust to pH 7.0 prior to autoclaving.
—Add filter-sterilized solutions of the test carbohydrates for a 1% final concentration.
—Dispense the medium in test tubes instead of in petri dishes.
—Record presence or absence of a yellow color indicating acid production.

Gluconate peptone broth

Dipotassium phosphate (K_2HPO_4)	1.0 g
Yeast extract	1.0 g
Peptone	1.5 g
Potassium gluconate	40.0 g
Distilled water	1.0 liter

Benedict's reagent

Copper sulfate ($CuSO_4$ $5H_2O$)	1.73 g
Sodium carbonate (Na_2CO_3)	10.0 g
Sodium citrate [$C_3H_4OH(COO)_3Na_3$]	17.3g
Distilled water	0.1 liter

Preparation:
1) Solution 1. Dissolve sodium carbonate and citrate in 60.0 ml of distilled water.
2) Solution 2. Dissolve copper sulfate in 20.0 ml of distilled water.
3) Add solution 2 to solution 1, while stirring constantly.
4) Adjust to a total volume of 100 ml with distilled water.

Hugh and Leifson's oxidation-fermentation medium (0-F)

Bromthymol blue	0.08 g
K_2HPO_4	0.3 g
Peptone	2.0 g
Agar	3.0 g
NaCl	5.0 g
Distilled water	1.0 liter

Add 10 ml of 10% aqueous D-glucose solution (filter-sterilized) to the sterile molten medium to obtain a final concentration of 1% glucose. Dispense in 6-ml volumes in sterile, narrow tubes. Seal one set of tubes with mineral oil.

King's medium B (KMB)

Glycerol	15.0 ml
K_2HPO_4	1.5 g
$MgSO_4 \cdot 7H_2O$	1.5 g
Agar	17.0 g
Peptone	20.0 g
Distilled water	1.0 liter

Kligler's iron agar, pH 7.4

Ingredients, BBL	
Phenol red	0.025 g
Ferric ammonium citrate (50:50 mixture)	0.5 g
a. Ferric citrate ($FeC_6H_5O_7$)	
b. Ammonium citrate [$(NH_4)_3C_6H_5O_7$]	
Sodium thiosulfate ($Na_2S_2O_3$)	0.5 g
Dextrose (glucose)	1.0 g
Sodium chloride (NaCl)	5.0 g
Lactose	10.0 g
Agar	15.0 g
Polypeptone	20.0 g
Distilled water	1.0 liter

Modified Wakimoto 's medium

Ferrous sulfate	0.05 g
Calcium nitrate	0.50 g
Sodium phosphate	0.82 g
Bacto peptone	5.00 g
Agar	17.00 g
Sucrose	20.00 g
Distilled water	1.00 liter

Nutrient agar (NA)

Beef extract	3.0 g
Peptone	5.0 g
Agar	17.0 g
Distilled water	1.0 liter

Nutrient broth (NB) is nutrient agar without the agar.

Peptone sucrose agar (PSA)

Sodium glutamate	1.00 g
Peptone	10.00 g
Sucrose	10.00 g
Agar	17.00 g
Distilled water	1.00 liter

Peptone sucrose broth (PSB) is PSA without the agar.

Potassium nitrate agar

Potassium nitrate (KNO_3)	1.0 g
Beef extract	3.0 g
Peptone	5.0 g
Agar	7.0 g
Distilled water	1.0 liter

Selective medium for P. fuscovaginae (Miyajima 1989)

Penicillin G	750,000 units
Novobiocin	45 mg
Cycloheximide	75 mg
75% ethanol	3 ml
Distilled water	50 ml
King's medium B	940 ml

Selective medium for P. glumae *PPGA + 0.1% $CaCl_2$* (Matsuda et al 1988)

KH_2PO_4	0.5 g
$CaCl_2$	1.0 g
NaCl	3.0 g
$Na_2HPO_4 \cdot 12H_2O$	3.0 g
Glucose	5.0 g
Peptone	5.0 g
Agar	20.0 g
Decoction of 200 g potato	1.0 liter

Selective medium for P. glumae *S-PG* (Tsushima et al 1986)

Cetrimide	0.01 g
EDTA-Fe	0.01 g
Phenol red	0.02 g
$Na_2MoO_4 \cdot 2H_2O$	0.024 g
$MgSO_4 \cdot 7H_2O$	0.25 g
Na_2HPO_4	1.2 g
KH_2PO_4	1.3 g
$(NH_4)_2SO_4$	5.0 g
D-sorbitol	10.0 g
Agar	20.0 g
L-cystine	10.0 g
	(0.1 ml of a 0.01% aqueous solution– dissolve 0.01 g in 100 tnl distilled H_2O by heating)
Methyl violet	1.0 mg
	(1 ml of a 0.01% aqueous solution (0.01 g dissolved in 100 ml distilled H_2O)
Distilled water	1.0 liter

After autoclaving the medium, add the following filter-sterilized antibiotic solutions (millipore filter 0.4 m pore diameter):

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

Starch medium (Lelliot and Stead 1987)

Soluble (potato) starch	2.0 g
Nutrient agar	28.0 g
Distilled water	1.0 liter

Dissolve the nutrient agar powder in 500 ml distilled water and heat into solution. Dissolve the starch in 10 ml distilled water by gently heating and add to the nutrient agar solution. Make up to 1 liter volume, Sterilize by autoclaving. Pour the medium into individual petri dishes.

Tryptic soy agar (TSA) (commercially available as powder preparation)

Bacto-dextrose	2.5 g
K_2HPO_4	2.5 g
Bacto-soytone	3.0 g
NaCl	5.0 g
Bacto-tryptone	17.0 g
Distilled water	1.0 liter

II. Stains

The following solutions are used for gram staining.

Acetone-alcohol decolorizer

Acetone	50.0 ml
Ethanol	50.0 ml

Crystal violet

Ammonium oxalate	0.8 g
Crystal violet	2.0 g
Ethanol 95%	20.0 ml
Deionized water	80.0 ml

Iodine solution

Iodine	0.33 g
Potassium iodide	0.66 g
Deionized water	100.00 ml

Safranin counterstain

Safranin 0	0.25 g
Ethanol 95%	10.00 ml
Deionized water	90.00 ml

III. Buffer

Phosphate-buffered saline (PBS)

Na_2HPO_4	1.15 g
KCl	0.20 g
K_2HPO_4	0.20 g
NaCl	8.00 g

IV. Others

Oxidase test

Tetramethylphenylene diamine dihydrochloride or Kovacs'reagent 1% solution

Appendix 3. **Glossary.**

achene = small, dry fruit with one seed

achlorophyllous = lacking chlorophyll

acervulus = a cushionlike mass of hyphae and palisadelike conidiophores and conidia (pl. acervuli)

acrogenous = borne at the tip

acropleurogenous = borne at the tip and along the sides

acuminate = pointed; tapering to point

aedeagus = in male insects, the penis or intromittent organ situated below the scophium and enclosed in a sheath

alternate host = one of two species of host plants upon which a parasitic fungus must develop to complete its life cycle

ampulliform = flask-shaped with the swollen part at the base

anamorph = the conidial or imperfect stage of fungi

appressorium = a swelling on a germ tube or hypha, attaching it to a host tissue in an early stage of infection (pl. appressoria)

ascomycetous = having characteristics of the ascomycetes, diagnostic character of ascomycetes is the ascus within which typically eight ascospores are developed by free cell formation

ascospore = spore produced in an ascus (perfect state); a meiospore borne in an ascus

ascus = a saclike cell generally containing a definite number of ascospores (typically eight) formed by free cell formation usually after karyogamy and meiosis; characteristic of the class Ascomycetes

aseptate = lacking cross walls

axenic = without another organism being present

awn = bristly fibers on the head of some cereal grains

bifid = having a crack or division near the middle; forked

binary fission = reproduction of a cell by division into two approximately equal parts

blast (rice) = disease in rice caused by *Pyricularia oryzae,* causing round to ob long (spindle-shaped) lesions on leaves and other plant parts

butyrous = butterlike

canker = a plant disease in which there is sharply limited necrosis of cortical tissue

chlamydospore = a thick-walled, nondeciduous, intercalary or terminal asexual spore made by the rounding up of a cell or cells

cicatrized (conidiogenous cell, conidiophore) = bearing scars

ciliate = edge with hairs

clavate = clublike; narrowing in the direction of the base

cleistothecium = a completely closed ascocarp

coccus = a spherical bacterium

colony = a group of individuals of the same species living in close association; in fungi, the term usually refers to many hyphae growing out of a single point and forming a round or globose thallus

columella = a sterile structure within a sporangium or other fructification; often an extension of the stalk (pl. columellae)

conidial chain = conidia developed/borne end to end to produce a chain

conidiophore = a simple or branched hypha arising from a somatic hypha and bearing at its tip or side one or more conidiogenous cells; sometimes used interchangeably with conidiogenous cell

conidium = a nonmotile asexual spore usually formed at the tip or side of a sporogenous cell; in some instances, a preexisting hyphal cell maybe converted to a conidium (pl. conidia)

dichotomous = pairwise forking; often repeatedly

distichous = in two lines

echinulate = having small pointed processes or spines

epiphytotic = a widespread occurrence of a plant disease; equivalent to epidemic on humans

epispore = the thick fundamental layer which determines the shape of the spore

eukaryotic = an organism composed of one or more cells with visibly evident nuclei

facultative anaerobe = an organism normally associated with aerobic condition but which can grow in anaerobic condition

facultative parasite = an organism capable of infecting another living organism or of growing on dead organic matter, according to circumstances

facultative saprobe = an organism capable of growing on dead organic matter, or of infecting another living organism, according to circumstances

fascicle = a litttle group or bundle

fascicular = having growth in fascicles

fistular = hollow, like a pipe

flagellum = a hair-, whip-, or tinsellike structure that serves to propel a motile cell (pl. flagella)

funiculose = occurring in ropes or bundles

fusiform = spindlelike, narrowing toward the end

fusoid = somewhat fusiform

geniculate = bent like a knee

hilum = a scar indicating the point of attachment

host = a living organism harboring a parasite; a living organism on or in which a parasite lives and from which the parasite obtains its sustenance

hyaline = colorless, transparent

hypha = one of the filaments of a mycelium (pl. hyphae)

imperfect stage = the asexual (usually conidial) stage of a fungus

infest = to introduce a pathogen into the environment of a host. Infestation should not be confused with infection.

inoculum = dispersal unit capable of initiating disease or introduced for that purpose

isolate = a single spore or pure culture and the subcultures derived from it

labial palp = feeler for touching or tasting, attached to thelip

lanceolate = narrow and tapering like the head of a lance

lesion = a discoloration of the host around the point of entry

macronematous = morphology very different from a vegetative hypha and usually erect

microconidium = a small conidium that often acts as aspermatium (pl. microconidia)

micronematous (conidiophore)= morphologically resembling a vegetative hypha but bearing conidia

smoniloid = having swellings at regular intervals like a string of beads

monotrichous = (of bacteria) having one polar flagellum

mucronate = pointed; ending in a short, sharp point

mycelium = a mass or group of hyphae making up the thallus of a fungus

mycoplasma = genus to which microorganisms with a unit membrane and no rigid cell wall belongs

obclavate = the shape of a club upside down, thickened towards the base

obligate aerobe = an organism that always needs oxygen for its growth

obligate parasite = an organism that can obtain food only from living protoplasm; it cannot be grown in culture on nonliving media

obligate saprobe = an organism that must obtain its food from dead organic matter and is incapable of infecting another living organism

osmophilic = growing under conditions of high osmotic pressure, as some yeast on concentrated sugar solutions

ostiole = opening of a true perithecium or a pycnidium, ending in a pore

parasite = an organism that lives at the expense of another, usually invading it and causing disease; an oganism that obtains its food or nutrients wholly or in part from another living organism, usually of a different species

pathogen = an organism able to cause disease

perfect stage = the sexual stage of a fungus; the state of a life cycle in which spores(such as ascospores and basidiophores) are formed after nuclear fusion or by parthenogenesis

peritrichous = having flagella all over the surface

petri dish = (named after R.J. Petri, a German scientist): a glass container consisting of a circular, flat dish with vertical sides, and a similar but slightly larger cover that fits over it; standard equipment for growing microorganisms in pure culture

phialide = a type of conidiogenous cell that produces blastic conidia in a basipetal fashion without detectably increasing in length

phytopathogenic = organisms characteristically either parasites of leaving plants or saprobes of plant material

pionnote = (of *Fusarium*) a spore mass having a fat- or greaselike appearance

polytretic = tretic with several channels or pores

predisposition = state or condition resulting from the act of inclining or conditioning beforehand, giving a tendency to, a propensity for

prokaryote = a cellular organism that does not have a distinct nucleus (e.g., bacterium or blue-green alga)

promycelia = a hypha of restricted growth bearing basidiospores, the epibasidium of the rusts and smuts

pronotum = the upper or dorsal surface of the prothorax

propagule = any unit in service of propagation, such as spore, sclerotium, mycelial fragment, etc.

pseudoseptum = a pluglike partition of cellulin or other substance in a hypha, resembling a septum (pl.pseudosepta)

pulverulent = powdered; as if powdered over

pycnidia = an asexual, hollow fruiting body in which conidia are produced, characteristic of Sphaeropsidales

pyriform = pearlike in form

resistance = the power of a host to overcome, completely or in some degree, the effect of a pathogen or damaging factor

reticulate = having the form of a net; covered with netlike ridges

rhizoid = a short, thin branch of thallus, superficially resembling a root

rust = a disease caused by one of the Uredinales; also a rust fungus

sagittate = shaped like an arrowhead

saprophyte = an organism using dead organic material as food and commonly causing its decay

sclerotium = a resting body of variable size composed of a hard mass of hyphae, with or without host tissue, from which fruit bodies, stromata, conidiophores, or mycelia may develop

septate = with more or less regularly occurring cross walls

septum = a cross wall in a hypha (pl. septa)

seta = a bristlelike hair (pl.setae)

sexual reproduction = reproduction involving nuclear fusion and meiosis

slant = sterile agar media in test tubes congealed in slanting position

slurry (seed treatment) = application of seed treatment fungicide by making watery paste of fungicide in a treatment jar (wide mouthed), seeds are poured in, covered, and shaken vigorously or jar is placed in roll mill for 5-10 min until the slurry is taken up evenly by the seed surfaces

smut = a disease caused by one of the Ustilaginales; also a smut fungus

solitary = arising singly at one point

spermatium = a nonmotile, sporelike gamete, e.g., a pycniospore in rust fungi or a microconidium in Discomycete

spinulose = covered with little spines

spiracle = an apparatus for breathing; any of the small openings of the tracheal respiratory system in most terrestrial arthropods

sporangiophore = a hypha that bears a sporangium

sporangiospore = a spore borne within a sporangium

sporangium = a saclike structure, the entire protoplasmic contents of which become converted into an indefinite number of spores (pl. sporangia)

spore = a minute propagative unit functioning as a seed but differing from it in that a spore does not contain a preformed embryo

sporidium = spore produced on the promycelium or basidium of smuts and rusts

sporiferous = carrying spores

sporodochium = a pulvinate stroma with closely packed, relatively short conidiophores

sporulation = producing spores or arrangement of produced spores

sterigma = a small hyphal branch or structure which supports a sporangium, a conidium, or a basidiospore (pl. sterigmata)

sterigose = having stiff hairs or bristles

systemic = (of a plant pathogen) occurring throughout the plant; (of a chemical) absorbed into the plant through roots or foliage

teleomorph = the perfect stage or sexual stage of fungi

teliospore = a thick-walled resting spore of rusts and smuts in which karyogamy occurs; it is part of the basidial apparatus

thallus = a relatively simple plant body devoid of stems, roots, and leaves; in fungi, the somatic phase (pl. thalli)

therapy = treatment of disease

treatment = mode of dealing with a person or thing; more specifically, intervention in the natural process in order to reduce disease

tretic (conidiogenous cell) = apparently enteroblastic, protrusion of the inner wall taking place through one channel or several channels in the outer wall. The channels (or pores) can be seen quite easily with an ordinary compound microscope and although some recent electron microscope studies indicate that conidium development is not necessarily strictly enteroblastic, their presence provides a character most useful to taxonomists. The continued use of the term tretic is recommended.

tubercle = wartlike growth

verrucose = warted

verruculose = finely warted

verticillate = arranged in whorls

wart disease = (of potato) caused by *Synchytrium endobioticum*

zygospore = a resting spore that results from the fusion of two gametangia in the Zygomycetes

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[a] Adopted from Zadoks and Schein (1979). Allexopoulus and Mims (1979). Ellis (1976). Neergaard (1979), Ainsworth and Bisby (1971).