REGIONAL WORKSHOP ON

CEREAL DISEASE METHODOLOGY

CENTRO INTERNACIONAL DE MEJORAMIENTO DE MAIZ Y TRIGO

INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER

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Organized jointly by:

The [illegible] Government of the Netherlands

International Maize and Wheat Improvement Center (CIMMYT)

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1. CEREALS: AN INTRODUCTION

Plants constitute 93 percent of the world's diet. Cereals contribute two-thirds of all food, and among the cereals, wheat is the largest crop. The eight major cereals—namely wheat, maize, rice, barley, sorghum, oats, millet and rye—cover 56 percent of the world's arable land and are the major source of calories and protein for most of the world's people. Wheat, maize and rice account for 80 percent of global cereal production.

The World Cereal Area and Production 1981, by Crop.

Crop	Area		Production	
	Ha (x 1000)	Per cent of Total	Mt (x 1000)	Per cent of Total
Wheat	239,381	33	458,195	28
Maize	134,024	18	451,704	27
Rice	144,915	20	413,785	25
Barley	79,751	11	158,488	10
Sorghum	47,762	7	71,984	4
Millet	43,203	6	29,652	2
Oats	26,810	3	44,024	3
Rye	15,302	2	24,443	1
Total	731,148	100	1,627,832	100

Source: FAO Production Yearbook 1981.

Developing country wheat production during the decade of the 1970s increased at a rate of 4.8 percent per annum, the highest of all the cereals. Most of this production growth has occurred in the five major developing country producers—China, India, Argentina, Pakistan and Turkey—which collectively account for 82 percent of all developing country production. Among these major producers, China has registered the greatest production gains.

	Production, Million Tonnes		Percent Annual Growth, 1970s
	1969-71	1979-81	
China	29,687	57,964	6.7
India	20,859	34,599	5.1
Turkey	11,423	17,054	4.0
Pakistan	6,796	10,698	4.5
Argentina	5,873	7,993	3.1

1.1 Wheat

The world's wheat acreage and production are clearly concentrated in the northern hemisphere. Bread wheat and durum wheat are the two principal commercial types of wheat. Bread wheat (*Triticum aestivum*) covers about 90 percent of the world wheat area and makes up about 94 percent of production. Durum wheat (*T. durum*) is less cosmopolitan in its growing distribution and is principally grown in North Africa, the Middle East, the USSR, India, Italy, France, northern USA and some areas of Canada.

As one of the world's most important staple foods, wheat is consumed in a variety of ways. Its most important use is, however, in the manufacture of flour, the basis of all bread, biscuit and pastry products. In addition, wheat is used extensively in breakfast cereals, bulgur, couscous, and macaroni products. Wheat is also a commercial source of starch, and thus finds use in a wide range of industries from food processing to paper manufacturing and from laundering to oil well drilling.

The continued reliance upon the wheat crop as one of the mainstays of world nutrition demands that both the stability and level of production are continually increased in order to minimize, as much as is possible, world malnutrition.

Wheat Classification

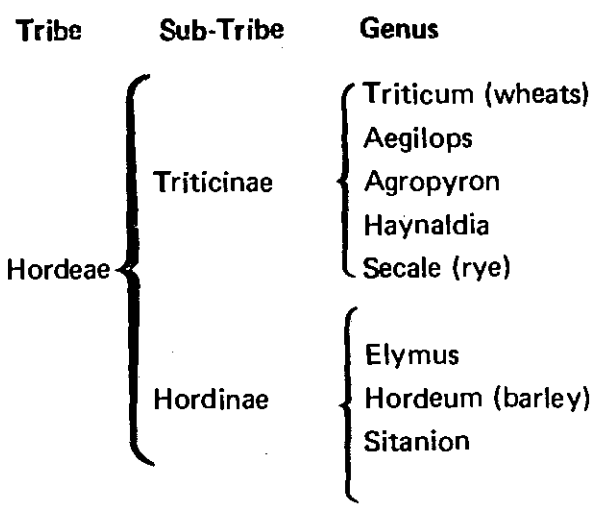
The wheat species of importance to agriculture are primarily *Triticum aestivum ssp. vulgare* (bread-wheat) and *T. turgidum ssp. durum* (durum wheat). Table 1.1 gives complete details of other species in the genus *Triticum*, which is part of the tribe *Hordeae* of the overall family *Graminae* (the grasses). Other members of this tribe are listed below:

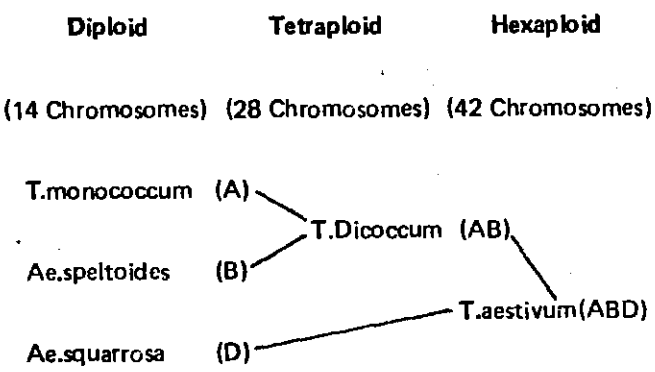
Table 1.1. SUBCLASSIFICATION OF THE GENUS TRITICUM

Name of Species	Common name	Chromo No.(n=)	Genome	Seed type**	Probable region of origin
Diploids					
T. monococcum	Einkorn	7	AA	H	Armenia, Georgia, Turkey
Tetraploids					
T. turgidum					
ssp. dicoccum	Emmer	14	AABB	H	Georgia, Abyssinia
ssp. durum	Durum	14	AABB	N	Abyssinia, Mediterranean
ssp. turgidum	Rivet	14	AABB	N	Abyssinia, S. Europe
ssp. polonicum	Polish	14	AABB	N	Abyssinia, Mediterranean
ssp. carthlicum	Persian	14	AABB	N	Georgia, Armenia, N.E. Turkey
ssp. turamcum	Khorasan	14	AABB	N	Mediterranean, Nr. East
T. timopheevii	–	14	AAGG	H	W. Georgia Abyssinia
Hexaploid					
T. aestivum					
ssp. spelta	Spelta	21	AABBDD	H	Austria, Germany
ssp. macha	Macha	21	AABBDD	H	W. Georgia
ssp. vavilovi	Vavilovi	21	AABBDD	H	Turkish Armenia
ssp. vulgare	Common wheat	21	AABBDD	N	S.W. Asia, Central Europe
ssp. compactum	Club	21	AABBDD	N	Afghanistan, Armenia
ssp. sphaexocuccum	Shot	21	AABBDD	N	N.W. India

** Seed Type: N = Naked; H = Hulled

Source: Principles of Field Crop Production, Martin, Leonard and Stamp (1976)



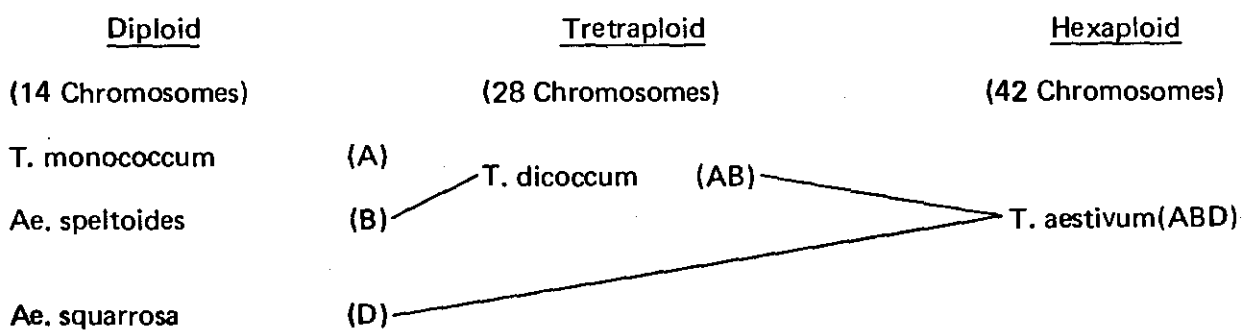


The Origin of Wheat

In common with all other crops, cultivated wheat has been derived from wild ancestors through a process of domestication by man. This probably commenced in the neolithic period. The first cultivated form of wheat appears to have been *Triticum monococcum* (einkorn), which is particularly close to the believed wild ancestor *T. boeoticum*. This species possesses seven pairs, or 14 chromosomes, thus being termed diploid. Pure stands of einkorn have been observed growing in close association with other members of their sub-tribe, namely *Aegilops speltoides*, in areas of Western Asia (Figure 1.1). Cytological studies have revealed that the second primary cultivated form of wheat, *Triticum dicoccum* (emmer), probably came into being through an inter-generic cross between these two individuals. As their two sets of chromosomes (seven pairs) are different, such a cross required chromosome doubling in order to be fertile. This accounts for the fact that emmer, a tetraploid, possesses 14 pairs or 28 chromosomes (four times the basic set of seven and double that of einkorn). A further inter-genetic cross between cultivated emmer and wild *Aegilops squarrosa* is believed to have been responsible, by the same process of chromosome doubling, for the evolution of the hexaploid or bread wheats (with six times the basic chromosome set, or 42 chromosomes). This evolutionary sequence is represented diagrammatically below:

1.2 Barley

Although of less importance than wheat when considered in the context of world cereal production, barley is a very important grain crop in many areas of the world. This is especially true where the production conditions are poor and the rainfall is low and erratic. Under these conditions barley, with its superior production per unit of moisture,



is often the only crop that is feasible or economic to produce.

The USSR, Europe and Asia together produce some 84 percent of the world barley grain on about 80 percent of the total acreage. The majority of this production is used for animal feed, especially in the more affluent countries. However, many of the countries of South and East Asia and North and East Africa use a substantial proportion of their barley production as a human food. It is, therefore, in these countries that barley assumes a particularly critical nutritional importance.

Barley Classification

The classification of barley is considerably simpler than that of wheat as there is only a single cultivated species, *Hordeum vulgare*. As with wheat the genus *Hordeum* belongs to the tribe *Hordeae* of the family *Graminae*.

H. vulgare is recognized as containing three sub-species:

ssp. hexastichum	six-rowed
ssp. distichum	two-rowed
ssp. irregulare	irregular

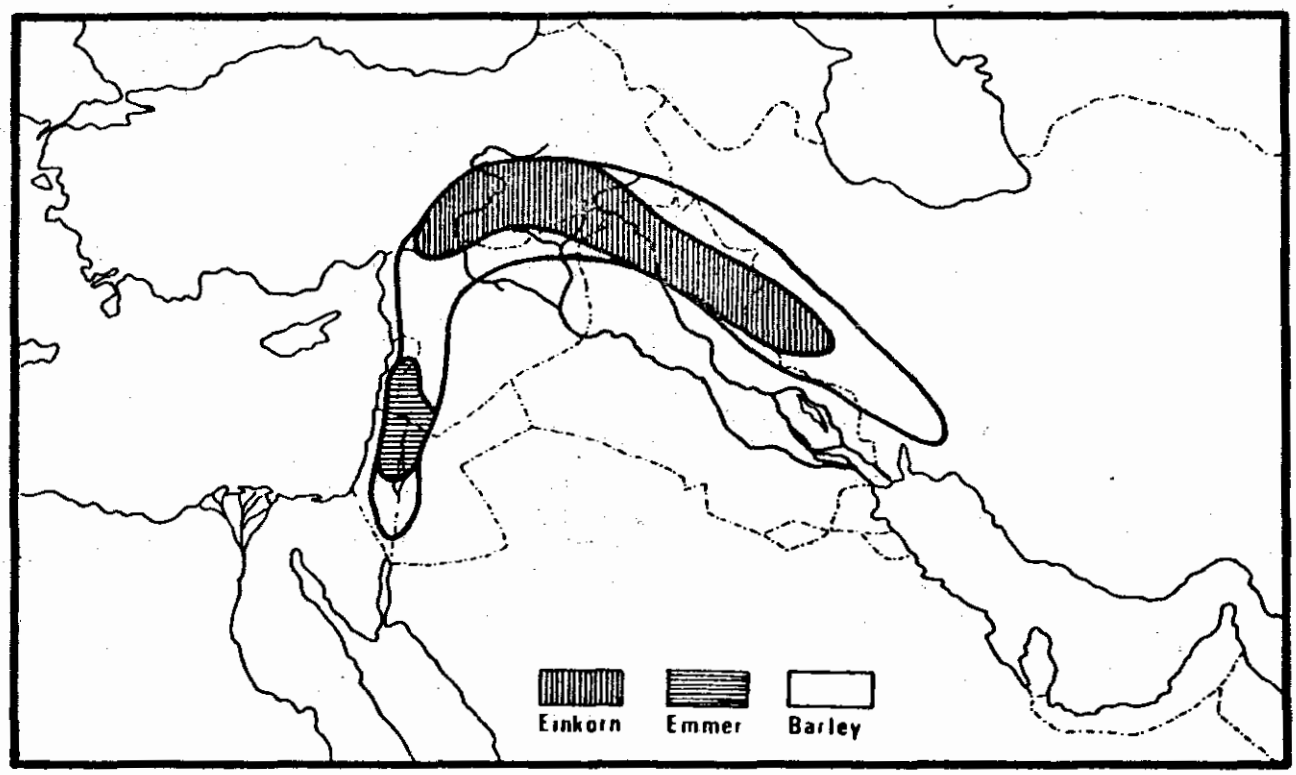
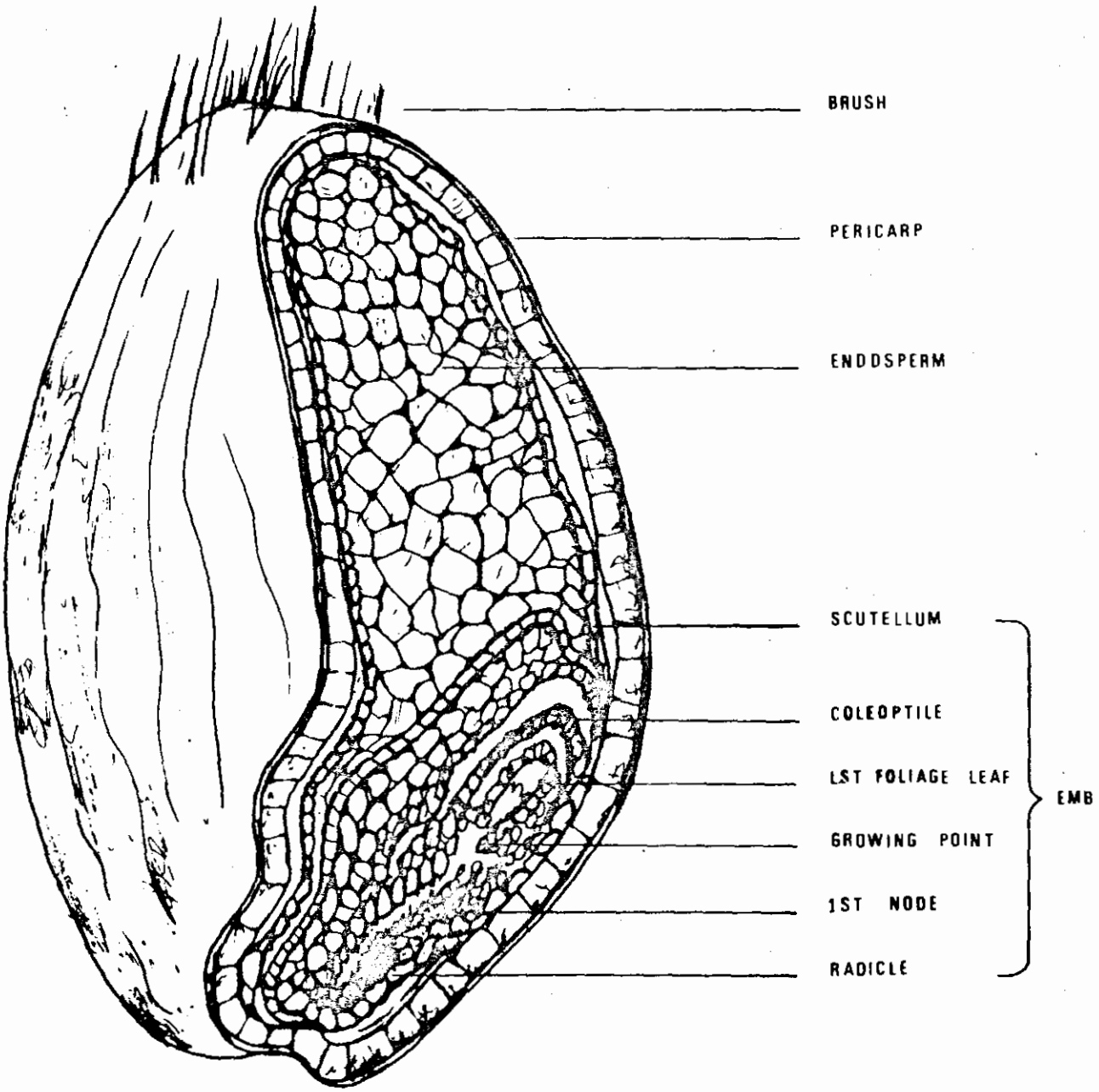


Fig 1.1 The Natural Distribution of Ancestral Wheat and Barley

(from 'Geography of Domestication' by E Isaac (1970) Prentice Hall N.J.)

FIG 1.2: WHEAT KERNEL



These are distinguished by the number of grain-producing, spikelets in each node of the ear; in two-rowed types only one of the three spikelets are fertile whereas in six-row varieties all three spikelets produce grains.

The Origin of Barley

The only truly wild forms of *Hordeum* are two-rowed. Although there has been much debate, it is now generally agreed that the presently cultivated barleys were domesticated, like wheats in the West Asian region. Massive and spontaneous stands of the wild two-rowed presumed ancestor are still found over much of this area today. Figure 1.1 shows the natural distribution of ancestral barleys and wheats, and the area in which they presumably evolved into the cultivated forms.

The earliest indications of cultivated two-rowed barleys have been found in the foothills of the Zagros mountains of Iraq and date back to about 7000 B.C. Six-rowed varieties apparently developed at a later stage and cyto-genetical studies have shown that such a development could be the product of a single recessive mutation. After about 5000 B.C. both types appear to have spread extensively throughout the Near East and thence to Europe at a later date.

1.3 Wheat and Barley Morphology and Growth

The cereal seed (or kernel) consists of three major parts: the *Pericarp* or protective covering, which surrounds and encloses the whole seed; the *Embryo* or germ, which is the young dormant plant; and the *Endosperm*, the source of stored food that the embryo utilizes for growth after germination and until the young plant has sufficient photosynthetic tissue to support itself (Figure 1.2).

In the process of germination, the embryo, which was dormant within the dry seed, resumes its growth with a suitable supply of water, oxygen, sufficient temperatures, and an absence of germination inhibitors. During germination the embryo swells and ruptures the pericarp, and from this rupture the radicle (roots) and pumule (shoot) grow (Figure 1.3).

Factors which influence germination include:
Water availability,
oxygen concentration,
soil temperature,
light, seed viability,
seed size,
degree of embryo maturity,
infections by micro-organisms,
damage to the pericarp, and
inherent seed dormacy (reflected in the presence of germination-inhibitory chemicals in the pericarp).

From the emergence of the young seedling at the soil surface to the production of mature seeds, the development of a wheat or barley plant may be seen as falling into a number of growth stages:

1. Seedling—from emergence to 3-leaf stage
2. Tillering—additional secondary shoots arising from the plant crown
3. Jointing—stem nodes can be felt on the lower parts of the plant; no head is prominent in the upper leaf sheath.
4. Boot—head is prominent in the sheath of the upper or flag leaf
5. Heading—head is emerging from the leaf sheath (flowering will commence in 1 to 2 days).
6. Flowering—florets (flowers) open and pollen is shed
7. Filling—fertilized ovary is enlarging into the seed and gradually attaining full size

A more detailed break down of these stages is given in Figure 1.4 and its key.

The head of a wheat or barley plant is composed of a number of flowers arranged along a stalk (rachis) and is referred to as a spike. The rachis supports a series of spikelets along both its sides, each of which is composed of a number of individual flowers (florets). Each floret is composed of

FIG 1.3: STAGES OF GERMINATION

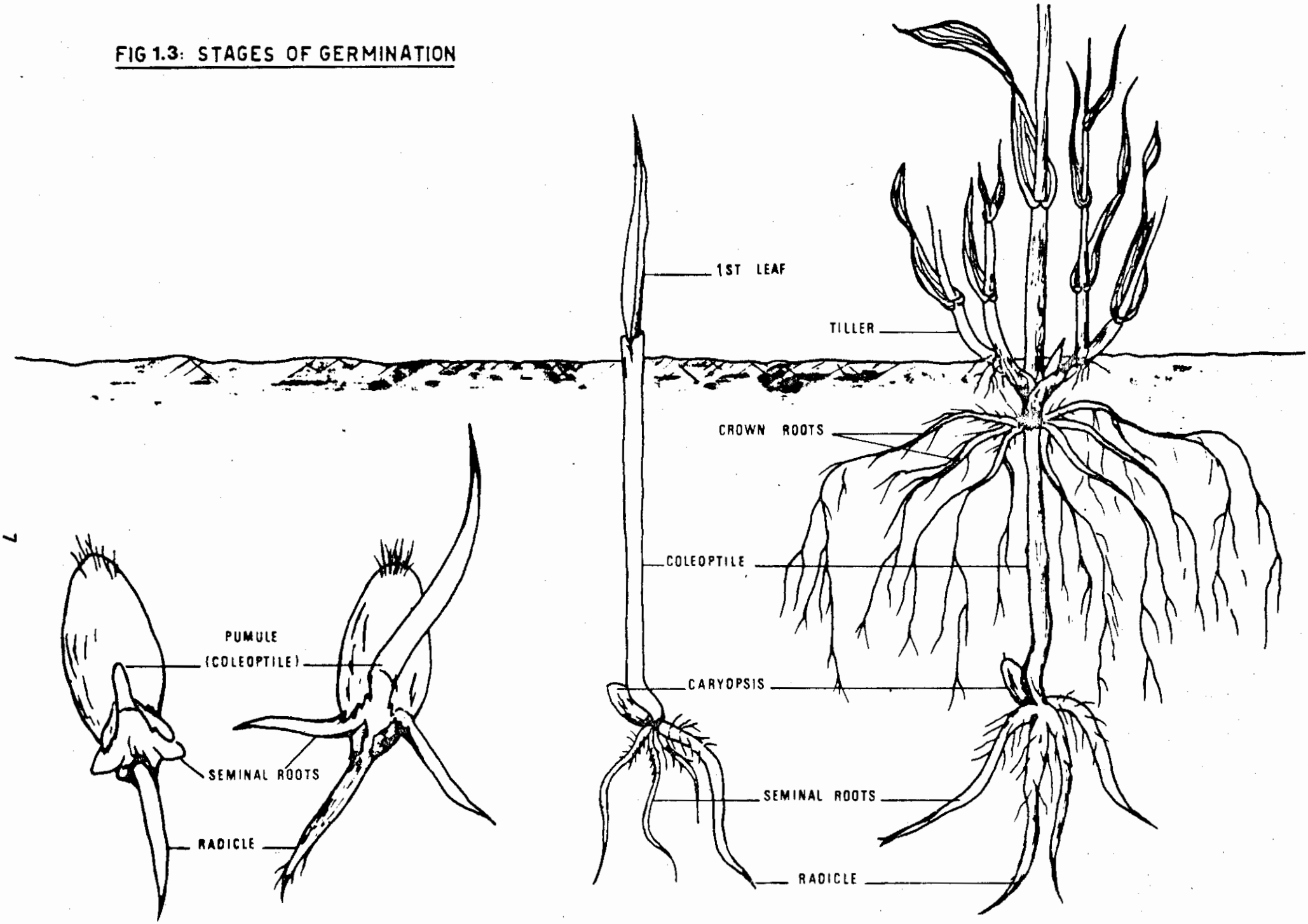
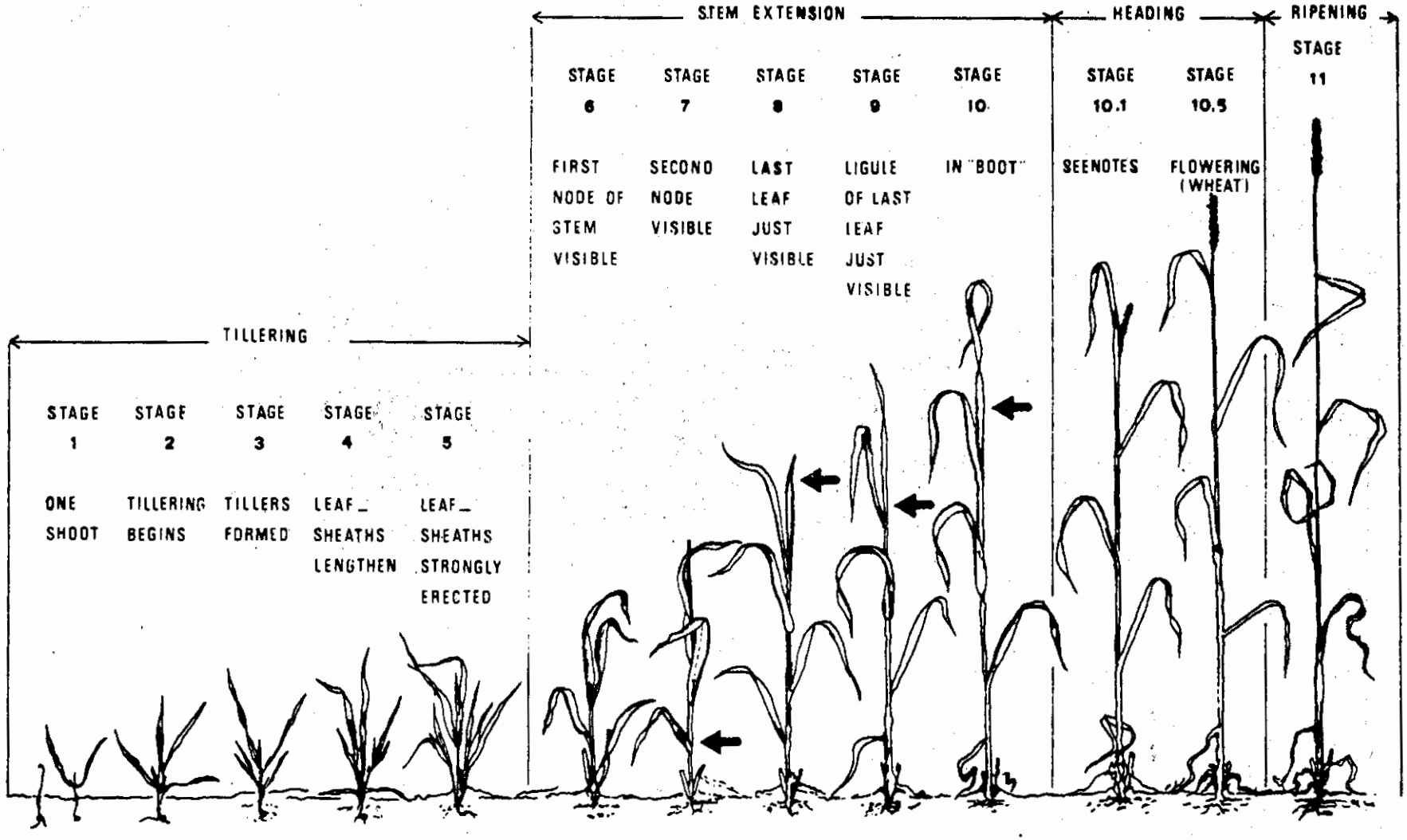


FIG 1.4 : GROWTH STAGES IN CEREALS

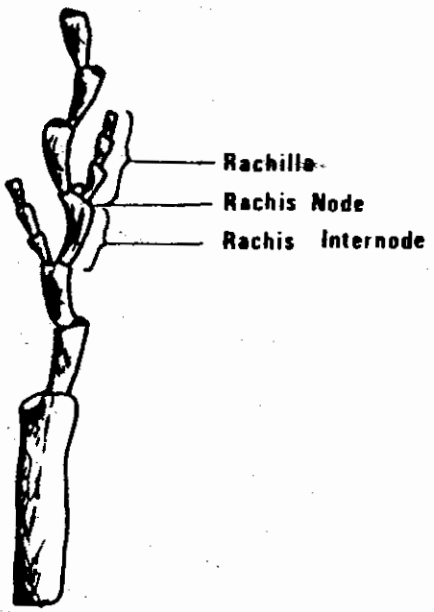


Key to Figure 1.4.

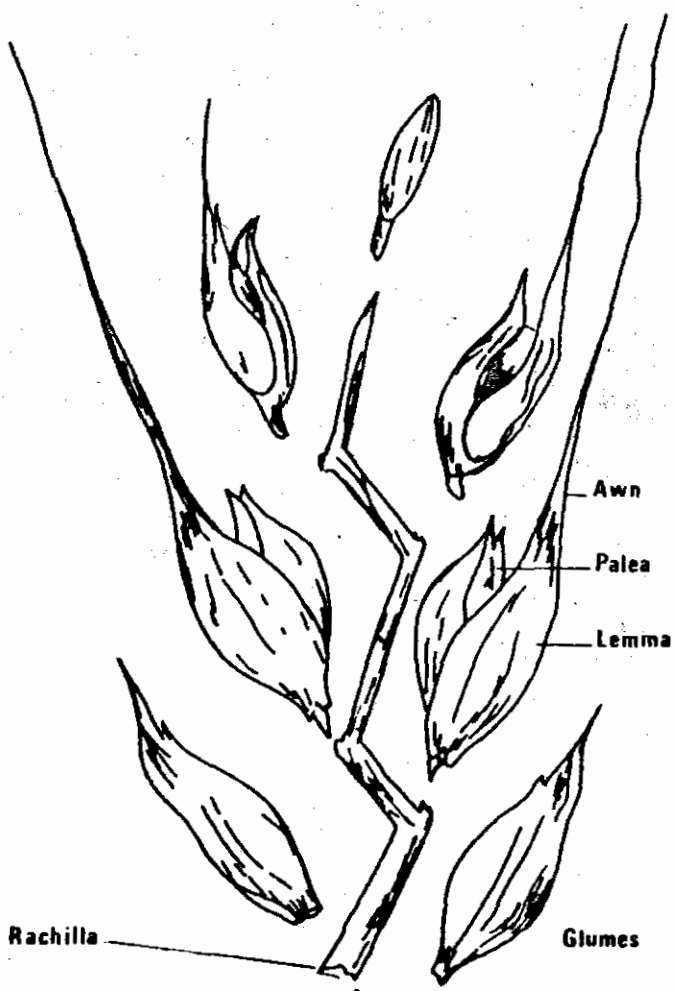
Stage		
1.	One shoot (number of leaves can be added) 'brairding'.	TILLERING
2.	Beginning of tillering.	
3.	Tillers formed, leaves often twisted spirally. In some varieties of winter wheats, plants may be 'creeping' or prostrate.	
4.	Beginning of the erection of the pseudo-stem, leaf-sheaths beginning to lengthen.	
5.	Pseudo-stem (formed by sheaths of leaves) strongly erected.	
6.	First node of stem visible at base of shoot	STEM EXTENSION
7.	Second node of stem formed, next-to-last leaf just visible.	
8.	Last leaf visible, but still rolled up, ear beginning to swell.	
9.	Ligule of last leaf just visible.	
10.	Sheath of last leaf completely grown out, ear swollen but not yet visible.	
10.1.	First ears just visible (awns just showing in barley, ear escaping through split of sheath in wheat or oats)	HEADING
10.2.	Quarter of heading process completed.	
10.3.	Half of heading process completed.	
10.4.	Three-quarters of heading process completed.	
10.5.	All ears out of sheath.	
10.5.1	Beginning of lowering (wheat)	FLOWERING (WHEAT)
10.5.2.	Flowering complete to top of ear.	
10.5.3.	Flowering over at base of ear.	
10.5.4.	Flowering over, kernel watery-ripe.	
11.1.	Milky - ripe.	RIPENING
11.2.	Mealy-ripe, contents of kernel soft but dry.	
11.3.	Kernel hard (difficult to divide by thum-nail)	
11.4.	Ripe for cutting. Straw dead.	

FIG 1.5 : Structure of Floret and Spikelet :

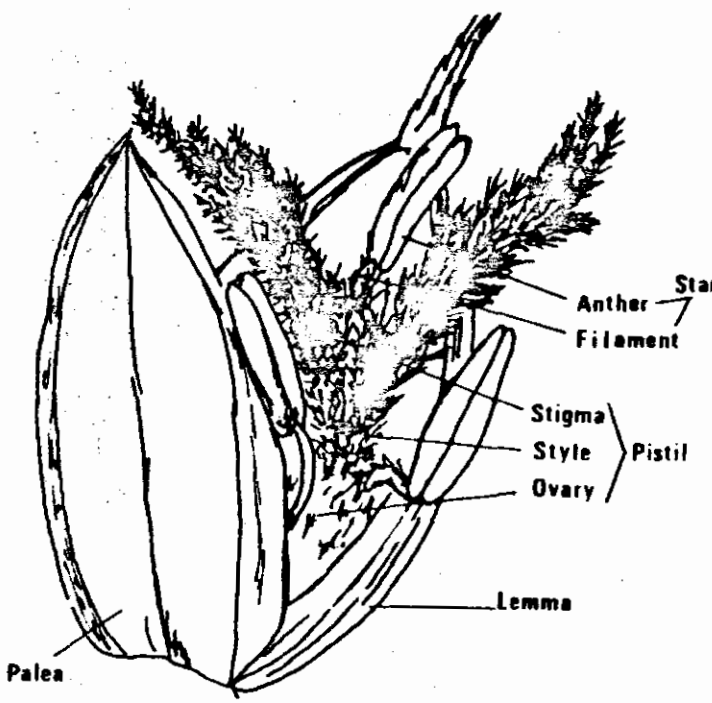
Spikelet



Rachis



Spikelet (Dissected)



Floret

female organs (the pistil) and male organs (the stamens) enclosed within an outer protective covering, the lemma and palea (Figure 1.5). At the flowering stage the floret opens and the pollen-containing parts of the stamens (the anthers) split to release their pollen. This may be transferred by the wind to other flowers (cross-pollination) or, as is more common in cereals, fall onto the stigma (receptive parts of the female organs) of their own flower (self-pollination). The pollen grain produces a tube which grows down the style (supporting the stigmas) and into the ovary where the female reproductive parts—the eggs—are located. The pollen nucleus penetrates an egg and fertilizes it, leading to the production and developrnent of the seed embryo, enclosed within the remains of the ovary, the endosperm, and the pericarp.

Growth Habit

Wheat and barley are grown under a wide range of environmental conditions. This is made possible by the considerable diversity of genetic material inherent in both the genera and their individual species. Both crops are in general considered to have one of three different growth habits —winter, spring or intermediate (also known as facultative).

Varieties with a winter growth habit are planted in the autumn/winter. The seeds germinate and the young seedlings emerge and develop vegetatively. Because these varieties require vernalization (exposure to cold conditions, usually for 1 to 2 months) to initiate the formation and growth of their reproductive organs, they remain in a vegetative state throughout the winter period. They are, thus, to a certain extent tolerant to frost and low temperatures. In the spring, when growth resumes, it is primarily vegetative (increase in leaf area), but reproductive growth, involving rapid stem extension or elongation and the emergence of the reproductive organs, rapidly overtakes and inhibits vegetative growth.

In contrast, spring growth habit varieties do not require vernalization to initiate reproductive growth and are, in general, much less tolerant to winter conditions. In areas where winter conditions are severe, these spring habit varieties can only be planted after the threat of frost is over. Under milder climatic conditions, spring habit wheats may be autumn or winter planted when there is little or no frost danger.

Wheats and barleys with facultative growth habits fall between the winter and spring types in characteristics, and may be autumn or winter planted in areas where winter temperatures are no lower than a few degrees below zero. They are, in general, more widely adapted than either of the other two types.

All three growth habits are found among commercial varieties of breadwheat, durum, and barley.

Apart from their adaptation to different temperature conditions, wheat and barley are also adapted to various conditions of day-length, requiring different 'photo-periods' for the onset of flowering and for optimum seed set. For practical purposes the 14-hour daylength (or photoperiod) has been found to be a convenient yardstick for the classification of plant types in terms of their response to light. Plants may either be:

1. Long-day varieties—which flower readily when the photoperiod exceeds 14 hours.
2. Short-day varieties—which only flower readily when the photoperiod is less than 14 hours.
3. Day neutral or intermediate varieties—which flower readily over a wide range of daylengths and do not appear to have a critical photoperiod.

The Genetic Determinants of Plant Growth

The basic constitution of any plant depends upon the characters that it inherits in its basic genetic make-up. These characters (such as plant height, growth habit, photoperiod response, yield potential, and disease resistance) are controlled by units of inheritance, or genes, which are located upon

the chromosomes of the plant cells. These genes differ widely and are inherited in various different combinations through the splitting of chromosomes in formation of gametes at meiosis, and their recombination when the eggs (female gametes) are fertilized by the pollen grains (male gametes). Some characters are controlled by either one or a relatively few genes and are termed qualitative (the inheritance of individual genes immediately confers certain characters upon the plant). Examples include growth habit and disease resistance. Other characters are controlled by a number of genes and are referred to as quantitatively inherited (the effect of the individual genes is relatively minor, but in combination they cause an increasing expression of the specific characters). Examples of quantitatively inherited characters include plant height and yield. The expression of this inherited genetic make-up or potential depends in turn upon the result of the interaction between these characters with the environment in which the plant grows.

The crop environment, which includes physical features (e.g. sunlight, temperature, topography, soils, etc.) and biological components (other plants, pests and diseases) thus imposes numerous constraints upon the way in which cereal crops grow and yield. The biological components of the environment have become particularly important as a result of the environmental disruptions caused by the expansion of monoculture and the progressive reduction of genetic diversity in both the wheat and barley crops. Diseases caused by pathogenic organisms now constitute perhaps the greatest single environmental constraint to increased cereal production and to the increasingly widespread cultivation of cereal crops throughout the world.

2. RECOGNITION AND IDENTIFICATION OF WHEAT DISEASES

Diseases represent one of the most severe constraints to increased and stable wheat production throughout the world. It is thus of fundamental importance that any program aimed at improving the production and stability of this crop has a strong pathology research input on a regular basis. In turn, a relevant and functional pathology input must be based upon a quick and accurate recognition and identification of diseases in the field to enable appropriate research activities to be initiated.

2.1 Fungal Diseases

Stem Rust

Stem rust of wheat, also known as black rust, is cosmopolitan in distribution, being found in all ecological locations occupied by wheat, its closely related species, and other grasses. Of the many diseases infecting wheat, stem rust is without doubt the most potentially devastating in any given year.

The disease, caused by the pathogen *Puccinia graminis* f. sp *tritici*, is characterized by the appearance of red/brown pustules, developing first as small chlorotic spots on all the above-ground parts of the plant: leaves, leaf sheaths, stems and spikes.

Severe infections result in considerable yield reductions mainly through a reduction in kernel number and serious grain shriveling.

Leaf Rust

This disease is also commonly known as brown or orange rust and, like stem rust, has a worldwide distribution. It is caused by *Puccinia recondita* f.sp. *tritici* and is characterized by small, round to oval, brown/red, isolated pustules occurring mainly on the leaves. However, under certain environmental conditions these pustules may also be found on the awns, glumes and leaf sheaths. Although little grain shriveling is evident, yield reduction through reductions in kernel number and size, may be very serious.

Some races have further been found to attack barley crops.

Yellow Rust

Often known as stripe rust, this disease is caused by the fungus *Puccinia striiformis* f. sp. *tritici* and occurs in many regions of the world. However, unlike the other rust diseases, it is generally confined to cooler higher elevations and higher latitude areas. The characteristic symptom of yellow rust is the linear arrangement (in stripes) of the yellow pustules on the leaves of the host plant. All above-ground plant parts, with the possible exception of stems, are subject to infection.

Infection affects the yield and quality of wheat by a reduction in the number of spikes/per plant, the number of kernels formed/per spike, and the size and weight of the kernels. Heavy infection results in severe kernel shriveling and reduced root development.

Speckled Leaf Blotch

Caused by the fungus *Septoria tritici*, this disease periodically infects the wheat crop throughout the world. Initial infections are normally confined to the lower leaves and appear as light green, slightly chlorotic spots, usually between the leaf veins. As the disease develops these lesions become light brown in color and necrotic, and take on a speckled appearance as the black resting bodies (pycnidia) are formed within the lesions. In particularly severe infections these lesions coalesce, completely killing the leaf.

Severe attacks cause a reduction in both the number and the size of kernels and may also cause seed shriveling.

Glume Blotch

This disease, better known by its imperfect form as *Septoria nodorum*, but also by its perfect or sexual form *Leptosphaeria nodorum*. Disease symptoms are typically found on the glumes, commonly on the leaves and leaf sheaths, and occasionally on the stems and are similar but somewhat darker in color than the lesions caused by *Septoria tritici*.

Yield reductions from infections of glume blotch are caused primarily by a reduction in the size and weight of kernels which may be accompanied by shrivelling.

Septoria Leaf Blotch

The fungus *Leptosphaeria avenaria* f. sp. *triticea*, commonly known by its imperfect form *Septoria avenae* f. sp. *triticea*, is the causal organism of this disease. It appears to be limited in occurrence to North America and northern Europe and produces similar symptoms as the other *Septoria* diseases. In common with these diseases a microscopic examination of the spores is necessary for positive identification.

Powdery Mildew

The causal agent of Powdery Mildew, *Erysiphe graminis* f. sp. *tritici*, is widely distributed in the humid and sub-humid wheat growing regions of the world and is favored by mild winters followed by cool, cloudy spring and summer weather. Infections, which appear as white/grey cottony masses on the plant surface, are commonly found on the leaves and may also be observed on the leaf sheaths and spikes. As the season progresses, small black resting bodies (Perithecia) develop in the cottony mass.

Heavy attacks of powdery mildew result in yield losses through reduced kernel size, which may be particularly severe with early infections.

Leaf Blotch

Widespread among the wheat growing regions of the world, this disease is caused by the fungus *Helminthosporium sativum*. Infections can be found on all parts of the plant (root rot, crown rot, leaf blotch, head blight or black point). The main symptom of the disease (leaf blotch) is characterized by dark brown spots, each surrounded by a chlorotic (yellow) margin. As the disease develops the leaves turn completely brown and die, severe infestations may thus result in total plant death.

Numerous other species of *Helminthosporium* have been reported attacking wheat crops throughout the world. Of these *H. tritici-repentis* is the most widely distributed and important on a global basis. The diseases caused by these pathogens all have similar symptoms, and thus precise identification requires microscopic examination.

Leaf Blight

This disease, caused by the pathogen *Alternaria triticina*, has been reported only from the Indian sub-continent. It is characterized by irregular green /grey oval lesions on the leaves. These lesions have yellow/green margins and in severe infections coalesce and become necrotic, causing the death of leaves and eventually the whole plant.

Flag Smut

Although only severe on a local basis, flag smut, caused by *Urocystis tritici*, has a worldwide distribution. In contrast to the other smut diseases, infections are confined to the leaves and stems where they appear as long grey/black stripes containing numerous black spores.

Infections cause dwarfing and stunting of leaves, stems and heads and finally plant death. Yield losses result from a failure to form heads, or a failure to produce grain if the heads are formed.

Cephalosporium Stripe

Found only on winter wheat in North America and northwestern Europe, this disease is caused by the fungus *Cephalosporium gramineum*. Symptoms appear in the spring as yellowing, which is resolved into chlorotic leaf stripes coupled with a brown discolouration of the vascular tissue at about heading. Infected plants are stunted, and most die prematurely with white heads or poorly filled grain.

Loose Smut

This disease occurs wherever wheat is grown, but is most common in humid, sub-humid, and irrigated areas. It is caused by the pathogen *Ustilago tritici* and is confined to the heads. Infection is through the florets and infected heads appear earlier than healthy heads, the florets being replaced to a varying extent by black masses of spores. Yield reduction is complete, as infected heads produce no grains.

Common or Stinking Bunt

Associated with two main pathogens, namely *Tilletia foetida* and *T. caries*, this disease is worldwide in its distribution. Infection occurs at the seedling stage and the fungus develops within the plant giving it a blue/green to dark green color and a slightly stunted appearance. The disease becomes evident at the heading stage with the seeds or kernels being replaced by a fungal structure resembling the kernel but containing a mass of black, odorous spores.

Dwarf Bunt

This disease is restricted in its distribution to the colder and/or higher elevation, higher latitude wheat producing areas. It is caused by *Tilletia controversa* and, similar to common bunt, results in the replacement of the seed in the head with fungal structures containing numerous black spores. It may be distinguished from the other bunt diseases by the severe dwarfing that it causes in growing plants.

Karnal or Partial Bunt

Caused by the fungus *Neovossia indica*, (also know as *Tillitia indica*) this disease is found mainly in the Indian sub-continent, but may also occur infrequently in other major wheat growing areas throughout the world. In contrast to the other smuts and bunts, infection occurs during flowering or grain filling and is usually confined to only a few kernels in each head, which are partially or totally destroyed.

Scab

This disease is associated with several species of the genus *Fusarium*, and is found predominantly in the warm and humid wheat growing areas of the world. The species *F. graminearum (Gibberella zeae), F. culmorum,* and *F. nivale* are commonly associated with the disease, which causes individual spikelets, florets or entire heads to turn white when inmature. This is often accompanied by the production of an external white/pink fungal mat on the surface of the glumes and the development of clusters of small black resting bodies.

Infections cause a great reduction in seed weight and a severe shriveling of kernels if they are produced at all, grain quality may also be seriously affected.

Ergot

Although considered to be of minor importance, ergot, caused by the fungus *Claviceps purpurea*, is occasionally found in the humid and subhumid regions of the world. The infected florets produce a sweet, colorless, and sticky exudate shortly after flowering. As the season progresses,

blue/black 'ergot' bodies (sclerotia), which are larger than the wheat kernel and thus protrude from the infected floret, are produced. These ergot bodies sclerotia are poisonous when consumed by humans or animals.

Common Root or Foot Rot

Associated with several different pathogens, namely *Helminthosporium sativum* (also causing leaf blotch and black point) and *Fusarium culmorum* and *F. graminearum* (also responsible for scab disease), this disease complex is cosmopolitan in its distribution although it is more widespread in the drier areas.

Infected seedlings appear blighted and have brown, rotted roots and sub-crown internodes. Infections in more mature plants are evident as a brownish cortical rot of roots, basal stem tissues and lower leaf sheaths. In addition, infected plants may be stunted or possess white, poorly-filled heads.

The protein content of the grain is reduced by infections of *H. sativum* and, together with the production of a toxin that interfers with nitrogen translocation, this may lead to increased incidence of 'yellow berry'.

Take All

This disease, caused by *Ophiobolus graminis*, has a worldwide distribution and, although it is primarily a disease of winter wheat, may also infect spring crops. Infected plants appear stunted and bleached, with a black discolouration of the crown, crown roots, and often also the lower stems. The root system is severely reduced and rotted and plants usually die prematurely. If they survive to maturity, infected plants usually produce heads which are empty or contain badly shriveled grains.

Cercosporella Foot Rot

Also known as eye-spot or strawbreaker, this disease, caused by the fungus *Cercosporella herpotrichoides*, is found in most winter wheat growing regions of the world. It is characterized by the appearance of oval/elliptical lesions, usually with dull brown/green margins and often marked by a dark colored dot in the center, on the leaf sheaths near the soil surface. In severe infections these lesions may coalesce and weaken the stem to such an extent that it collapses, and also cause the production of premature and/or empty and white heads.

Sharp Eye-spot

This disease is widely distributed throughout the world, but is generally considered to be a relatively minor disease. In Australia, for example, it causes the severe stunting, stiffening and discoloration of wheat seedlings known as 'purple patch'. It is caused by the fungus *Rhizoctonia solani* and is characterized by light brown elliptical lesions with dark brown margins on the basal leaf sheaths.

Browning or Pythium Root Rot

Caused by a group of pathogens of the genus *Pythium*, this disease is found in almost all the major wheat producing regions of the world. It causes distinct brown, watersoaked lesions and rotted areas on the roots, which result in yellowing, stunting, reduced tillering and delayed maturity in infected plants.

Downy Mildew

Downy Mildew is associated with the fungus *Sclerospora macrospora* and is widely distributed wherever wheat is grown. However, it is only severe on a very local basis, usually being prevalent where flooding has occurred in the early stages of crop development. Severely infected plants show excessive tillering, dwarfing and failure to produce heads.

They may also develop striped, yellowed, thickened or distorted parts. As the fungal mycelium is entirely internal, there are rarely any visible 'mildew' symptoms such as those evident in powdery mildew infections.

Snow Mold

This disease is caused by several fungi, of which the most important are *Fusarium nivale* (pink mold) and *Typhula idahoensis* (speckled snow mold). Although it is found throughout the world, the disease is considered to be of only minor importance.

Snow molds are more prevalent in areas with harsh winter conditions, where they become very distinct as the snow cover recedes. The abundance of fungal growth on the surfaces of rotting plant tissue shows as a pink coloration in the early spring in the case of pink mold. Infections of speckled mold develop slightly later in the season and are characterized by numerous black sclerotia in the mold tissue. Damage from mold infections may be severe under certain conditions and ranges from complete yield loss to meager grain yield if infected plants recover.

Black Point

Helminthosporium sativum together with several species of *Alternaria*, especially (*A. tenuis*), are the main causal agents of this disease, which is worldwide in distribution. Infections appear as irregular black or brown blotches in the outer covering of the kernel. These discolorations are commonly concentrated near the germ end, but may be found distributed over the whole seed coat.

H. sativum infections result in poor germination of seed, poor stands, reduced yields and increased levels of root rotting; infections of *Alternaria* cause little or no loss in seed viability.

2.2 Bacterial Diseases

Black Chaff

Although occurring widely on wheat plants, this disease, caused by the bacteria *Xanthomonas translucens*, usually causes only slight damage. Attacks are characterized by brown to purple/black blotches on the glumes and on the stems immediately below the head. Severe infections reduce the number of kernels formed and may cause kernel shriveling.

Yellow Ear Rot

This disease is also commonly known as bacterial spike blight and is caused by *Corynebacterium tritici*. It is often associated with the wheat nematode *Anguina tritici* and has been reported from a number of different countries (e.g., in North America, India, and Pakistan). Infections result in the appearance of a yellow, slimy bacterial growth on the head at the 'boot' stage of growth and may either prevent the head from emerging or cause severe head distortion. The damage caused by this disease is seldom widespread, but may result in appreciable yield reductions on a local scale.

Basal Glume Rot

Caused by the bacteria *Pseudomonas atrofaciens*, this disease occurs in wheat crops throughout the world, especially when high moisture conditions prevail at the time of heading. The germ-end tips of infected kernels become blackened and water-soaked lesions appear at the base of the glumes. Yield losses are rarely severe.

2.3 Viral Diseases

Wheat Soil-Borne Mosaic Virus (WSBMV)

This disease appears in North America, Japan, Egypt, Turkey, Brazil, Italy and Argentina. It is favored by low temperatures and short days and

usually develops in the autumn, winter or spring. The symptoms vary with the strain of virus, ranging from rosetted plants with blue/green leaves and white mottling to dwarfed plants with excessive tillering and light green leaves mottled with yellow. If the infection is heavy and the prevalence high, crop losses may exceed 50 percent.

This virus can be transmitted by sap, but more often by the fungus, *Polymyxa graminis*. It also infects wheat, barley, rye and other grasses.

Wheat Striate Mosaic Virus

This disease is widely distributed in North America, Europe, Australia and the Indian subcontinent, and mainly infects winter wheats although spring sown crops may also be attacked. It is transmitted by several species of leaf hoppers (*Endria* and *Elymana spp.*) and causes narrow, threadlike yellow/white streaks along the leaf veins, leaf yellowing and necrotic blotching, plant stunting, reduced head size and shriveling of kernels. Barley, oats and a number of grasses are also affected.

Wheat Streak Mosaic Virus (WSMV)

This disease is found on winter wheat and winter-planted spring wheat in North America, Europe and several Middle Eastern countries. It is transmitted by the mite *Aceria tulipae* and, depending upon the viral strain, the host variety and the environmental conditions, causes a range of leaf symptoms from mild light green to yellow chlorotic streaks and mottling. Most diseased plants are also stunted and, if seed is produced, it is usually shriveled. The disease also affects barley, oats, rye, maize and several annual grasses.

Enanismo (Dwarfing) Virus

This disease is important in South America and is transmitted by a tropical leaf hopper. Early infection causes severe plant dwarfing (or even plant death); later infections are less serious, causing poor ear development and chlorotic leaf blotching.

Barley Yellow Dwarf Virus

BYDV has worldwide distribution and is transmitted exclusively by aphids. Early infection causes stunting and dwarfing, reduced tillering, a variety of leaf discolorations, ranging from chlorotic striping to total leaf chlorosis, and reduced head size and emergence. Later attacks have less effect, only causing leaf yellowing and reddening together with slight stunting and seed reduction in head and grain development. Most cereals and grasses are attacked by this disease.

2.4 Diseases Caused by Nematodes

Root Cysts

Root cysts are caused by the feeding of nematodes of the genus *Heterodera*, of which *H. punctata* and *H. avenae* are important in wheat. These plant parasites have been reported infesting cereal crops throughout the world, causing stunting and some chlorosis in attacked plants. Examination of the roots of such plants shows excessive branching and root deformation in the form of numerous gall-like cysts.

Ear Cockle

Caused by *Anguina tritici*, known as the wheat nematode, this disease also has a low incidence but worldwide distribution. Infested plants are stunted and the leaves wrinkled, rolled, twisted or curled (foliar symptoms are most severe in young plants). Diseased heads appear shorter and thicker than healthy ones and contain galls (within which are nematode larvae) in place of the kernels. In some cases the symptoms of wheat nematode attack resemble those of Downy Mildew, bunt or 2,4-D damage.

3. RECOGNITION AND IDENTIFICATION OF BARLEY DISEASES

Although diseases tend to be more of a production problem in the wheat crop, they also constitute an increasingly recognized threat to barley production in all but the driest countries of the world. A strong and well-developed pathology input, based upon rapid and accurate disease identification is therefore of great importance for increase world barley production.

3.1 Fungal Diseases

Stripe Rust

Caused by *Puccinia striiformis* f. sp. *hordei*, this disease is of particular importance in the countries of northern Europe, northern Asia and South America and in India, Pakistan, Nepal, Afghanistan and the higher elevations of the Arabian Pennisula. Like yellow rust of wheat it tends to be confined to the cooler, higher altitude areas of these countries.

Stripe rust produces symptoms and effects similar to those of wheat yellow rust.

Leaf Rust

This disease is caused by the pathogen *Puccinia hordei* (=*P. anomala*). It is widely distributed within the Mediterranean countries and is known to cause particularly serious yield losses in the countries of North Africa and in Pakistan.

The disease is characterized by small round yellow/brown pustules occurring mainly on the leaves or leaf sheaths. Later in the season round/oblong, dark brown telial pustules develop.

A leaf rust with darker brown/red pustules may also result from infections of *P. recondita tritici.*

Yield reductions result primarily from reductions in kernel number and seed shriveling.

Stem Rust

Barley stem rust may be caused by a number of barley-specific races of either *Puccinia graminis tritici* (wheat stem rust) or *P. graminis secalis* (rye stem rust). Although present throughout the humid and semi-humid areas of the world, stem rust is not of major importance to barleys as they, through their early maturity, tend to escape severe losses.

Powdery Mildew

In contrast to stem rust, this disease, caused by infections of *Erysiphe graminis hordei*, is of major importance wherever barley is produced. High humidities favor its development but powdery mildew precipitates serious losses even under relatively low rainfall conditions, especially in cool, cloudy weather.

The disease symptoms are, in general, similar to those caused by powdery mildew of wheat. However certain host/pathogen race combinations result in light to dark brown discolourations, which may indicate a type of resistance.

If the disease develops early (in the seedling stage), it may seriously reduce the root system and have consequently severe effects on seed yield, especially in drier areas. Severe disease development before heading tends to reduce kernel number, whereas infections occurring after heading only affect kernel weight.

Downy Mildew

Sclerospora macrospora is the causal organism of this disease, which is restricted to areas where flooding occurs in the early stages of crop development. It is thus of only limited importance, especially on barley which is grown under predominantly dry conditions.

Net Blotch

Occurring widely throughout the temperate and humid areas of the world, net blotch, caused

by Helminthosporium teres and sometimes known by its perfect stage as *Pyrenophora teres*, may precipitate serious yield losses, especially in North Africa, Mediterranean Europe and the Caspian areas of west Asia.

Infections in the seedling stage cause brown reticulate blotches to develop at or near the tip of the leaf blade. These develop into the characteristic dark brown netted blotch which may finally cover the whole of the leaf blade but never extend to the leaf sheath. Further lesions may also develop on the kernels, where they appear as a light brown, discoloration of the lemma rather than as the characteristic netting of leaf infections.

Net blotch causes grain shriveling which may be severe, especially in early infections. In this case both the number of tillers/per plant and the number of kernels/per tiller may be seriously reduced.

Spot Blotch

This disease is caused by *Helminthosporium sativum (=Cochliobolus sativus)*. It is widespread within the countries of North Africa, West Asia and Mediterranean Europe where its development and spread is favoured by warm and moist conditions.

Seedling infections cause a seedling blight which frequently results in pre-or port-emergence seedling death. Infections of mature plants appear as characteristic dark brown, round/oblong lesions which may coalesce, covering the whole leaf blade. The kernel and floral bracts may also be affected by spot blotch, in which case it is more commonly known as black point.

Stripe Disease

Stripe disease, caused by *Helminthosporium gramineum*, is almost invariably present on barley crops throughout the world. However, it only results in serious crop losses in certain areas (especially in the low rainfall areas of Iran, Syria and Turkey). In these areas it is recognized as one of the most important barley diseases.

Disease symptoms appear at about the tillering stage of crop growth. At first they are confined to the older leaves and leaf sheaths as parallel yellow stripes, but as the disease develops the symptoms become brown and may extend over all the leaves. Infected plants tend to be stunted and may split, fray out, or collapse. In severe infections the spike fails to emerge and in cases where it actually emerges tends to be blighted and brown in colour.

Scald

This disease results from infections of the pathogen *Rhynchosporium secalis* and is of major importance in cooler humid and sub-humid regions. It causes considerable yield losses in Afghanistan, Turkey, Ethiopia, Kenya, Tanzania and in many of the Mediterranean countries.

It occurs mainly on winter barleys and appears first as irregular, blue/green, water-soaked lesions on the leaf blades and sheaths. As the disease progress, these symptoms develop into bleached areas with brown margins.

Infections cause a similar damage to those of net blotch.

Loose Smut

Ustilago nuda is the causal organism of this disease, which is widespread throughout areas in which humid, cool and showery conditions prevail during heading.

The symptoms, which result from the replacement of the floral tissues by spores, appear at flowering when the covering fragile membrane ruptures, revealing a dark brown spore mass. Infected heads usually emerge from the sheath a few days earlier than healthy ones but may be difficult to distinguish until flowering.

Black or Semi-loose Smut

Similar to loose smut, this disease is caused by the pathogen *Ustilago nigra*. It is of importance in Europe, Asia and in some parts of the Near and Middle East. The symptoms of infection resemble those of loose smut, except that the spore mass tends to be less compact. Infected heads tend to emerge later than those infected with loose smut.

Covered Smut

This disease, caused by *Ustilago hordei*, is considered to be one of the most serious and widespread diseases of barley throughout North Africa, the Near and Middle East and South and Southeast Asia.

The spore masses produced completely replace the kernel and are covered by a membrane which persists until the spores are fully mature. Unlike the wheat smut diseases, covered smut of barley is readily identifyable as soon as the ear has emerged from the sheath.

Fungal Root Rot

As with root rot in wheat, this disease is primarily caused by *Pythium* species and results in root necrosis, which in turn causes infected plants to become chlorotic and stunted. It may be very damaging if infection occurs during the seedling stage; it is of less significance in adult plants. The most common species infecting barley is *P. graminicale*.

Scab or Fusarium Blight

The causal organisms, symptoms and damage of this disease are identical to those of the similar wheat disease.

Ergot

Here again the characteristics of the disease are the same as in wheat.

3.2 Bacterial Diseases

Bacterial Blight

This is the only bacterial disease of any importance occurring in barley. It is caused by *Xanthomonas translucens* and is most prevalent in northern Europe, Asia, North Africa and various countries of the Near East.

Early infections appear as minute, linear, water-soaked lesions on both leaf blades and sheaths. These lesions elongate and may coalesce into narrow and irregular glassy-surfaced stripes, which may be light-yellow, light brown or dark brown according to age. In the later stages of infection the centers of the lesions become translucent, and small drops of white exudate may be seen.

Infections are, in general, only serious if they start at an early stage of crop growth, in which case they can cause severe and sometimes complete crop loss.

3.3 Viral Diseases

In addition to the viral diseases discussed in relation to wheat, two further species deserve particular mention:

Barley Stripe Mosaic Virus (BSMV)

This disease has been found in North America, Europe, Japan, Korea, Pakistan, the USSR, Australia and some West Asian countries.

Its symptoms vary from yellow/brown striping to chlorotic spotting and general mottling. Little is known about its affect upon the host plant.

Cereal Tillering Virus (CTV)

This disease attacks barley exclusively, resulting in a variety of mosaic, mottling, yellowing and stunting symptoms.

3.4 Diseases Caused by Nematodes

In general, barley is attacked by the same groups of nematodes that commonly infest wheat crops. Nematodes of the genus *Heterodera* are particularly important in this respect and *Anguina tritici* has also be found to cause ear cockle in barley.

As with wheat, the volume of work carried out to date on nematodes in particularly dry climates is limited. Their importance may thus be considerably greater than is currently supposed.

4. THE MAJOR DISEASES OF WHEAT AND BARLEY

Pathogenic fungi are by far the most important and yield-limiting of the many disease-causing organisms which attack cereal crops. Of these the general *Puccinia* (rusts), *Ustilago* (smuts), *Tilletia* (bunts), *Erysiphe* (powdery mildews), *Septoria, Alternaria, Helminthosporium, Fusarium* and *Pythium* are the most widespread, regularly occurring and potentially dangerous throughout the world.

Studies of the distribution and ranked importance of these diseases reveal the tantamount importance of the rusts, especially in wheat (Tables 4.1 and 4.2). Such studies also show the increasingly recognized importance of other pathogens such as *Helminthosporium* spp. (causing leaf blotch, foot rot and black point), *Erysiphe* spp. (causing powdery mildew), *Ustilago* spp. (causing loose smuts) and *Tilletia* spp. (causing bunt diseases).

Table 4.1 Distribution and Importance of Wheat Diseases

Area	Disease and Ranked Importance SR	YR	LR	Sm	B	PM	S	A	H	RR
Afghanistan	2	1	5	4	7	6	3	–	–	B
Turkey										
Plateau	4	1	6	3	2	–	–	–	–	5
Coast	1	1	6	5	7	4	3	10	9	8
S.East	5	3	4	2	1	–	–	–	–	6
Cyprus	1	3	2	Tr	Tr	5	4	Tr	Tr	Tr
Syria	2	5	3	4	1	–	–	–	–	–
Iraq	–	2	1	–	–	–	Tr	–	Tr	–
Lebanon	3	1	2	–	–	–	–	–	–	–
Jordan	2	–	–	–	1	–	–	–	–	–
Saudi Arabia	1	4	3	2	–	–	–	–	–	–
Egypt	3	1	2	–	–	–	–	–	–	–
Libya	1	–	2	3	–	–	–	–	–	–
Algeria	4	2	3	6	5	7	1	10	9	8
Tunisia	3	7	6	2	1	7	4	–	–	5
Morocco	3	–	1	1	5	4	2	–	6	–

Table 4.2 Distribution and Importance of Barley Diseases.

Area	Disease and Ranked Importance SR	YR	LR	PM	H	Sc	Sm
Afghanistan	4	1	6	1	3	2	4
Turkey	6	7	4	3	2	1	5
Cyprus	–	–	1	3	2	–	4
Syria	–	–	–	1	–	–	–
Iraq	–	–	–	–	1	–	–
Lebanon	–	–	2	1	4	–	1
Jordan	6	7	3	2	4	–	1
Saudi Arabia	–	–	–	–	–	–	–
Egypt	4	–	2	3	1	–	–
Libya	–	–	–	2	–	3	1
Algeria	–	3	2	5	1	5	4
Tunisia	7	6	4	3	1	5	2
Morocco	–	–	3	4	1	–	2

(Numbers refer to how disease ranks in importance, 1= most important)

SR = Stem Rust
YR = Yellow Rust
LR = Leaf Rust
Sm = Smut
B = Bunt
PM = Powdery Mildew
S = Septoria
A = Alternaria
H = Helminthosporium
Sc = Scald
RR = Root Rot and Fusarium

4.1 The Cereal Rusts

Puccinia graminis (causing stem or black rust), *P. recondita* (causing leaf or brown rust), *P. striiformis* (causing yellow or stripe rust), *P. hordei* (causing dwarf or leaf rust) and *P. coronata* (causing crown or leaf rust) regularly precipitate serious losses of wheat barley, oats and rye throughout the world. Owing to their prime role in limiting the productivity of these cereal crops in almost every major cereal-producing country, the rust diseases deserve special and detailed attention in research designed to minimize production losses from cereal diseases.

Life Cycle

The rust fungi are, in general, obligate parasites, being unable to complete their life cycle in the absence of a living plant host. They have developed a very complex life cycle involving, in many cases, an alternation between two host species and a number of different spore types. The full life cycle is illustrated in Figure 4.1. It should be stressed, however, that the complete two-host, five-spore type cycle is not commonly found in cereal rusts except under rather specific environmental conditions. In most areas, where environmental conditions are favourable, the fungi reproduce almost exclusively through the asexual urediospore cycle on the crop itself, on volunteer plants, and on related plant species. The complete life cycle may be encountered in a few areas where the primary crop or grass host and the alternate host are found in close proximity to one another. The following plant species are known to act as alternate hosts for the principal cereal rust pathogens:

P.graminis—*Berberis spp. Mahonia spp.*

P.striiformis—Unknown

P.recondita—*Thalictrum spp. Isopyrum spp.*

P.hordei—*Ornithogalum spp.*

P.coronata—*Rhamnus spp.*

In many regions the graminaceous host (or hosts) may be found throughout the year allowing the rusts to survive locally, a situation referred to as endemic. However, there are a number of areas (especially where hot and dry conditions prevail for some months) where the host species and consequently the fungi do not survive from one season to another. Where these conditions occur the rust infections initially arise from inoculum reintroduced from some distant source and are termed exodemic.

Taxonomy

The classification of rust fungi into family and genus is based upon the morphological features of the teliospores, and the species within genera are distinguished on the basis of their host range and urediospore characteristics (Figure 4.2).

In the genus *Puccinia*, however, specialization is highly developed and a number of *formae speciales*, based upon host range, may be distinguished within each species. *Formae speciales* (f.sp.) are names referring to the primary host species attacked; each *forma speciale*, in general, being able to attack several closely related grasses. The major recognized *formae speciales* infecting cereal crops are listed below:

Pathogen Species	Primary Host Formae speciales	
P.graminis Pers.	*tritici*	Wheat, Barley
	secalis	Rye, Barley
	avenae	Oats
P.recondita Rob ex Desm	*tritici*	Wheat
P.striiformis Westend	*tritici*	Wheat
	hordei	Barley
P.hordei Orth.		Barley
P.coronata Cda.		Oats

Within each *forma speciale* the pathogens may further be divided into physiological races and/or biotypes. A biotype is defined as a population of individuals with identical genotypes, and a physiological race as a group of biotypes similar in morphology but differing in physiological, biochemical, pathological and other characters. Physiological races (designated by race numbers) are identified on the basis of differential responses on selected host varieties. While this system of nomenclature serves a very useful taxonomic purpose, it cannot be used to completely identify any rust collection.

The indentification of physiological races of rusts may be made on the basis of observations of the infection response of different wheat and barley varieties to a purified rust culture. A fixed number

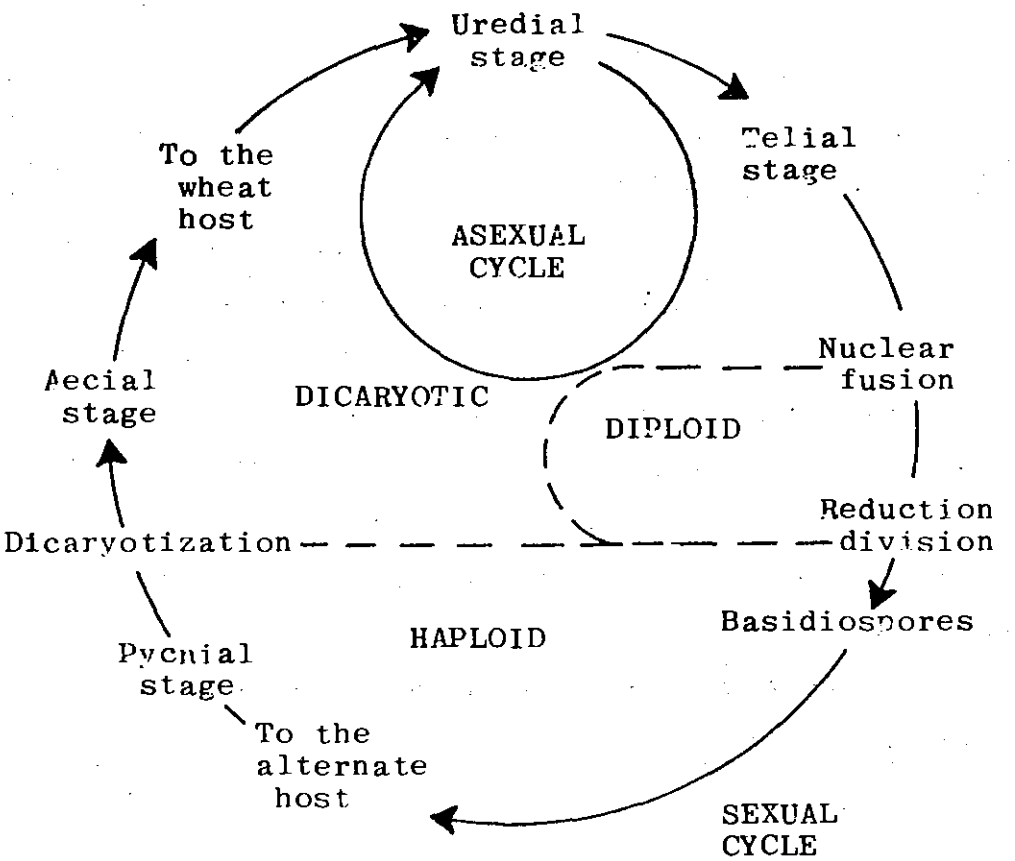


Fig. 4.1.: Generalized life cycle of the wheat rust fungi (Loegering, Johnston and Hendrix)

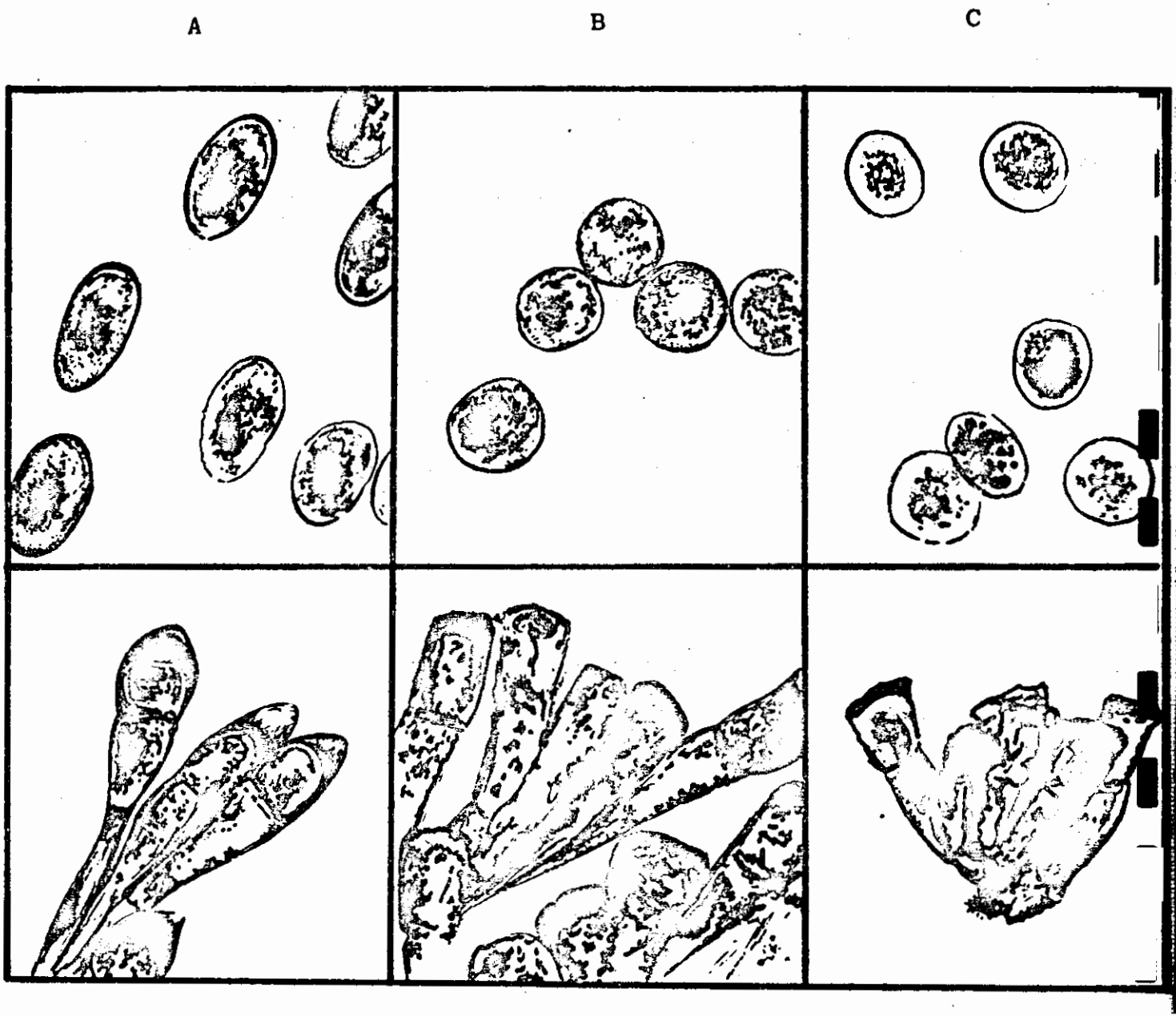


Fig.4.2.: Urediospores (top row) and teliospores (bottom row) of stem (A), leaf (B), and stripe (C) rust fungi magnified about 875 times. The spores of the stem rust fungus are easily differentiated from the other two when observed under the microscope. The spores of the leaf and stripe rust fungi are difficult to distinguish.

of varieties in the seedling stage are used, and infections will be fully developed at about 10-15 days after inoculation if conditions are optimal. (The procedures and techniques of this process will be elaborated in the glasshouse practical sessions.)

Based upon the host response classification, a race number can be either determined from published reports or assigned if a new race is isolated. The method for coding infection types for stem and leaf rusts is given in Table 4.3. Although there is no unanimous agreement among rust workers on coding, the majority of the yellow rust researchers now classify infection types according to the general scheme given in Table 4.4. European yellow rust workers have further developed a binary notation system for race nomenclature. This allocates to every differential host a fixed value (decanery value). Race reactions are classified as either resistant (binary score=0) or susceptible (score=1) and in this way a decanery value may be obtained for each differential. These are then added to give a decanery total which then becomes the race number (see below). Such a system allows the susceptible varieties to be identified from the decanery total or race number.

The Use of Binary Notation in Naming a Physiological Race

Differential Host	G	F	E	D	C	B	A
Decanery Value	2^6	2^5	2^4	2^3	2^2	2^1	2^0
Race Reaction	R	R	S	R	S	S	R
Binary Score	0	0	1	0	1	1	0
Race Decanery Value-	-	-	16	-	4	2	-

Decanery Total = 22 = Race Number

Development of New Biotypes

Rust fungi may evolve new variants or biotypes in a number of different ways. If the alternate host full life-cycle occurs, new variants can arise in profusion through sexual hybridization (the production of gametes at meiosis and their recombination at fertilization). However, the full sexual cycle is not a common feature in rust fungi in many parts of the world (there is for example no known alternate host for *P. striiformis*). Mutation is a further mechanism through which pathogen variability may be created. Even at low mutation frequencies, the very high rate of urediospore production observed in these pathogens would allow mutations to be one of the major source of variants. Other mechanisms recognized as being operative in rusts are heterokaryosis and somatic hybridization (parasexuality). Little evidence of the importance of these two mechanisms under field conditions is currently available.

Studies of the genetics of rust pathogens have revealed the presence of a number of genes for virulence (the inherent ability of a pathogen to cause disease), many of which exist in a heterozygous and recessive state. A single mutation of such a gene in this case would allow the full expression of the virulence character in a new biotype. It is, therefore, not surprising that investigation has indicated that many virulence genes may arise through mutation. Studies for host:parasite interactions in the wheat/rust system have further indicated a very close relationship between the genetics of pathogen virulence and host resistance. A gene-for-gene association, implying a direct relationship between host genes for resistance and parasite genes for virulence on a one-to-one basis, has thus been postulated and has gained considerable acceptance.

Table 4.3 The Basic Infection Types for Classifying Cultures of Puccinia graminis tritici in the glasshouse: (Stakman et al)

0 — **Immune:** No signs of disease at all
0 — **Practically Immune:** No pustules but hypersensitive flecking (small flecks of dead host tissue) observable
1 — **Very Resistant:** Rust pustules extremely small and isolated; often surrounded by sharp and continuous hypersensitive flecking
2 — **Moderately Resistant:** Pustules small to medium in size; usually occurring in green islands of host tissue surrounded by bands of yellow, chlorotic or dead tissue
3 — **Moderately Susceptible:** Pustules medium in size; usually separate; no areas of dead tissue; yellowish (chlorotic) areas may be evident
4 — **Susceptible:** Pustules large, numerous and often united (confluent); no dead tissue, chlorosis may occur under unfavourable growing conditions
X — **Reaction Heterogenous (Mesothetic):** Pustules variable in size; all types of infection may be found on a single leaf blade; no mechanical separation possible. On isolation and re-inoculation small pustules may produce large ones and vice-versa

Table 4.4 General Classification of Infection Types for Yellow Rust

Description of Infection Type	Code Symbol*	Index Value
No Data		
No Visible Infection	0	0
Necrotic/Chlorotic Flecks—No Sporulation	VR	1
Necrotic/Chlorotic Stripes—No Sporulation	R	2
Necrotic/Chlorotic Stripes—Trace of Sporulation	MR	3
Necrotic/Chlorotic Stripes—Light Sporulation	LM	4
Necrotic/Chlorotic Stripes—Intermediate Sporulation	M	5
Necrotic/Chlorotic Stripes—Moderate Sporulation	HM	6
Necrotic/Chlorotic Stripes—Abundant Sporulation	MS	7
Chlorosis Behind Sporulating—Area: Abundant Sporulation	S	8
No Necrosis/Chlorosis—Abundant Sporulation	VS	9

* R=Resistant
M=Moderate
S=Susceptible

5. EPIDEMIC DEVELOPMENT

The cycle of pathogen and disease developmet –from primary inoculum through plant infection and back to primary inoculum–varies considerably between different pathogenic organisms. Plant pathogenic fungi can be divided into two groups on this basis: viz., single and multiple cycle types (Figure 5.1).

Single Cycle Diseases

Fungal pathogens with a single cycle have no mechanisms for secondary spread within their host crop. Thus all disease expression results from infections caused by primary inoculum, with increased levels of disease resulting from increased primary infections as the environment becomes more favorable. Diseases which fall into this category include the bunts, smuts and foot rots, which are primarily soil-borne.

Multiple Cycle Diseases

Pathogens with a multiple disease cycle are able to spread within an infected crop through the continuous production of re-infective spores (secondary inoculum). Thus even very low levels of primary infection can result in severe crop damage. As a result, pathogens of this type (e.g., rusts, *Helminthosporium,* and *Septoria*) are considered to possess an inherently higher disease-causing potential than those with a single cycle. Discussions will therefore, at this stage, be confined to multiple cycle diseases.

5.1 Disease Establishment

Inoculum

The first necessity in the establishment of a disease is for the *inoculum* (that part of the pathogen which carries infection to a host) to come into contact with the surfaces of a suitable host. Inoculum particles may be in a number of different forms (e.g., spores, mycelial masses, bacterial cells, or viral particles).

Disease inoculum is generated by previous infections and liberated into the environment, constituting a reservoir of potential infection. It may originate from a few infected plants or from many; it may come from the same location or have travelled over great distances; and it may be primary–resulting from infections in a previous season–or secondary–arising from earlier infections in the same season. Rust infections, for example, may result from spores produced or surviving locally or from spores carried long distances by the wind.

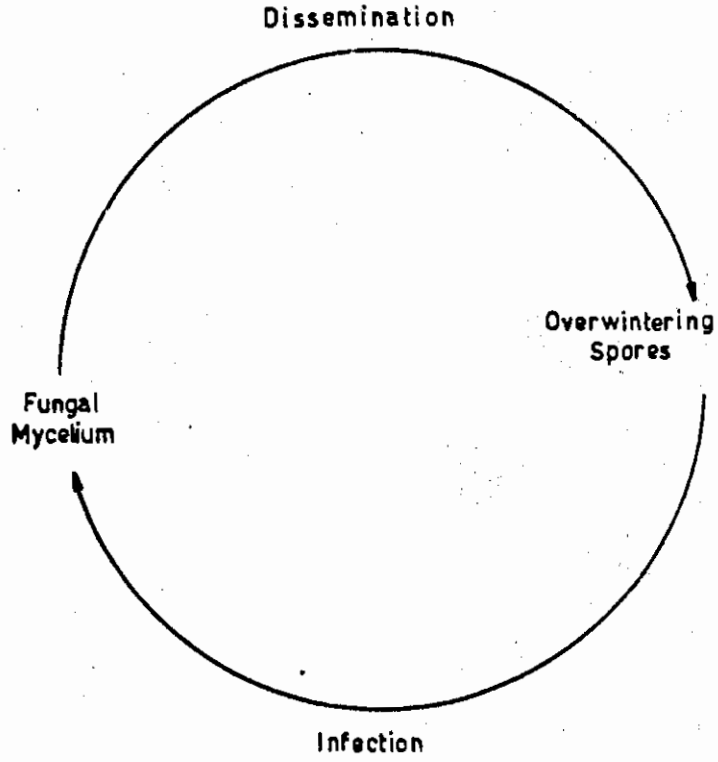
Wind is perhaps the major way in which multiple disease cycle fungal spores are disseminated over greater or lesser distances. Water may also play an important role in the dissemination of the spores of some fungal pathogens (e.g. *Septoria*), especially over short distances. Other mechanisms of transferring pathogenic inoculum to host plants include insects and nematodes (especially with viruses), other pathogens (bacteria and viruses are sometimes carried by fungi), and the activities of man and animals.

In general only a small percentage of inoculum survives, reaches a suitable host plant at a suitable time, and is able to infect it. This is perhaps the primary reason for the large amount of inoculum (and consequent crop damage) produced by most pathogenic organisms. Numerous factors affect inoculum survival, including dormancy, spore structure, dispersal mechanisms, and environmental conditions.

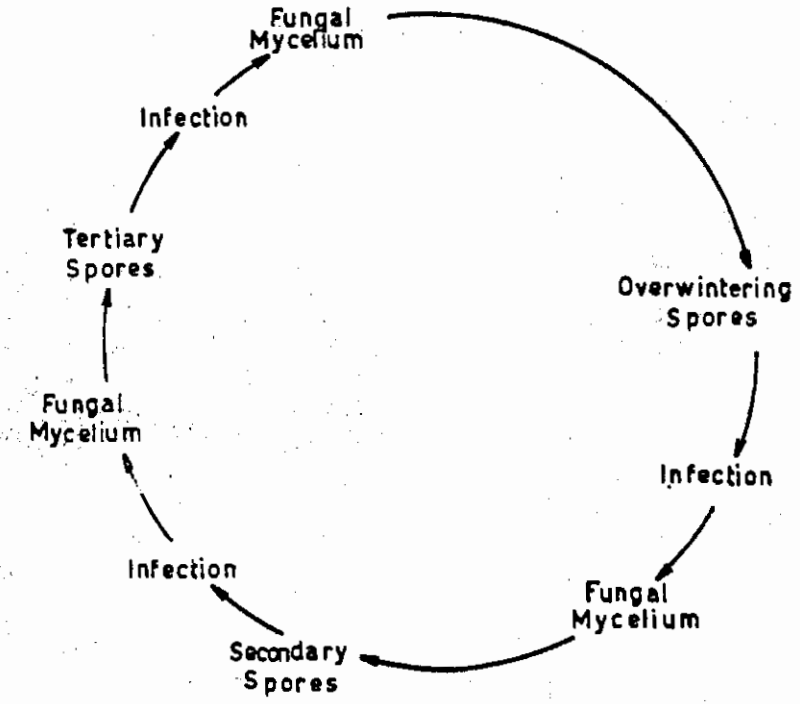
Rust urediospores, for example, are well adapted to survival, being able to travel over long distances and through adverse conditions while still retaining a fairly high viability.

Fig 5.1

DISEASE CYCLES



SINGLE CYCLE



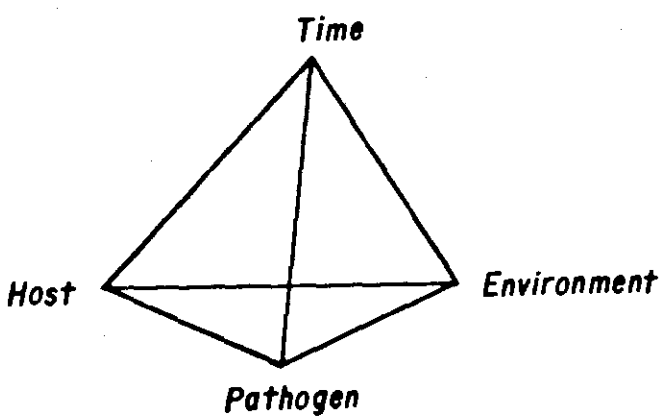
MULTIPLE CYCLE

Once in contact with the host surface, and providing that environmental conditions are favorable, the pathogenic inoculum enters into a phase of rapid growth supported by its own food reserves. This growth phase, which results in the formation of a germ tube responsible for the actual penetration of the host surface, is termed 'germination'. It is during this time that the pathogen is most vulnerable to desiccation from adverse conditions and thus most dependent upon the environment; after penetration has occurred the pathogen is once again protected from external conditions.

The sequence of disease establishment, development and spread in relation to plant growth is illustrated in Figure 5.2.

5.2 Disease Spread

Traditionally the major factors affecting disease spread and development have been linked together in a 'disease triangle' of interaction between the virulence of the pathogen, the susceptibility of the host, and the favorability of the environment. Time, however, is a further and very important consideration in this regard (e.g. the time period during which the host and pathogen are in contact, the time and duration of optimal infection conditions, the time necessary for infection, etc.). The insertion of a time-factor into this interaction equation results in its quantification as a 'disease pyramid' rather than triangle.



The amount of disease caused by a pathogen on a particular host, in a specific environment and over any particular time is thus represented by the volume of the pyramid resulting from the interaction of these factors. The development of diseases which rely upon biological vectors for their transmission (e.g. viruses) is further complicated by interactions involving these vectors.

Disease spread involves the continuous multiplication of infections at progressively greater distances from the original foci of infection until the established infections are so close together and produce such a large amount of inoculum that no healthy susceptible plant within the particular area can escape infection. The multiplication of infections reflects the influence of all the factors of the disease pyramid and their component parts.

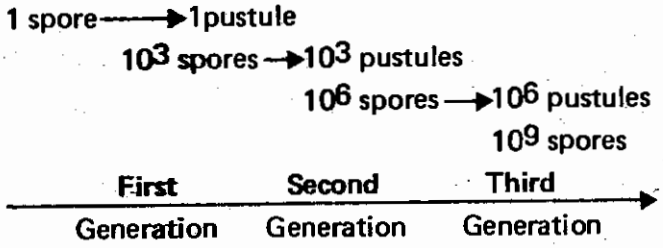
Host Factors

The size, distribution and genetic diversity of host populations are of great importance in determining the degree and rate of epidemic development. Large areas of genetically uniform host plants constitute an ideal medium for disease infections reaching epidemic proportions. The risk of major crop losses in such areas (e.g. the wheat producing areas of the American Great Plains) would be very high were it not for the genetic diversity for rust resistance in the cultivars grown. A high degree of genetic diversity in resistance is imperative in order to reduce the likelihood of the sudden appearance of new physiological races capable of overcoming the resistance and the disastrous losses that would thus result.

In order to develop high yielding cultivars with a broad-based resistance to various diseases, all known sources and types of resistance must be utilized in breeding efforts. Systems of multi-location testing are a further asset in the selection of cultivars with this type of resistance.

Pathogenic Factors

The primary components of disease spread are the abundance of inoculum, its virulence and its reproductive ability. Epidemics will, in general, occur when large amounts of vigorously growing and rapidly reproducing pathogenic particles come into contact with highly susceptible hosts. Assuming 100 per cent inoculum viability and infection, 10^6 rust pustules can theoretically result from one initial rust urediospore infection after only three generations:



Although this situation never occurs in nature, the example serves to illustrate the enormous infection potential of some fungi, especially when the rate of generation turn-over can be as rapid as 14 days. With this rate of reproduction the effective rate of mutation may be very high, further underlining the danger inherent in growing large areas of cultivars with a narrow base of resistance.

Environmental Factors

Favorable environmental conditions are an essential prerequisite for epidemic development. Of major importance in this regard are moisture and temperature. Although pathogens differ in their environmental requirements for optimal infection, leaf-and stem-infecting fungi, in general, and rust species, in particular, require sufficient free moisture on the plant surfaces and adequate temperatures before they will germinate and infect. (See Table 5.1).

Time Factors

In the case of the cereal rusts, and indeed most foliar pathogens, infections may occur at any time during the crop growth period, provided, of course, that environmental conditions are favorable. This means that explosive epidemics may develop at any time during the growing season. In contrast the bunt and loose smut fungi, among others, are able to infect their hosts only at specific growth stages (i.e. the germinating seed and the developing flower respectively). Epidemics of these pathogens thus build up slowly over a number of seasons rather than occurring explosively.

The Extent of Disease Spread

The distance over which infective inoculum can travel is of great importance in the spatial expansion of infection centres. Rust diseases, as already mentioned, are able to spread over very considerable distances, and are thus particularly dangerous as outbreaks are difficult to contain.

Epidemics begin with a localized disease spread around the primary foci of infection, and then diffuse rapidly outwards, producing secondary foci. (This holds true whether considering an individual field or a whole region.) The degree and rapidity of spread depends upon the pathogen (its rate of spore production and growth), the environment, the availability of susceptible hosts, and time—the four factors of the disease pyramid. Under optimum conditions of these factors the disease spreads until the primary and secondary foci coalesce and the epidemic continues until there is no more susceptible tissue to infect.

In an epidemic the following conditions are evident:

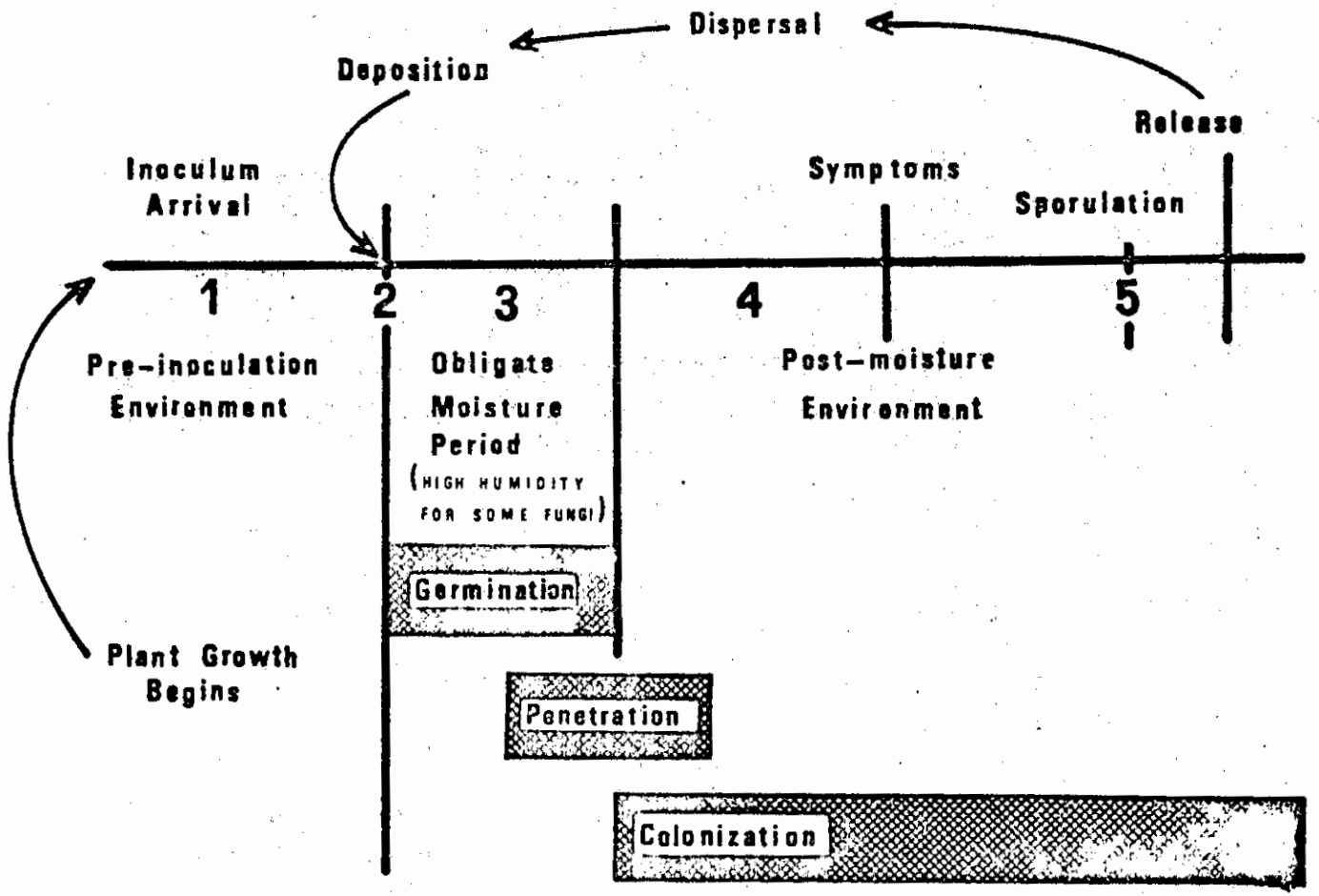
- The rate of disease multiplication increases with increasing numbers of infections.
- The amount of infection due to a single disease focus at any time decreases with the distance from that focus.
- The chance that a given plant will be infected thus also decreases with increasing distance from the inoculum focus.

Table. 5.1 Relationship between Environmental Conditions and the Establishment of Rust Diseases

Disease	Growth Stage	Free Water	Temperature	Light	Humidity
Black Rust *P. graminis*	Germination	Essential: 2 hr dew period gives high rate	Minimum 10°C Optimum 15-24°C Maximum 30°C	Max. Intensity 11 x 10^3 lux	
	Germ-tube growth and appressorium formation	Essential Optimal with 10 hr dew 70% with 4 hr dew	Optimum 16-27°C		Slow drying in the dark maintains viability /appressorium
	Penetration and formation of sub-stomatal vesicle	Dew necessary	Minimum 15°C Optimum 19°C Maximum 35°C	Min. Intensity 5.5 x 10^3 lux	
Brown Rust *P. recondita*	Germination	Essential	Minimum 2°C Optimum 15-20°C	May retard germination under certain conditions	High humidity essential
	Germ-tube growth and apressorium formation		Minimum 5°C Optimum 15-20°C Maximum 31°C		High humidity essential
	Penetration and formation of sub-stomatal vesicle		Optimum 20°C	No effect.	
Yellow Rust *P. striiformis*	Germination	Essential	Minimum 0°C Optimum 9-13°C Maximum 23°C		
	Germ-tube growth		Optimum 10-15°C		
	Penetration and formation of sub-stomatal vesicle		Minimum 2°C Optimum 8-13°C Maximum 23°C	Little or no effect	6 hr high humidity essential/opt.temp.

Fig 5.2 : Sequence of development of a typical foliar fungal plant disease

(*after R . D . Schein* 1963)



5.3 A Simple Way to Measure Disease Increase

Close to 20 years ago, investigations by Van der Plank helped to transform plant pathology into a more quantitative science. Through these studies he showed that many epidemics–as typified by rust epidemics–build-up as does money in a bank–through compound interest. Thus the phrase "compound interest disease" was coined for epidemics caused by the multiplication of a pathogen through succeeding generations. The "compound interest formula," derived by Van der Plank, gives an estimate of the infection rate (r) per unit time and thus a measure of the disease increase.

$$r = \frac{1}{t_2 - t_1}\left(\log_e \frac{x_2}{1 - x_2} - \log_e \frac{x_1}{1 - x_1}\right)$$

where t_1 and t_2 are the dates at which disease measurements were made and x_1 and x_2 the amounts of disease recorded on these dates.

The observed infection rate (r) is a result of the interaction of all the factors affecting disease development. As these factors become more optimum, 'r' increases.

$\log_e \left(\frac{x}{1-x}\right)$ is termed the logit of x.

Logistic increase implies that there is a limit to the amount of infection that a population can sustain and that infection increases at a constant rate. The development of an epidemic may thus be plotted as a straight line according to the equation $y=a+bx$ (where b=slope=infection rate and a=infection level at the first observation).

Example: Calculation of the infection rate of the wheat rust epidemic in Sonora, Mexico 1976-1977.

Date:	18/II	10/III	18/III
Growth Stage:	Heading	Flowering Complete	Kernels Half Formed
o/o rust on flag leaves (Cobb Scale)	7.3	35	100
$\log_e \left(\frac{x}{1-x}\right)$	−2.54	+0.62	+6.9

Using the equation

$$r = \frac{1}{18}\quad (6.9 + 2.54) = 0.52 \text{ units/day}$$

(See Figure 5.3 for graphical plotting of these values)

5.4 Development of Rust Epidemics in the Field

The primary rust inoculum, which establishes the initial infections, is carried to the host population by wind or water. If the urediospores arrive in a viable state they will germinate when the environmental conditions become favorable. Only those races which possess the virulence factors allowing the rust pathogen to establish a successful parasitic relationship with the host can infect its tissues. The environmental conditions following infection and the interaction between the genetic make-up of the pathogen and the host then determine the time elapsed until the production of new urediospores (the latent period). Temperature is particularly important in this regard, and its relationship with the latent time period for primary infections of yellow, leaf, and stem rusts is illustrated in Figure 5.4:

As constantly optimum temperatures do not occur in nature, the production of new pustules from initial infections will generally take between

Fig. 5.3 LOGIT PLOT : APPARENT INFECTION RATE OF LEAF RUST EPIDEMIC

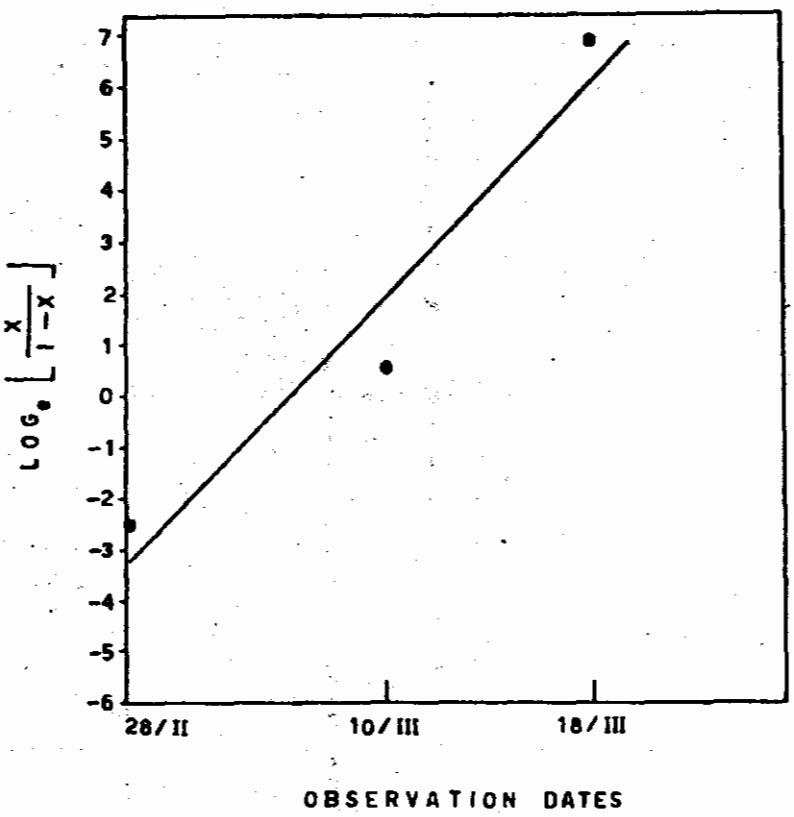
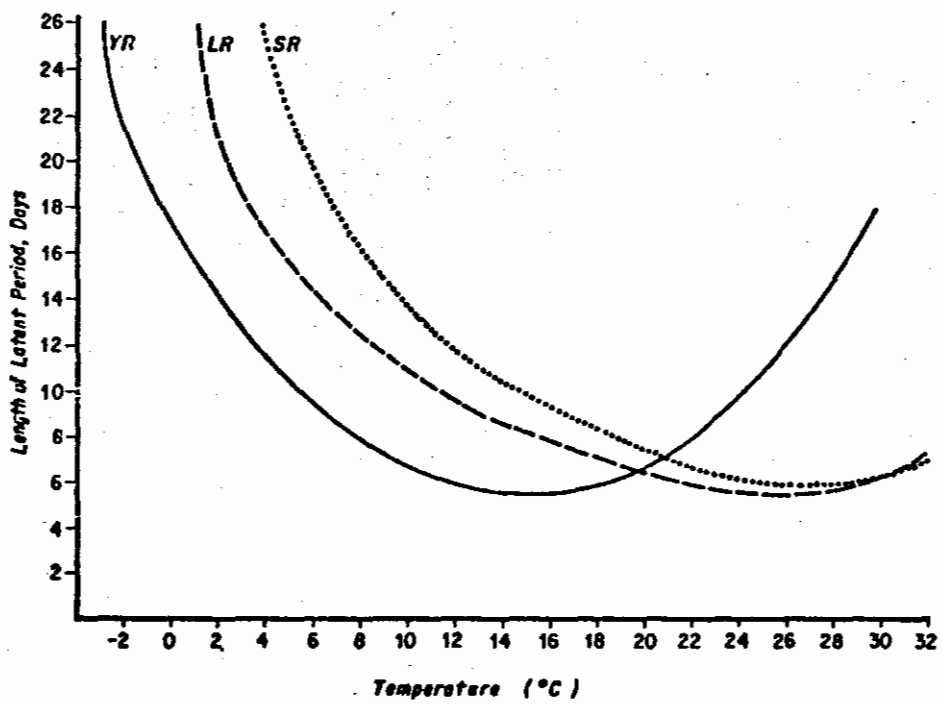


Figure 5.4 Latent Time Period for Primary Infections of Yellow Leaf and Stem Rusts



12 and 14 days. If the spores produced by these pustules cause a re-infection every day thereafter, then secondary pustules will appear about 24 to 28 days after the initial infection. From the 24th day onwards new uredia will be developing every day.

Following the eruption of the first pustules on the host leaves or stems, secondary infections can occur on the same or neighbouring plants whenever environmental conditions are favorable. Pustules will, under good conditions, continue to produce spores for a period of 16-21 days.

The number of spores produced per uredium under field conditions varies between rust species and with ambient temperature. Data below indicate the approximate scale of spore production and its relationship to temperature for a stem rust infection.

The Production of Urediospores from a Single Rust Pustule over an Eleven Day Period (from Prabhu and Wallin)

Temperature °C	Nos. of Urediospores (1,000)
13	40
18	83
24	206
29	218

(A brief summary of simple relations between environmental conditions and disease establishment is given for each of the major rusts in Table 5.1).

5.5 Long Distance Transport of Disease Organisms

The important role of long distance transport in establishing infections in areas where the pathogen cannnot survive from one season to the next is self-evident. The distance over which pathogenic inoculum can travel varies greatly between pathogens; the majority have only limited mobility. However, the spores of certain species are capable of traveling over very considerable distances through the action of specific vectors. Man and wind play particularly important roles in long distance transport.

Transport by Man

Many plant pathogens, especially smuts, are disseminated in or on host seed or together with contaminating plant or soil debris. The large volume of plant material currently transported throughout the world thus constitutes a serious danger of the simultaneous and widespread transport of pathogenic inoculum. Indeed, numerous examples may be cited of severe crop losses arising from the introduction of diseases into areas where they were hitherto unknown.

In order to minimize the risk of disease introduction on imported plant material, most countries have evolved detailed, and sometimes complex, plant introduction and quarantine regulations. These regulations are often over-enforced on the exchange of scientific materials, whereas commercial channels, through which the bulk of plant imports flow, often have few restrictions placed upon them. In this way the free exchange of cereal germplasm, and through it the rapid dissemination of improved crop varieties, may be seriously hindered.

The quarantine regulations of many countries would benefit from a more rational application, based upon a realistic study of the dangers and benefits of the facilitated exchange of germplasm of a number of important crops.

Wind Transport

Wind is without doubt the most important factor in the long distance movement of the inoculum of many foliar diseases; as such it often negates the consideration of quarantine regulations for the control of such diseases. The effects of wind transport have been particularly well-illustrated in studies of the cereal rusts.

The limits of dissemination are governed primarily by weather patterns and the resistance of the urediospores to adverse environmental conditions. Movement usually takes place in a stepwise fashion, but single flights of over 1,000 kilometers have been reported for spores of *Puccinia graminis*

tritici; extensive travel can take place over a very short time period given favourable conditions. In addition movements may take place either regularly on a seasonal pattern or gradually over a period of years. A number of well-documented reports of migrations and migration routes throughout the world illustrate the various patterns of movement.

Annual Migration

The best known and documented annual rust migration route stretches from the southern USA to the northern states and southern Canada. It is commonly known as the *'Puccinia Pathway'* and covers more than 3,000 kilometers. The migration usually involves a number of steps, but with severe infections in the south and two to three days of strong winds, the number of steps may be reduced, and the area and distance covered may be very extensive. Movement is primarily in a northward direction following the development of the wheat crop in the different states. However, as the wheat in the northernmost areas of Canada is maturing, the inoculum once again moves south to infect the early-sown crops of the Great Plains.

Other well documented pathways of cereal rust migration occur in Europe, Australia, and the Indian subcontinent. In Europe *P.graminis* regularly moves northwards each spring after surviving the winter period in North Africa and Mediterranean Europe. A similar long distance movement of stem rust can be seen in Australia, and there is evidence of dissemination from there to New Zealand, across some 2,500 kilometers of ocean. The new yellow rust disease in Australia took less than two years to reach New Zealand. In the case of India, stem rust occurs all year round in the southern hills where conditions allow the year-round production of wheat and barley. These areas thus constitute the reservoirs of inoculum for the regular infection of the newly-sown crops in the plains. The occurrence of infections is closely correlated with the time at which mean monthly minimum temperatures reach 14°C, the minimum temperature for the establishment of stem rust infections.

The situation in Egypt, although much less clearly understood than the other migration pathways, is interesting in that all three major rust diseases are commonly found attacking wheat. Each year the rusts die out after the wheat harvest (May-June) and re-infection occurs after sowing in November. This re-infection must be exodemic as the rusts cannot survive the hot Egyptian summer in the absence of their host. However, in order to reach the Nile Valley, spores must travel extensively either over vast areas of desert or across the Mediterranean. Such regular movement implies that the spores have a considerably greater resistance to environmental conditions than is usually assumed.

In general it appears that the stem rusts regularly travel over greater distances than the leaf rusts and that the yellow rusts tend to be only short distances travelers. However, as is illustrated by the Egyptian situation, yellow rusts can move over considerable distances under certain conditions.

Gradual Introduction

In addition to the regular seasonal migrations outlined above, new races of a disease may spread steadily over great distances. For example, a new virulent race of yellow rust, capable of attacking hitherto resistant varieties of wheat (e.g. Mexipak), appears to have migrated in a stepwise fashion from Turkey across to India over a three year period. Similarly, a new and virulent race of barley yellow rust, first recorded in Colombia in 1976, had spread to Ecuador in 1977, southern Peru by 1978, Bolivia by 1979, and Chile by late 1980. The distance from Colombia to Chile is ca. 4,500 km. Such a rapid spread of virulent strains creates particular problems for the wide-

spread use of genetically similar crop varieties.

The Mechanism of Wind Transport

The long distance movement of pathogenic spores by wind involves three distinct stages: uplift, transport, and deposition. First the spores must be lifted from their source and carried to heights exceeding 1000 metres. Thermal updrafts and a reasonable wind velocity are essential for this process. Once they have achieved these altitudes the spores are carried by the various moving air masses that comprise the atmosphere. For example, the low pressure system which periodically builds up over western Turkey and moves eastwards, eventually breaking up in central Asia and northern India, appears to be the primary cause of the spread of new yellow rust races detailed earlier. Finally, as the air masses break-up or as periodic downdrafts or rain storms occur, the spores are redeposited on the earth's surface. The movement of spores is thus dependent on a number of meteorological factors and may be closely correlated with patterns of air movements. This immediately suggests the use of satellite weather photography as a means of tracking long distance disease spread.

Rain sampling has indicated that precipitation is an effective and important means of depositing pathogenic spores. As rainfall also provides the free moisture essential for the infection process, it is considered to be especially important in the establishment of exodemic disease infections.

In many areas both exodemic and endemic disease infections occur; this fact tends to confuse and confound accurate recording of spore movements. Therefore, in tracking the spread of diseases, the contribution of each system must be clearly distinguished.

6. SURVEYING PLANT DISEASES

Disease surveying is basic to all effective disease control and research programs. Surveys are essential in the development of such programs in order to determine their emphasis and direction. They are equally important throughout research as a means of assessing the effectiveness of control measures.

6.1 Basic Survey Techniques

Organizing Surveys

Surveys may be made for either regulatory or non-regulatory purposes. Regulatory surveys usually aim to delimit known infestations and determine the spread of new ones (often for plant quarantine purposes), whereas non-regulatory surveys are primarily geared towards the assessment of actual disease levels (frequently for the planning of control programs). In organizing a survey it is essential to first identify its purpose. Definite objectives can then be established based upon this purpose. Once the objectives have been made clear the survey can be planned with regard to the known characteristics of the pathogen (its reproductive rate, virulence, transport, etc.), the host (its stage of maturity, defence mechanisms, nutritional status, etc.), and the environment (both physical and chemical).

There are two basic systems of survey. These involve the use of either mobile units (observers travelling between large numbers of sites) or static units (e.g. trap nurseries-which may have a wide geographic distribution). Each system has its advantages and disadvantages and a choice of system must be made in order to minimize the disadvantages for a given set of objectives. It is usual to achieve this by adopting a combination of the two systems.

It should be noted that, in general, the broader the objectives of the survey the more difficult it will be to carry out and the less reliable will be the data collected. This underlines the need for firm and well-defined survey objectives.

Sampling

Whatever system of surveying is adopted it is physically impossible, except in a few, specific instances, to survey every single unit (plant-part, plant, field, area, country, etc.) in a given population. Systems by which the true disease levels can be estimated with as much accuracy and from as few observations as possible are thus necessary. Such systems involve sampling; taking samples from amongst a population and using them to estimate its true mean level. The sampling procedures normally used include:

- Random sampling (e.g., appraising fields at every 10th kilometer as indicated by the car speedometer)
- Area sampling (e.g., examining all fields in selected areas)
- Stratified sampling (e.g., sampling ten wheat fields for every field of barley if the wheat area is 10 times that of barley)
- Purposive sampling (e.g., appraising only the fields of growers producing certified seed).

Although the other methods may be used for rather specific cases, random sampling is the most widely used procedure. This is primarily because plant diseases are rarely distributed uniformly throughout a unit of crop (field, area, country, region) and thus any structuring of the sampling would tend to result in inaccurate estimations. However, random sampling must be carried out intelligently so that obviously atypical areas and areas known to be subject to biassed disease development are avoided. For example, samples should not be taken from the edges of crop areas as these are subject to considerable bias, commonly known as 'border effects'. Having deliniated obviously atypical areas, random sampling based upon the use of random number tables or other methods of randomization may then be undertaken.

In certain cases, especially when the emphasis is on disease discovery rather than measurement, non-random sampling is desirable. This is particularly applicable when surveying for new pathogenic races in a field sown to resistant varieties or in a nursery in which resistant varieties are incorporated.

When making estimates of disease occurence and/or severity it is usual to employ aids, such as quadrates, or procedures, such as meter row counts. Quadrates are square, rectangular, U-shaped or round structures, usually made from wire, which encompass a known area. They are dropped over growing plants in randomly selected locations and all the plants within the quadrate sampled. Measures of disease/per unit area may thus be derived. Meter row counts involve the sampling of measured lengths of crop rows, also at locations in a plot selected at random. A knowledge of the number of crop rows and row length in a given area will also enable disease/per unit area to be estimated by this procedure.

The sampling of individual plants within a selected area usually involves making leaf collections. These collections may be total (of all the leaves) or only partial (for example, of the flagleaf or the flagleaf and the first beneath it only).

Disease assessment sampling thus combines a number of different levels of sampling, sample locations within an area, sample plots within locations, and sample plants within plots. Although these levels do not apply to all sampling situations, they serve to illustrate the complexity of making large scale estimates.

The geography and varietal composition of an area to be sampled greatly affect the number of samples that must be taken to give an accurate estimate of the true disease situation. For example, there tends to be a rather low variation inherent in crops grown in areas of homogenous geography or where only a few different varieties are involved. Such areas will thus require considerably less sampling for accurate assessment than will areas of very varied geographical or varietal composition. The timing and frequency of surveys are also major factors governing the intensity and accuracy of sampling required and achieved. Single recordings may suffice if made at the time of maximum disease expression but, as this is difficult to judge, a number of observations over a period of time may result in greater accuracy.

In general, the cost of sampling increases with the accuracy and reliability of data required. The most economic sample will thus be the smallest one which can give the required level of accuracy and reliability.

6.2 Principles of Disease Assessment

The interaction between plant host, pathogen and environment is visibly expressed in characteristic symptoms and in the severity and prevalence of such symptoms. Disease symptoms (termed infection types) may vary from non-existant (host immune) to a maximum expression of pathogen reproduction (host highly susceptible). Together with disease severity (the degree or number of infections on a given plant or plant part) and prevalence (a measure of the number of diseased plants or plant parts in a given area), disease symptoms may be used to quantify the level of plant/disease interactions. Prevalence is frequently used as the major criterion for forecasting epidemics, of particular importance in the correct and economic application of chemical control measures. Overall levels of disease are, however, normally measured by a combination of all three characteristics of disease expression.

A number of scales have been developed in order to quantify infection types, disease severity and prevalence. While these are basically descriptive and rely upon subjective observations, they have in most cases been transformed into coded scales for ease of use and time-saving.

Infection types for rust diseases are normally coded either in Roman numerals (i, 0, I, II, III and IV) or in Arabic numbers (0, 1, 2, 3, 4, 5, 6, 7, 8, 9). These scales, known as basic, may be expanded for more detailed evaluations. For example, the value 0 may be expanded into 00, 0 ⁻ and 0+.

In contrast, severity and prevalence are normally recorded in percentages (0 to 100%). A distinction is made between observed and actual percentage of the leaf surface affected by disease (e.g. a visual rating of 20% may in fact represent an actual infection of 7.4%). Such a difference depends upon the subdivision of the particular percentage scale and the infection levels to which it refers. Percentage scales may also be transformed into coded linear scales for ease of recording. An example of this is the logarithmic international yellow rust scale (0.001, 0.01, 0.1, 1, 5, 10, 25, 50, 75 and 100%), where the prime importance is attached to the lower values. This is often written as a 1-10 linear scale.

When assessing any plant material for disease it is essential that the growth stage of the plant at which the measurements are made be recorded (using scales such as those illustrated in Figure 1.4). This will enable meaningful comparisons to be made with other varieties and between locations and years.

6.3 Recording Wheat and Barley Rust Diseases

The intensity of rust diseases on wheat and barley plants is commonly measured by infection type and severity. Numerous, highly specific scales have been developed as aids to rust assessment. The most generally useful of these methods are outlined below.

Greenhouse studies

The uredial stage is commonly used for rust assessment in the greenhouse. Infection types for use in the identification of resistance and susceptibility to *Puccinia graminis tritici* in wheat seedlings were first described by Stakman and Levine. These have been suitably adapted to form the basis of assessments of other cereal rusts. Infection types applicable to stem rust in wheat are given in Table 4.3.

Varieties are considered resistant when the rust is unable to grow and sporulate extensively. As is indicated in types 1 and 2 in the table, the pustules will be small and may be surrounded by discoloured or dead host tissue. The area actually killed always remains small so that the actual damage to the plant is slight.

In contrast, susceptible varieties (producing infection types 3 and 4) allow extensive fungal growth and sporulation. Necrotic areas (areas of dead tissue) are seldom produced as these tend to effectively limit fungal growth by cutting off the source of nutrients. In general the production of necrotic flecks is characteristic of some level of resistance (usually known as hypersensitivity). A pictoral scale for determining infection types of *Puccinia striiformis* is given in Figure 6.1.

Recording in the Field

Studies of seedlings under greenhouse conditions are relatively simple, as the environment can be controlled to favor maximum disease expression and the leaf area varies little between individuals. The measurement of rusts in the field is, however, complicated by variations in the environment (which affect disease expression) and by variations in the leaf area between individual plants. Diagrammatic scales are thus essential aids to field assessments of rust intensities.

Perhaps the most widely used scales stem from the original concept of N.A. Cobb in 1892. He published a scale representing five degrees of rust severity—1, 5, 10, and 50 per cent of the leaf area actually occupied by rust pustules. In 1917 a modification of this original scale was adopted by the

U.S. Department of Agriculture. This modified Cobb's scale is now used widely as the basis for the assessment of cereal rust intensities throughout the world. It classifies rusted plants into six categories and takes an actual 37o/o of the leaf area covered by pustules to represent 100o/o infection. This assumption is based upon the fact that mycelial development is always more extensive than that of pustules and that at this level of sporulation the development and destructiveness of the underlying mycelium is almost at its maximum. The remaining percentage classes are also divided in this way and a new diagram (representing 65o/o infection) added. Although this scale has proved of inestimable value to rust investigators, it has several inadequacies. To overcome these inadequacies, further modifications were proposed by Peterson et al (1948). In order to take into account the different sizes of pustules and their distribution, this scale provides four series of diagrams (each series containing twelve actual diagrams) covering a wide range of pustule size/ distribution combinations (Figure 6.2). Such a scale enables considerably greater objectivity and accuracy to be achieved in assessment. A scale for field use in recording wheat yellow rust infections is illustrated in Figure 6.3.

Detailed outlines for recording stem, leaf and crown rust intensities in cereals, based upon severity (percentage of rust infection on the plants) and response (type of disease reaction), have been developed by Loegering (1959).

Severity is recorded as a pecentage, according to the modified Cobb's scale, As this relies upon observation it cannot be absolutely accurate. Thus it is common to use the following intervals: Trace, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent infection.

Response refers to the infection type and has been classified into the following scale:

0 —No visible infection

R —Resistant; necrotic areas with or without small pustules

MR —Moderately Resistant; small pustules surrounded by necrotic areas

MS —Moderately Susceptible; medium-sized pustules; no necrosis, but some clorosis possible

S —Susceptible; large pustules, no necrosis or chlorosis

X —Intermediate; pustules of variable size; some necrosis and/or chlorosis.

Severity and Response readings are usually combined together. For example:

tR = Trace severity of a resistant type infection

5MR= 5o/o severity of a moderately resistant type

60S = 60o/o severity of a susceptible type

It appears that there may occasionally be an obvious variability in disease reaction between plants within a line. This may occur in several ways:

- A clear-cut separation of plants into classes (5R, 40S)
- A range of reactions without clear separation (15R-5S)
- A range of reactions on each plant

The first two reactions may result from either segregation or a mixture of seed, whereas the third reaction is probably due to either a race mixture or an X reaction of the variety.

A further practical method of disease assessment is provided by a manual prepared by Clive James (1971). All the keys in this manual, which covers a wide range of plant diseases, are based upon percentage scales. Only a few degrees of infection (representing the actual area covered) are given, and interpolations should be made between these levels in recording.

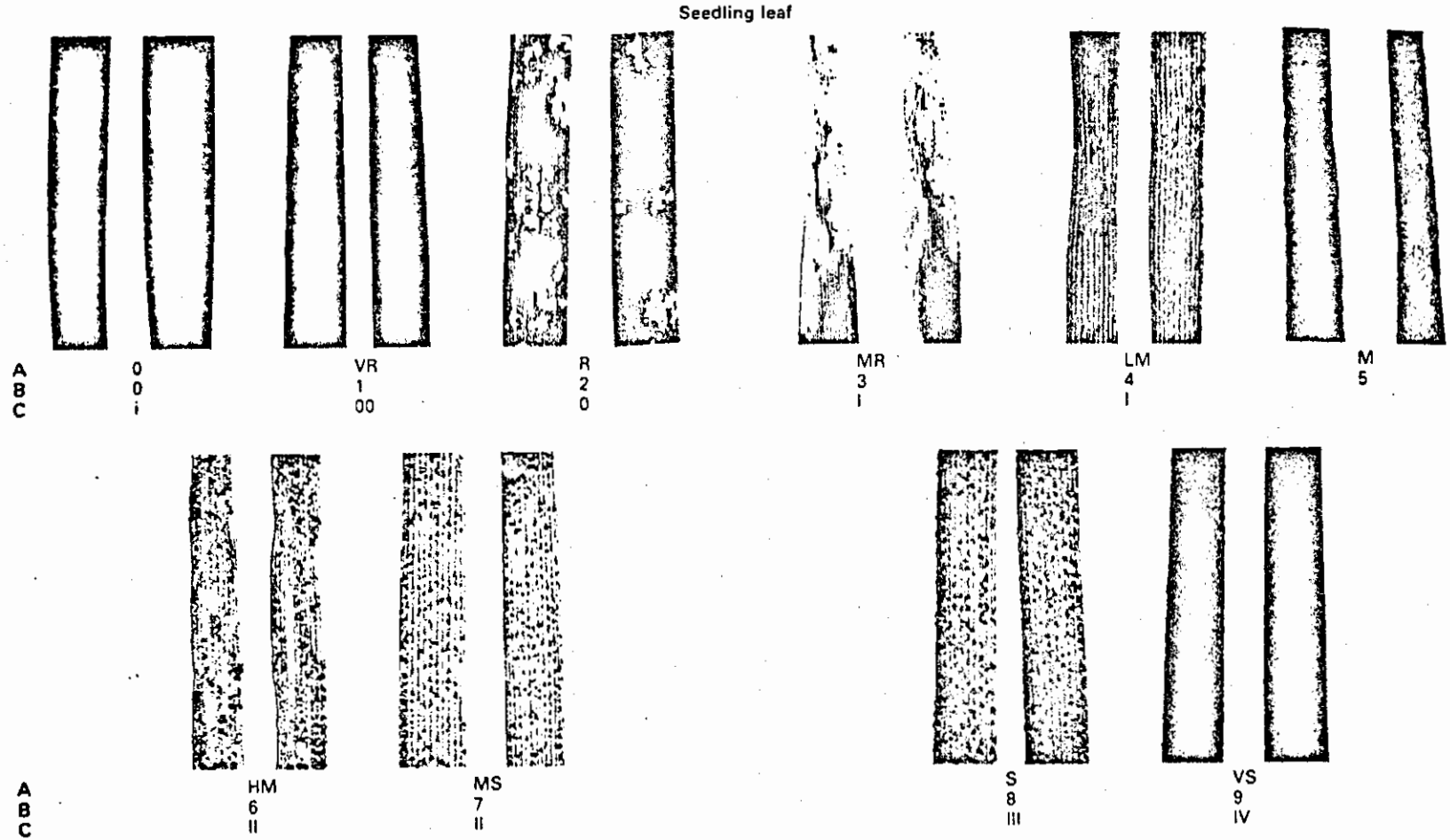
6.4 Recording Other Cereal Diseases

A number of pictoral/diagramatic scales have been developed for scoring both foliar and ear diseases of cereals. Some of these are illustrated in Figures 6.4, 6.5 and 6.6.

Other scales, which are more detailed, have also been developed to facilitate the scoring of a number of foliar diseases. One particularly useful aid is the scale developed by Saari and Prescott for recording infections of powdery mildew (*Erysiphe graminis*), *Helminthosporium* and *Alternaria* blights and *Septoria* leaf blotch. The basic scale, illustrated in Table 6.1 and Figure 6.6, is applied to the whole plant and hinges on the value of 5, which has been defined as the mid-point of the plant. A scale specific to *Septoria* is further illustrated in Figure 6.7 Table 6.2.

Figure 6.1

Pictorial scale for infection types of Puccinia striiformis Westend.
(A = code symbol, B = index value, C = Gassner and Straib code)



Composed by R.W. Stubbs and H. Vecht

Printed by Pudoc Centrale Offsetdrukkerij, Wageningen, The Netherlands.

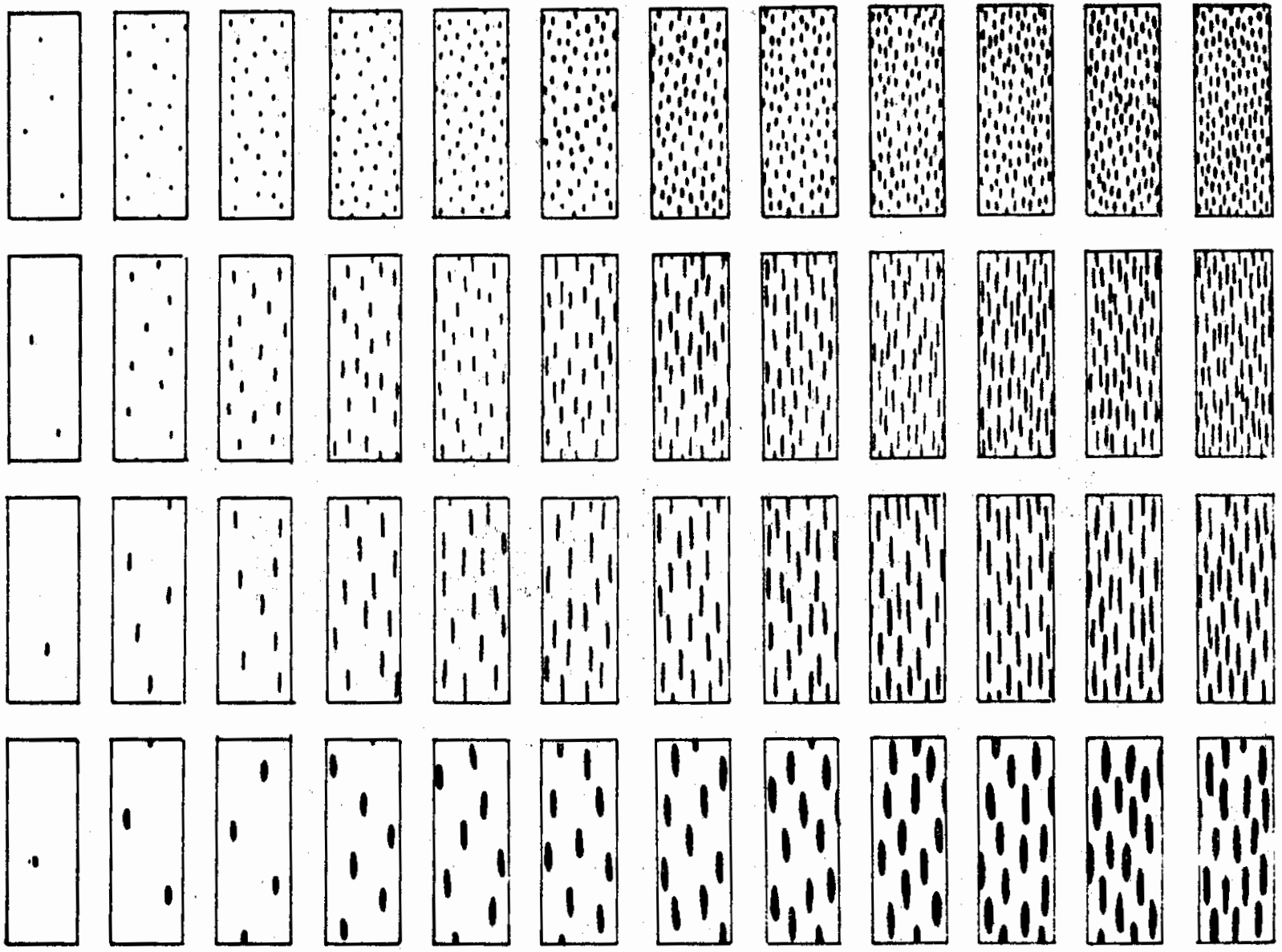
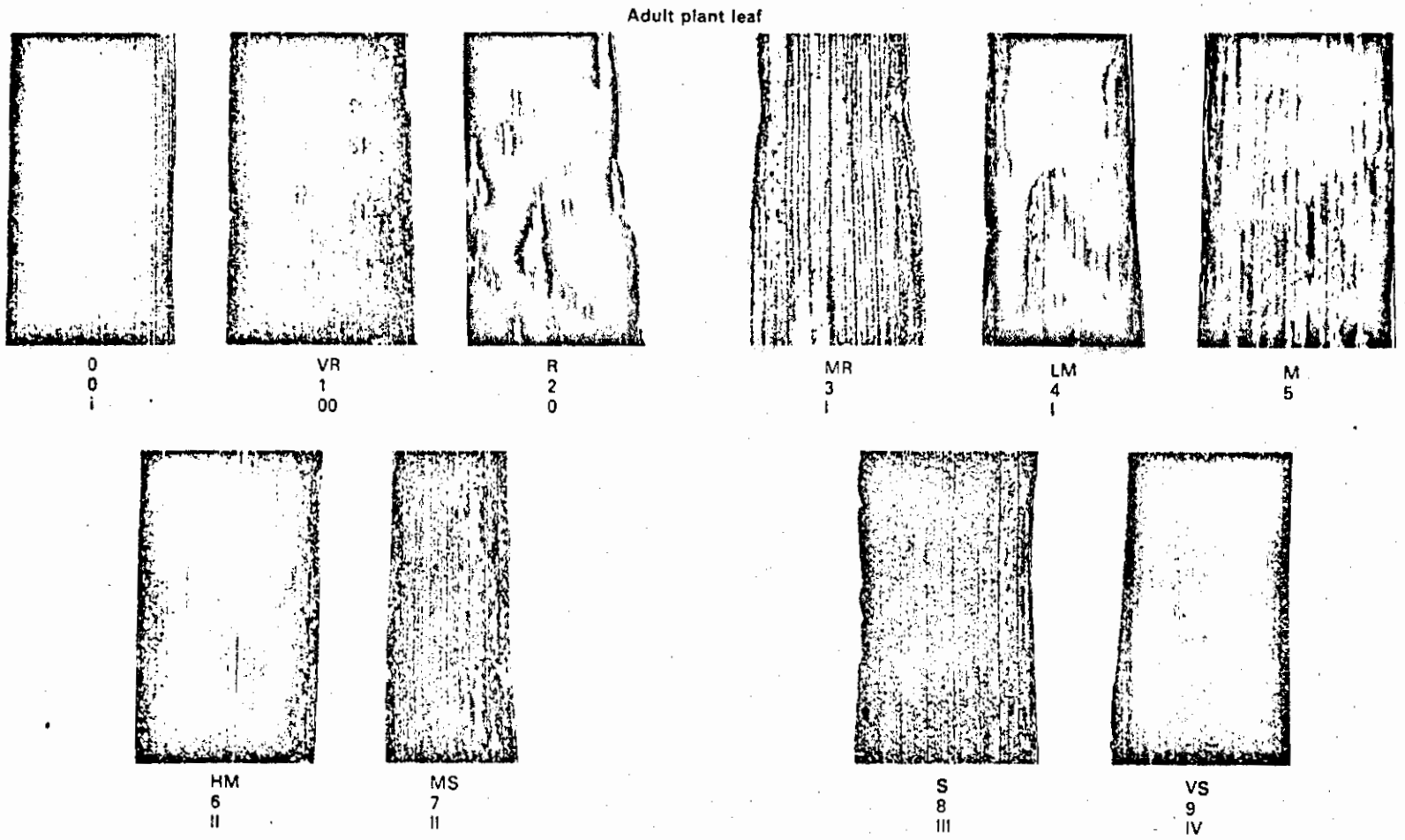


Fig 6.2 Diagrams to illustrate degrees of rust severity when the uredia are of different sizes (after Peterson, Campbell, and Hannah, 1948) A is the actual percentage of the surface covered by lesions, and B is the visual percentage

Figure 6.3.

Pictorial scale for infection types of Puccinia striiformis Westend.
(A = code symbol, B = index value, C = Gassner and Straib code)



Composed by R.W. Stubbs and H. Vech.

Printed by Pudoc Centrale Offsetdrukkerij, Wageningen, The Netherlands.

Fig. 6.4 : Diagrammatic Scales for Assessing the Intensity of Various Cereal Diseases

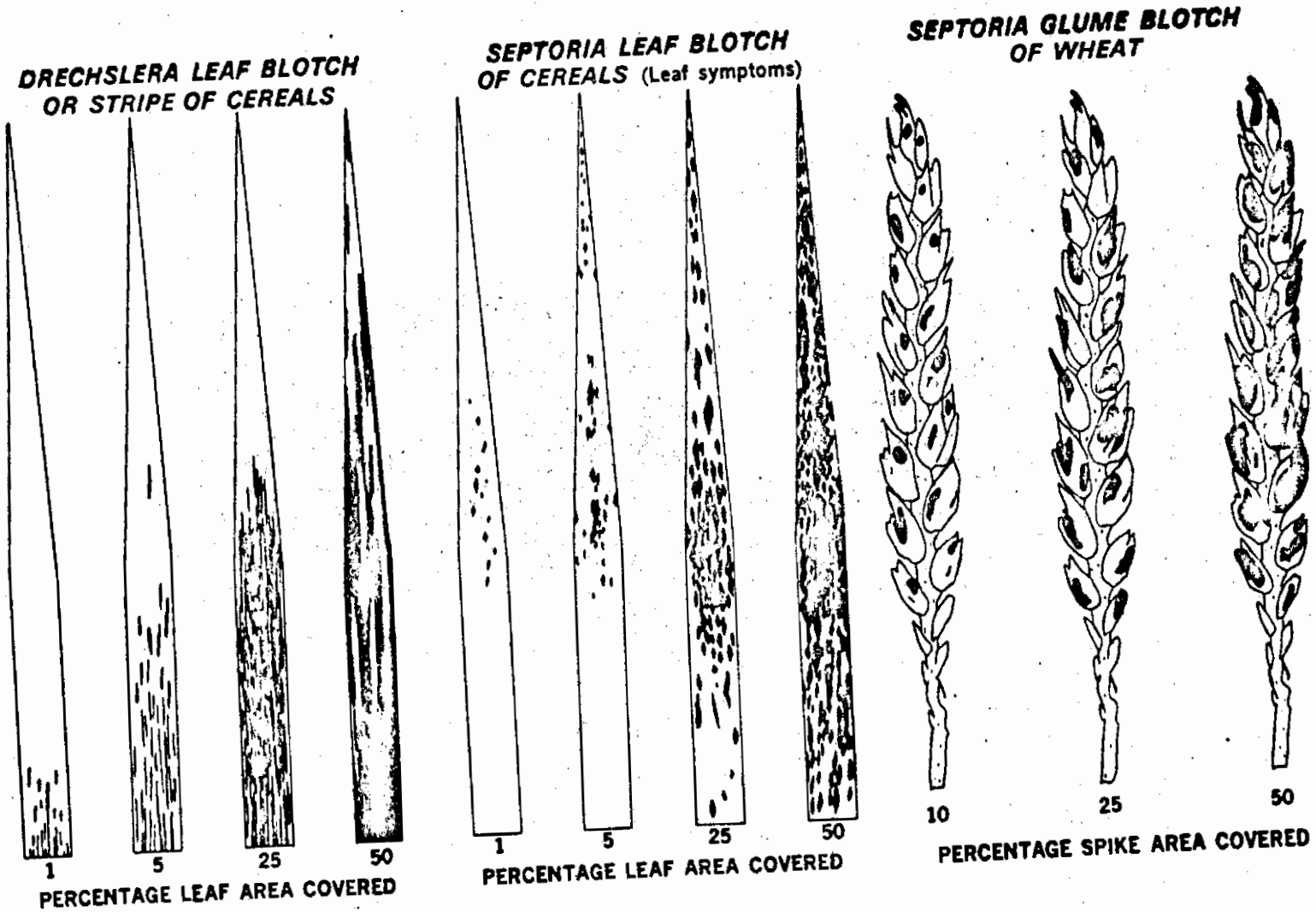


Fig. 6.5 : Diagrammatic Scales for Assessing the Intensity of Various Cereal Diseases

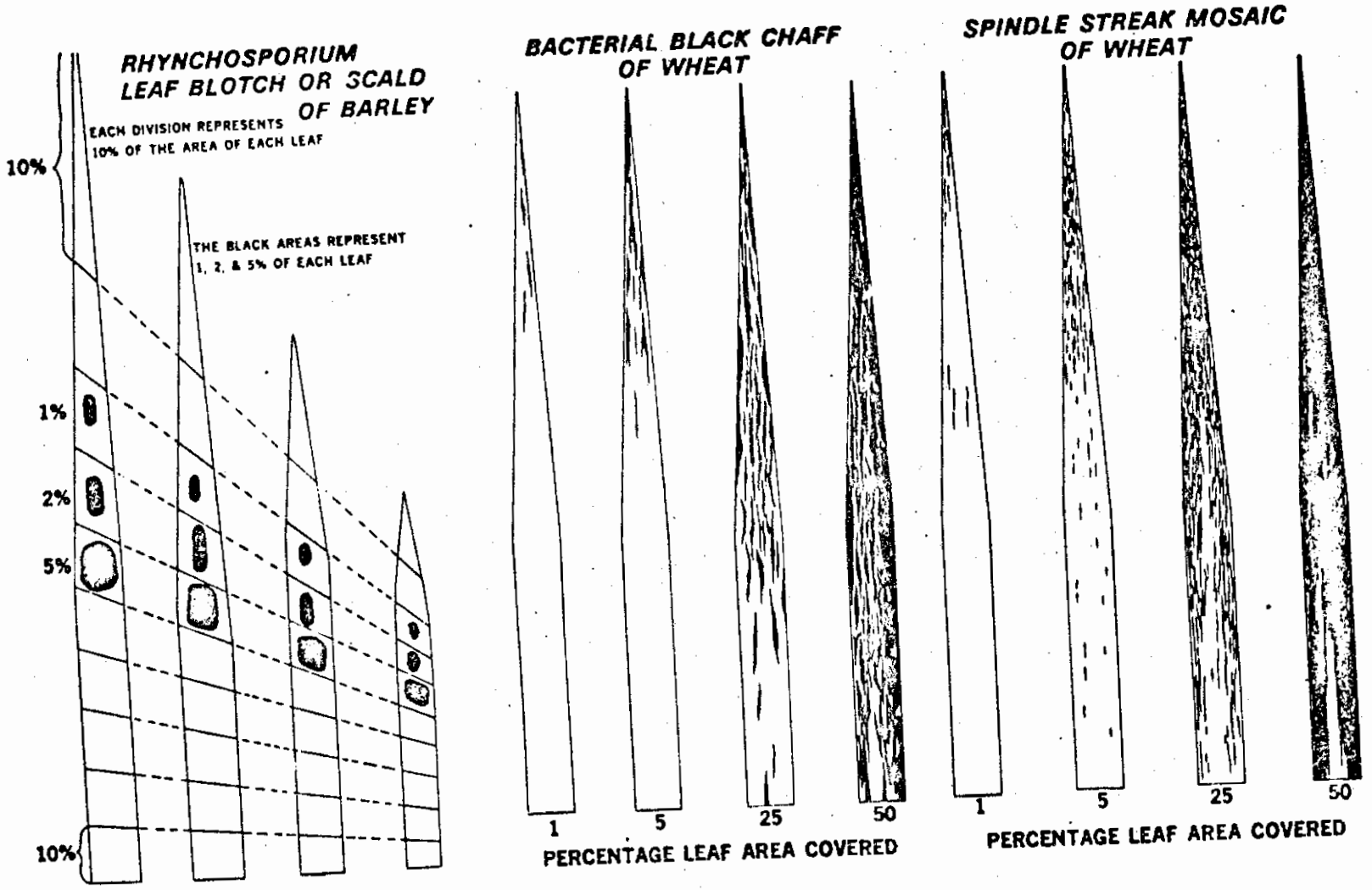


Fig. 6.6 : Scale for Appraising the Intensity of Foliar Diseases in Wheat and Barley

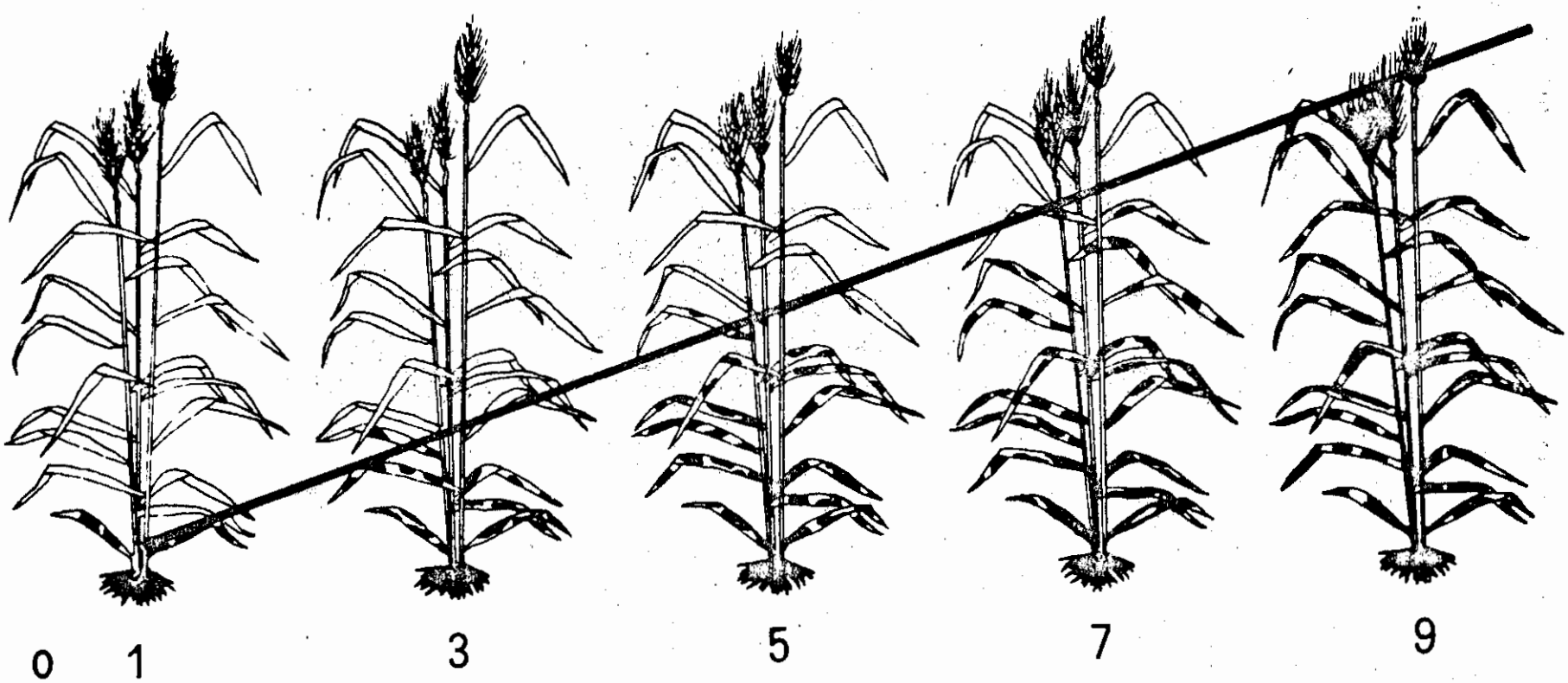


Fig 6.7 : Diagrammatic Scale for determining the Intensity of Septoria Leaf Spot Infections on Wheat and Barley (K . Harrower)

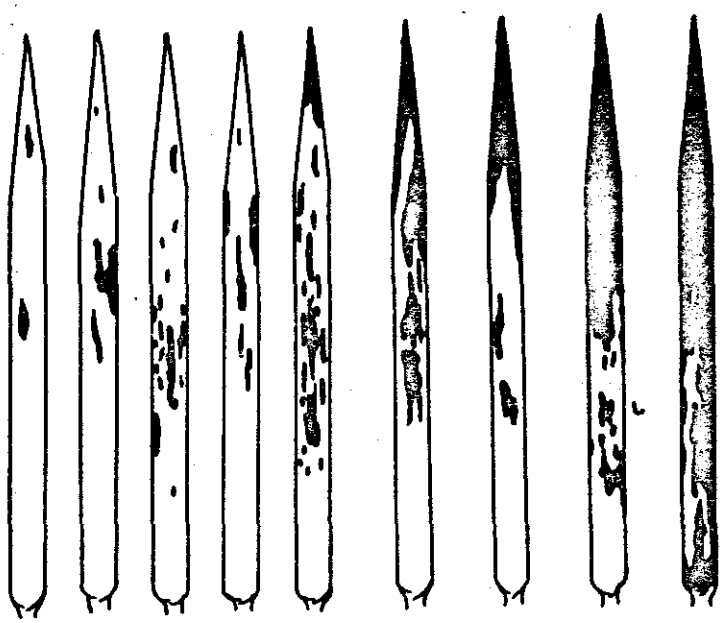


Table 6.1. Foliar Disease Scoring Scale (0-9) for Wheat and Barley

0= Free from infection.

0E= Free from infection, but probably represents an escape.

1= Resistant: A few isolated lesions on only the lowest leaves.

2= Resistant: Scattered lesions on the second set of leaves with first leaves lightly infected.

3= Resistant: Light infection of lower third of plant, lowermost leaves infected at moderate to severe levels.

4= Moderately Resistant: Moderate infection of lower leaves with scattered to light infection extending to the leaf immediately below the mid-point of the plant.

5= Moderately Susceptible: Severe infection of lower leaves. Moderate to light infections extending only to the mid point of the plant.

6= Moderately Susceptible: Severe infection on lower third of plant, moderate degree on middle leaves and scattered lesions beyond the mid point of the plant.

7= Susceptible: Lesions severe on lower and middle leaves with infections extending to the leaf below the flag leaf, or with trace infections on the flag leaf.

8= Susceptible: Lesions severe on lower and middle leaves. Moderate to severe infection of upper third of plant. Flag leaf infected in amounts more than a trace.

9= Highly Susceptible: Severe infection an all leaves; the spike may also be infected to some degree. (Spike infections are scored as a modified scale of the percentage of the total area covered; the percentage figure follows the numerical leaf infection score and is separated by a "I".)

N= Used to indicate no scoring possible due to necrosis as a result of other disease factors.

Table 6.2. The Rosielle Scale for Determining the Infection Types of Septoria Leaf Spot on Wheat and Barley

0=Immune: No pycnidial formation, no symptoms or occasional hypersensitive fleck.

1=Highly Resistant: No or only occasional isolated pycnidium formed, particularly in older leaf tissue; hypersensitive flecking in younger leaf tissue.

2=Resistant: Very light pycnidial formation; some coalescement of lesions mainly towards the leaf tip and in older leaf tissue.

3=Intermediate: Light pycnidial formation; coalescement of lesions normally evident towards the leaf tip and elsewhere on the leaf blade.

4=Susceptible: Moderate pycnidial formation, lesions much coalesced.

5=Very Susceptible: Large abundant pycnidia, lesions extensively coalesced.

7. PRINCIPLES OF BREEDING FOR DISEASE RESISTANT CEREALS

Plant pathogens constitute a major constraint to cereal production in almost every part of the world. Agriculturalists have thus placed considerable emphasis upon the development of 'disease control mechanisms' in an attempt to minimize the serious crop losses that frequently result from diseases caused by these pathogens.

Of the three main determinants of disease (pathogen, host and environment), only the characteristics of the host and the environment can, to a certain extent, be altered. Disease control mechanisms can, therefore, be basically divided into two categories: (1) those which involve a modification of the environment, making it less suitable for the pathogen (i.e., the use of toxic chemicals, modified agronomic practicies, etc.), and (2) those that involve alterations to the genetic make-up of the plant, making it less suitable as a host (through plant breeding). Undoubtedly the best control for any particular disease will involve an integration of both these approaches, based upon an understanding of the host and the pathogen and their interaction.

Studies of the interaction between cereal rusts and their hosts indicate a very close relationship between the genetics of the pathogen and the host in the expression of disease. In this case then there would appear to be a considerable potential for minimizing disease losses through plant breeding. In recognition of this potential, the development of cereal varieties resistant to the various rust diseases constitutes perhaps the major focus of research into disease control. Comprehensive integrated control strategies may then be built from this background of strong resistance.

7.1 Types of Resistance

Both the pathogen and the host differ in their ability to cause and to withstand disease, respectively. Plants may respond to a given pathogen (one with a given disease-causing ability) in a number of ways:

1) **Susceptibility:** Infection results in rapid disease development and spread within the host tissue and appreciable yield reductions.

2) **Tolerance:** Of two plants with the same apparent levels of infection one survives to produce a considerably higher yield than the other.

3) **Resistance:** The pathogen is unable to colonize the host, or its growth and development is restricted so that damage is reduced.

4) **Immunity:** No observable signs of disease are apparent. Care should be taken that this is not taken to mean complete resistance when escape (inadequate exposure to the pathogen) is the real cause.

Of the favorable plant reactions detailed above, tolerance and resistance are considered to be the most desirable and achievable. Immunity, which may appear to be very desirable, is rarely encountered. In addition, it exerts a very strong selection pressure on the pathogenic population and thus often results in the very rapid appearance of new biotypes able to overcome the frequently rather specific resistance barriers.

Tolerance

Studies conducted on hybrid wheat families have indicated that under levels of infection of between 65 and 100% some families suffered a yield reduction of about 44.5%, whereas others only lost 9.5% of their yield. This example illustrates the potential importance of tolerance as a mechanism for minimizing disease losses. To date, however, only limited research has been carried out on the overall subject of tolerance, and the mechanisms conferring tolerance are not well understood. For this reason, varietal improvement efforts have tended to focus upon the development of resistant cultivars. Without implying that tolerance is, or will prove to be, of lesser overall importance, the discussions of this section will be predominantly concerned with disease resistance.

One of the major shortcomings of tolerance as a mechanism for minimizing disease losses is the fact that tolerant cultivars are still able to produce large amounts of disease inoculum. This may create considerable problems of disease spread in other varieties grown in the same location and should be seen as being of particular significance.

Resistance

Plant pathologists commonly divide resistance into two main categories: Vertical and Horizontal or specific and non-specific.

Vertical resistance, also known as perpendicular, racial or specific resistance, is said to occur when a cultivar is resistant to some physiological races of the pathogen but susceptible to others. Vertical resistance thus reduces the amount of initial inoculum able to infect the host. However, since races or strains of the pathogen not limited by this resistance are still able to colonize the cultivar, vertical resistance does not serve to reduce either the infection or the spore production rates.

Horizontal resistance, on the other hand, involves a resistance which is equally effective against all the races of a pathogen. A multitude of terms, including field resistance and generalized resistance, have also been used to describe this type of resistance. By reducing the number of spores that cause lesions, increasing the time lag between infection and sporulation, and reducing the number of secondary spores generated by each infection (among other effects), horizontal resistance mechanisms serve to reduce the rates of pathogen infection and reproduction.

Resistance Mechanisms

During their long evolution, cereal plants have developed a multitude of mechanisms which reduce the numbers of pathogens able to penetrate and infect their tissues. Resistance to penetration is usually accomplished by features of plant structure, such as a thick epidermis, narrow stomatal openings, and the presence of specialized protective layers. These are termed mechanical or passive resistance mechanisms and, together with elements of functional resistance (represented, for example, by the timing of stomatal closure), they constitute broad-based, rather horizontal types of resistance. In general, however, such passive defense mechanisms develop only as plants grow and mature. Thus, at an early stage of crop development, most varieties possessing this type of resistance tend to be more susceptible to infection—a phenomenon which has given rise to the designation of 'mature plant resistance' as a specific resistance form.

In addition to these passive mechanisms, plants possess a number of active resistance mechanisms which are initiated only in response to the presence of certain pathogenic races. These mechanisms vary widely and are often highly specific to particular races; therefore they usually (but not always) confer a vertical-type resistance.

7.2 Modes of Inheritance

Resistance to plant pathogens may be conferred by a number of different genes or gene combinations. Genetic studies have resulted in the inheritance of resistance being classed into two main groups: Monogenic—or resistance controlled by the inheritance of a single gene; and Polygenic—or resistance controlled by the inheritance of more than one gene.

If disease resistance is controlled by a single gene, it usually has a substantial effect and can be studied and designated with relative simplicity. However, the inheritance of polygenes is normally more vague, and it is often impossible to isolate the effects of particular genes or estimate the number involved. This arises from the fact that polygenes tend to be additive in character, producing an increasing level of resistance with increasing number. Plants possessing polygenic resistance thus do not form discrete classes in segregating populations, but rather demonstrate a continuous range of variation.

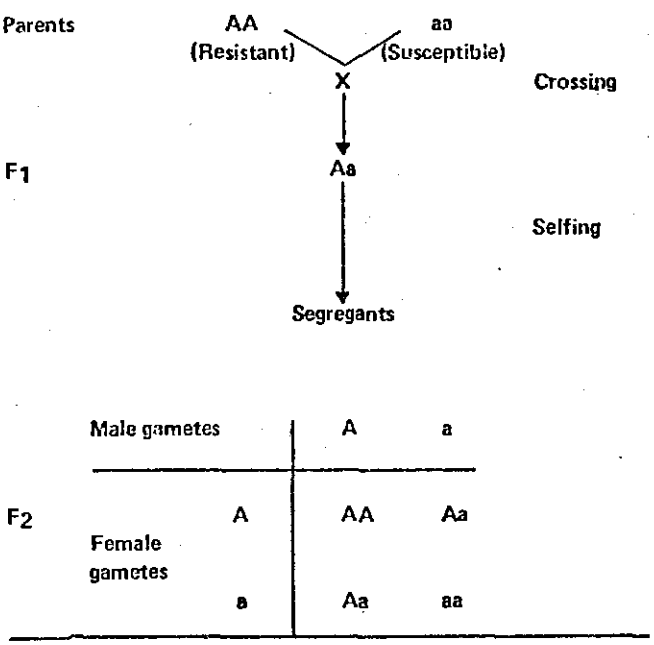
As instances are known in which resistance is conferred by two or three identifiably separate genes, multi-gene resistance might, perhaps, be better classified into: **Oligogenic**—resistance controlled by few genes; and **Polygenic**—resistance determined by many. Oligogenic resistance is often termed **major-gene** resistance, whereas polygenic resistance is referred to as **minor gene** resistance. This terminology, while in common usage, may be very misleading as not all oligogenes are major genes, in the sense of having a large effect; likewise a single identifiable gene, inherited in a Mendelian fashion, may in certain instances confer only slight disease resistance.

Vertical resistance is generally (but not always) conferred by major-genes of a large effect, and as a result tends to be relatively easy to select and thus to breed for. In contrast, horizontal resistance, normally thought to involve primarily minor-genes, is generally more difficult to identify.

The majority of crop improvement efforts have thus tended to focus on the development of varieties with a vertical-type resistance. While such a resistance is often very complete, it places an appreciable selection pressure upon pathogen populations, and thus, pathogenic races capable of overcoming the rather specific resistance barriers usually evolve rapidly and become dominant in these populations. This situation is particularly well-documented in the case of the cereal rusts and necessitates the continual development of varieties with new resistance genes or gene combinations in order to keep ahead of the appearance of newly virulent pathogenic races. Major-gene resistance, however, remains of considerable importance in most crop improvement programs, and plant breeders are continually on the look out for new major-gene sources.

While polygenic resistance is generally much less dramatic in its effect than major-gene resistance, it tends to confer a fairly broad-based, horizontal type of resistance. Horizontal resistance places considerably less selection pressure on pathogen populations, especially as it is rarely complete. Varieties with this type of resistance cause much less disruption in pathogen populations and therefore tend to possess an increased stability. An increasing emphasis is currently being placed upon the development of methods for identifying useful levels of polygenic resistance.

A Typical Scheme Illustrating Monogenic Inheritance



Assuming that resistance is inherited as a dominant character, all the F_1 individuals, although heterozygous, will appear resistant. However, with segregation occurring in the F_2 generation, three plants out of every four will be resistant:

1 AA Resistant (Homozygous)

2 Aa Resistant (Heterozygous)

1 aa Susceptible (Homozygous)

If, however, resistance is inherited as a recessive character, all the F_1 individuals will be susceptible and only one plant (AA) out of every four F_2 segregants will exhibit resistance.

Male Gametes	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

Female Gametes

Assuming the gene A confers resistance to one disease and is dominant and gene B confers resistance to a second disease, also being inherited as a dominant character, all the F_1 individuals will be resistant to both diseases. However, upon segregation in the F_2 generation the following phenotypic ratio will be observed:

1 AABB
2 AABb
2 AaBB
4 AABb — Resistant to both diseases (9)

1 AAbb
2 Aabb — Resistant to first disease only (3)

1 aaBB
2 aaBB — Resistant to second disease only (3)

Resistant to first or second diseases or both (15)

1 aabb — Susceptible to both diseases (1)

A similar situation will hold if the genes A and B confer resistance to two races of the same disease.

As increasing numbers of genes are involved the number of possible genotypes increases and so too does the phenotypic ratio (the ratio between the number of resistant plants and the number of susceptible ones). For example one gene gives a ratio of 3:1, two genes 15:1, three genes 63:1, etc.

Gene/Resistance Interactions

If there are four different resistance genes (A, B, C and D) and six prevalent pathogenic strains (1, 2, 3, 4, 5 and 6) a number of possible gene combinations may result:

	Genes for resistance	Strains of Pathogen 1	2	3	4	5	6	Outcome
1)	A	S	R	S	R	S	R	Gene C confers resistance to all strains of the pathogen
	B	R	R	S	S	R	R	
	C	R	R	R	R	R	R	
	D	S	S	S	R	R	R	
	Total	R	R	R	R	R	R	
2)	A	S	R	S	R	S	R	Genes A and D combined confer resistance to all strains of the pathogen
	B	R	S	S	S	S	R	
	C	R	S	R	R	S	R	
	D	R	S	R	S	R	S	
	Total	R	R	R	R	R	R	
3)	A	S	R	S	R	S	R	No single gene or gene combination gives resistance to all pathogenic strains
	B	R	R	S	R	R	S	
	C	R	S	S	S	R	S	
	D	S	S	S	R	S	S	
	Total	R	R	S	R	R	R	

7.3 Resistance Breeding Strategies

The majority of disease resistance breeding programs are based upon a simple procedure: the identification of a source of resistance followed by the incorporation of that source into a genetic background which is suitably adapted and high yielding. This procedure may be accomplished by breeding strategies involving either single plants (i.e., pedigree breeding, backcross breeding) or populations of plants (i.e., mass selection, bulk population breeding).

As the cereals in general and wheat in particular are predominantly self-pollinated crops, single-plant breeding strategies are almost exclusively used. These involve the exposure of a large number of individual lines to pathogenic races in order to identify specific individuals with resistance. Suitably resistant sources are then crossed with plants exhibiting other desirable characteristics and the segregating generations tested for resistance, all the susceptible individuals being discarded. In this way a large number of sources of resistance may be

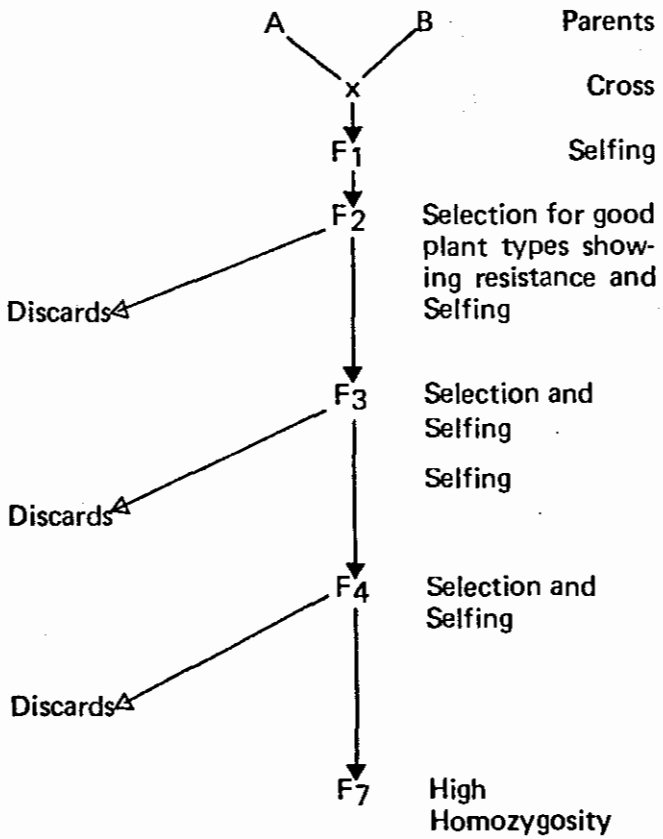
combined into specific individuals and the individuals stabilized by repeated cycles of selfing and selection for the desired characters (leading to a rapid increase in homozygosity-uniformity).

Single-plant screening for disease resistance may be carried out at the seedling stage in a greenhouse, but the complex and involved testing procedures necessary when large volumes of material are being screened against numerous pathogenic races necessitate field-screening.

Pedigree Breeding Strategies

Pedigree breeding involves simple single, double or triple crosses followed by repeated selection cycles to eliminate undesirable individuals. The use of different rust races in successive testing will enable the selection of varieties with a rather wider resistance. A typical pedigree breeding scheme is illustrated below:

A Scheme for a Pedigree Breeding Program



As each cycle of selection and selfing progresses, the degree of homozygosity rapidly increases as illustrated by the following simple example:

Generation	Genotype	%Homozygosity
F_1	Aa	0
F_2	AA Aa Aa aa	50
F_3	4AA AA Aa Aa aa AA Aa Aa aa 4aa	75

If A is considered to be the resistant gene and is dominant, the percentage of resistant homozygous plants increases from 33% to 60% if all susceptible types (i.e. aa) are discarded.

Backcross Breeding Strategies

When it is desired to transfer a character, such as disease resistance, to an otherwise good genotype a backcross strategy is commonly used. This involves making the original cross, growing out the segregating F_2 population (by selfing in the F_1), and then crossing again all those segregants showing disease resistance with the good genotype. This cycle of selection and back-crossing may be carried out for several generations, after which the population is selected for a combination of the characters desired in the normal way. The mechanics of this breeding strategy will vary considerably, depending upon the character(s) involved.

Multi-line Varieties

Also known as composite or artificial varieties, multi-lines may be successfully used to produce a commercial 'variety' in cases where individual lines possess no genes or gene combinations conferring resistance to all the prevalent pathogenic races of a disease. Such 'varieties' are artificially produced from a combination of different lines, each with its own resistance genes. Thus, each individual is not itself resistant to the whole disease spectrum. How-

ever, when attacked by a given pathogenic race, only those lines without specific resistance to that race will be affected; the others will remain undamaged. In this way, although the variety as a whole is invariably infected, the pathogen is unable to infect more than a few individuals and the overall damage is slight.

The development of effective multi-line varieties demands considerable skill and a very good knowledge of the prevalent pathogenic races. The components of such varieties require continual assessment and replacement to keep pace with the changing pathogenic spectrum.

It must be emphasized that in practice breeding programs usually involve a multiplicity of objectives, a large volume of plant material, and the consideration of numerous characters. The discussions of this section have thus been considerably over-simplified in order to underline the basic features of breeding strategy.

However, these considerations have served to illustrate the critical importance of selection throughout all breeding programs. In selecting desirable types from a population, plant breeders must rely upon observations of plant growth in the field (a product of the interaction between plant genotype and environment) as indicators of the actual plant genetic make-up. Differences in environmental conditions affect the expression of genetic characters considerably. For example, if no rust pathogens are present then all plants will appear resistant (e.g., they will not be affected by rust). Equally, if the concentration of rust pathogens varies appreciably across a nursery, some varieties (not exposed to high concentrations) will appear resistant when in fact they are not. These simple examples highlight the importance of achieving uniform environmental conditions designed to favour maximum disease expression throughout nurseries from which selections are made. Only in this way will selections made on the basis of plant phenotype accurately reflect the actual characteristics of the plant, and only in this way will breeding programs be successful.

A strong pathology input in disease resistance breeding programs is therefore of tantamount importance.

8. PLANT PATHOLOGY IN CEREAL BREEDING

A good pathological input is fundamental to the whole aspect of disease loss minimization and the development and operation of research programs aimed at achieving this. Such an input can be conceived as falling into a number of distinct but closely interrelated areas.

Extensive Disease Survey

The emphasis and direction of disease control research programs relies, in the first instance, upon accurate assessment of the importance and occurrence of specific pathogens in specific locations. In addition, plotting of the long distance movement of specific diseases provides an effective 'early warning' system, so that susceptible varieties may be exploited to their maximum without the danger of falling victim to disease. Surveys are also vital in order to identify new virulent races or biotypes as soon as they arise and to keep track of the fluctuations in pathogen populations that occur from year to year and place to place.

Without the continuous input of survey data disease breeding programs would fast become obsolete. Extensive survey systems, such as the Regional Disease Trap Nursery, are of great importance in providing up-to-date information on the state of pathogen populations over a wide area, and thus in keeping breeding programs focussed on the real problems.

Intensive Pathogenic Studies

The identification of physiological races and the factors affecting their interaction with cereal hosts is another important area of pathological studies. A developing understanding of host:pathogen relationships and specificities will enable strategies designed to minimize the effects of such parasitic relationships to be put on an increasingly more solid physiological basis. Such studies will thus make a significant contribution to the evolution of breeeding strategies in the future.

Nursery Management

Perhaps one of the most important roles of plant pathology in developing disease-resistant varieties involves ensuring adequate and uniform disease development in screening nurseries. In many cases, with the careful selection of site and control of environmental conditions, it may prove sufficient simply to rely upon natural infection. However, where environmental conditions do not favor disease development, the creation of artificial epiphytotics will be necessary. In this regard, the various aspects of inoculum collection, mutiplication, storage and inoculation assume particular importance alongside the direct considerations of nursery design, location and management.

Disease Assessment

Closely allied to the aspects of nursery management outlined above is the whole sphere of disease assessment. As outlined earlier, accurate assessment (based on adequate and uniform nursery conditions) is vital to all breeding efforts. Disease assessments are very subjective, relying exclusively upon field observations and the skill of assessors in making these observations. Pathology thus has an important role to play in the development of more objective, accurate and rapid scoring and screening techniques.

8.1 Establishing Disease Nurseries

There is considerable scope for flexibility in the design and planning of disease nurseries. Plot size can be varied, depending upon the availability of seed and sowing equipment and plots of one or two rows, one or two meters in length, are adequate. It is essential, however, that agronomic practices (fertilizer applications, weeding, irrigation, etc.) which ensure optimum plant growth and development are followed. In addition, planting date and plant density should be regulated to favor maximum disease development. These conditions are necessary to ensure that (1) plants express their genetic resistance potential to the maximum (this cannot occur when plant growth is suboptimal), and (2) the selection pressure applied is also maximum (selections made under a very light selection pressure tend to be highly unreliable).

In general, disease nurseries should include susceptible check cultivars, often as frequently as every twentieth row. Such check rows act as 'spreaders' for the build-up and dispersal of inoculum within the nursery and as yardsticks against which a relative scoring of disease severity can be made in cases of low disease incidence. The use of locally-grown susceptible cultivars as checks is highly recommended since agronomic characteristics can be simultaneously compared using this standard.

However, it should be recognized that the inclusion of susceptible check cultivars has the disadvantage of causing interplot interference (i.e., the inoculum produced on the susceptible row will increase the amount of disease on adjacent entries being tested). The non-uniform disease conditions created by this interference may be of little consequence in testing for specific, major-gene resistance, but can cause severe problems in nurseries where the emphasis is placed upon the identification of moderate levels of resistance.

8.2 Techniques for Enhancing and Creating Epidemics

Techniques designed to ensure adequate and uniform development of disease epidemics so essential in screening nurseries are primarily based upon ensuring sufficient levels of pathogenic inoculum together with favourable environmental conditions. There are a number of methods, some simple and some complex, by which a rapid inoculum build-up may be achieved in the field. The choice of method depends, to a large extent, upon the characteristics of the individual pathogen involved, the prevailing environmental conditions, and the objectives of the particular nurseries.

Enhancing Natural Epidemics

The simplest disease screening method available is the exposure of plant material to natural infection. However, adequate and uniform infection levels can rarely be achieved in most locations and years. Thus some degree of enhancement is almost inevitable.

The first consideration in this respect is the location of nurseries. By locating screening nurseries in areas where the disease incidence is known to be consistantly high every year (e.g., Izmir in western Turkey, Njoro in the Kenyan highlands, the Yangtze river valley in China), high disease levels can regularly be obtained. In order to ensure that the material is exposed to the widest possible pathogenic population, it is necessary to establish a number of nurseries at such sites over as large a geographic area as possible. This consideration forms the rationale for the wide distribution of Disease Screening Nurseries throughout the region.

Within the nursery itself, naturally occurring disease almost invariably appears at a few isolated foci within the susceptible check rows . Tissue from these isolated initial infections may be used to spread the disease more widely and evenly within

the spreader rows. With the rust diseases this may be accomplished simply by shaking the infected plant parts over the healthy rows. Such a procedure should be repeated several times early in the season in order to be effective. It should also be accompanied by adequate watering (preferrably sprinkler irrigation if necessary) to keep the plant surfaces moist prior to inoculation.

Perhaps the major advantage of natural disease testing (especially if carried out at a number of different locations) is that the plant material is exposed to the full variability of the pathogenic population over a larger geographic area.

Creating Artificial Epidemics

Environmental conditions favourable for optimal disease development rarely occur every year even at the most suitable locations. Furthermore, there may be a great variation in the severity of individual diseases between locations and within one location in each year. Under these conditions of uncertainty, and especially when the season is drier than normal, the only way of ensuring adequate epidemic development is to create epidemics artificially.

This may be achieved through the use of inoculum collected from locations where disease occurs earlier in the season or inoculum preserved from infections of the previous season. Techniques for inoculum collection, storage, multiplication and inoculation will be considered in detail in later sections. Suffice it to say at this stage that it is essential to provide adequate moisture conditions in the nurseries and to inoculate several times early in the season.

Although artificial inoculation often results in good disease development if carried out correctly, there is a considerable danger inherent in this method in exposing the material to only a limited range of the prevalent pathogenic races.

8.3 Justifying the Creation of Artificial Disease Epidemics

The whole concept of artificially creating disease epidemics has been, and continues to be, subject to continuous and often unfairly harsh criticism from a large number of people. Such criticism is, in general, based upon an over-estimation of the dangers involved and little appreciation of the benefits that may accrue. It is thus worth considering both these aspects before embarking upon a study of the topic in greater depth.

Benefits of Epidemic Creation

It is generally agreed that one of the most effective ways of minimizing the losses resulting from infections of plant pathogens is to build into crop varieties some form of inherent resistance. This is known as "genetic insurance". This is especially true in developing countries, where factors related to rural incomes and infrastructure effectively prevent the use of the more costly chemical control measures .

Due to the immense capacity of some pathogens (e.g., the cereal rusts) to mutate and produce new virulent strains or biotypes, the average life-span of a disease-resistant variety (the time between introduction and the widespread appearance of pathogenic races capable of overcoming this resistance) is often short (for rust-resistant cereals about five years). Some varieties have, however, survived considerably longer, although this is not common. Indeed, many rust-resistant varieties have proven to be commercially useful for only one or two seasons, possibly a result of ineffective screening in the developmental stage. This problem means that breeding programs must continually be developing new resistant varieties, and varieties with an increasingly broader and more stable level of resistance.

From previous considerations we have seen that the only way to ensure effective screening with a high and broad selection pressure on a regular basis is through the use of artificially created epidemics. The importance of such practices, especially in developing countries where "genetic insurance" constitutes such a vital part of agricultural production improvement, thus becomes particularly clear.

Dangers of Epidemic Creation

The main danger of creating artificial epidemics and the basis of all the recurrent criticism is that inoculum produced in the nurseries will be dispersed into surrounding crops and lead to severe epidemics in the locality. This is considered by the critics to be of particular importance in testing for resistance against new virulent races, which are not, as yet, of widespread prevalence. For this reason, there is often appreciable reluctance to use new pathogenic races in screening programs. And yet the whole point of resistance screening is to identify sources of resistance to such races *before* they become widespread enough to consitute a major production threat.

In this context it is worth considering a number of past experiences in epidemic development. Studies have shown that the majority of rust inoculum produced in an inoculated crop remains within the boundary of that crop (90% of the inoculum never moves more than 100 meters from the inoculation site during the early stages of an epidemic). It is only when epidemic development nears its maximum that the urediospores are present in sufficient quantity to be effectively lifted from the crop for longer distance transport. On certain occasions neighboring crops have been found to be infected. However, since epidemic development only reaches the stage at which wide dispersal of inoculum occurs when the crops are reaching maturity, the damage is negligible and the likelihood of pathogenic build-up and further spread is extremely low.

Even given the exceedingly low risk of contamination from disease nurseries, what harm can come contamination actually do? Knowing how widely and rapidly new races of rust can spread, it will not be long before such races are well established in all but a few areas anyway. When these considerations are taken into account it appears that concern over the danger of the very low levels of contamination that could be caused by artificially created epidemics is much greater than is actually warranted.

When the benefits of such practices are analyzed alongside the dangers inherent in them, it becomes obvious that the criticisms voiced should in no way be allowed to prevent the widespread use of artificial epidemics in cereal breeding. The potential for serious crop losses and consequently widespread social and economic problems is extremely high if we are unable to produce effectively resistant varieties. This threat is much higher than the potential losses which may arise from contamination. It would thus be criminal to restrict the use of such practices on the basis of the dangers involved. It should be emphasized, however, that every care must be taken in disease screening work to prevent contamination and perhaps more importantly every care should be seen to be being taken.

8.4 Some Important Technical Considerations in Creating Epidemics

Virulence Spectrum

All pathogens are populations of many physiologically different races or biotypes. As already mentioned, when using artificially created epidemics as a basis for selection, the danger of exposing the plant material to only a very narrow range of the pathogen population is considerable. In ensuring

that the nursery is exposed to the broadest possible spectrum of races, collection and inoculation techniques deserve particular attention.

Collection Methods

Each host variety tends to favor the development of a single specific race of a disease. This fact is well illustrated by rust surveys which show that 85% of collections from a single variety represent only one race, only 10% represent two races and three races are only found in 5% of the samples. In addition the pathogenic population tends to vary between locations.

Thus inoculum collections should be made from the largest possible number of different host varieties (including commercial ones) and from the widest possible range of locations in a given area in an attempt to combine a large number of different races into one bulk population for inoculation purposes.

If greenhouse and laboratory facilities are available, the virulence spectrum of a population may be broadened further by the multiplication of races with a low natural frequency of occurrence. This will guard against the possibility of an inadequate screening resulting from these particular races being unable to build up to sufficiently high levels during the screening period.

Inoculation Procedures

The method by which a nursery is inoculated may also influence the virulence spectrum to which plant material is exposed.

If epidemics are created primarily by inoculating the spreader rows, the varietal composition of these rows can have a strong selective effect upon the race spectrum. Experiments, for example, have shown that if a mixture of rust races is inoculated onto a single variety, one race will come to dominate after only a few pathogen generations and many other races may be almost eliminated. For this reason it is essential that spreader rows be composed of a mixture of different varieties. This mixture should be reviewed regularly so that varieties of known importance in relation to certain virulent races are always included.

Perhaps a better way of ensuring that every opportunity is given for each race to find a compatible host is by inoculating the entire nursery with a large inoculum bulk using a sprayer or duster. This method exposes all the genotypes in a nursery to the same infection conditions and is thus likely to result in a more uniform selection pressure than may be achieved through only spreader row inoculations. It is, however, more time-consuming—appreciably so when large nurseries are concerned—and therefore often conflicts with the time considerations outlined below.

Timing of Inoculation

The early establishment of primary infections will considerably enhance the development of epidemics and thus the selection pressure exerted. Inoculations made early in the season increase the number of disease generations occurring prior to screening (provided, of course, that environmental conditions are favorable). When it is considered that one rust infection can produce between 50,000 and 250,000 new spores it is obvious that one extra generation will allow a considerable increase in disease severity.

It is also essential that inoculations coincide with environmental conditions favorable for infection. The first inoculation should thus be undertaken when environmental conditions first become favorable. Once the pathogen is well established, it will be able to generate its own far-superior, secondary inoculations. The cycle from infection to spore production is also affected by the environment. At constant optimum temperatures, new rust spores may be produced in one week. However, under field conditions, 10-14 days will be required under favorable conditions and the generation time may exceed three weeks if sub-optimum tempera-

tures prevail. If reactions are to be scored at the mid-dough stage of cereal development, the primary infections must be established at least five weeks in advance of this stage and preferably earlier. In the case of yellow rust, areas with rapidly increasing spring temperature conditions will require the establishment of infections even earlier than this. The question of when to start inoculating will be determined by the requirements of the fungus concerned, the weather conditions and the stage of plant development in relation to the time remaining for epidemic development. In some instances it may be desirable to establish initial infections prior to the first optimal weather conditions in order to maximize secondary disease spread. Such a practice will provide a continuous supply of inoculum for each favorable infection period and sufficient time for epidemics to develop to a level which will allow adequate and accurate screening.

Another important consideration is the number of times that a nursery should be inoculated. Many environmental factors affect the dispersal of inoculum and disease establishment. For example, winds may remove spores from the vicinity of the nursery, or sudden reductions in free moisture availability may cause the death of spores which have started to germinate. These and other factors diminish the chances of establishing good infections from one initial inoculation. For this reason at least five inoculations are usually considered to be essential for rust diseases.

Amount of Inoculum

The quantity of spores required to inoculate a given area is not fixed. In general, the mottou 'excess helps to guarantee success," is a good rule of thumb. To quantify this in broad terms, each hectare of wheat or barley contains approximately 2.5 million plants (of course the number of actual culms is considerably more as each plant has several). A good coverage of plant surfaces will require about 1,000 rust spores per plant. A gram of spores contains an estimated 500 million individual cells. Thus a simple calculation tells us that about 2,500 million spores will be needed for every hectare of nursery. However, this simple calculation ignores several important points: many spores fall on bare ground and thus never reach the plant; many spores may be non-viable; many spores are unable to germinate on the plant surfaces due to micro-environmental differences; of the spores that do germinate many are unable to find stomata through which to penetrate. Hence, the chances of a given viable spore actually penetrating the plant and proving compatible are also low. These considerations make it obvious that no firm recommendation can be made to cover every situation. Experience, however, suggests the use of about 5 gm of spores per hectare (diluted in talc or oil/water) as the minimum level, given reasonably favorable conditions and five inoculations.

8.5 Techniques for Rust Urediospore Collection

There are a number of ways by which spores of Yellow Rust, Leaf Rust and Stem Rust may be collected for use in artificial inoculation work. The most effective of these are outlined below.

Dried Leaf Collection

Rusted leaves are removed from plants, placed in glassine envelops, pressed and left to dry. No more than 6-8 leaves should be placed in each envelope so that drying is rapid and effective. Twenty-four hours will usually be sufficient for adequate drying at room temperature. This collection method is particularly useful in making field collections.

Leaf Tap Collection

This method involves tapping heavily rusted leaves while they are held over a suitable container. Urediospores collected in this way can then be stored in vials. Leaf tap collection is used almost exclusively for collecting spores from greenhouse raised plants.

Cyclone Collection

By using special cyclone collectors (different sizes are available) rust spores from both the greenhouse and the field may be collected and inoculated with the greatest of ease. Large collections of urediospores will require drying before storage.

When making collections by whatever method, it is essential that the material be adequately indexed and catalogued so that it is readily identifiable when required for inoculation.

8.6 Storing Rust Urediospores

As referred to earlier, the viability of rust spores is a very important factor determining the quantity of inoculum required for the adequate establishment of disease epidemics. Freshly collected urediospores generally have a high germination percentage. This decreases with time, regard less of environmental conditions. However, the rate of loss of viability varies appreciably with conditions; spores can remain viable for periods of up to one year given suitable environments.

It is essential to ensure optimal storage conditions in storing urediospores between seasons so that they may be used in the initiation of artificial epidemics. In this way the loss of viability may be kept to a minimum and the chances of effective artificial inoculation enhanced. Of the four main factors affecting urediospore viability —temperature, moisture, light and atmospheric oxygen—the first two are the most important. The maintenance of adequately low temperature and moisture levels are thus vital considerations in spore storage, especially as, being single-celled, the spores are extremely sensitive to environmental conditions.

Examples of the effect of temperature and moisture content on the length of viable storage of yellow and stem rust spores are given in Tables 8.1 and 8.2.

Storage Methods

Perhaps the simplest method of storing urediospores is to lower their moisture content by about 10o/o and maintain them at a temperature of between 2 and 4°C. This allows viability to be retained for between three and twelve months. The spores may be air dried for 24 to 36 hours to lower their moisture content. While this may be particularly successful in dry climates, a desiccator (with either calcium chloride or silica gel as the desiccant) may be required in more humid areas. Freshly collected urediospores will not dry adequately en-mass and so they should be spread thinly on a plate, sheet of aluminium foil, or petridish. It is also preferable that drying be carried out in the laboratory and away from direct sunlight. After drying, the spores should be placed in a sealed vial or bottle (dark containers appear to be best in many cases) and stored at the correct temperature and under dark conditions in a refrigerator. Care should be taken to prevent excessive drying as this tends to be detrimental to spore viability (Table 8.2).

If the equipment is available, a more effective method of storage involves the evacuation of the storage environment in order to remove atmospheric oxygen and thus lower spore respiration. Both partial and high vacuum storage systems are possible. Under a partial vaccuum system, the spores should be spread evenly and fairly thinly on a petridish which is then placed in a dessicator with facilities for evacuation and sealing. A small air pump is used to create the partial vaccum and the dessicator sealed and placed in a darkened refrigerator at 2-4°C. Under these conditions the period of viable storage can be doubled.

If a high vaccuum system is used the spores must be stored in narrow pyrex tubes (5 - 22 mm diameter). About five milligrams of inoculum can be stored in each container. The atmospheric pressure should be lowered to 1 mm of mercury

(Mcleod Gauge) or 0.1 ton (Edwards Speeclivar Gauge) and the tubes sealed using a gas burner. Care must be taken to avoid heat build-up in the glass tubing in areas close to where the spores are located. The spores will remain viable for several years under these conditions if stored at 2-4°C.

Dried or vaccum dehydrated spores will usually germinate poorly unless rehydrated before inoculation. It is thus normal practice to place spores in a moist chamber for between 12 and 24 hours prior to inoculation to enable adequate rehydration. The effects of rehydration are illustrated by the following example:

Type of Storage	Duration of Storage (Weeks)	Percentage Germination Before Storage	Dehydrated	After 24 Hours Rehydration
Air Dried	42	72	4	21
Vaccuum	62	72	4	60

Summary of Important Steps in Spore Storage

1) Use freshly collected spores.
2) Establish germination percentage by experimentation.
3) a)Air dry for 24-48 hours, place in sealed vial, and refrigerate at 2-4°C, or
   b)Place in dessicator with calcium chloride or silica gel in petridishes or open vials if air dried. Seal dessicator and store at 2-4°C, or
   c)Place in dessicator with a vaccuum petcock as above. Evacuate partially with small air pump or water aspirator, seal and store at 2-4°C, or
   d)Place 5 mg of spores in pyrex tube, plug opening with cotton, and attach manifold/vaccuum pump. Draw vaccuum for one to two hours. Seal tube using a gas-air torch, taking care not to heat spores. Store under refrigeration between 2-4°C, or
4) Rehydrate before use.

Table 8.1. The Effect of Temperature on the Viability of Yellow Rust Spores Maintained at 40% Relative Humidity

Temperature °C	Days of Viable Storage
0	433
5	179
15	50

Table 8.2. The Effect of Relative Humidity and Temperature on the Days that Stem Rust Spores can be Viably Stored

Relative Humidity (Percent)	Temperature °C 5	10	15	20
90	7	7	7	7
81	14	14	7	7
70	112	112	14	7
61	112	98	98	7
49	112	112	105	7
38	105	98	98	7
30	28	21	7	7
22	28	14	7	7
11	7	7	7	7

8.7 Techniques for Multiplying Rust Inoculum

Field collections of rust spores may not always be sufficient, either in volume or in composition (the relative proportion of different biotypes), for reliable nursery screening. Perhaps the best way of ensuring both the quantity and quality of collections is to supplement the inoculum gathered from the field with artificially multiplied spores. Various techniques have been developed to enable this to be carried out on a sufficiently large scale.

Seedling Methods

Traditionally, rust inoculum has been multiplied on seedlings grown in 10 cm clay pots in greenhouses. Between 15 and 20 plants are grown in each pot. They are inoculated at the first leaf stage and the inoculum is collected when sporulation reaches a maximum, usually 10-14 days under optimum conditions. This method is, however, very wasteful of greenhouse space and rarely generates

sufficient inoculum for field-scale use.

Perhaps a more efficient multiplication method involves growing seedlings in rectangular pans instead of clay pots. Over 150 seedlings can be raised in each 25 x 10 cm pan. It is thus possible to grow about four times the number of plants per unit area of greenhouse. In addition, sowing and inoculum-collecting operations are considerably facilitated. However, this method is still only applicable when small quantities of inoculum are required.

Using Maleic Hydrazide

Maleic hydrazide can be effectively used for the enhanced multiplication of *Puccinia graminis, P. recondita, P. striiformis* and *P. hordei.* It has been shown that the growth response of varieties to applications of the chemical (suppression of secondary and tertiary leaves and darker leaf pigmentation) is very closely connected to the degree of pathogen sporulation, so much so, that treated plants will yield between three and five times as much inoculum as non-treated ones. By preventing the growth of other leaves, maleic hydrazide provides a longer base life for the primary leaf (and thus the rust infections as well) and also simplifies collection procedures.

This method has been found particularly useful for the multiplication of nucleus inoculum or for the maintenance of single spore cultures of a race or biotype.

Detached Leaf Culture

Considerable savings in greenhouse space can be achieved using this method, but laboratory facilities are also required. The procedure basically involves inoculating plants as above and then removing leaves as they show the first sign of infection (flecking). The cut ends of these leaves are then immediately placed in a solution of sucrose (100o/o), kinetin (40 ppm), and benzimidazole (50 ppm), or in either kinetin or benzimidazole alone. The leaf cultures are kept in the laboratory or in growth chamber. After a few days sporulation begins and several spore crops may be taken from each set of leaves if the cultures are carefully maintained.

Using this method, it is possible to maintain and culture different races with more certain isolation to maintain purity.

Adult Plant Methods

Raising inoculum on susceptible adult plants has been found to be considerably more rapid and efficient than seedling methods. Adult plants are grown in 25 cm pots (each pot can support 9 plants -approximately 30 tillers), and are inoculated at either the tillering or the boot leaf stage. The inoculated pots are kept in a greenhouse and inoculum is collected periodically using a large collector. In this way one or two grams of spores may be obtained from each well-maintained pot.

Greenhouse space is frequently the limiting factor in the production of the large quantities of inoculum required for screening nurseries. It is especially limiting due to the high capital and maintenance costs involved. Studies have shown that considerably cheaper plastic-houses can be effectively be used in place of greenhouse if ventilation is adequate. Thus the multiplication of rust inoculum is possible even where physical facilities for research somewhat lacking.

8.8 Collecting, Storing and Multiplying the Inoculum of Other Cereal Diseases

Powdery Mildew of Wheat and Barley

Spores from infected plants should be collected late in the season after the small black resting-bodies have developed in the surface mycelium. The collected plant material should then be dried well and stored at a low temperature (2-4°C) in the laboratory. Under these conditions inoculum can be preserved in a viable state for use the following season.

Inoculum of powdery mildew may also be multiplied in culture in the laboratory using the detached leaf method. This enables identification of pathogenic races and the bulking of small amounts of inoculum.

Helminthosporium and Rhynchosporium Diseases

Plants infected with net blotch, spot blotch, leaf blotch, stripe disease or scald should be collected, dried for 24-48 hours, and stored at low temperatures to provide inoculum for the following season.

As these fungi are saphrophytic in nature, they can also be preserved and multiplied on natural and artificial media. In this way the inoculum can be considerably purified and increased if necessary.

Septoria Diseases

All Septoria diseases of wheat and barley (including speckled leaf blotch, glume blotch and Septoria leaf blotch) are collected on infected plant material and stored dry under cold conditions as are the powdery mildews.

Pathogens of the genus *Septoria* can also be successfully cultured and thus preserved and increased on artificial media (e.g. potato dextrose agar).

Bunt of Wheat, Covered Smut of Barley and Flag Smut

All these diseases are characterized by pathogenic spores which are very long-lived. Collected plant material with the disease can thus be kept without refrigeration for up to a year. Storage for longer periods, however, demands refrigeration.

Loose Smut of Wheat and Barley

In contrast to the other smut and bunt diseases, loose smut spores remain viable for only short periods of time under normal temperatures. Refrigeration or vacuum storage methods are thus necessary to preserve this inoculum.

Bacterial Blight of Barley

The same collection, storage and multiplication procedures used for powdery mildews are successful for this disease.

Foot and Root Rots

As with other diseases, inoculum is collected by removing infected material. However, the pathogens involved in these diseases are almost exclusively saprophytic and must therefore be preserved and propogated on artificial media.

8.9 Inoculation Techniques for Rust Diseases

Under Greenhouse Conditions

Plants to be incoulated should be grown in good soil, under good light conditions, at suitable temperatures and in a rust-free environment. It is advisable to use both treated seed and soil to reduce the danger of root rots. Inoculation can be carried out when the plants are between 5 and 10 cm tall.

All surface inoculations should be preceded by a gentle rubbing of the leaf surfaces with moistened fingers or by spraying with a wetting agent (e.g. Tween 20). This helps to remove the outer waxy coating of the leaves and thereby increases the number of spores able to adhere to them. In addition, the leaves should be atomized with distilled water both before and after inoculation in order to produce favorable moisture conditions for infection.

The inoculum may be applied in one of the following ways:

By Spatula or Lancet Needle

Spores are removed from individual pustules or storage containers on a wet spatula or needle, which is then run gently across the leaf surface of recipient plants so that the inoculum is distributed evenly. This method is particularly suitable when the initial inoculum is scarce or when inoculations

are to be made with spores from different pustule types in a mixed infection.

By Toothpick

Another precise inoculation method (also suitable if inoculum is scarce) involves the use of a toothpick with a small piece of cotton around one end. The spores are picked up on the dry cotton. A small drop of water or light mineral oil is then added and the cotton rolled gently across the surface of the recipient seedling leaves.

By Finger Rubbing

Similarly, clean fingers may also be used to transfer inoculum.

Using a Fine-Hair Brush

Spores alone or mixed with talcum powder (if only small amounts of inoculum are available) are picked up on a fine camel-hair brush and dusted over dry recipient seedlings or brushed directly onto the leaves.

Pot Brushing

This method is convenient for inoculating large numbers of plants when inoculum is abundant. A rusted plant is held close to a group of recipient plants and shaken gently to produce a uniform shower of spores. It is then brushed gently over the plant surfaces several times to ensure adequate inoculation.

Using a Multiple Inoculator

The multiple inoculator was developed by M. B. Moore at the University of Minnesota. It consists of a large paper clamp fastened to a metal sheet with fingers to which small pieces of foam rubber are attached. Spore suspensions (in water or mineral oil) are placed upon the sponges, which are then carefully pressed against the leaf surface. In this way it is possible to simultaneously inoculate single leaves with several different races or species of pathogen fairly rapidly.

With a Cyclone Duster

Small cyclone separators have proved valuable in making inoculations when inoculum is scarce. A glass cyclone (about 8 cm in length and 2 cm in diameter) is filled with 0.5 gm of talc. Spores are sucked from the source into the cyclone. A further 0.5 gm of talc is added and the contents throughly mixed. The mixture is then dusted onto the recipient plants by reversing the air flow through the cyclone.

With an Atomizer

The spores are suspended either in water (with a small amount of surfactant) or in a light mineral oil. They can then be sprayed onto plants using an atomizer. Care should be taken when using oil as a suspension medium as excess oil on the leaf surface tends to interfere with spore germination. In addition, some seedlings such as Barley are rather sensitive to damage from mineral oils.

Following inoculation, by whatever procedure, the inoculated plants should be placed in a moist atmosphere for 24 to 48 hours.

In the Field

Inoculation methods on a field scale are rather different to those detailed above, as the objective is to create epidemic conditions in thousands of plants over a large area. Furthermore, there is no control over environmental conditions.

Dusting

One of the simplest and most effective ways of inoculating large numbers of plants in the field is through the use of a spore/talc mixture. The ratio of spores to talc will depend upon considerations of effective cover, the quantity of inoculum available, and the area to be inoculated. There are many different types and sizes of dusters available for applying inoculum in this way. Small hand dusters may be sufficient if it is only necessary to inoculate border rows; power-operated units are more suitable if the area to be inoculated is large.

Dusting should always be carried out in the late evening, preferably just prior to dew formation, and when the air is still. This gives the best possible chance of achieving high levels of infection.

Injection

This is perhaps the most reliable inoculation method in drier climates where the chances of surface spore dessication due to adverse environmental conditions are high. Spore suspensions in water plus the surfacant should be injected into the leaf sheaths, using a hypodermic syringe, at either the late tillering or the stem extension stage of plant growth. Such a procedure has been found to result in good disease establishment. However, it is very time-consuming and is usually confined to border-row inoculation, as it may prove impossible to inoculate large numbers of plants in the limited time available. The development of automatic syringes has done much to make this method more popular.

Oil Inoculation

The feasibility of inoculating field plots with rust spores carried in non-phytotoxic oils was illustrated by Rowell and Hayden in 1956. They used Mobilsol 100, an isoparaffinic spray oil, and a spore concentration of 6 gm/gallon/acre. The application was made with a two-gallon capacity knapsack sprayer with a low-volume nozzle (Teejet No. 730039). In this way the inoculation of a 1/3 acre plot could be completed in just 30 minutes. As the spores disperse readily and uniformly in oil, a relatively small spore concentration could be used and a good cover still obtained. This method has been found to be effective and its simplicity recommends it where large scale inoculations are necessary.

Planting Infected Material

When border/spreader row inoculation is used rather than whole nursery methods, it is possible to transplant material previously infected in a greenhouse between these rows. It is essential to provide adequate watering following transplanting in order to ensure good establishment of the plants. Good results have been obtained using this method as the infection sources produce spores rapidly and survive for three to four weeks, supplying inoculum continuously.

8.10 Inoculation Techniques for Other Cereal Diseases

Foliar Diseases

Epidemic conditions of most foliar diseases may be successfully created by the simple expedient of chopping previously collected plant material and spreading it throughout the nursery. Inoculations of this type must be timed to coincide with environmental conditions favouring infection (which vary with each pathogen) and carried out on several occassions if a high success rate is to be achieved.

However, more specific inoculation techniques are required for a number of diseases. This is particularly true with those diseases that are soil-borne and those which require artificial multiplication prior to inoculation.

Flag Smut

This is a soil-borne disease. Epidemics are thus created through the development of a 'sick plot'. This may be achieved by growing a susceptible variety in the same plot for several years and continually burying infected plants in order to allow a natural inoculum build-up. Varieties to be tested may then be sown in the plot.

Artificial disease conditions may be created by spraying the soil of a test plot with a spore suspen-

sion at the rate of 2gm/litre of water per five metre row. The varieties to be tested are then sown and the soil turned over to mix seed and spores.

Helminthosporium Diseases

Considerable difficulties are experienced in increasing *Helminthosporium* inoculum in the laboratory. However, a technique involving multiplication on sterilized wheat seed has now been developed. Inoculum produced in this way can be successfully inoculated in spray suspension with water, a wetting agent, and a surfactant.

Diseases of the Spike

These diseases demand considerably more specific infection conditions than do those that affect the leaves. Thus a number of specific inoculation techniques have had to be evolved for the successful creation of epidemics of these diseases.

Loose Smut

A number of inoculation methods have been developed since Maddox (1895) first demonstrated that the disease could be produced by dusting wheat florets with spores of *Ustilago nuda tritici*. The most common of these are detailed below:

a) Partial Vacuum Method—An apparatus was deviced by Moore in 1936, with which individual cereal heads could be subjected to a partial vacuum while completely submerged in an aqueous suspension of loose smut spores. Using this apparatus, as many as 30 heads can be inoculated per hour. The basic operation of the device involves the creation of an alternating partial vacuum/normal pressure regime around the enclosed head, causing the air within the florets to be replaced with the spore suspension. Such a method can also be used for inoculating partial bunt disease.

b) Injection—A spore suspension, made by dissolving one smutted ear head in 100 ml of water with 1 gm of dextrose, is injected into the two main florets of each spikelet with a hypodermic syringe. Great care must be taken that the developing ovary is not injured during injection.

c) Needle Method—Dry spores may also be blown (puffed) onto the stigmas of the developing ovaries using a hypodermic needle attached to a rubber bulb reservoir. When using this inoculation technique, the spores must first be well sieved to remove material likely to block the needle. Again care is necessary to prevent ovary damage.

d) Using Forceps—This method involves the use of sharp forceps to collect spores and then pierce the developing floret and deposit them on the stigma.

Of these techniques the dry spore methods have generally proved superior to those involving spore suspensions. The needle method is perhaps the easiest to handle and thus the most efficient. However the partial vacuum method has also been reported as giving very good results.

Karnal or Partial Bunt

Moore's vacuum method has traditionally been used to inoculate wheat plants with this disease, although boot leaf inoculations with spore suspensions using a hypodermic syringe may also be used with good success.

Stinking Smut or Bunt

Bunt pathogens are carried naturally on the outer seed coat and infected cereal plants at germination. Thus inoculation is achieved by mixing spores with seed prior to planting at a rate of about 0.5 to 1%/o of the seed weight.