PN-AAG-665
ISN = 46660

Cereal Disease Methodology Manual

R.W. Stubbs

Institut voor Plantenziektenkundig Onderzoek (IPO)
Wageningen, The Netherlands

and

J.M. Prescott
E.E. Saari
H.J. Dubin

Centro Internacional de Mejoramiento de
Maíz y Trigo, (CIMMYT) México

Table of Contents

			Page
Preface			**iii**
Part I. Introduction			
Section	**1**	**Cereals: An Overview**	**1**
	1.1	Wheat	1
	1.2	Barley	2
	1.3	Wheat and Barley Morphology and Growth	4
Part II. Diseases and Disease Development			
Section	**2**	**The Major Diseases of Wheat and Barley**	**8**
	2.1	The Cereal Rusts	8
Section	**3**	**The Development of Natural Epidemics**	**12**
	3.1	Disease Establishment	12
	3.2	Disease Spread	13
	3.3	A Simple Way to Measure Disease Increase	14
	3.4	Development of Rust Epidemics in the Field	16
	3.5	Long Distance Transport of Disease Organisms	17
Section	**4**	**Surveying Plant Diseases**	**19**
	4.1	Basic Survey Techniques	19
	4.2	Principles of Disease Assessment	20
	4.3	Recording Wheat and Barley Rust Diseases	20
	4.4	Average Coefficient of Infection	22
	4.5	Recording Other Cereal Diseases	22
Part III. Developing Disease-Resistant Varieties			
Section	**5**	**Principles of Breeding for Disease-Resistant Cereals**	**25**
	5.1	Types of Resistance	25
	5.2	Modes of Inheritance	26
	5.3	Resistance Breeding Strategies	27
	5.4	Breeding in Practice	29
Section	**6**	**Plant Pathology in Cereal Breeding**	**30**
	6.1	Establishing Disease Nurseries	30
	6.2	Techniques for Enhancing and Creating Epidemics	30
	6.3	Justifying the Creation of Artificial Disease Epidemics	31
	6.4	Some Important Technical Considerations in Creating Epidemics	32
	6.5	Techniques for Rust Urediospore Collection	34
	6.6	Storing Rust Urediospores	34
	6.7	Techniques for Multiplying Rust Inoculum	35
	6.8	Collecting, Storing and Multiplying the Inoculum of Other Cereal Diseases	36
	6.9	Inoculation Techniques for Rust Diseases	37
	6.10	Inoculation Techniques for Other Cereal Diseases	38

Literature Cited 40

Appendices

Appendix	**1**	**Characteristics of Wheat Diseases**	**41**
	A1.1	Fungal Diseases	41
	A1.2	Bacterial Diseases	44
	A1.3	Viral Diseases	44
	A1.4	Diseases Caused by Nematodes	44
Appendix	**2**	**Characteristics of Barley Diseases**	**45**
	A2.1	Fungal Diseases	45
	A2.2	Bacterial Diseases	46
	A2.3	Viral Diseases	46
	A2.4	Diseases Caused by Nematodes	46

Preface

There are 27 countries in the developing world that grow more than 100,000 hectares of wheat, with more than 95 percent of this production destined for direct human consumption. While average yields and total production have registered important gains in recent years, wheat diseases are still one of the major contributors to year-to-year yield instability in developing countries.

The major wheat disease pathogens are obligate parasites that can mutate into new virulent forms capable of attacking varieties that were formerly resistant. To offset this danger, plant pathologists and plant breeders, working cooperatively, must continually develop new varieties with resistance to the prevalent disease races. Unfortunately, in many countries a strong linkage has not been established between the practioners of these two disciplines. Moreover, there has been a tendency on the part of pathologists toward more basic research, rather than toward the application of the latest field techniques to the development of epidemics to screen breeding materials.

Beginning in 1976, the Research Institute for Plant Protection (IPO), Wageningen, The Netherlands, and the International Maize and Wheat Improvement Center (CIMMYT), Mexico, initiated the Regional Workshops in Cereal Disease Methodology to develop or strengthen plant pathology research activities in national wheat improvement programs in developing countries. These workshops, usually lasting two weeks, have now been held in 11 locations around the world. Approximately 290 scientists from 40 developing countries have participated in the series.

This manual is an outgrowth of the workshop series; in it are presented the principal topics developed in the workshops. It is hoped that it will be widely read and consulted not only by future workshop participants but also by other pathologists in the developing countries. By providing a concise review of principles and clear descriptions of methodologies appropriate to research conditions in the developing world, it is hoped that it will promote the practical application of plant pathology to breeding programs in wheat and other small grain cereals.

The authors wish to thank the Ministry of Foreign Affairs of the Government of The Netherlands and the International Maize and Wheat Improvement Center (CIMMYT) of Mexico for the financial, logistic and material support which made the workshops, and later this manual, possible. The comments and questions of the workshop participants were extremely useful in improving the workshop presentations from which the manual was developed.

Part I. Introduction

1. Cereals: An Overview

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, 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 (Table 1.1). Wheat, maize and rice account for 80 percent of global cereal production.

Wheat production in the developing countries 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 growth occurred in the five major developing country producers, China, India, Pakistan, Turkey and Argentina (Table 1.2), which collectively account for 82 percent of all developing country producti Among these major producers, China registered the greatest gains.

1.1 Wheat

The world's wheat acreage and production are clearly concentrated in the northern hemisphere. Bread and durum are the two principal commercial types of wheat. Bread wheat covers about 90 percent of the world wheat area and makes up about 94 percent of production. Durum wheat is less cosmopolitan in its distribution, being grown principally in North Africa, the Near and Middle East, the USSR, India, Italy, France, the northern USA and some areas of Canada.

As one of the world's most important staple foods, wheat is consumed in variety of ways. Its most important use is 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 wheat as one of the mainstays of world nutrition requires that both the stability and level of production continue to increase in order to minimize malnutrition as much as possible.

Wheat classification—The wheat species of importance to agriculture are primarily *Triticum aestivum* ssp. *aestivum* (bread wheat) and *T. turgidum* ssp. *durum* (durum wheat). Table 1.3 (page 3) gives complete details of the species and subspecies in the genus *Triticum*, which is part of the tribe Triticeae of the family Gramineae (the grasses). This and other members of this tribe are listed below:

Tribe	Subtribe	Genus
Triticeae	Triticinae	*Triticum* (wheat)
		Aegilops
		Agropyron
		Haynaldia
		Secale (rye)
	Hordinae	*Elymus*
		Hordeum (barley)
		Sitanion

Table 1.1. World cereal area and production for 1981, by crop

Crop	Area: Hectares (thousands)	Area: Percent of total	Production: Tons (thousands)	Production: Percent 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.

Table 1.2. Wheat production increases of the major developing producers

Country	Production (thousands of tons) 1969-71	Production (thousands of tons) 1979-81	Percent annual growth (1970s)
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

The origin of wheat—In common with all other crops, cultivated wheat was 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). This species possesses seven pairs of chromosomes (14 in total), and is termed diploid. Pure stands of einkorn have been observed growing in close association with other members of their subtribe, namely *Aegilops speltoides*, in areas of western Asia (Figure 1.1). Cytological studies have revealed that the second primary cultivated form of wheat, *Triticum turgidum* ssp. *dicoccum* (emmer), probably came into being through an intergeneric cross between these two species. As their two sets of chromosomes (each with 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 intergeneric cross between cultivated emmer and the 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, which form 21 pairs). This evolutionary sequence is represented diagrammatically below:

Diploid (14 chromosomes)	**Tetraploid** (28 chromosomes)	**Hexaploid** (42 chromosomes)
T. monococcum (A)	*T. turgidum* ssp. *dicoccum* (AB)	
Ae. speltoides (B)		
Ae. squarrosa (D)		*T. aestivum* (ABD)

A, B and D represent genomes

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 production conditions are poor and 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.

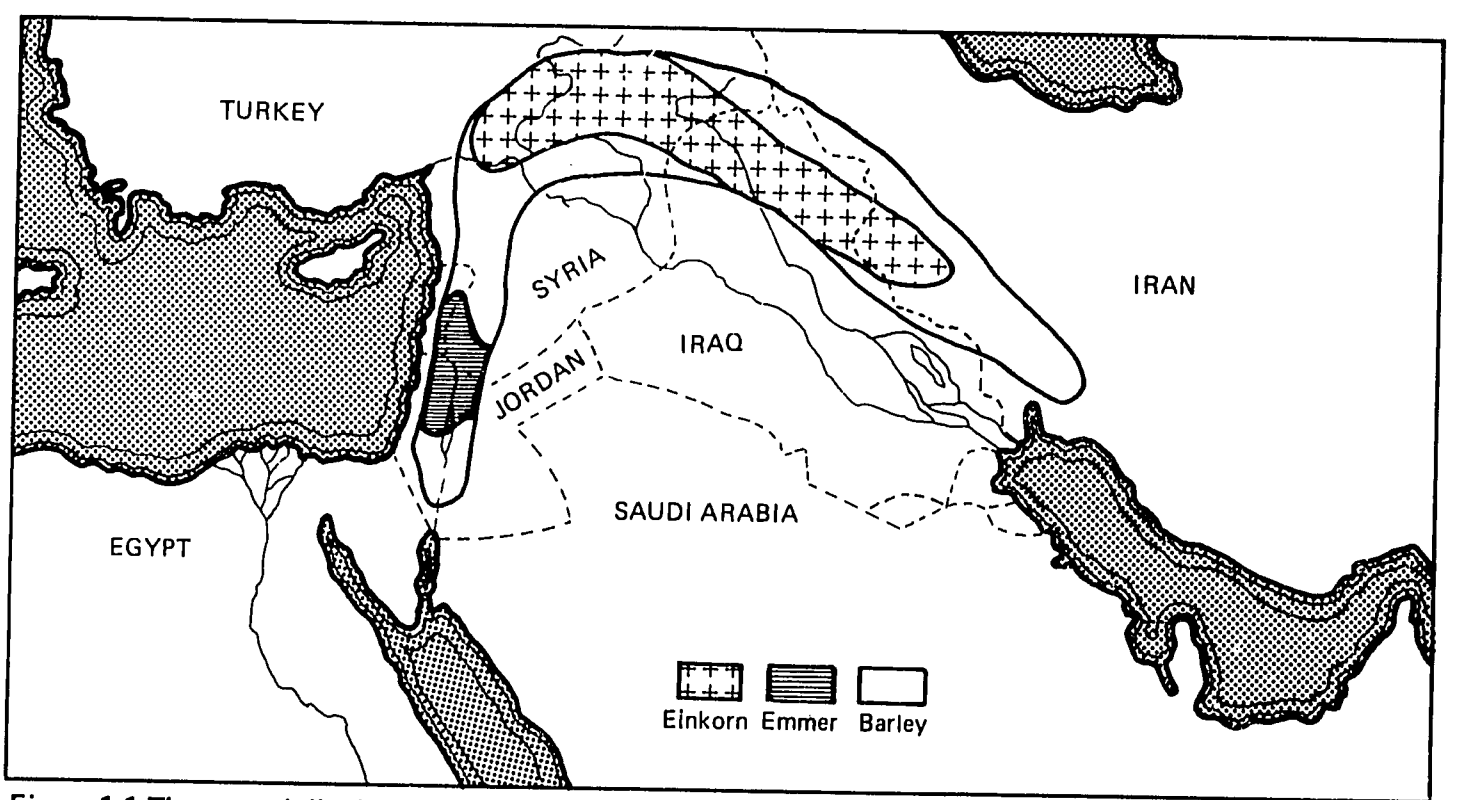


Figure 1.1 The natural distribution of ancestral wheats and barleys (from Isaac 1970).

The USSR, Europe and Asia together produce some 84 percent of the world barley grain on about 80 percent of the total world 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 role.

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 Triticeae of the family Gramineae. *Hordeum vulgare* is considered to contain three subspecies:

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

These are distinguished by the number of grain-producing spikelets at each node of the ear; in two-rowed types only one of the three spikelets is 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. Large, self-seeding stands of wild two-rowed barley are still found over much of this area. Figure 1.1 shows the natural distribution of ancestral barleys and wheat. It was in this same area that the cultivated forms presumably evolved.

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 cytogenetic 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.

Table 1.3. Features of the genus *Triticum*

Scientific name	Common name	Chromosome number (*n*)	Genome formula	Seed type*	Probable region of origin
Diploids					
T. monococcum	Einkorn	7	AA	H	Armenia, Georgia, Turkey
Tetraploids					
T. turgidum					
spp. *dicoccum*	Emmer	14	AABB	H	Georgia, Abyssinia
spp. *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, NE Turkey
ssp. *turanicum*	Khorasan	14	AABB	N	Mediterranea, Near East
T. timopheevii	—	14	AAGG	H	W Georgia, Abyssinia
Hexaploids					
T. aestivum					
ssp. *spelta*	Spelt	21	AABBDD	H	Austria, Germany
ssp. *macha*	Macha	21	AABBDD	H	W Georgia
ssp. *vavilovii*	Vavilov	21	AABBDD	H	Turkish Armenia
ssp. *aestivum*	Bread wheat	21	AABBDD	N	SW Asia, Central Europe
ssp. *compactum*	Club	21	AABBDD	N	Afghanistan, Armenia
ssp. *sphaerococcum*	Shot	21	AABBDD	N	NW India

From Martin, *et al.* 1976

* N = naked; H = hulled

1.3 Wheat and Barley Morphology and Growth

The cereal seed or kernel (technically a caryopsis), 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, which is the source of stored food that the embryo utilizes for growth after germination 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 when it is exposed to suitable environmental conditions. During germination the embryo swells and ruptures the pericarp, and from this rupture the radicle (root) and plumule (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-inhibiting chemicals in the pericarp).

From the emergence of the young seedling at the soil surface to the production of the mature seed, the growth of the wheat or barley plant can be divided into a number of (sometimes simultaneous) stages. These have been codified by Zadoks *et al.* (1974) as follows:

1. Seedling growth—leaves unfold, from the first one breaking through the coleoptile to the appearance of the flag leaf ligule.

2. Tillering—additional (secondary) shoots arise from the plant crown.

3. Stem elongation—the first pseudostem is erected and the nodes become visible; upper leaf sheath is not swollen by the head.

4. Booting—head is evident in the upper or flag leaf.

5. Ear emergence—head emerges from the sheath.

6. Flowering—florets and flowers open; pollen is shed.

7. Milk development—fertilized ovary enlarges to mature seed size; its contents become increasingly white and opaque.

8. Dough development—ovary contents solidify.

9. Ripening—seed becomes hard; harvest.

A more detailed breakdown of the stages recognized in the Zadoks' scale is given in Figure 1.4 and its key (page 6).

The head of a wheat or barley plant is composed of a number of spikelets arranged along both sides of a stalk (rachis) and is referred to as a spike. Each spikelet is composed of a number of individual flowers (florets). Each floret is composed of female organs (the pistil) and male organs (the stamens) enclosed within outer protective coverings, the lemma and palea (Figure 1.5, page 7). 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 its own flower (self-

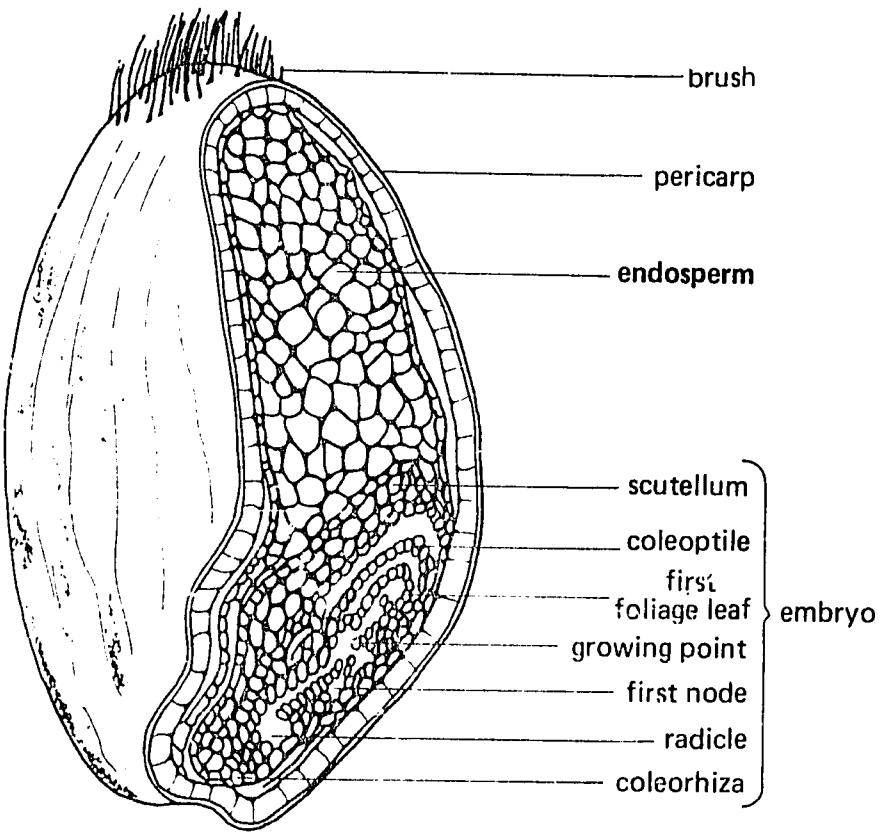


Figure 1.2. The wheat kernel.

pollination). The pollen grain produces a tube which grows down the style (structure supporting the stigmas) and into the ovary where the female reproductive part (the egg) is located. The pollen nuclei penetrate the egg and fertilize it, leading to the production and development of the seed, which includes the embryo and endosperm and is enclosed in the matured ovary, which becomes 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 or 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. Thus, they are to a certain extent tolerant to frost and low temperatures. In the spring, when growth resumes, it is at first 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 cold 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 planted in the autumn or winter if 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 freezing. They are, in general, more widely adapted than either of the other two types.

All three growth habits are found among commercial varieties of bread wheat, durum, and barley. Apart from their adaptation to different temperature conditions, wheat and barley are also adapted

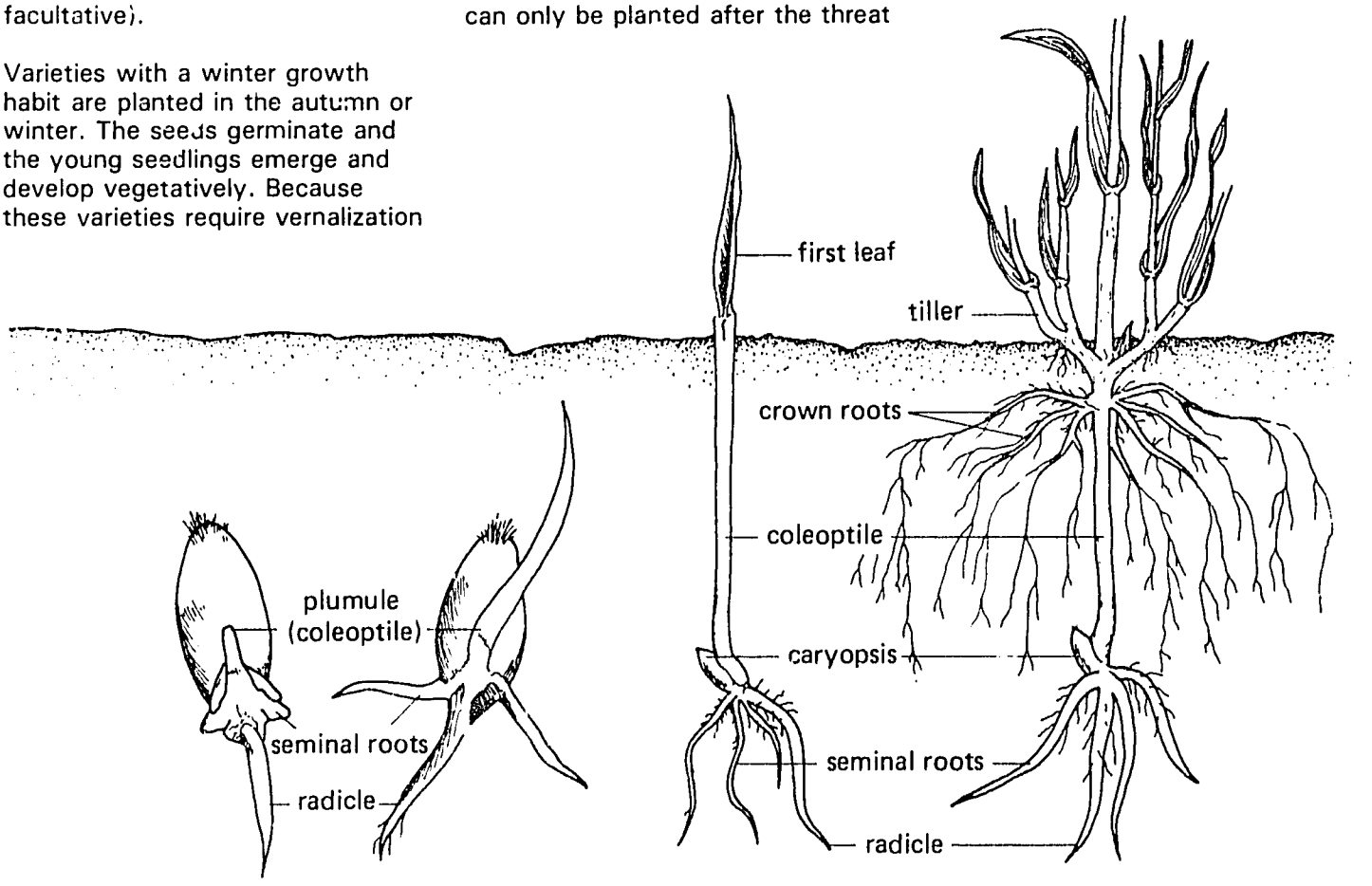


Figure 1.3 The stages of germination.

Key to figure 1.4. Descriptions of the principal and secondary growth stages of the Zadoks' scale, as modified by Tottman and Makepeace (1979).

Code	Stage
0	**Germination**
00	Dry seed
01	Start of imbibition
03	Imbibition complete
05	Radicle emerged from seed
07	Coleoptile emerged from seed
09	Leaf just at coleoptile tip
1	**Seedling growth**
10	First leaf through coleoptile
11	First leaf unfolded
12	2 leaves unfolded
13	3 leaves unfolded
14	4 leaves unfolded
15	5 leaves unfolded
16	6 leaves unfolded
17	7 leaves unfolded
18	8 leaves unfolded
19	9 or more leaves unfolded
2	**Tillering**
20	Main shoot only
21	Main shoot and 1 tiller
22	Main shoot and 2 tillers
23	Main shoot and 3 tillers
24	Main shoot and 4 tillers
25	Main shoot and 5 tillers
26	Main shoot and 6 tillers
27	Main shoot and 7 tillers
28	Main shoot and 8 tillers
29	Main shoot and 9 or more tillers
3	**Stem elongation**
30	Pseudostem erection (winter cereals only)
31	1st node detectable
32	2nd node detectable
33	3rd node detectable
34	4th node detectable
35	5th node detectable
36	6th node detectable
37	Flag leaf just visible
39	Flag leaf ligule just visible
4	**Booting**
41	Flag leaf sheath extending
43	Boots just visibly swollen
45	Boots swollen
47	Flag leaf sheath opening
49	First awns visible
5	**Ear emergence**
51	First spikelet of ear just visible
53	One-fourth of ear emerged
55	One-half of ear emerged
57	Three-fourths of ear emerged
59	Emergence of ear complete
6	**Flowering**
61	Beginning of flowering
65	Flowering halfway complete
69	Flowering complete
7	**Milk development**
71	Kernel water ripe
73	Early milk
75	Medium milk
77	Late milk
8	**Dough development**
83	Early dough
85	Soft dough (fingernail impression not held)
87	Hard dough (fingernail impression held; head losing chlorophyll)
9	**Ripening**
91	Kernel hard (difficult to divide by thumbnail)
92	Kernal hard (can no longer be dented by thumbnail)
93	Kernel loosening in daytime
94	Overripe; straw dead and collapsing
95	Seed dormant
96	Viable seed giving 50 percent germination
97	Seed not dormant
98	Secondary dormancy induced
99	Secondary dormancy lost

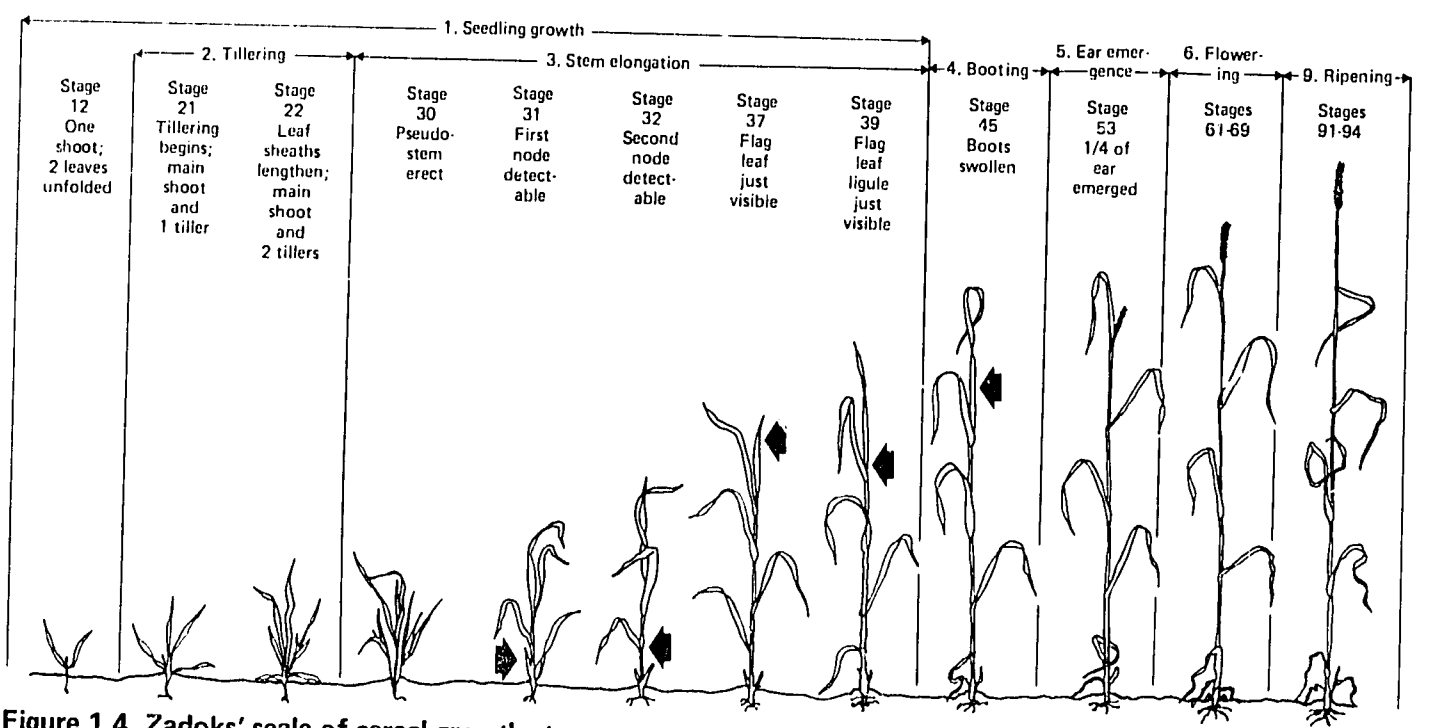


Figure 1.4. Zadoks' scale of cereal growth stages.

to various conditions of day length, requiring different photoperiods for the onset of flowering and for optimum seed set. For practical purposes the 14 hour day length (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 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 day lengths 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 on the chromosomes of 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 tube nuclei (male gametes). Some characters are controlled by one or a relatively few genes and are termed qualitative (the inheritance of individual genes immediately confers certain characteristics 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 an individual gene is relatively minor, but increasing numbers of them cause an increasing expression of specific characters). An example of a quantitatively inherited character is yield.

The expression of this inherited genetic make-up or potential depends in turn upon the result of the interaction between these characters and 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), imposes numerous contraints upon the way in which cereal crops grow and yield. The biological components of the environmnt have become particularly important a a result of 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 most important single environmental constraint to increased cereal production and to the increasingly widespread cultivation of cereal crops throughout the world.

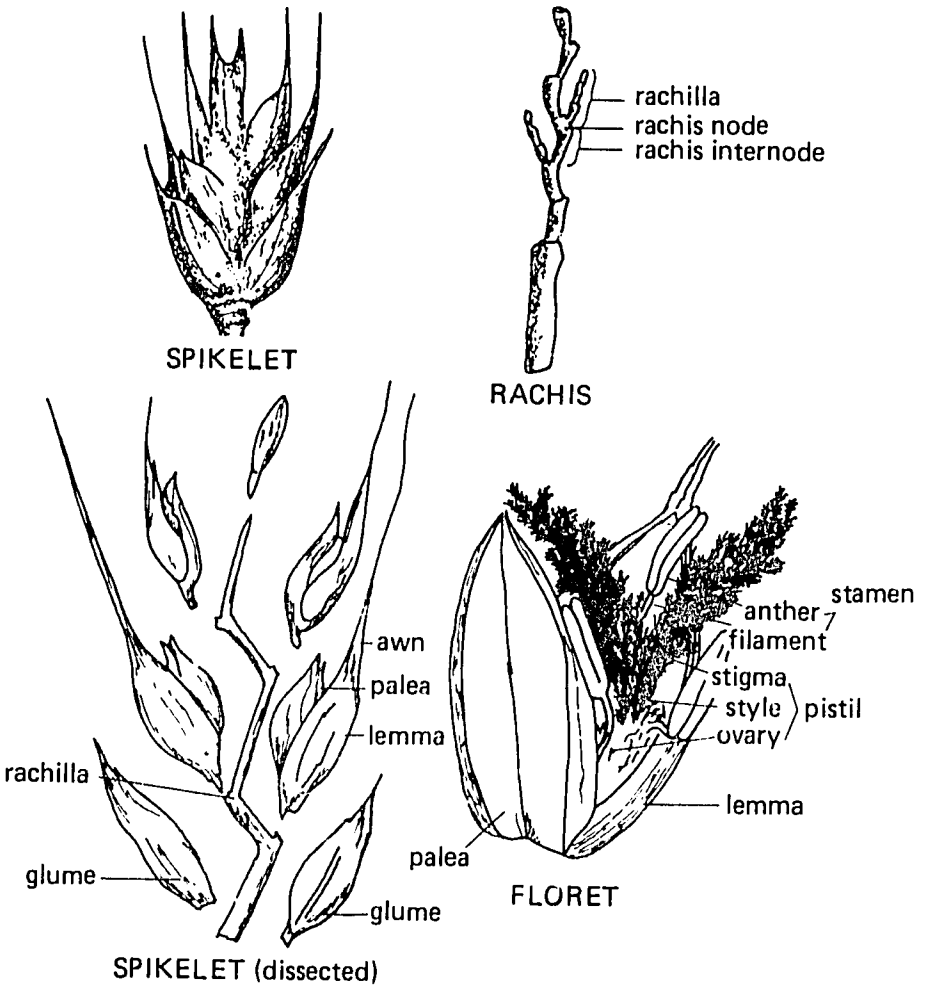


Figure 1.5. Structure of the spike, spikelet and floret of wheat.

Part II. Diseases and Disease Development

2. 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 genera *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 relative importance of these diseases reveal the paramount importance of the rusts, especially in wheat (Tables 2.1 and 2.2). Such studies also demonstrate 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).

2.1 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 dwarfing or leaf rust) and *P. coronata* (causing crown or leaf rust) regularly cause 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.

Table 2.1. Distribution and importance of wheat diseases in the Middle East and North Africa

Area	Disease* and relative importance									
	SR	YR	LR	Sm	B	PM	S	A	H	RR
Afghanistan	2	1	5	4	7	6	3	–	–	8
Turkey										
Plateau	4	1	6	3	2	–	–	–	–	5
Coast	1	1	6	5	7	4	3	10	9	8
Southeast	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	–

Numbers indicate the importance of the disease, with 1 being most important; tR = trace; dash = not present

*A = alternaria, B = bunt, H = helminthosporium, LR = leaf rust, PM = powdery mildew, RR = root rot and fusarium, S = septoria, Sm = smut, SR = stem rust, YR = stripe rust

Table 2.2. Distribution and importance of barley diseases in the Middle East and North Africa

Area	Disease* and relative 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 indicate the importance of the disease, with 1 being most important; dash = not present

* H = helminthosporium, LR = leaf rust, PM = powdery mildew, Sc = scald, Sm = smut, SR = stem rust, YR = stripe rust

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 a number of different spore types and in many cases, alternation between two host species. The full life cycle is illustrated in Figure 2.1. It should be stressed, however, that the complete two-host, five-spore type of cycle is not commonly found in cereal rusts except under rather specific environmental conditions. In most areas where environmental conditions are favorable, the fungi reproduce almost exclusively through the asexual urediospore cycle on the crop itself, on volunteer plants, or 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. and *Mahonia* spp.

P. striiformis—unknown

P. recondita—*Thalictrum* spp., *Isopyrum* spp. *Anchusa* spp., and *Anemonella* spp.

P. hordei—*Ornithogalum* spp.

P. coronata—*Rhamnus* spp.

In many regions a graminaceous host (or hosts) may be found throughout the year, which allows the rusts to survive in the area. In this situation the rusts are 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 initally arise from inoculum introduced from some distant source and are termed *exodemic*.

Taxonomy—The classification of rust fungi into families and genera 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 2.2, page 10).

In the genus *Puccinia* host specificity 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 specialis*, in general, is able to attack several closely related grasses. The major recognized *formae speciales* infecting cereal crops are listed in Table 2.3 (page 10).

Within each *forma specialis* the pathogens may be further 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/or other characters. Physiological races (designated by race numbers) are identified on the basis of differential responses on selected host varieties. While this system of classification is very useful, it cannot be used to identify completely any rust collection.

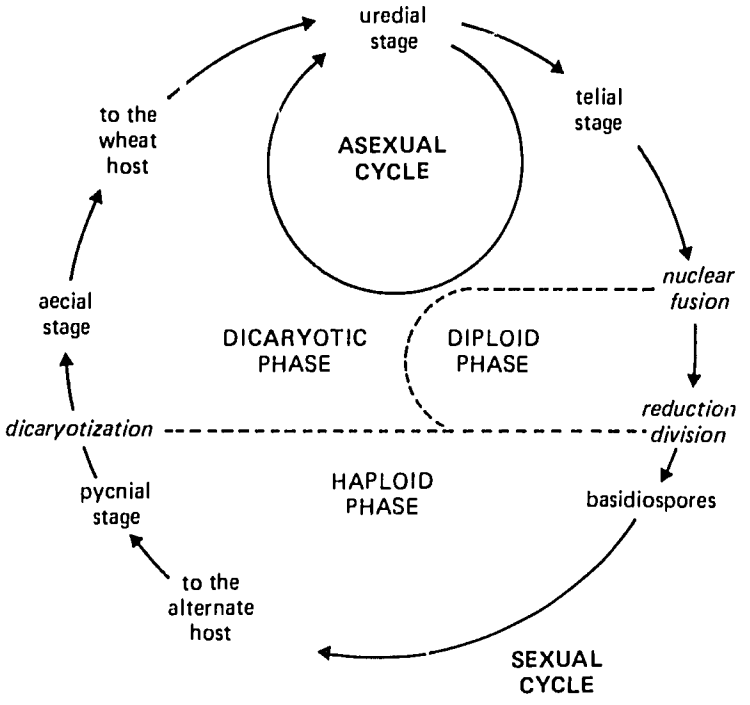


Figure 2.1 Generalized life cycle of the wheat rust fungi (from Loegering *et al.* 1967).

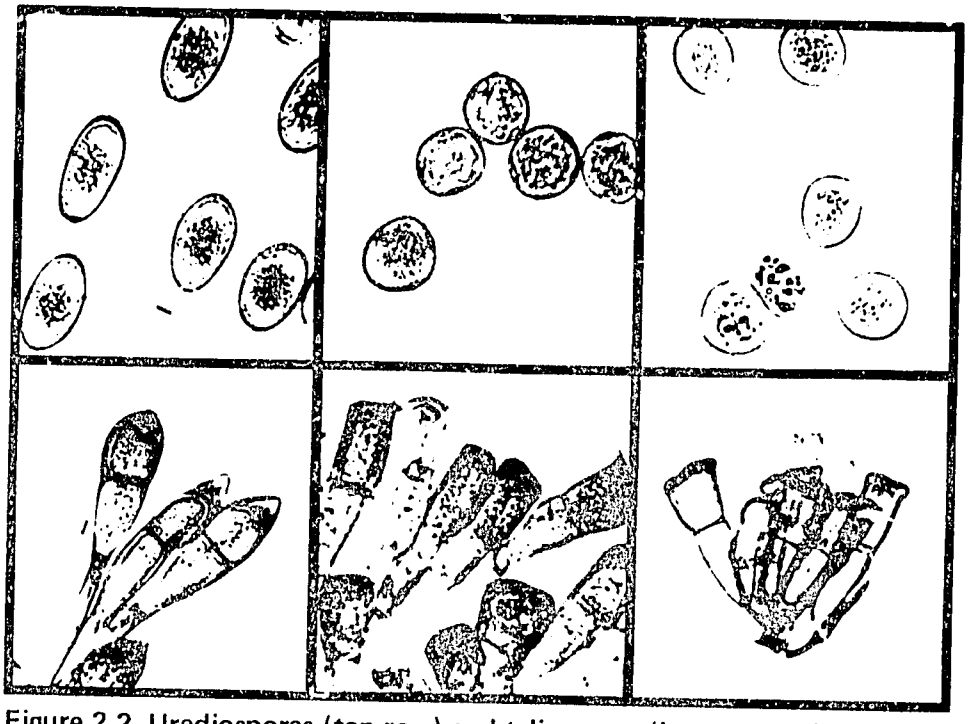


Figure 2.2. Urediospores (top row) and teliospores (bottom row) of stem (A), leaf (B), and stripe (C) rust fungi magnified about 875 times. The spores of the stem rust fungus are easily differentiated from the other two when observed under the microscope. Spores of leaf and stripe rust fungi are difficult to distinguish (USDA photo courtesy of W.Q. Loegering).

Table 2.3. The major species and *formae* of cereal rusts

Pathogen		Primary host
Species	*Forma specialis*	
Puccinia 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

Table 2.4. The basic infection types for classifying cultures of stem and leaf rusts in the greenhouse

0 **Immune:** No signs of disease.

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 separated; 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 unfavorable 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 viceversa.

From Stakman, *et al.* 1962

The identification of physiological races of rusts may be made on the basis of the response of different wheat and barley varieties to infection by a purified rust culture. Seedlings of a fixed number of varieties are used; infections will be fully developed about 10-15 days after inoculation if conditions are optimal.

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 2.4. Although there is no unanimous agreement on coding among rust workers, the majority of stripe rust researchers now classify infection types according to the general scheme given in Table 2.5.

European and Indian stripe rust workers have developed a binary notation system for race nomenclature. This allocates to every differential host a fixed exponential value (decanery value). Host reactions are classified as either resistant (binary score = 0) or susceptible (score = 1). By multiplying the binary score and the decanery value, a decanery value may be obtained for each differential host. 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. An example of the use of binary notation in naming a physiological race is given in Table 2.6.

Table 2.5. General classification of infection types for stripe 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

From Mc Neal, *et al.* 1971

* H = high, L = light, M = moderate, R = resistant, S = susceptible, V = very

Table 2.6. Using binary notation to name 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
Host reaction	R	R	S	R	S	S	R
Binary score	0	0	1	0	1	1	0
Host decanery value	0	0	16	0	4	2	0

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 full life cycle, involving the alternate host, occurs, new variants can arise in profusion though sexual recombination (the production of gametes at meiosis and their random fusion 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 an additional mechanism through which pathogen variability may be created. Even at low mutation frequencies, the very high rate of urediospore production observed in these pathogens allows mutation to be one of the major sources of variants. Other mechanisms recognized as being operative in rusts are heterokaryosis and somatic hybridization (parasexuality). Little evidence about the importance of these two mechanisms under field conditions is 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 are recessive and exist in a heterozygous state. A single mutation leading to dominance of such a gene 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 on 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 been hypothesized and has gained considerable acceptance.

3. The Development of Natural Epidemics

The cycle of pathogen and disease development, from primary inoculum through plant infection and back to primary inoculum, varies considerably among different pathogenic organisms. Plant pathogenic fungi can be divided into two groups on this basis, viz., single and multiple cycle types (Figure 3.1).

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 the 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 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., *Puccinia, Helminthosporium* and *Septoria* spp.) are considered to possess an inherently higher disease-causing potential than those with a single cycle. Discussion will therefore be confined to multiple cycle diseases.

3.1 Disease Establishment

Inoculum—The first requirement 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 surface of a suitable host. Inoculum particles may be in a number of different forms, such as spores, mycelial masses, bacterial cells, or viral particles.

Disease inoculum is generated by previous infections and liberated into the environment, forming a reservoir of potential infection. This 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 most important way in which multiple disease cycle fungal spores are disseminated over greater or lesser distances. Water may also play an important role in 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 produced by most pathogenic organisms. Numerous factors affect inoculum survival, including dormancy, spore structure, dispersal mechanisms, and environmental conditions. Rust urediospores, on the other hand, are well adapted to survival, being able to travel over long distances and to endure adverse conditions while still retaining a fairly high viability.

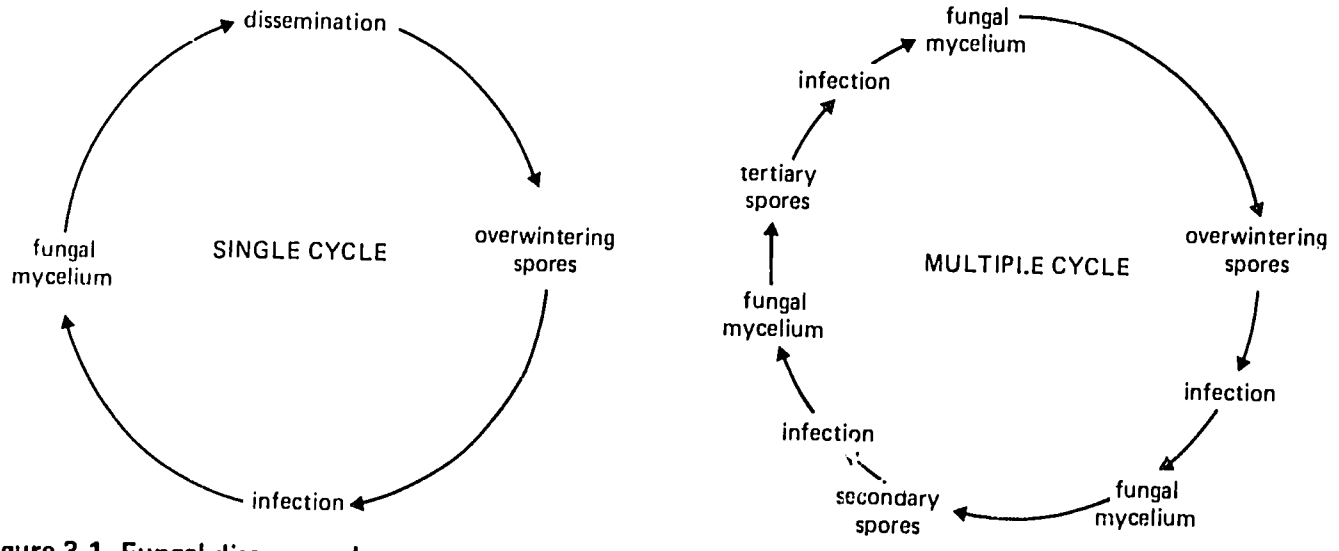


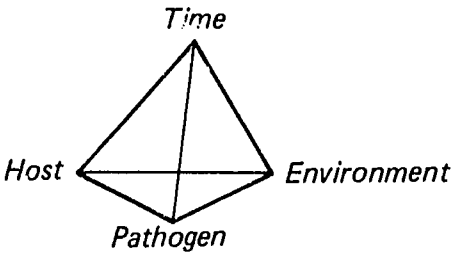
Figure 3.1. Fungal disease cycles.

Once in contact with the host surface, and provided that environmental conditions are favorable, the pathogenic inoculum enters into a phase of rapid growth supported by its own food reserves. This growth phase results in the formation of a germ tube that actually penetrates the host surface and is termed germination. It is during this time that the pathogen is most vulnerable to adverse conditions (e.g., desiccation) and thus is 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 3.2.

3.2 Disease Spread

Traditionally the major factors affecting disease spread and development have been linked together in a "disease triangle" of interaction among the virulence of the pathogen, the susceptibility of the host, and the favorability of the environment. Time, however, is an additional and very important consideration in this regard (i.e., the time period during which the host and pathogen are in contact, the timing and duration of optimal infection conditions, the time necessary for infection, etc.). The insertion of a time factor into this interaction relationship results in its representation as a "disease pyramid" rather than a 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 focus 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. The multiplication of infection 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 infection 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 in 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 existing resistance and causing disastrous losses.

In order to develop high-yielding cultivars with broad-based resistance to various diseases, all known sources and types of resistance must be utilized in breeding efforts. Systems of multilocation testing are an aid 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 inoculum of vigorously growing and rapidly reproducing pathogens

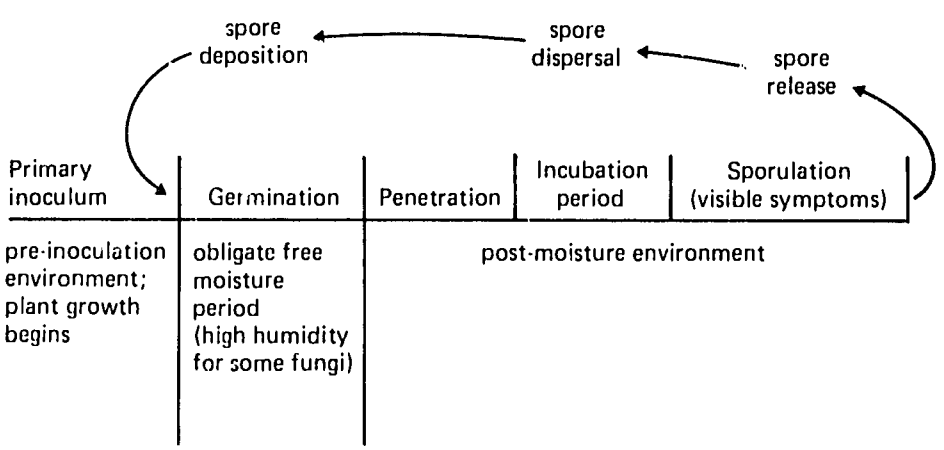


Figure 3.2. Developmental sequence of a typical fungal disease.

come into contact with highly susceptible hosts. Assuming one percent viability and infection, a million rust pustules can theoretically result from one initial rust urediospore infection after only three generations of 10 days each:

	1 spore
First generation	1 pustule 10^5 spores
Second generation	10^3 pustules 10^8 spores
Third generation	10^6 pustules 10^{11} spores

Although this situation never occurs in nature, the example serves to illustrate the enormous infective potential of some fungi, especially when the rate of generation turnover can be as rapid as 10 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 (Table 3.1).

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 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 (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.

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

Epidemics begin with the disease spreading locally around the primary focus of infection and then diffusing 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 rate of spore production and growth rate of the pathogen, the environment, the availability of susceptible hosts, and time—the four factors of the disease pyramid. Under optimum conditions the disease spreads until the primary and secondary foci coalesce and the epidemic continues until there is no more susceptible tissue to infect.

Concerning epidemics the following generalizations can be made:

- The rate of disease multiplication increases with the increasing number of foci.
- The amount of infection due to a single disease focus decreases with the distance from that focus.
- The chance that a given plant will be infected also decreases with increasing distance from the focus.

3.3 A Simple Way to Measure Disease Increase

During the 1960s, 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 exponential 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 (t) and thus a measure of the disease increase:

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

where t_1 and t_2 are the dates at which disease measurements were made and x_1 and x_2 are the amounts of disease recorded on these dates. The observed infection rate (r) is the result of the interaction of all the factors affecting disease development. As these factors approach the optimum, r increases.

Table 3.1. The influence of environmental factors on the germination of urediospores and the infection process of the wheat rust fungi

Fungus	Process	Moisture	Temperature (°C)	Light
Puccinia graminis	1. Initiation of germination	Free water required for 2 or more hours	Minimum > 5 Optimum 15-24 Maximum 30	Strong light may inhibit stored spores; less effect on fresh spores
	2. Germ tube growth	Free water required	Optimum *ca.* 15-20	As above
	3. Appressorium formation	Free water required	Optimum *ca.* 20	Favored by dark conditions
	4. Penetration	Not necessary	Optimum *ca.* 30	Favored by bright light
	5. Urediomycelium development	Adequate moisture required by host	Optimum *ca.* 20	Adequate light favors host and pathogen
Puccinia recondita	1. Initiation of germination	Free water required for 2 or more hours	Minimum 2-3 Optimum 8-28 Maximum 32	Strong light may retard process
	2. Germ tube growth	Free water required	Optimum *ca.* 15-20	As above
	3. Appressorium formation	Free water required	Optimum *ca.* 18-25	Favored by dark conditions
	4. Penetration	Not necessary	Optimum *ca.* 20	No effect
	5. Urediomycelium development	Adequate moisture required by host	Optimum *ca.* 20	Adequate light favors host and pathogen
Puccinia striiformis	1. Initiation of germination	Free water required for 2 or more hours	Minimum 0 Optimum 7-15 Maximum 23-26	Variable; may be favorable at > 15°C
	2. Germ tube growth	Free water required	Optimum 10-15	As above
	3. Appressorium formation does not occur	Not applicable	Not applicable	Not applicable
	4. Penetration	Not necessary	Optimum 8-13	No effect
	5. Urediomycelium development	Adequate moisture required by host	Optimum 12-15	No effect

From Chester (1946), Hassebrauk and Schroeder (1964), Hogg, *et al.* (1969), Rowell (1984), Sharp (1964), Staples and Macke (1984), and Togashi (1949).

Epidemics develop in a logistic fashion. This implies that there is a limit to their growth and that their increase is exponential. However, using a logarithmic function ($\ln[x/(1-x)]$, which is termed the *logit* of x), the development of an epidemic can be plotted as a straight line according to an equation of the form $y = a + bx$, where y is the logit of the amount of infection, a is the infection level at the first observation, b is the slope or the infection rate (r) and x is the number of days after the first observation (Figure 3.3).

3.4 Development of Rust Epidemics in the Field

The primary rust inoculum, which establishes the initial infection, 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 at this time, and its relationship with the latent period for primary infections of stripe, leaf, and stem rusts is illustrated in Figure 3.4.

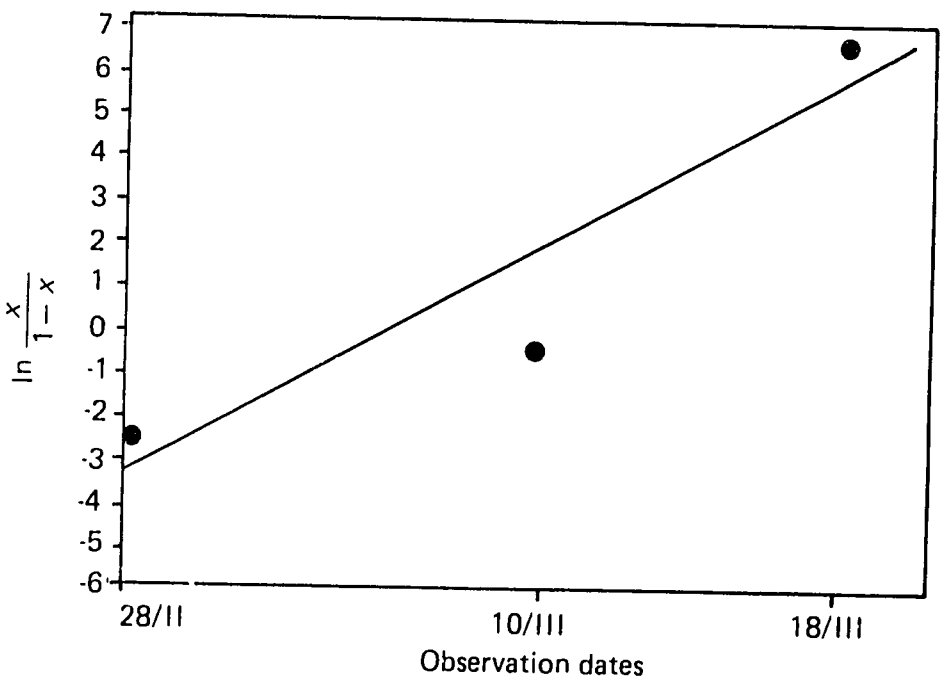
As constantly optimum temperatures do not occur in nature, the production of new pustules from initial infections generally takes between 10 and 14 days. If the spores produced by these pustules cause a re-infection every day thereafter, then secondary pustules will appear about 24 to 28 days after the 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 neighboring plants whenever environmental conditions are favorable. Pustules will, under good conditions, continue to produce spores for a period of two to three weeks.

The number of spores produced per uredium under field conditions varies among rust species and with ambient temperature. The data below indicate the approximate scale of stem rust spore production from a single pustule over an 11 day period and its relationship to temperature:

Temperature (°C)	Number of urediospores (thousands)
13	40
18	83
24	206
29	218

From Prabhu and Wallin 1971



Date:	28/II	10/III	18/III
Growth stage:	Heading	Flowering complete	Kernels half-formed
Percent rust on flag leaves (Cobb scale):	7.3	35	100
$\ln[x/(1-x)]$:	-2.54	-0.62	+6.9

Using the equation, $r = 1/18(6.9 + 2.54)$, the infection rate is 0.52 units per day.

Figure 3.3. Data and logit plot for the apparent infection rate of a leaf rust epidemic in Sonora, Mexico, 1976-77.

A brief summary of simple relations between environmental conditions and disease establishment is given for each of the major rusts in Table 3.1 (page 15).

3.5 Long Distance Transport of Disease Organisms

The important role of long distance transport in establishing infections in areas where the pathogen cannot survive from one season to the next is self-evident. The distance over which pathogenic inoculum can travel varies greatly among pathogens. The majority have only limited mobility, but 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 contaminated plant or soil debris. The large volume of plant material currently transported throughout the world thus presents the serious danger of simultaneously transporting pathogenic inoculum over large distances. 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, while simultaneously propitiating the spread of diseases.

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 effect of quarantine regulations for the control of such diseases. The effects of wind transport have been particularly well documented 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 1000 kilometers have been reported for spores of *Puccinia graminis* f. sp. *tritici*. Extensive travel can take place over a very short time period, given favorable conditions. In addition, movements may take place regularly, seasonally, 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 south-central USA to the northern United States and southern Canada. It is commonly known as the "Puccinia Pathway" and covers more than 3000 kilometers.

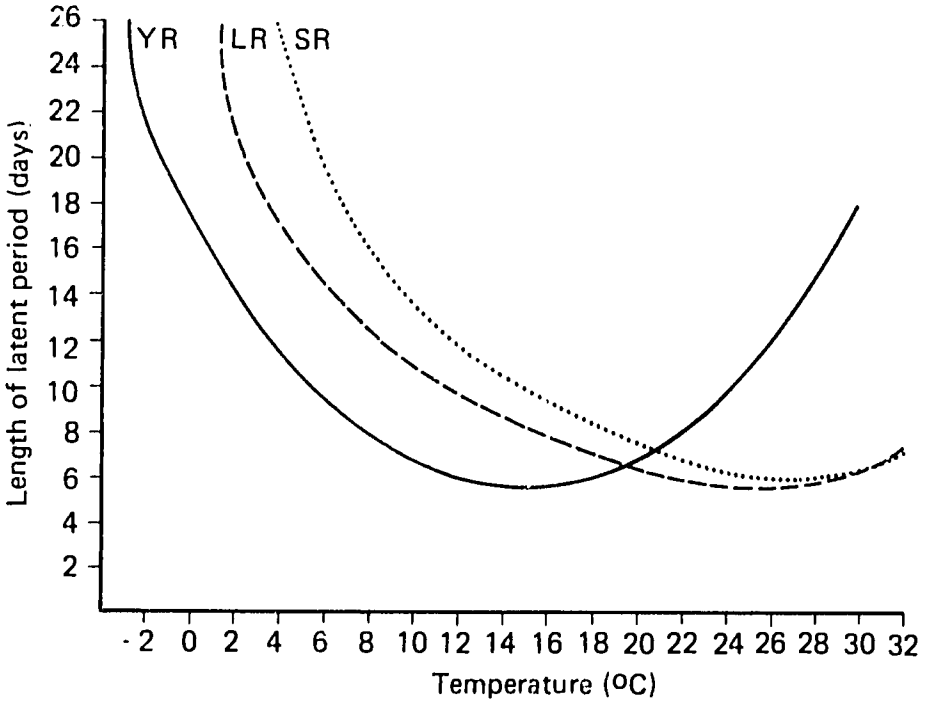


Figure 3.4. Latent period of primary infections of stripe (YR), leaf (LR) and stem (SR) rusts.

The migration usually involves a number of steps, but with severe infections in the south and two to three days of strong wind, the number of steps may be reduced, and the area and distance covered increased considerably. Movement is primarily in a northward direction, following the development of the wheat crop. 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 2500 kilometers of ocean. The recent introduction of stripe rust into Australia took less than two years to reach New Zealand. In the case of India, stem rust occurs all year 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 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 well 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 to 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 stem rust regularly travels over great distances; leaf rust and stripe rust tend to travel short distances only. However, as is illustrated by the Egyptian situation, leaf and stripe 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 stripe rust, capable of attacking hitherto resistant varieties of wheat (e.g., Mexipak), appears to have migrated in a stepwise fashion from Turkey and Bulgaria across to India over a three-year period. Similarly, a new and virulent race of barley stripe rust, first recorded in Colombia in 1975, had spread to Ecuador by 1976, to southern Peru by 1977, to Bolivia by 1978, and to Chile by late 1980. The distance from Colombia to Chile is about 4500 km. Such a rapid spread of virulent strains creates particular problems for the widespread use of genetically similar crop varieties.

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 meters. 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 that 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 the new stripe rust race detailed earlier. Finally, as 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. Because 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 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.

4. Surveying Plant Diseases

Disease surveying is basic to all effective 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 while research is in progress as a means of assessing the effectiveness of control measures.

4.1 Basic Survey Techniques

Organizing surveys—Surveys may be made for either regulatory or nonregulatory purposes. Regulatory surveys usually aim to delimit known infestations and to follow the spread of new ones (often for plant quarantine purposes), whereas nonregulatory surveys are primarily geared towards the assessment of actual disease levels (frequently for planning 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, mode of dispersal, etc.), the host (its stage of maturity, defense 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 among large numbers of sites) or stationary units (e.g., trap nurseries, which may have a wide geographic distribution). Each system has its advantages and disadvantages and the 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 carry out and the less reliable the data collected will be. 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 within a population and using them to estimate its level. The sampling procedures normally used are of four types:

- Random sampling (e.g., appraising fields at every tenth kilometer as indicated by the car odometer),
- 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 ten times that of barley), and
- Purposive sampling (e.g., appraising only the fields of growers producing certified seed).

Although the other methods may be used in 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, or 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 biased 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 delineated 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, nonrandom sampling is desirable. This is particularly applicable when surveying for new pathogen races in a field sown to resistant varieties or in a nursery in which resistant varieties are incorporated.

When making estimates of disease occurrence 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 examined. Disease frequency per unit area may thus be measured. Meter-row counts involve the sampling of measured lengths of crop rows, also at randomly selected locations in a plot. A knowledge of the number of crop rows and row length in a given area will 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 complete (all of the leaves) or only partial (for example, the flag leaf or the flag leaf 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 in crops grown in areas of uniform 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 determining the intensity of sampling required and the accuracy 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.

4.2 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 nonexistent (host immune) to the maximum expression of pathogen reproduction (host highly susceptible). Together with disease severity (the amount 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 and is 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 describe infection types and to quantify 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 saving time.

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, known as basic scales, 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 percent may in fact represent an actual infection of 7.4 percent). Such a difference depends upon the subdivisions of the particular percentage scale and the infection levels to which it refers. Percentages scales may also be transformed into coded linear scales for ease of recording. An example of this is the international stripe rust scale in which the scale numbers 1-10 refer to 0.001, 0.01, 0.1, 1 (trace), 5, 10, 20, 40, 60, and 100 percent, respectively. This is often called the 1 to 10 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 that illustrated in Figure 1.4, page 6). This will enable meaningful comparisons to be made with other varieties and among locations and years.

4.3 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 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* f. sp. *tritici* in wheat seedlings were first described by Stalkman and Levine. These have been suitably adapted to form the basis for assessment of other cereal rusts. Infection types applicable to stem rust in wheat are given in Table 2.4 (page 10).

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), which tend to effectively limit fungal growth by cutting off the source of nutrients, are seldom produced. In general the production of necrotic flecks is characteristic of some level of resistance (usually known as hypersensitivity).

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 leaf area between individual plants. Diagrammatic scales are thus essential aids to field assessment 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—0, 1, 5, 10, and 50 percent 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 assessment of cereal rust intensities throughout the world. It classifies rusted plants into six categories and takes an actual 37 percent of the leaf area covered by pustules to represent 100 percent infection. This relationship is based upon the fact that mycelial development is always more extensive than that of the 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 related in this way and a new diagram (representing 65 percent infection) was 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 combinations of pustule size and distribution (Figure 4.1). Such a scale permits considerably greater objectivity and accuracy to be achieved.

Detailed outlines for recording stem, leaf and crown rust intensities in cereals, based upon severity (percentage of the plant infected) and response (type of disease reaction), have been developed by the U.S. Department of Agriculture for use with their international rust nurseries. They use the following system:

- Severity is recorded as a percentage, according to the modified Cobb scale. As this relies upon observation it cannot be absolutely accurate. Thus it is common to use the following intervals: Trace, 5, 10, 20, 40, 60, and 100 percent infection.

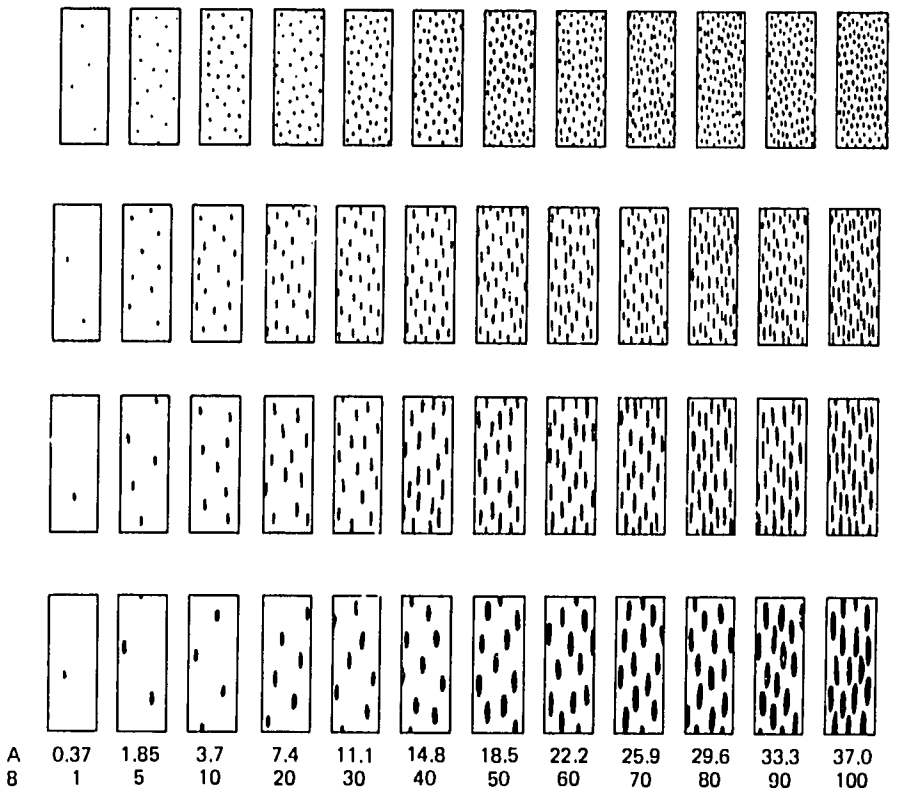


Figure 4.1. Diagrams illustrating the degrees of rust severity when the uredia are of different sizes; A is the actual percentage of the surface covered by lesions and B is the visual percentage (from Peterson, *et al.* 1948).

- Response refers to the infection type and is classified according to 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

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

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

  S—Susceptible; large pustules, no necrosis or chlorosis

Severity and response readings are usually combined, for example:

tR = trace severity of a resistant type infection

5MR = 5 percent severity of a moderately resistant type

60S = 60 percent severity of a susceptible type

It appears that there may occasionally be obvious variation in disease reaction among 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), or
- A range of reactions on each plant (plant 1: 10R-S; plant 2: 20MR).

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 "M" reaction of the variety.

An additional practical method of disease asessment is provided by a manual prepared by 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 (Figures 4.2 and 4.3).

4.4 Average Coefficient of Infection (ACI)

CIMMYT has found the ACI to be a useful method for ranking or rating varieties. This method of analysis was developed for the International Rust Nurseries distributed by the U.S. Department of Agriculture. In brief, the field scores for the three rusts are recorded in the classical manner, giving the severity on the modified Cobb scale, along with the field response. These scores are then converted to a coefficient of infection by multiplying severity by an assigned constant value for the field response. The following field responses have been assigned the constant values shown below:

Field response	Symbol	Constant value
Resistant	R	0.2
Moderately Resistant	MR	0.4
Intermediate or M	M	0.6
Moderately Susceptible	MS	0.8
Susceptible	S	1.0

Applying this to the scores noted for a variety at four different locations, the calculations are as shown in Table 4.1.

4.5 Recording Other Cereal Diseases

Other pictorial or diagrammatic scales have been developed for scoring both foliar and ear diseases of cereals.

One that is particularly useful is the foliar scale developed by Saari and Prescott (1975) for recording infections of powdery mildew (*Erysiphe graminis*), helminthosporium and alternaria blights, and septoria leaf blotch. The basic scale, illustrated in Figure 4.4 and its key (page 24) is applied to the whole plant and hinges on the value of 5, which has been defined as the midpoint of the plant.

Table 4.1. Calculating coefficients of infection

Location	Response	Severity x constant	Coefficient of infection
1	R*	1 x 0.2	0.2
2	5MR	5 x 0.4	2.0
3	10MS	10 x 0.8	8.0
4	20S	20 x 1.0	20.0

Average (ACI) = (0.2 + 2.0 + 8.0 + 20.0)/ 4 = 7.6

* In case of trace infection (R), a severity of one percent is assigned for convenience.

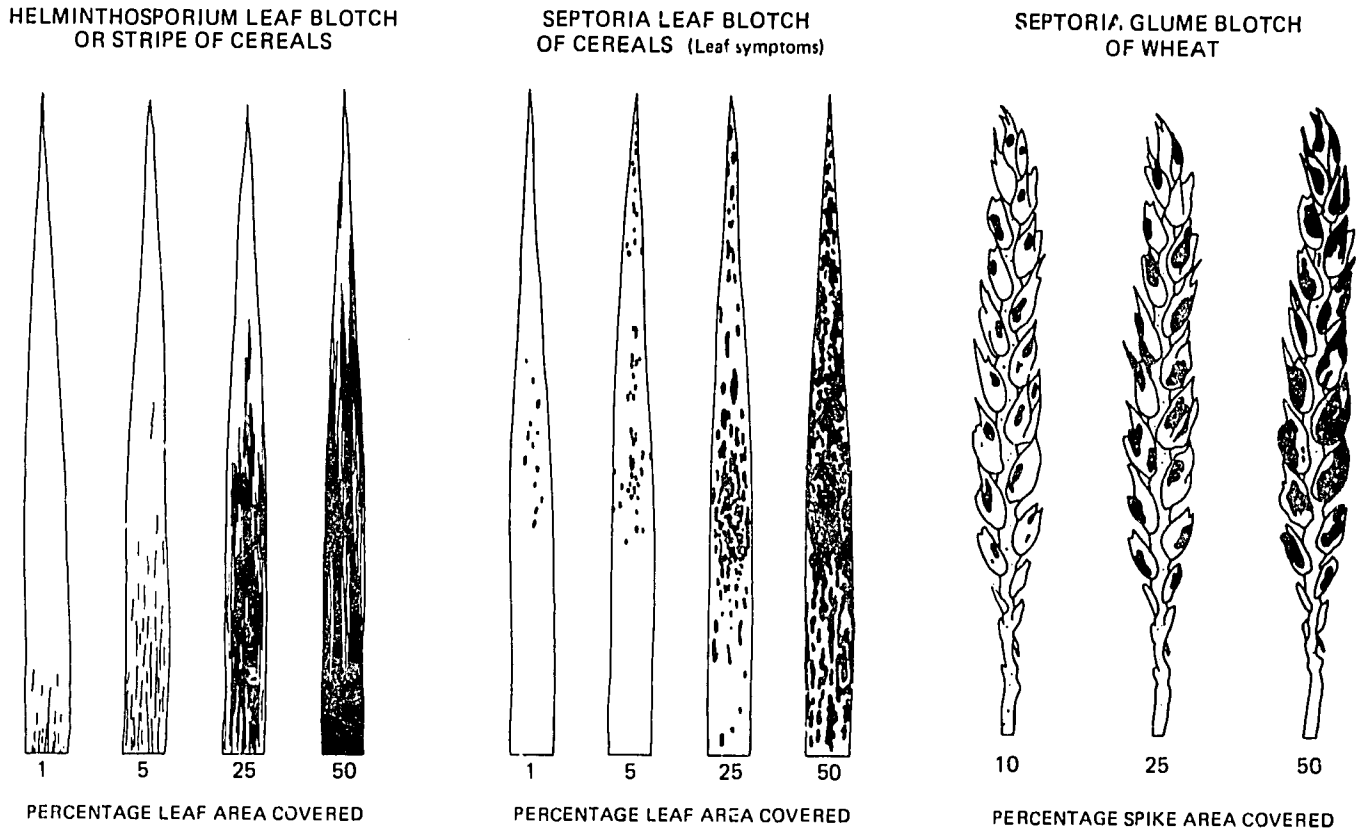


Figure 4.2. Diagrammatic scales for assessing the intensity of various cereal diseases (from James 1971).

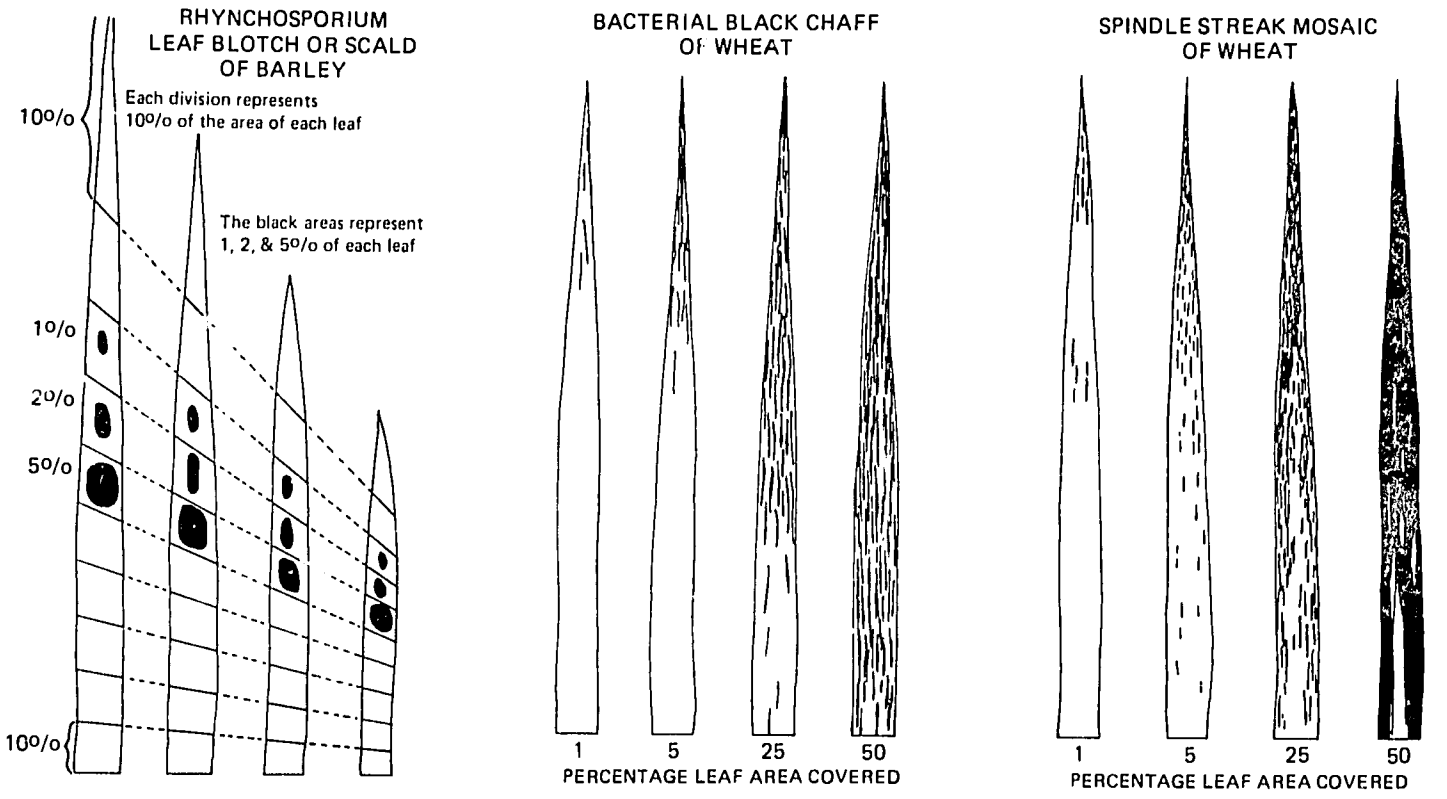


Figure 4.3. Diagrammatic scales for assessing the intensity of various cereal diseases (from James 1971).

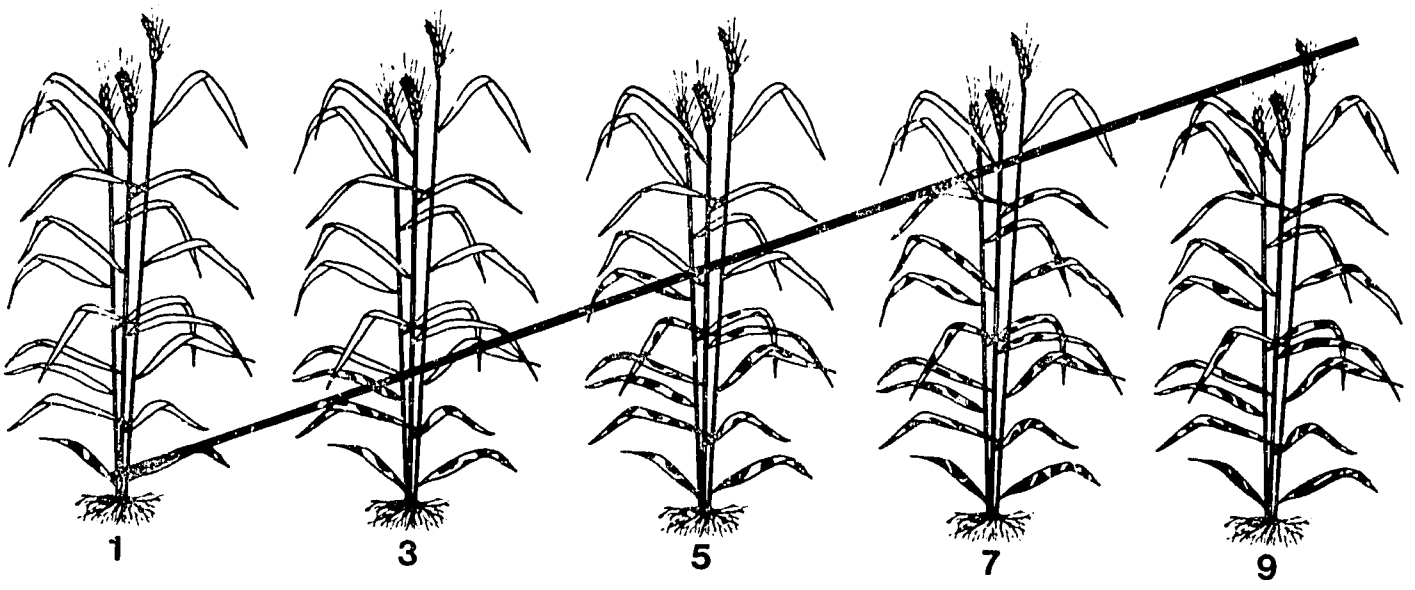


Figure 4.4. Scale for appraising the intensity of foliar diseases in wheat and barley (Saari and Prescott 1975).

Key to Figure 4.4. Descriptions of severity levels

0 **Free from infection.**

OE **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 middle of the plant.

5 **Moderately susceptible:** Severe infection of lower leaves; moderate to light infection extending only to the middle of the plant.

6 **Moderately susceptible:** Severe infection on lower third of plant moderate on middle leaves and scattered lesions beyond the middle of the plant.

7 **Susceptible:** Lesions severe on lower and middle leaves with infection extending to the leaf below the flag leaf, or with trace infection 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 on all leaves; spike also infected to some degree. [Spike infection is scored on a modified scale based on the percentage of the total area covered; the percentage figure follows the numerical leaf infection score and is separated by a slash (/)].

N **No scoring possible** due to necrosis as a result of other disease factors.

From Saari and Prescott 1975.

Part III. Developing Disease-Resistant Varieties

5. 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 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 that involve a modification of the environment, making it less suitable for the pathogen (e.g., the use of toxic chemicals, modified agronomic practices, etc.), and 2) those that involve alterations in 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 of these approaches, based upon an understanding of the host, the pathogen and their interactions.

Studies of the interactions between cereal rusts and their hosts indicate a very close relationship between the genetics of the pathogen and of the host in the expression of disease. Thus there would appear to be 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 on this background of strong resistance.

5.1 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 that has the ability to cause a particular disease in a number of ways:

- *Susceptibility*: Infection results in rapid disease development, spread within the host tissues and appreciable yield reductions.
- *Tolerance*: Different plants with the same apparent levels of infection are affected differently so that some survive to produce considerably higher yields than others.
- *Resistance*: The pathogen is unable to colonize the host, or its growth and development are restricted so that damage is reduced.
- *Immunity*: No observable signs of disease are apparent. [Care should be taken not to confuse this with *escape* (inadequate exposure to the pathogen)].

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 pathogen population. This often results in the very rapid appearance of new biotypes that are able to overcome the frequently rather specific resistance barriers.

Tolerance—Studies conducted on hybrid wheat families have indicated that with levels of infection between 65 and 100 percent, some families suffered yield reductions of about 44.5 percent, whereas others only lost 9.5 percent 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 subject of tolerance, and the mechanisms conferring it are not well understood. For this reason, variety 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 to 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 nonspecific.

Vertical resistance, also known as perpendicular, racial or specific resistance, occurs 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 the particular resistance are still able to colonize the cultivar, vertical resistance does not serve to reduce either the infection or the spore production rates of these races or strains.

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. 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, the cereals have developed a multitude of mechanisms which reduce the number 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 (e.g., timing of stomatal closure), they constitute broadly based, rather horizontal types of resistance. In general, however, such passive defense mechanisms develop only as plants grow and mature. Thus, at early stages of plant development, most varieties possessing this type of resistance tend to be more susceptible to infection, a phenomenon which has given rise to the designation "mature plant resistance" as a specific type of resistance.

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 of resistance.

5.2 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 classified into two main groups: 1) *monogenic*, or resistance controlled by the inheritance of a single gene, and 2) *polygenic*, or resistance controlled by the inheritance of more than one gene.

If disease resistance is controlled by a single gene, the gene's effect is usually clear, and can be studied and detected relatively easily. In contrast, the inheritance of polygenes is normally not so clear, and it is often impossible to isolate the effects of particular genes or to estimate the number involved. This arises from the fact that polygenes tend to be additive in character, producing an increasing level of resistance with an increasing number of genes. 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, polygenic resistance might perhaps be better divided into two types, *oligogenic* (resistance determined by few genes) and *polygenic* (resistance determined by many genes). 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 with a large effect and, as a result, tends to be relatively easy to select and thus to breed for. In contrast, horizontal resistance, normally involving 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 of resistance. While such resistance is often very complete, it places appreciable selection pressure upon pathogen populations, and thus, pathogen 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 it necessitates the continual development of varieties with new resistance genes or gene combinations in order to keep ahead of the appearance of newly virulent pathogen races. Major gene resistance, however, remains of considerable importance in most crop improvement programs, and plant breeders are continually on the lookout for new sources of major genes.

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 increased stability. Increasing emphasis is being placed upon the development of methods for identifying useful levels of polygenic resistance.

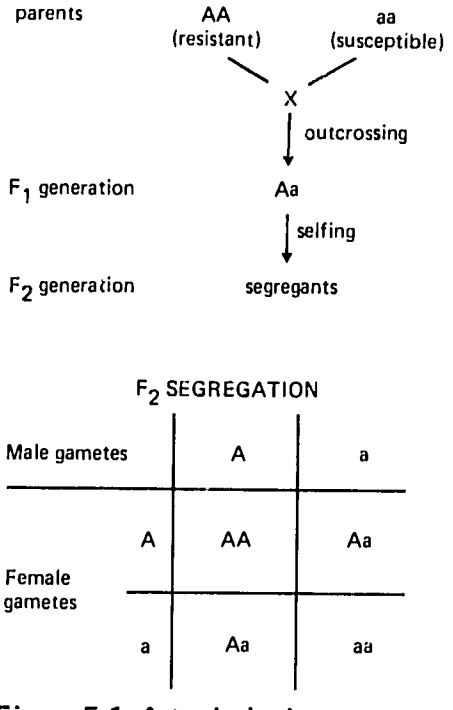


Figure 5.1. A typical scheme illustrating monogenic inheritance.

Inheritance patterns—A diagram illustrating the pattern of monogenic inheritance is presented in Figure 5.1. From that figure it can be seen that if resistance is inherited as a dominant character, all the F_1 individuals, although heterozygous, will be resistant. However, with segregation occurring in the F_2 generation, only three plants out of every four will be resistant:

1 *AA* (homozygous resistant)
2 *Aa* (heterozygous resistant)
1 *aa* (homozygous susceptible)

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.

In the case of two genes, and assuming that gene *A* confers resistance to one disease and is dominant and that gene *B* confers resistance to a second disease and also 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 genotypic and phenotypic ratios will be as in Figure 5.2. 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-pathogen interactions—If there are four different resistance genes (*A, B, C* and *D*) and six prevalent pathogen strains (1, 2, 3, 4, 5 and 6) a number of possible gene-pathogen interactions may result, three of which are shown in Table 5.1 (page 28).

5.3 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 and backcross breeding) or populations of plants (i.e., mass selection and bulk population breeding).

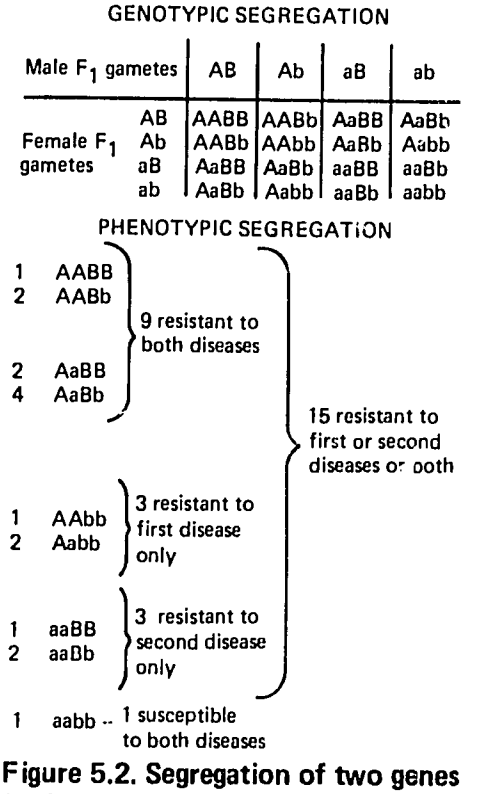


Figure 5.2. Segregation of two genes in the F_2 generation.

As the cereals in general and wheat in particular are predominantly self-pollinated crops, single-plant breeding strategies are used almost exclusively. These involve the exposure of a large number of individual lines to pathogen races in order to identify specific individuals with resistance. Suitably resistant individuals 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 lines and the lines stabilized by repeated cycles of selfing and selecting for the desired characters (leading to a rapid increase in homozygosity and 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 pathogen races necessitate field screening.

Pedigree breeding strategies—Pedigree breeding involves single, double or triple crosses followed by repeated selection cycles to eliminate undesirable individuals. The use of different rust races in successive testing cycles will permit the selection of varieties with a rather wider resistance. A typical pedigree scheme is illustrated in Figure 5.3.

As the cycles of selection and selfing progress, the degree of homozygosity rapidly increases as illustrated by Figure 5.4. In this example, if *A* is considered to be the resistant gene and is dominant, the percentage of homozygous, resistant plants increases from 33 percent to 60 percent if all susceptible types (*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 (produced by selfing the F_1), and then crossing again all those segregants showing disease resistance with the good genotype (or recurrent parent). This cycle of selection and backcrossing is carried out for several generations, after which the population is selected in the normal way for a combination of desired characters. The mechanics of this breeding strategy will vary considerably, depending upon the character(s) involved.

Multiline varieties—Also known as composite or artificial varieties, multilines may be successfully used to produce a commercial "variety"

Table 5.1. Three types of gene-pathogen interactions

Genes for resistance	Pathogen strain 1	2	3	4	5	6	Conclusion
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	
Host reaction	**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	
Host reaction	**R**	**R**	**R**	**R**	**R**	**R**	
3) A	S	R	S	R	S	R	No single gene or gene combination gives resistance to all pathogen strains
B	R	R	S	R	R	S	
C	R	S	S	S	R	S	
D	S	S	S	R	S	S	
Host reaction	**R**	**R**	**S**	**R**	**R**	**R**	

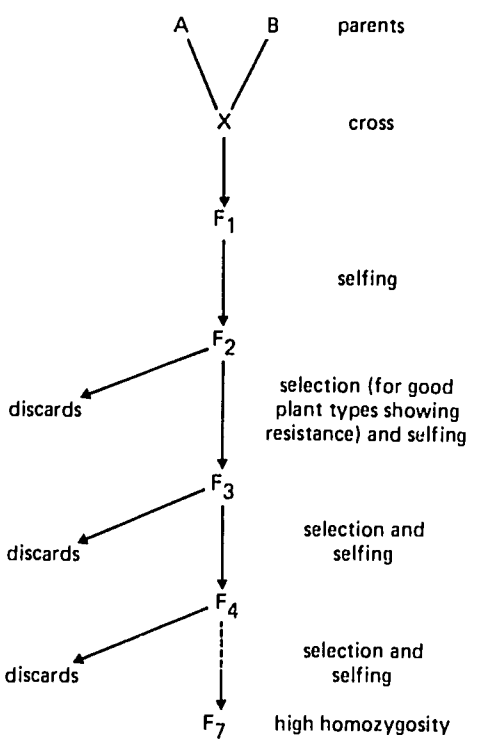


Figure 5.3. A scheme for a pedigree breeding program.

in cases where individual lines possess no genes or gene combinations conferring resistance to all the prevalent races of a disease. Such varieties are artificially produced by mixing seed of different lines, each with its own resistance genes. Thus, each individual is not itself resistant to the whole disease spectrum. However, when attacked by a given pathogen 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 multiline varieties demands considerable skill and a very good knowledge of the prevalent pathogen races. The components of such varieties require continual assessment and replacement to keep pace with the changing pathogen spectrum.

5.4 Breeding in Practice

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 oversimplified in order to underline the basic features of breeding strategy.

The importance of selection throughout all breeding programs cannot be overemphasized. 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 the indicator of the actual plant's 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 (i.e., not be affected by rust). Similarly, if the concentration of rust pathogens varies appreciably across a nursery, those 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 favor 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 paramount importance.

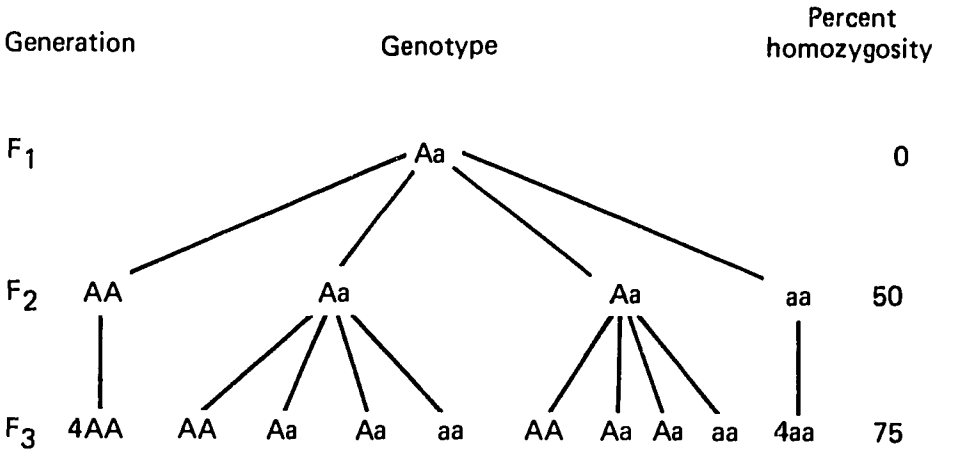


Figure 5.4. Increase in homozygosity with repeated selection.

6. Plant Pathology in Cereal Breeding

A good pathology input is fundamental to the whole effort to minimize disease loss and to 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 rely, in the first instance, upon accurate assessment of the importance and occurrence of specific pathogens in specific locations. In addition, plotting 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 risk 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 from place to place.

Without the continuous input of survey data, disease breeding programs would fast become obsolete. Extensive survey systems, such as the International Disease Trap Nursery (IDTN), 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 focused on the real problems.

Intensive pathogenic studies—The identification of physiological races and the factors affecting their interaction with their 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 solid physiological basis. Such studies will thus make a significant contribution to the evolution of breeding strategies in the future.

Nursery management—One of the most important roles for 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 be 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 connection, the various aspects of inoculum collection, multiplication, storage and application 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 (together with 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 the assesors 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.

6.1 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; 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.) that 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) the 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 very light selection pressure tend to be highly unreliable).

In general, disease nurseries should include susceptible check cultivars, sometimes 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 disease severity can be measured in cases of low disease incidence. The use of locally grown, susceptible cultivars as checks is highly recommended since agronomic characteristics can be compared at the same time.

It should be recognized that the inclusion of susceptible check cultivars has the disadvantage of causing interplot interference (i.e., the inoculum produced in the susceptible row will increase the amount of disease on adjacent entries). The nonuniform 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 on the identification of moderate levels of resistance.

6.2 Techniques for Enhancing and Creating Epidemics

Techniques designed to ensure the adequate and uniform development of disease epidemics so essential in

screening nurseries are primarily based upon ensuring sufficient levels of pathogenic inoculum, together with favorable 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 enviromental 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 always required.

The first consideration in this respect is the location of nurseries. By placing screening nurseries in areas where the disease incidence is known to be consistently 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 most diverse pathogen population possible, it is necessary to establish a number of nurseries over as large a geographic area as possible. This consideration is the rationale for the wide distribution of disease screening nurseries throughout a region.

Within the nursery itself, naturally occurring disease almost invariably appears at a few isolated foci within the susceptible check rows. Material 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 (preferably by sprinkler) 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 pathogen population over a large geographic area.

Creating artificial epidemics—Environmental conditions favorable for optimal disease development rarely occur every year, even at the most suitable locations. Furthermore, there may be great variation in the severity of individual diseases among locations and within one location in any year. Under these uncertain conditions, 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 of inoculum preserved from infections of the previous season. Techniques for inoculum collection, storage, multiplication and application 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 considerable danger of exposing the material to only a limited number of the prevalent pathogen races.

6.3 Justifying the Creation of Artificial Disease Epidemics

The whole concept of artificially creating disease epidemics has been, and continues to be, subject to continual and often unfairly harsh criticism from some people. Such criticism is, in general, based upon an overestimation of the dangers involved and little appreciation of the benefits that may accrue. It is thus worth considering both of 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 by 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 pathogen races capable of overcoming the 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 as a result of ineffective screening in the developmental stage. This means that breeding programs must continually be developing new

resistant varieties with an increasingly broad and stable type of resistance.

From previous considerations we have seen that the only way to ensure effective screening with consistently high and broad selection pressure 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 in 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 that are not as yet widespread. For this reason, there is often considerable reluctance to use new pathogen races in screening programs. However, the whole point of resistance screening is to identify sources of resistance to such races before they become widespread enough to constitute a major production threat.

In this context it is worth considering past experience 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 percent of the inoculum moves less 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 and transported longer distances. 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 pathogen build-up and further spread is extremely low.

Even given the exceedingly low risk of contamination from disease nurseries, what harm can some contamination actually do? Knowing how widely and rapidly new races of rusts can spread, it is clear that 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 from 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 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 greater than the potential losses which may arise from contamination of adjacent fields. It would be incorrect to restrict the use of such practices on the basis of the dangers involved. It should be emphasized, however, that every precaution must be taken in disease screening work to prevent contamination and, perhaps more important, every care should be taken to ensure that the precautions are noticed.

6.4 Some Important Technical Considerations in Creating Epidemics

Virulence spectrum—All pathogen populations include many physiologically different races or biotypes. As already mentioned, when using artificially created epidemics as a basis for selection, the risk of exposing the plant material to only a very small part of the pathogen's variation is considerable. In order to ensure 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 percent of the collections from a single variety include only one race, only 10 percent include two races and three races are found in only 5 percent of the samples. In addition the pathogen 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 order to 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 the inoculum may be broadened further by the multiplication of races with a low natural frequency of occurrence. This will guard against the possibility of 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 have shown that if a mixture of rust races, for example, is inoculated onto a single variety, one race will come to dominate after only a few pathogen generations and many other races will 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 known to be susceptible 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 bulk inoculum using a sprayer or duster. This metnod expqses all the genotypes in a nursery to the same inoculum mixture and is thus likely to result in more uniform selection pressure than can be achieved through spreader row inoculations only. It is, however, more time consuming—appreciably so when large nurseries are involved—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) and, considering that one rust infection can produce between 50,000 and 250,000 new spores, it is obvious that this extra generation will allow a considerble increase in disease severity.

It is also essential that inoculations coincide with environmental conditions favorable for infection. The first inoculation should 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 time 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 favorable field conditions, 10 to 14 days will be required, but the generation time may exceed three weeks if suboptimum temperatures prevail. If reactions are to be scored at the mid-dough stage of kernel development, the primary infections must be established at least five weeks before this stage and preferably earlier. In the case of stripe rust, areas with rapidly increasing spring temperatures will require the establishment of infections even earlier than this.

Thus, the time at which 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 earliest optimal weather conditions in order to maximize secondary disease spread. This will provide a continuous supply of inoculum for each favorable infection period and allow sufficient time for epidemics to develop to a level that will allow adequate and accurate screening.

Another important consideration is the number of times 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 essential for rust diseases.

Amount of inoculum—The quantity of spores required to inoculate a given area is not fixed. In general, the motto "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 1000 rust spores per plant. A gram of spores is estimated to contain 500 million individual cells. Thus a simple calculation tells us that about 2500 million spores, or 5 grams, will be needed for every hectare of nursery. The large number of spores per plant is necessary for several reasons: many spores will fall on bare ground and thus never reach the plant; many spores may be nonviable; many spores will be unable to germinate on the plant surfaces due to local micro-environmental conditions; many of the spores that do germinate will be unable to find stomata through which to penetrate. Hence the chances of a given viable spore

actually penetrating a plant and establishing an infection are low. These considerations make it obvious that no firm recommendation can be made to cover every situation, but experience suggests the use of about 5 grams of spores per hectare (diluted in talc, oil or water) as the minimum level, given reasonably favorable conditions and five inoculations.

6.5 Techniques for Rust Urediospore Collection

There are a number of ways by which spores of stripe 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 envelopes, pressed and left to dry. No more than six to eight 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 any method, it is essential that the material be adequately labelled and catalogued so that it is readily identifiable when required for inoculation.

6.6 Storing Rust Urediospores

As mentioned 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, regardless of environmental conditions, but the rate of loss of viability varies appreciably with storage conditions; spores can remain viable for periods of up to one year, given a suitable environment.

Of the four main factors affecting urediospore viability, temperature, moisture, light and atmospheric oxygen, the first two are the most important. The maintenance of sufficiently low temperatures and moisture levels are vital in spore storage, especially as, being single celled, they are extremely sensitive to environmental conditions. Examples of the effect of temperature and moisture content on the length of viable storage of stripe and stem rust spores are given in Tables 6.1 and 6.2.

Storage methods—Perhaps the simplest method of storing urediospores is to lower their moisture content by about 10 percent and maintain them at a temperature of between 2° and 4°C. This allows viability to be retained for between three and twelve months. In dry climates, the spores may be air dried for 24 to 36 hours; in more humid areas, a desiccator (with either calcium chloride or silica gel as the desiccant) may be required. Freshly collected urediospores will not dry adequately unless they are spread thinly on a plate, sheet of aluminium foil, or petri dish. It is also preferable that drying be carried out in the laboratory and away from direct sunlight. After

Table 6.1. Effect of temperature on the time vacuum-stored stripe rust spores can be kept without loss of viability

Temperature (°C)	Days stored
0	433
5	179
15	50

Table 6.2. Effect of relative humidity and temperature on the time stem rust spores can be stored without loss of viability

Relative humidity (percent)	Time (days)			
	5°C	10°C	15°C	20°C
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

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 6.2).

If the equipment is available, a more effective method of storage involves the evacuation of the storage container in order to remove atmospheric oxygen and thus lower spore respiration. Either a partial or a high vacuum system can be used. Under a partial vacuum system, the spores should be spread evenly and fairly thinly on a petri dish which is then placed in a desiccator with facilities for evacuation and sealing. A small air pump is used to create the partial vacuum and then the desiccator is sealed and placed in a darkened refrigerator at 2° to 4°C. Under these conditions the period of storage without loss of viability can be doubled.

If a high vacuum system is used the spores must be stored in narrow pyrex tubes (5 to 22 millimeters in diameter). About 5 milligrams of inoculum can be stored in each container. The atmospheric pressure should be lowered to approximately 0.1 millimeter of mercury (McLeod gauge) or 0.1 torr (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 the spores. The spores will remain viable for several years under conditions if stored at 2° to 4°C.

Dried or vacuum-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 permit adequate rehydration. The effects of rehydration are illustrated in Table 6.3.

Preservation of urediospores for longer periods of time can be achieved by placing them in high quality glass vials or in polyethylene strips and storing them in a liquid nitrogen refrigerator (-196°C). This ultralow temperature storage induces a domancy in the urediospores. Restoration of high germinability can be effected by thawing or heat shocking the urediospores in a water bath for 2 to 5 minutes at 40° to 45°C.

Summary of important steps in spore storage:

1) Use freshly collected spores.

2) Establish the germination percentage by experimentation.

3) Store, using one of the following methods:

   a) Air dry for 24 to 48 hours, place in sealed vial and refrigerate at 2° to 4°C or at -196°C in a liquid nitrogen refrigerator; or

   b) place in a desiccator with calcium chloride or silica gel in petri dishes or, if air dried, in open vials; seal the desiccator and store at 2° to 4°C; or

   c) place in a desiccator with a vacuum petcock as above, evacuate partially with a small air pump or water aspirator, and seal and store at 2° to 4°C; or

   d) place 5 milligrams of spores in a pyrex tube, plug the opening with cotton, and attach it to the manifold of a vacuum pump; operate the vacuum pump until the desired pressure is obtained; seal the tube using a gas-air torch, taking care not to heat the spores; and store under refrigeration at 2° to 4°C.

4) Rehydrate or apply heat shock (if stored in liquid nitrogen) before use.

6.7 Techniques for Multiplying Rust Inoculum

Field collections of rust spores may not always be sufficient, either in volume or in the relative proportions 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:

- *Without special treatment*: Traditionally, rust inoculum has been multiplied on seedlings grown in 10-centimeter clay pots in the greenhouse. Between 15 and 20 plants are grown in

Table 6.3. Effect of rehydration on the germination of air and vacuum-dried rust urediospores

Type of storage	Duration of storage (weeks)	Percent germination		
		Before storage	Dehydrated	After 24 hours' rehydration
Dry air	42	72	4	21
Vacuum	62	72	4	60

each pot. They are inoculated at the first leaf stage and the inoculum is collected when sporulation reaches its maximum, usually after 10 to 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-centimeter 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 useful only when small quantities of inoculum are required.

- *Using maleic hydrazide*: Maleic hydrazide can be effectively used to enhance multiplication of *Puccinia graminis*, *P. recondita*, *P. striiformis* and *P. hordei* on seedlings. A water solution of maleic hydrazine (40 to 100 parts per million) is applied to the soil when the primary leaf begins to emerge from the coleoptile. It has been shown that the growth response of seedlings to applications of this chemical (suppression of the second and third leaves and darker leaf pigmentation) is so closely related to the degree of pathogen sporulation that treated plants will yield between three and five times as much inoculum as untreated ones. By preventing the growth of other leaves, maleic hydrazide extends the life of the first 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 required. The procedure basically involves inoculating seedlings as above and then removing leaves as they show the first signs of infection (flecking). The cut ends of these leaves are then immediately placed in a solution of sucrose (1 gram per milliliter) and kinetin (40 parts per million) and/or benzimidazole (50 parts per million). The leaf cultures are kept in the laboratory or in a 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 in isolation to maintain purity.

Adult plant methods: Raising inoculum on susceptible adult plants has been found to be considerably faster and more efficient than the seedling methods. Adult plants are grown in 25-centimeter pots (each pot can support nine plants or approximately 30 tillers) and are inoculated at either the tillering or the booting 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, due to high capital and maintenance costs involved. Studies have shown that considerably cheaper plastic houses can be effectively be used in place of greenhouses if ventilation is adequate. Thus the multiplication of rust inoculum is possible even where physical facilities for research are somewhat lacking.

6.8 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° to 4°C) in the laboratory. Under these conditions, inoculum can be preserved in a viable state for use in the following season.

Inoculum of powdery mildew may also be multiplied in culture in the laboratory using the detached leaf method. This permits identification of pathogen 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 to 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 or defined media. In this way the inoculum can be 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 from infected plant material and stored dry under cold conditions, like 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 a short period of time at 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 propagated on artificial media.

6.9 Inoculation Techniques for Rust Diseases

Under greenhouse conditions—Plants to be inoculated 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 centimeters tall.

All surface inoculation should be preceded by gentle rubbing of the leaf surfaces with a moistened finger 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 misted with distilled water both before and after inoculation and then placed in a moist atmosphere for 24 to 48 hours, 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 pustules or storage containers with 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 at one end. The spores are picked up on the dry cotton, a small drop of water or light mineral oil is added and the cotton is rolled gently across the surface of the recipient seedling leaves.

- *By finger rubbing*: Similarly, clean fingers may 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.

- *By 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. The rusted plant is then brushed gently over the recipient plant surfaces several times to ensure adequate spore distribution.

- *Using a multiple inoculator*: The multiple inoculator was developed by M.B. Moore at the University of Minnesota (Browder, 1972). It consists of a piece of sheet metal fastened to one finger tab of a large spring clamp (the type used in offices to hold thick stacks of papers) in which several "fingers", each tipped with a small piece of foam rubber, are held. Spore suspensions (in water or mineral oil) are placed on the sponges, which are then carefully pressed against the leaf surface. In this way it is possible simultaneously to inoculate single leaves with several different races or species of pathogen fairly rapidly.

- *With a cyclone collector-duster*: Small cyclone collector-dusters have proved valuable in making inoculations when inoculum is scarce. A glass cyclone (about 8 centimeters in length and 2 centimeters in diameter) is filled with 0.5 gram of talc. Spores are sucked from the source into the cyclone. An additional 0.5 gram of talc is added and the

contents are 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 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, seedlings of some plants, such as barley, are rather sensitive to damage from mineral oils.

In the field—Inoculation methods on a field scale are rather different from 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 with the use of a spore-talc mixture. The ratio of spores to talc will depend upon factors such as 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 desiccation due to adverse environmental conditions are high. A spore suspension in water plus a surfactant is injected into the leaf sheaths, using a hypodermic syringe, at either the late tillering or 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 be 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 efficient.

- *Oil inoculation*: The feasibility of inoculating field plots with rust spores carried in nonphytotoxic oils was demonstrated by Rowell and Hayden (1956). They used Mobilsol 100, an isoparaffinic spray oil, and a spore concentration of 6 grams per 10 liters per hectare. 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 0.1 hectare plot could be completed in just 30 minutes. A battery-powered ultra low-volume sprayer (ULVA) can also be used. As the spores disperse readily and uniformly in oil, a relatively small spore concentration can be used and a good cover still be 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 and spreader row inoculation is used rather than whole nursery methods, it is possible to transplant material previously infected in a greenhouse into 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.

6.10 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 favoring infection (which differ among pathogens) and be carried out on several occasions if a high success rate is to be achieved.

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 produced by growing a susceptible variety in the same plot for several years and continually burying infected plants in order to build up a natural inoculum. Varieties to be tested may then be sown in the plot.

Artificial disease conditions may also be created by spraying the soil of a test plot with a spore suspension at the rate of 2 grams per liter of water per 5-meter row. The varieties to be tested are then sown and the soil turned over to mix seed and spores.

- *Helminthosporium diseases*: Considerable difficulty is 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 applied by spraying a suspension of spores in water with a wetting agent and surfactant.

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

- *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* f. sp. *tritici*. The most common of these are detailed below:

  a) *Partial vacuum method*—An apparatus was devised by Moore (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 device operates by alternately applying and releasing a partial vacuum 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 dispersing the spores from one smutted head in 100 milliliters of water with 1 gram of dextrose, is injected into the two main florets of each spikelet with a hypodermic syringe. Great care must be taken not to injure the developing ovary.

  c) *Needle method*—Dry spores may be blown (puffed) onto the stigmas of the developing ovaries using a hypodermic needle attached to a rubber bulb reservior. 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 to pierce the developing floret and deposit them on the stigma.

  e) *Dry twist method*—Spikes to be inoculated are trimmed or cut in a way similar to preparing a spike to be the pollen source in making a cross. The spike is them enclosed in a glassine pollination bag. When ready to inoculate, the bag is opened at one end and a smutted spike is inserted and twisted around to dislodge the smut spores. The smutted spike is them removed and the bag closed. A label or tag is attached to the plant giving the inoculation date and other pertinent information.

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

- *Karnal or partial bunt*: Moore's vacuum method can be used inoculate wheat plants with this disease. However, recent experiments at CIMMYT have shown that injection of a water suspension of secondary sporidia into the boot is a much more efficient means of inoculation. Alternatively, whenever the relative humidity can be maintained at very high levels for at least 12 hours, a simple spray application of a water suspension of primary and secondary sporidia can be the most effective means of inoculation. The time to spray-inoculate wheat plants is between head emergence and flowering.

- *Stinking smut or bunt*: Bunt pathogens are carried naturally on the outer seed coat and infect 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 percent of the seed weight.

Literature Cited

Browder, L.E. 1972. A multi-culture inoculation system for host-parasite relationships. Plant Dis. Reporter 56:847-849.

Chester, K.S. 1946. The Nature and Prevention of Cereal Rusts as Examplified in the Leaf Rust of Wheat. Waltham, Massachusetts, USA: The Chronica Botanica Co.

Hassebrauk, K. and J. Schroeder. 1966. Studies on the germination of yellow rust urediospores. Pp. 12-18 *in* R.C.F. Macer and M.S. Wolfe, eds. Cereal Rust Conferences, 1964, Cambridge. Cambridge, England: Plant Breeding Institute.

Hogg, W.H., C.E. Hounam, A.K. Malik and J.C. Zadoks. 1969. Meteorological factors affecting the epidemiology of wheat rusts. World Meteorological Organization Tech. Note No. 99.

Isaac, E. 1970. Geography of Domestication. New York: Prentice Hall, Inc.

James, W.C. 1971. An illustrated series of assessment keys for plant diseases: their preparation and usage. Canad. Pl. Dis. Survey 51:39-65.

Loegering, W.Q., C.O. Johnston and J.W. Hendrix. 1967. Wheat rusts. Pp. 307-335 *in* K.S. Quisenberry and L.P. Reitz, eds. Wheat and Wheat Improvement. Madison, Wisconsin, USA: American Society of Agronomy.

Maddox, F. 1895. Experiments at East Field: smut, bunt, rust. Tasmania: Report of the Department of Agriculture.

Martin, J.H., W.H. Leonard and D.L. Stamp. 1976. Principles of Field Crop Production. New York: McMillan Publ. Co.

Mathre, D.E., editor. 1982. Compendium of Barley Diseases. St. Paul, Minnesota, USA: American Phytopathology Society.

McNeal, E.H., C.S. Konzak, E.P. Smith, W.S. Tate and T.S. Russell. 1971. A uniform system for recording and processing cereal research data. Agricultural Research Service Publ. ARS 34-121. Washington, D.C: U.S. Dept. of Agriculture.

Moore, M.B. 1936. A partial-vacuum method for the inoculation of wheat and barley with loose smuts. (Abstract). Phytopathology 26:103.

Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Canad. J. Res. C-26: 496-500.

Prabhu, A.S. and J.R. Wallin. 1971. Cyclical variations in spore production by *Puccinia graminis tritici* (Pers.) Erikss. and Henn. (Abstract). Page 45 *in* Proceedings of a Symposium on Epidemiology, Forecasting and Control of Plant Diseases. New Delhi, India: Indian National Science Academy.

Rowell, J.B. 1984. Controlled infection of *Puccinia graminis* f.sp. *tritici* under artificial conditions. Pp. 292-332 *in* W.R. Bushnell and A.P. Roelfs, eds. The Cereal Rusts. Vol. 1. New York: Academic Press.

Rowell, J.B. and E.B. Hayden. 1956. Mineral oils as carriers of urediospores of the stem rust fungus for inoculating field grown wheat. Phytopathology 46:267-268.

Saari, E.E. and J.M. Prescott. 1975. A scale for appraising the foliar intensity of wheat diseases. Plant. Dis. Reporter 59:377-380.

Sharp, E.L. 1964. Some effects of environment on the infection process of *Puccinia striiformis* West. Pp. 32-38 *in* R.F.C. Macer and M.S. Wolfe, eds. Cereal Rust Conferences 1964, Cambridge. Cambridge, England: Plant Breeding Institute.

Stakman. E.C., D.M. Stewart and W.Q. Loegering. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agricultural Research Service Tech. Bull. No. E-617. Washington, D.C: U.S. Dept. Agriculture.

Staples, R. and V. Macke. 1984. Germination of urediospores and differentiation of infection structures. Pp. 225-229 *in* W.R. Bushnell and A.P. Roelfs, eds. The Cereal Rusts. Vol. 1. New York: Academic Press.

Togashi, K. 1949. Biological Characters of Plant Pathogens—Temperature Relations. Tokyo: Meibundo.

Tottman, D.R. and R.J. Makepeace. 1979. An explanation of the decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol. 93:221-234.

Wiese, M.V., editor. 1977. Compendium of Wheat Diseases. St. Paul, Minnesota, USA: American Phytopathology Society.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14:415-421.

Zillinsky, F.J. 1983. Common Diseases of Small Grain Cereals. Mexico City, Mexico: Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

Appendices

Appendix 1. Characteristics 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 have a strong pathology research input on a regular basis. In turn, a relevant and functional pathology input must be based upon quick and accurate recognition and identification of diseases in the field to enable appropriate research activities to be initiated.

A1.1 Fungal Diseases

Stem rust—Stem rust of wheat, also known as black rust, is cosmopolitan in distribution, being found in all locations occupied by wheat, its close relatives, and other grasses. Of the many diseases infecting wheat, stem rust is without doubt potentially the most devastating. 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, the leaves, leaf sheaths, stems and spikes. Severe infections result in considerable yield loss, 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* 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 loss through reduction in kernel number and size may be very serious. Some races have been found to attack barley crops.

Stripe rust—Also known as yellow rust, this disease is caused by the fungus *Puccinia striiformis* and occurs in many regions of the world. However, unlike the other rust diseases, it is generally confined to the cooler higher elevations and higher latitudes. The characteristic symptom of stripe 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 reducing the number of spikes per plant, the number of kernels per spike, and the size and weight of the kernels. Heavy infection results in severe kernel shriveling and reduced root development.

Septoria tritici leaf blotch—Caused by the fungus *Mycosphaerella graminicola* (*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. The disease can be found on all above-ground parts of the plant. Severe attacks cause a reduction in both the number and the size of kernels and may also cause seed shrivelling.

Septoria nodorum glume blotch—This disease is best known in its imperfect form as *Septoria nodorum*; its perfect or sexual form is named *Leptosphaeria nodorum*. Disease symptoms are typically found on the glumes, commonly on the nodes, leaves and leaf sheaths, and occasionally on the stems. They are similar to, but somewhat darker in color than, the lesions caused by *Septoria tritici*. Yield reduction from infections of glume blotch is caused primarily by reduction in the size and weight of kernels, and may be accompanied by shrivelling.

Septoria avenae leaf blotch—The fungus *Leptosphaeria avenaria* f. sp. *triticea*, commonly known in its imperfect form *Septoria avenae* f. sp. *triticea*, is the causal organism of this disease. It appears to be limited in occurrence to North and South America and northern Europe and produces symptoms similar to 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 subhumid wheat-growing regions of the world and is favored by mild winters followed by cool, cloudy spring and summer weather. Infections, which appear as grayish, 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 sexual bodies (cleistothecia) develop in the cottony masses. Heavy attacks of powdery mildew result in yield losses through reduced kernel size, which may be particularly severe in early infections.

Leaf blotch—Widespread in the wheat-growing regions of the world, this disease is caused by the fungus *Cochiobolus sativus* (*Helminthosporium sativum*).

Infection can be found on all parts of the plant, as indicated by the common names root rot, crown rot, leaf blotch, head blight or black point. The main symptom of the disease (leaf blotch) is dark brown spots, each surrounded by a chlorotic margin. As the disease develops, the leaves turn completely brown and die; severe infestations may result in total plant death.

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

Leaf blight—This disease, caused by the pathogen *Alternaria triticina*, has been reported only from the Indian subcontinent. It is characterized by irregular, green to gray, oval lesions on the leaves. These lesions have yellow-green margins and in severe infections coalesce, causing necrosis, the death of leaves and eventually of the whole plant.

Flag smut—Although only severe on a local basis, flag smut, caused by *Urocystis agropyri*, has a worldwide distribution. In contrast to the other smut diseases, infections are confined to the leaves and stems where they appear as long, gray to black stripes containing numerous black spores. Infection causes 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 evolves into chlorotic leaf stripes coupled with a brown discoloration of the vascular tissue at about heading time. Infected plants are stunted, and most die prematurely with white heads or poorly filled grains.

Loose smut—This disease occurs wherever wheat is grown, but is most common in humid, subhumid, 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 various degrees 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 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, higher elevation or higher latitude wheat-producing areas. It is caused by *Tilletia controversa* and, like 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 *Tilletia indica* (also known as *Neovossia indica*), this disease is found mainly on the Indian subcontinent and in Mexico, 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 the heading to flowering period and is usually confined to only a few kernels in each head, which can be 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* (*Giberella zeae*), *F. culmorum*, and *F. nivale* are commonly associated with the disease, which causes individual spikelets, florets or entire heads to turn white. This is often accompanied by the production of an external, white to pink fungal mat on the surfaces of the glumes and by 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, 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 are poisonous when consumed by humans or animals.

Common root or foot rot—Several different pathogens are associated with this disease complex. The most important are *Helminthosporium sativum*, which also causes a leaf spot and black point of the seed, *Fusarium culmorum* and *F. graminearum*, both of which also cause head scab. This disease complex is cosmopolitan in its distribution although it is more serious in drier areas.

Infected seedlings appear blighted and have brown, rotted roots and subcrown internodes. Infection in more mature plants is 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 interferes with nitrogen utilization, this may lead to increased incidence of "yellow berry."

Take all—This disease, caused by *Gaeumannomyces graminis* f.sp. *tritici* (previously known as *Ophiobolus graminis*), has a worldwide distribution. Although primarily a disease of winter wheat, it may also infect spring crops. Infected plants appear stunted and bleached, with a black discoloration 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 *Pseudocercosporella herpotrichoides*, is found in most winter wheat-growing regions of the world. It is characterized by the appearance of oval or elliptical lesions, usually with dull, brown-green margins and often marked with 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. The disease also causes the production of premature and/or empty, white heads.

Sharp eye-spot—This disease is widely distributed throughout the world, but is generally considered to be relatively minor. 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 distinctive brown, water-soaked 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 *Sclerophthora macrospora* (*Sclerospora macrospora*) and is widely distributed wherever wheat is grown. However, it is severe only 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 *Calonectria nivalis* (*Fusarium nivale*, pink mold) and *Typhula idahoensis* (speckled snow mold). Although it found throughout the world, this disease is considered to be of only minor importance.

Snow molds are must prevalent in areas with harsh winter conditions, where they become apparent as the snow cover recedes. In the case of pink mold the abundant fungal growth on the surface of rotting plant tissue has a pink coloration and the disease appears in the early spring. Infections of speckled mold development slightly later in the season and are characterized by numerous black sclerotia in the mold tissue. Damage from the infection may be severe under certain conditions and ranges from complete loss to meager grain yield, if the infected plants recover at all.

Black point—*Helminthosporium sativum*, together with several species of *Fusarium* and *Alternaria*, especially *A. alternata*, 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.

Helminthosporium 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.

A1.2 Bacterial Diseases

Black Chaff—Although occurring throughout the world on wheat plants, this disease, caused by the bacteria *Xanthomonas campestris* pv. *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 *Aguina tritici* and has been reported from a number of different areas (e.g., 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 syringae* pv. *atrofaciens*, this disease occurs in wheat crops throughout the world, especially when high moisture conditions prevail at the time of heading. The germ ends of infected kernels become blackened at the tip and water-soaked lesions appear at the bases of the glumes. Yield loss are rarely severe.

A1.3 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 early spring. The symptoms vary with the strain of virus, ranging from rosetted plants with blue-green leaves mottled with white 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 the fungus *Polymyxa graminis*. It 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, yellowing and necrotic blotching of the leaves, plant stunting, reduced head size and shriveling of kernels. Barley, oats and number of other 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 streaks and mottling ranging in color from light green (mild infections) to yellow and chlorotic (severe infection). 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 other annual grasses.

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

Barley yellow dwarf virus—BYDV has a 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 a lesser effect, only causing leaf discoloration together with slight stunting. Most cereals and grasses are attacked by this disease.

A1.4 Diseases Caused by Nematodes

Root cysts—Root cysts are caused by 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 deformation in the form of numerous gall-like cysts.

Ear cockle—Caused by *Anguina tritici*, also known as the wheat nematode, this disease also has a low incidence but a 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 (containing 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.

Appendix 2. Characteristics of Barley Diseases

Although diseases tend to be more of a production problem in wheat, 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 increased world barley production.

A2.1 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 Peninsula. Like stripe rust of wheat, it tends to be confined to the cooler, higher elevation areas of these countries. Stripe rust produces symptoms and effects similar to those of wheat stripe rust.

Leaf rust—This disease is caused by the pathogen *Puccinia hordei*. It is widely distributed in 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 to oblong, dark brown, telial pustules develop. Yield losses result primarily from a reduction in kernel number and shrivelling.

A leaf rust with darker, brown-red pustules may also result from infections of *P. recondita* f. sp. *tritici*.

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

Powdery mildew—In contrast to stem rust, this disease, caused by infections of *Erysiphe graminis* f. sp. *hordei*, is of major importance wherever barley is produced. High humidity favors its development but powdery mildew causes 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 discolorations, which may indicate a type of resistance.

If the disease develops early (in the seedling stage), it may seriously reduce the root system and consequently have severe effects on 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—*Sclerophthora macrospora* (*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* (sometimes known by its perfect stage as *Pyrenophora teres*), may precipitate serious yield losses, especially in North Africa, Mediterranean Europe and the areas of West Asia around the Caspian Sea.

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 leaf blade, but they never extend to the leaf sheath. Additional lesions may develop on the lemmas, where they appear as a light brown discoloration rather than as the characteristic netting of leaf infections. Sometimes *H. teres* causes leaf spots similar to those of *H. sativum*.

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 *Cochiobolus sativus* (*Helminthosporium sativum*). It is widespread in the countries of North Africa, West Asia and Mediterranean Europe where its development and spread is favored by warm and moist conditions.

Seedling infections cause a blight which frequently results in pre- or post-emergence seedling death. Infections of mature plants appear as characteristic dark brown, round to oblong lesions which may coalesce to cover 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 results in serious crop losses only in certain areas (especially the low rainfall areas of the Near and Middle East). 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 affected areas become brown and may extend over all the leaves. Infected plants tend to be stunted and the leaves may split, fray or collapse. In severe infections the spike fails to emerge or, if it does emerge, tends to be blighted and brown in color.

Scald—This disease results from infections of the pathogen *Rhynchosporium secalis* and is of major importance in cooler humid and subhumid regions. It causes considerable yield losses in Afghanistan, Turkey, Ethiopia, Kenya, Tanzania and in many of the Mediterranean countries. It occurs on both winter and spring barleys, appearing first as irregular, blue-green, water-soaked lesions on the leaf blades and sheaths. As the disease progresses, these lesions develop into bleached areas with brown margins. Infections cause damage similar to that of net blotch.

Loose smut—*Ustilago nuda* is the causal organism of this disease, which is widespread in areas in which 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 fragile covering 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 semiloose 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 resemble those of loose smut, except that the spore mass tends to be more compact and enclosed by a loose, nonpersistent membrane. 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. Spore masses 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 identifiable 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* spp. 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 effects 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.

A2.2 Bacterial Diseases

Bacterial blight—This is the only bacterial disease of any importance occurring in barley. It is caused by *Xanthomonas campestris* pv. *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 their age. In the later stages of infection the centers of the lesions become translucent, and small drops of yellowish 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.

A2.3 Viral Diseases

In addition to the viral diseases discussed in relation to wheat, two additional 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 effect upon the host plant. It is both seed borne and pollen transmitted.

Cereal tillering virus (CTV)—This disease attacks barley exclusively, resulting in a variety of mosaic, mottling, yellowing and stunting symptoms.

A2.4 Diseases Caused by Nematodes

In general, barley is attacked by the same groups of nematodes that commonly infest wheat. 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 amount of work carried out to date on nematodes, particularly in dry climates, is limited. Their importance may thus be considerably greater than is currently supposed.