

A Survey of the Diseases of Wild Rice in Manitoba

by

David Andrew Ross McQueen

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
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ABSTRACT

During the summers of 1979 and 1980 natural stands of wild rice in Manitoba were surveyed for disease. Of the five disease syndromes identified; three, brown spot, leaf and stem smut, and ergot, were already described in the literature; the other two, anthracnose and leaf blotch, have not been reported previously. Fungi isolated from diseased tissues were identified and subjected to pathogenicity testing. In addition to Helminthosporium sativum and Fusarium spp. which are normally associated with brown spot syndrome, Dichotomophthoropsis sp. and Sclerotium hydrophilum were isolated and found to be pathogenic. Colletotrichum sp. and an identified pycnidial fungus were shown to be the causes of anthracnose and leaf blotch respectively. Microdochium sp. was also found to be associated with anthracnose and there is a strong likelihood that it is associated in some way with the disease.

Quantitative measurements of incidence and intensity revealed that although brown spot is widely distributed it posed no threat to production. The amount of anthracnose on floating leaves raised questions as to the vulnerability of this stage and what effect pathogenesis of floating leaves may have on the overall development of the plant.

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Chapter I

GENERAL INTRODUCTION

The unique and fascinating plant that has formed the centre of my thesis is Zizania aquatica L. or , as it is more commonly known, wild rice. Wild rice is a member of that small group of plants which did not require domestication to be of use to man. Wild rice grows in lush stands in eastern Manitoba and has provided a valuable source of nutrition to Canada's native peoples for centuries. Only recently has wild rice attracted the eye of the entrepreneur. The desire for higher and more stable yields has stimulated a greater interest in the biology of the plant.

Wild rice has evolved in a unique way that enables it to exploit a niche unsuitable for most other species of plants. It is an aquatic annual growing in depths of water unsuitable for all but a few competitors. To do this it produces a specialized leaf, the floating leaf, that grows to the surface and lies on the water. The floating leaf is thin and fragile and large amounts of carbohydrates probably are not required for its development. Therefore, it is able to reach from the bottom to the surface in waters as deep as six feet, with the seed as its main source of carbohydrates.

The floating leaf stage consists of a number of leaves and can last up to six weeks. The role of the floating leaf stage is to produce carbohydrates necessary for the development of the inflorescence. When conditions are satisfactory aerial leaves are formed and internodal elongation occurs. This is the aerial leaf stage. The plant next enters the reproductive phase.

The culture of wild rice is in a transition phase. Historically, the North American native peoples did not domesticate wild rice, as they did corn, but they knew where it grew and when it should be harvested. Today, wild stands still exist in their natural state and very few show any signs of domestication. However, in some areas, paddy culture used by the orientals in the culture of Oryza sativa have been adapted for the cultivation of wild rice. The paddy culture of wild rice is now common in the state of Minnesota and is becoming more and more specialized with the introduction of genetically improved varieties, chemicals, and sophisticated machinery. Therefore, it seems that the culture of wild rice has taken on a dual nature with two groups of people, each having their own unique background, exploiting this plant to suit their own particular needs. There are the people of the Manitoba and Ontario northlands who harvest the natural stands to supplement other sources of income, and there are those in the south, who are domesticating wild rice; looking to it as their sole source of income.

This thesis is concerned with the natural stands of wild rice in Manitoba. In the past, yields from the natural stands in this province have varied enormously. Wild rice has provided an erratic source of income to those who harvest it, and crop failures have resulted in considerable hardship for those already living at a subsistence level. The question remains as to the role of disease in the fluctuating yields of wild rice.

When studying the pathology of a plant there are three questions of a basic nature that should be asked. First, what diseases are present, secondly, what is the cause; and thirdly how much disease is present? The object of this study was to examine the natural stands of wild rice in Manitoba and attempt to provide suitable answers to these questions.

Chapter II

LITERATURE REVIEW

The discovery by Fyles(1915) of ergot bodies infecting the inflorescence of wild rice was the first significant contribution to the pathology of wild rice. Since then there has been a limited number of reports. The latest publication dealing with this subject was by Kernkamp, Kroll, and Woodruff (1976). They listed five diseases known to affect wild rice growing under paddy conditions in Minnesota: helminthosporium blight, stem rot, ergot, leaf and stem smut, and bacterial streak.

Helminthosporium blight was first identified by Preston and Dosdall (1955) on herbarium specimens at the University of Minnesota. An epidemic of brown spot was reported by Bean and Schwartz(1961) and they determined that the pathogen causing the disease was Helminthosporium oryzae.

Gilbert (1974) conducted the first extensive study of brown spot in Manitoba. He examined several stands, the most northerly being the experimental paddies at Fort Alexander. Other stands sampled were the commercial paddies at Brokenhead and Sprague and experimental paddies at Glenlea, as well as natural stands in the LaSalle R. and Lone Isle L.

There were two primary objectives in his study. First, to determine the range of symptom expression of brown spot and correlate lesion type with the causative pathogen and secondly to determine the effect of brown spot on the yield of wild rice.

During 1971 and 1972 Gilbert sampled the selected stands and damp-chambered the lesions found on leaves. He isolated any fungi that appeared on the damp-chambered leaves and later tested their pathogenicity by inoculating greenhouse and field grown plants, measuring the differences in yield between inoculated and uninoculated plants.

His pathogenicity tests showed that isolates identified at the Commonwealth Mycological Institute (CMI) as Drechslera bicolor (Mitra) Subram. and Jain were the most damaging to wild rice based on their ability to cause significant yield losses. However, it appears now that these isolates should be referred to the Drechslera state of Cochliobolus miyabeanus (Ito and Kuribayashi) Drechslera ex Dastur (= Helminthosporium oryzae). (Punter, personal communication).

Gilbert also isolated what he considered to be several other distinct "races" of the Drechslera state of Cochliobolus sativus (Ito and Kurabayashi) Drechslera ex Dastur = Helminthosporium sativum. These were also shown to be capable of causing significant loss to greenhouse and field grown plants.

Gilbert found that H. oryzae and H. sativum were not the only fungi contributing to the brown spot syndrome. He was able to isolate several morphologically distinct Fusarium species from necrotic spots and, although the Fusaria did not cause losses comparable to those of the Helminthosporium species, they were definitely pathogenic. Because of this Gilbert preferred the use of the name brown spot to describe the entire syndrome rather than the more specific 'Helminthosporium blight'.

Little information is available in the literature on the other three known diseases of wild rice. Stem rot was first reported by Morrison and King in 1971, but no estimate of severity was given.

The first report of leaf and stem smut was by Preston and Dosdall (1955) who identified Entyloma lineatum (Cke.) Davis on herbarium specimens at the University of Minnesota. Gilbert (1974) found E. lineatum to be only weakly pathogenic when inoculated on greenhouse grown wild rice. However Kernkamp, Kroll, and Woodruff (1976) suggested that even though smut does little damage, the striking differences in susceptibility among individual lines and selections indicated that susceptible lines should be avoided in developing cultivars of wild rice.

The early reports of ergot were mostly concerned with the taxonomy of the causative fungus Claviceps zizaniae (Fyl-

es, 1915; Pantidou, 1959). Connors (1939) indirectly reported an epidemic of ergot in Minnesota. He referred to the increased value of the crop in Manitoba due to a heavy infection of ergot in Minnesota.

Kernkamp, Kroll, and Woodruff (1976) isolated Xanthomonas translucens from elongate lesions stretching 1 to 2 centimeters along leaf veins. Reinfection experiments were successful.

There is no report in the literature of quantitative measurements of the diseases of wild rice apart from the statement that during the epidemics of brown spot, losses reached 100% (Bean and Schwartz, 1961).

Most of the information needed in relation to sampling techniques and disease measurement was taken from Chester (1950) who appears to have produced the only monograph devoted entirely to disease measurement. He reviewed all facets of disease measurement including sampling methods, estimation of severity and interpretation of severity measurements. Methods for measuring disease were first developed in the late 1800's by Cobb (1890). He provided a standardized key from which the percentage of the leaf area infected could be estimated. Since then there have been essentially no modifications to his method.

Chapter III

OCCURENCE AND CAUSES OF DISEASE

The first stage in elucidating the pathology of any plant is to describe the nature of the disease syndromes and their causative agents. In the following chapter this aspect of the pathology of wild rice will be discussed. Chapter IV will describe the amount of disease found to occur in the sampled stands.

3.1 DESCRIPTION OF THE SAMPLING RANGE

The sampling range extended from 49° N to 53° N and from 96° W to 100° W. The sample sites used in this study are presented on Figure 1.

For convenience the sample range has been divided into four zones. The Alluvial Zone is that area to the south-west of the Canadian Shield. The Whiteshell and Nopiming Zones are defined by the Whiteshell and Nopiming Provincial Parks. The Northern Zone is the area north of the Nopiming Provincial Park.

As would be expected there is a large amount of geographical and climatical variation within the sampling range. The most obvious physical differences relate to superficial geology (Figure 2).

FIGURE 1
Sample Locations

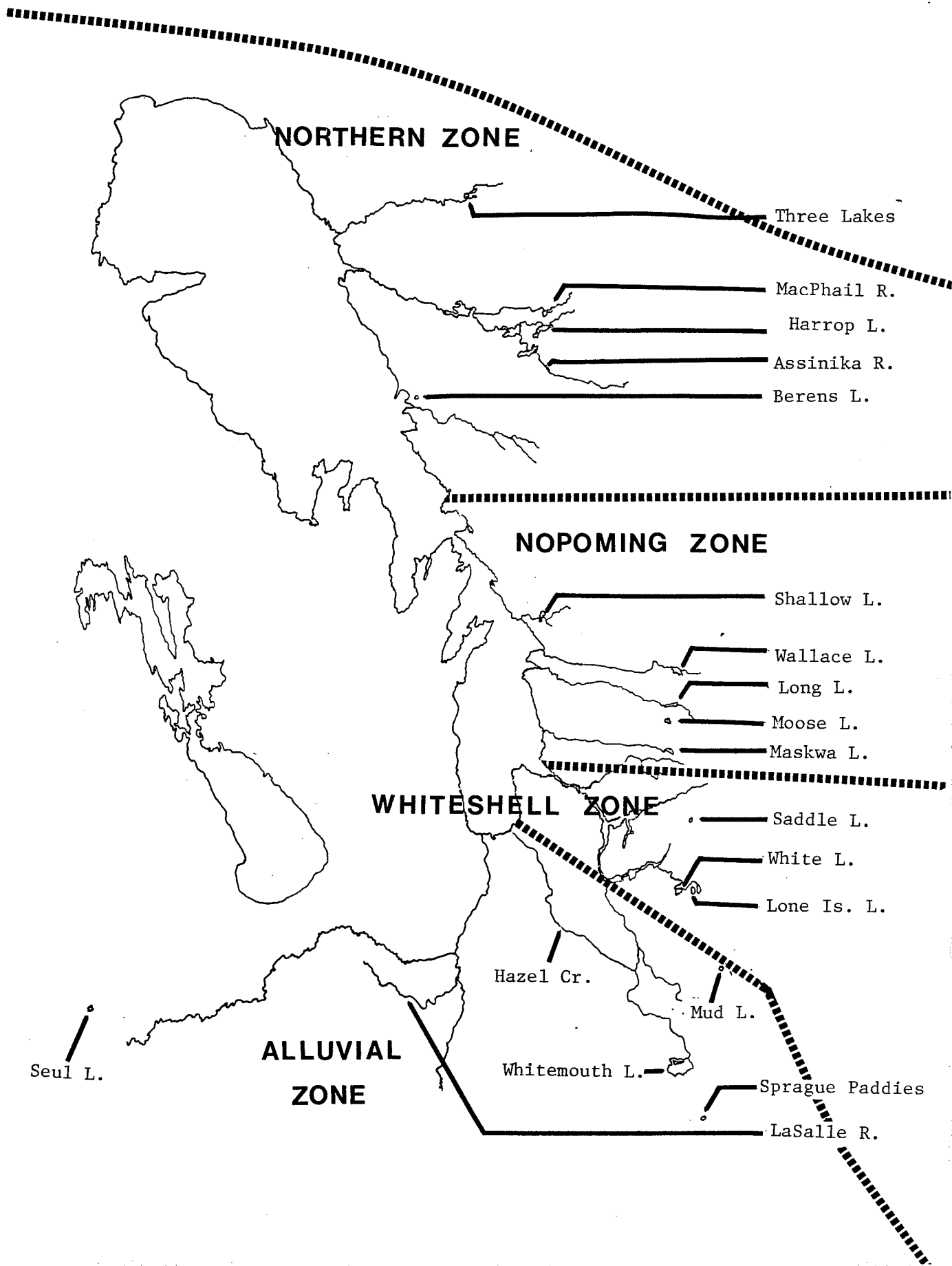


FIGURE 2
Surface Deposits

Redrawn from the Manitoba Economic Atlas.

Legend:



Granitic Materials



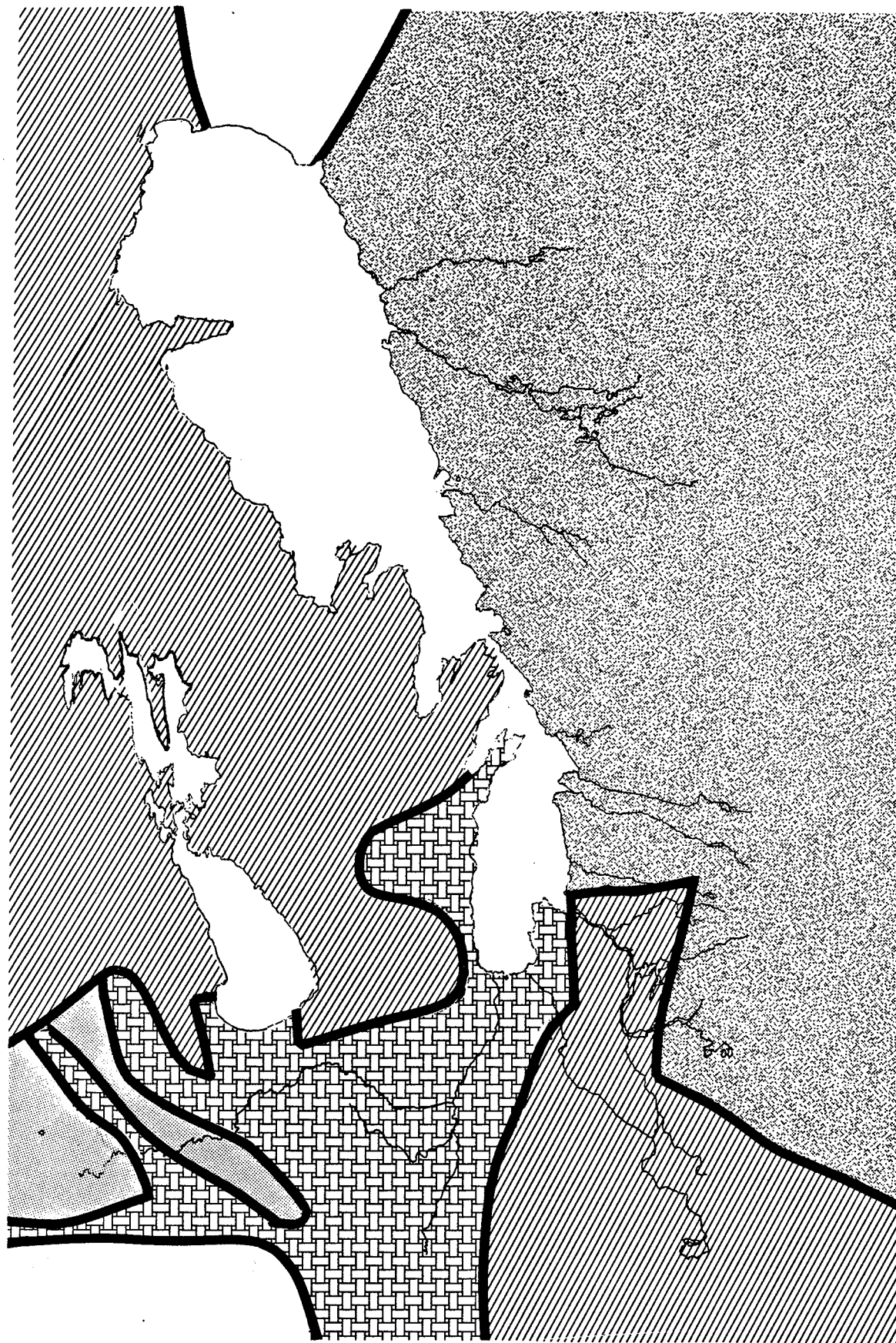
Predominately Limestone



Clay and Silt



Sands and Sandy Loams



In eastern Manitoba there is mostly granitic material. Southeastern Manitoba is predominately limestone and in the Winnipeg area the surface deposits are clay and silt. Seul L. is situated in an area with sands and sandy loams as surface materials.

There are many climatical factors that may have significance in the development of the diseases of wild rice. Those parameters which have been chosen to describe the climate of the study area integrate many of the factors which may be of importance to wild rice and its pathogens. One such parameter is potential evapotranspiration (Figure 3) or water loss from soil, plants, and surface areas.

One would expect to find differences in temperature in a northward direction. Figure 4 shows that the highest average July temperature for the sampling range is 68° F. in the south and 63° F in the north. It is likely that July is the month of greatest vegetative activity of wild rice.

FIGURE 3

Potential evapotranspiration

Isolines indicate potential water loss from soil, plants and surfaces in an average year in millimeters. Redrawn from the Manitoba Economic Atlas.

Legend:

Millimeters

1.....	500
2.....	525
3.....	550
4.....	575
5.....	600

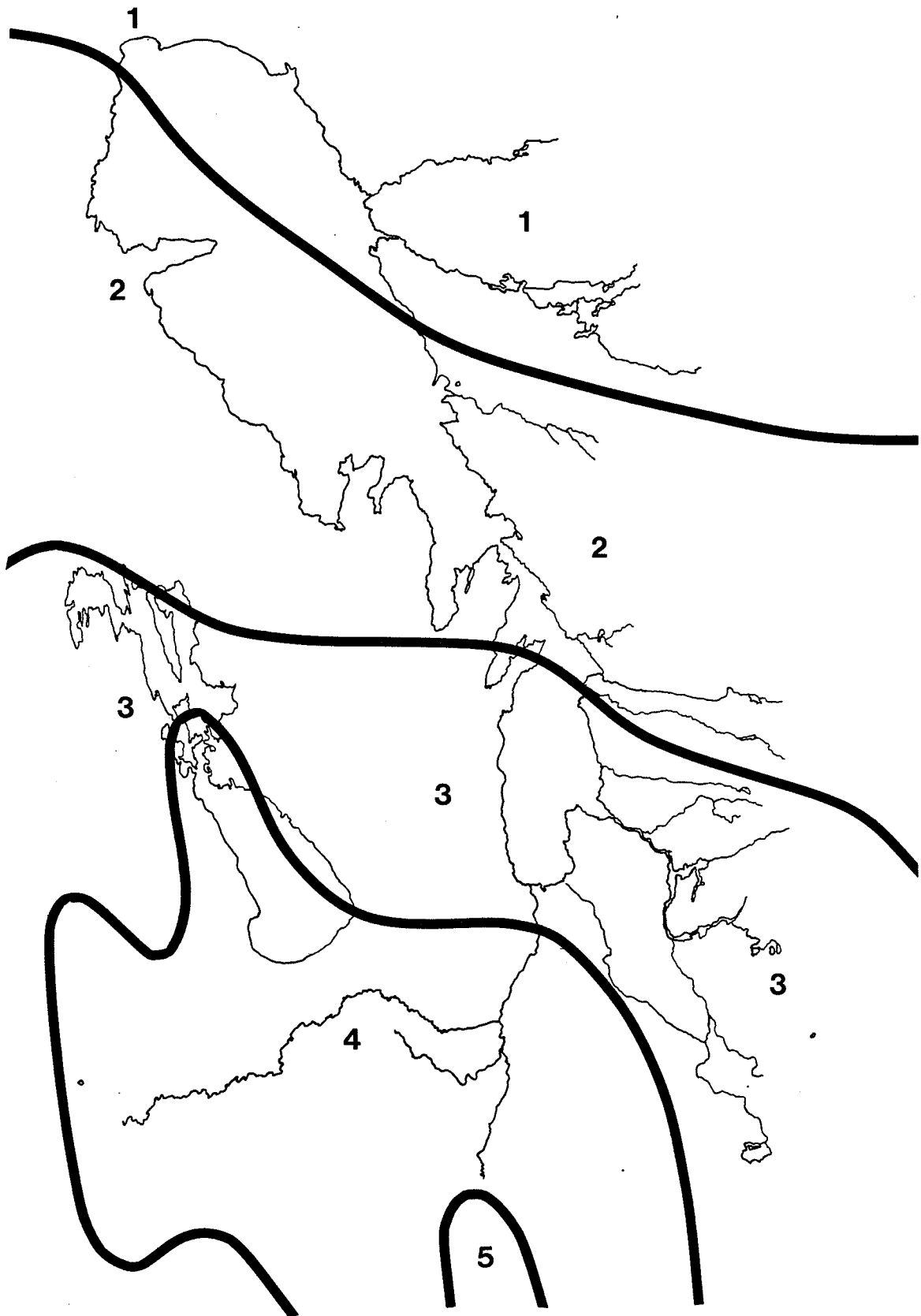
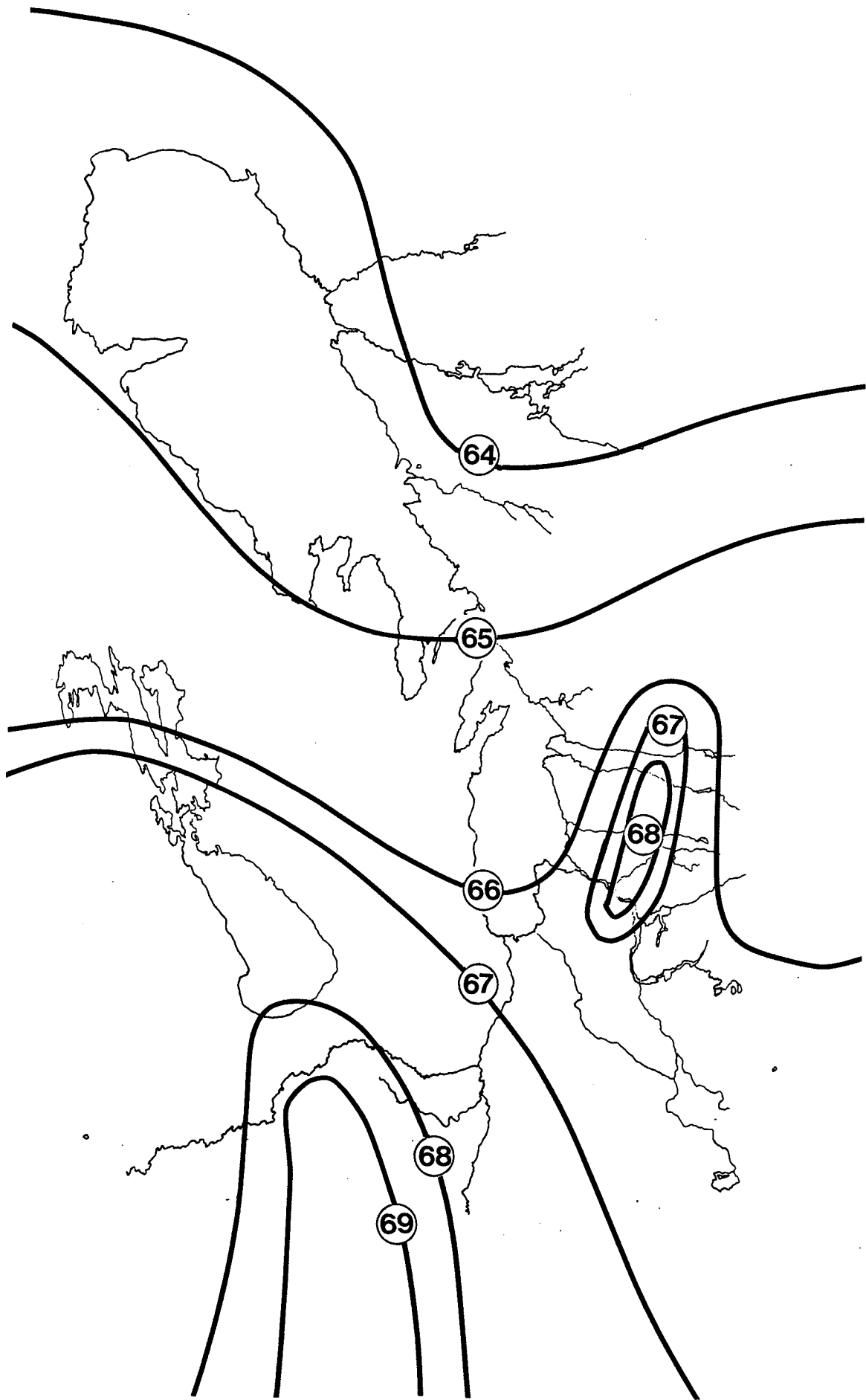


FIGURE 4

Average July Temperature

Average July temperature in degrees fahrenheit. Redrawn from the Manitoba Economic Atlas.



3.2 METHODS AND MATERIALS

3.2.1 Sampling

Sample sites were chosen to represent an even distribution along the north-south axis. Sampling times coincided with the developmental stages of the plant. During the sampling period the goal was to collect at least one sample when the plants were in the floating stage, one sample in the harvest stage, and one sample in between. To provide a continuum of samples during the life cycle, as many samples as possible were taken from each stand with at least two weeks between samples. Figures 5 and 6 present several aspects of the samples taken in 1979 and 1980 respectively. First the date of sampling and the sample size taken from each location is indicated. Secondly, the stage of the stand when sampled is indicated by the position of the sample date within the table. The stage can be determined by looking vertically above the information.

Except for those samples taken from Harrop L., Long L. and Three Lakes all were taken from the same transect in 1979 and 1980. Three Lakes was sampled in different locations in 1979 and 1980. In 1979 the sample was taken from a stand growing in the river mouth, while in 1980 the sample was taken from a nearby bay. The 1979 sample from Harrop L.

FIGURE 5

Summary of the Samples Collected 1979

The sample size, in brackets, is indicated under the sample date. The position of the sample date is relative to the stage when it was sampled. This is, however, only approximate because of the wide variation between plants.

	FLOATING LEAF	FLOWERING	HARVEST
Three Lakes		13/8/80 (50)	12/9/80 (50)
MacPhail R.			12/9/80 (50)
Harrop L.		13/8/80 (50)	12/9/80 (50)
Assinika R.	12/6/80 (48)	13/8/80 (40)	
Beren's L.			12/9/80 (50)
Shallow L.	26/6/80 (50)	10/7/80 (50)	
Wallace L.	11/6/80 (50)	26/6/80 (50)	10/7/80 (50)
Long L.	11/6/80 (50)	28/6/80 (49)	10/7/80 (50)
Moose L.	11/6/80 (50)		
Maskwa L.			
Saddle L.	11/6/80 (47)	11/8/80 (50)	
Lone Is. L.	11/6/80 (50)		3/9/80 (50)
White L.			28/8/80 (49)
Mud L.	26/6/80 (50)		28/8/80 (50)
LaSalle R.	30/6/80 (50)		23/9/80 (50)

FIGURE 6

Summary of the Samples Collected 1980

The sample size, in brackets, is indicated under the sample date. The position of the sample date is relative to the stage when it was sampled. This is, however, only approximate because of the wide variation between plants.

FLOATING LEAF	FLOWERING-DOUGH STAGE	HARVEST
---------------	-----------------------	---------

Three Lakes		15/8/79 (50)	
Harrop L.		15/8/79 (50)	17/9/79 (50)
Assinika R.			17/9/79 (50)
Beren's L.			
Shallow L.	31/7/79 (50)	15/8/79 (48)	19/9/79 (37)
Wallace L.	31/7/79 (50)		19/9/79 (50)
Long L.	31/7/79 (50)		19/9/79 (50)
Shoe L.			
Saddle L.			20/9/79 (50)
Lone Is. L.	10/7/79 (50)	8/8/79 (50)	10/9/79 (50)
White L.	10/7/79 (50)	8/8/79 (44)	10/9/79 (50)
Mud L.			18/9/79 (47)
Birch L.	22/6/79 (25)		
Whitemouth L.	22/6/79 (50)		
Sprague Paddies	21/6/79 (50)		13/9/79 (50)
Hazel Cr.		8/8/79 (48)	10/9/79 (48)
LaSalle R.	8/7/79 (50)		7/9/70 (50)
Seul L.	12/7/79 (50)	13/8/79 (49)	

was taken from a bay in front of the lodge and in 1980 from a large stand in the open water.

Beren's L. was the only stand sampled that had been artificially seeded. In 1978 lake rice seed was spread over much of the lake. In 1979 a small stand had developed in the middle of the lake and in 1980 the stand had covered most of the lake. In 1979 and 1980 the sample was taken from a transect just off the north-south median.

Fifty random plants from each sample site were collected along a transect bisecting the stand. Entire plants were collected in all cases except the 1979 Sprague Paddies harvest sample. In that case the large size of individual plants made it impossible to collect and transport 50 plants. Consequently, the tallest tiller from each plant was selected and placed into plastic bags for transport.

3.2.2 Detection of Potential Pathogens

Following collection, plants were placed into plastic bags and stored at 2°C. Examination of samples always took place within 10 days of collection. It consisted of visually inspecting leaves for the presence or absence of lesions. Representative and unusual lesions were cut from the leaf along with 1 to 2 centimeters of lamina. To stimulate growth and sporulation of fungi, the leaf segment, including the lesion, was placed into a damp-chamber. The type of damp-

chamber differed between 1979 and 1980. During the first year it consisted of damp filter paper in the bottom of a petri plate, neither of which had been sterilized. The system was changed in 1980 to washed, sterilized sand in a sterile petri plate. This provided better air circulation around the leaf segment and reduced overgrowth by contaminating surface saprophytes.

Before identification of a fungus and tests for its pathogenicity could be carried out, cultural conditions required for sporulation had to be determined. Single spore isolates were cultured on several media representing a variety of nutritional levels. Difco Potato Dextrose agar provided a media rich in carbohydrates. A weak potato carrot agar(WPCA) was chosen for a media weak in carbohydrates. The potato carrot agar, one-quarter the strength recommended by Tuite(1969), consisted of 5 grams each of potato and carrot autoclaved for 20 minutes and strained through 4 layers of cheesecloth. Twenty grams of agar was added and a second autoclaving of 20 minutes followed. In some cases, where sporulation did not occur on more conventional media, autoclaved wild rice leaves resting on sterile distilled water agar were used.

If sporulation was observed on a leaf segment, single spore isolates were made in one of several ways depending on the size of the spore. Large spores were streaked across

WPCA with 0.3 grams per litre of penicillin and streptomycin added to suppress bacterial growth. The plate was allowed to incubate overnight at room temperature (22°C). Well separated germlings were then removed along with a small amount of agar and placed on a suitable medium.

If the spore size was small and not amenable to streaking, an alternative method was used. Spores were transferred on a needle into 5 ml of sterile distilled water (SDW). The suspension was agitated on a vortex mixer, poured on SDWA, swirled for 10 seconds and allowed to settle for 20 seconds after which the suspension was poured on a fresh plate of SDWA. This process was repeated a second and third time. The suspension was drained from the third plate and all three plates were placed on their edges and incubated overnight or until germination took place. Germlings were transferred as before.

3.2.3 Testing Pathogenicity

Plants used for testing the pathogenicity of suspected parasites were grown in one of two ways depending on whether the parasite was a floating or aerial leaf pathogen. Seed stocks were collected from greenhouse grown plants of the variety 'Netum'. They were stored under water at 2 C. Before planting, seeds were germinated on a lab bench under 2-3

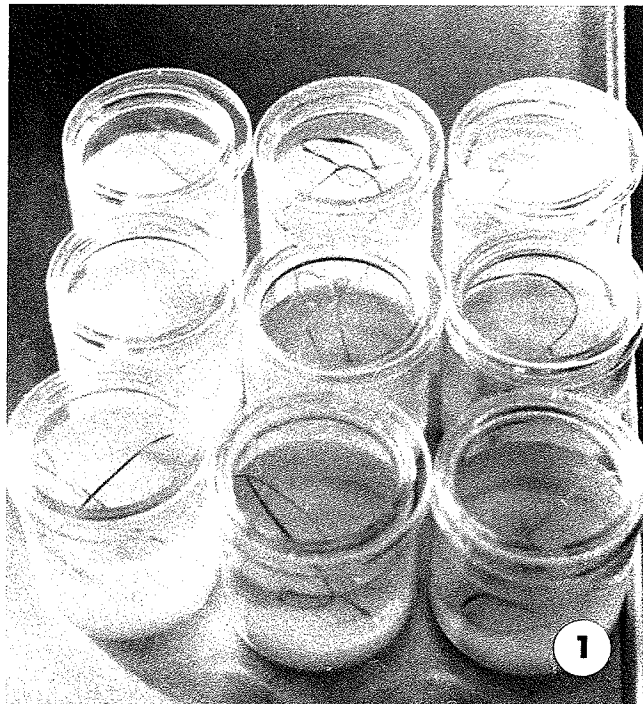
inches of distilled water in 4 litre plastic buckets. Planting took place when enough seedlings had produced 2-3 submerged leaves.

If floating leaves were to be inoculated, a system of soilless culture was used. Sixteen ounce glass jars were used as containers. One hundred mls of nutrient amended agar (see appendix) was added and 250 mls of sterile distilled water provided the aquatic environment needed by the wild rice plant. All components were sterilized for 20 minutes at 120°C. To maintain sterility the jar was covered with the bottom of a petri plate.

After planting, seedlings grown in soilless culture were kept on a lab bench until floating leaves began to emerge (Plate 1.1). At that time, they were transferred to a growth chamber. Conditions in the growth chamber were maintained at 20°C with a daylength of 15 hours. Twelve Gro-lux and 4 cool-white fluorescent tubes provided 30 Watts M of light. If aerial leaves were to be inoculated plants were grown in four litre plastic buckets filled to within 9 cm. of the rim with a 2:1:1 mixture of loam, peat and sand (Plate 1.2). To ensure that there would be at least one plant in each pot, two seedlings were planted in each. Once one of the plants had begun to grow, the weaker plant was removed. The buckets were kept filled with water until the aerial leaf stage was established, after which the soil was kept in a saturated state.

Plate 1: Methods Used to Culture Wild Rice

1. Plants growing in soilless culture.
2. Plants growing in greenhouse culture.



Inoculum was prepared in a fashion that depended on the cultural and morphological characteristics of the suspected parasite. To avoid contamination all procedures were carried out under aseptic conditions. Two suspected pathogens produced spores in slimy masses. In these cases a two week old culture was flooded with 5 ml SDW. The surface of the agar was then gently scraped to dislodge the spore masses without breaking up the substrate mycelium.

Another suspected pathogen produced pycnidiospores only when cultured on detached leaves. In this case, after 3-4 weeks of incubation, leaf segments were removed from the surface of the agar and placed in tubes containing 7 ml SDW. Spores were dislodged from their pycnidia by vigorous agitation on a vortex mixer. Filtering through two layers of cheesecloth removed the larger pieces of leaf debris.

After suspension, spores in both cases were washed three times by centrifugation at 3000 RPM for 3 minutes followed by resuspension in 7 ml of SDW. One drop of Tween-20 per 10 ml of spore suspension was added to improve spreading qualities. Spore concentrations were determined by averaging three replicate measurements made on a haemocytometer cell and then adjusting each suspension to a concentration of 500,000 spores per ml.

Control and inoculated plants were randomly selected. A minimum of 20 plants were used in each test. Each plant was

assigned a random number. The first ten numbers taken from a table of random numbers (Snedecor and Cochran, 1972) were used to determine those plants to be inoculated.

Spores were applied to the leaf surface in the form of an aerosol generated by a manually operated glass atomizer. The aerosol was sprayed on to the leaf surface until large droplets were formed or runoff was initiated. Control plants received SDW containing one drop of Tween-20 per 10 ml.

The large size and density of the spores of one suspected pathogen necessitated that alternative means of inoculation be used. Rather than using an atomizer, floating leaves were inoculated with individual spores. A small group of spores was spread over the surface of a SDWA plate and allowed to germinate overnight at room temperature. Germinated spores, along with a minimum amount of agar were transferred to the appropriate leaf. Each leaf was inoculated only once. Controls consisted of a corresponding cube of SDWA.

After inoculation, plants were returned to either the growth chamber if floating leaves were being inoculated or into a dew chamber if aerial leaves were being inoculated. Floating leaf plants remained in the growth chamber for the remainder of the experiment while aerial leaf plants were removed from the dew chamber after three days. The dew chamber consisted of 6 mil plastic hung over a bank of lights. A mister provided moisture.

3.3 RESULTS AND DISCUSSION

3.3.1 Introduction

Close visual inspection of the samples collected during 1979 and 1980 revealed that at least 5 disease syndromes could be clearly distinguished. A description and discussion of each will follow. Three: brown spot, leaf and stem smut, and ergot were already described in the literature. The other two have not previously been reported to affect wild rice.

3.3.2 Brown spot

The most widespread disease syndrome was that which Gilbert (1974) termed brown spot. It consisted of scattered necrotic lesions variable in size and found on floating and aerial leaves. Gilbert was able to isolate Fusarium spp. in addition to Helminthosporium spp., and was not able to correlate the appearance of the lesion with the fungus isolated.

The present study continued Gilbert's (1974) investigation into the brown spot syndrome. However, certain difficulties arose that prevented further elucidation of the causes of this disease.

To continue Gilbert's (1974) inquiry into the causes of brown spot would not have been compatible with the overall

objectives of this study. First of all it would have been necessary to ensure that lesions collected in the field be free of contaminants from water, air and other plants. Therefore, extensive precautions would have been needed to prevent contamination of lesions before being placed into sterile damp-chambers. Secondly, when a damp-chambered lesion produces no fungal reproductive structures it is not possible to determine if there was indeed no pathogen or if the pathogen caused the lesion but is not responding to the high humidity of the damp-chamber. The exacting methods needed to overcome these difficulties would have thwarted the overall objectives of this study.

In spite of these limitations, necrotic lesions representing the range of lesion types were taken from each sample and damp-chambered to provide some information on the range of Helminthosporium sativum and other pathogens that may be involved in the brown spot syndrome. Tables 1 and 2 show the number of lesions damp-chambered in each sample along with the types and numbers of pathogens isolated in 1979 and 1980 respectively. The information in these two tables shows that H. sativum is the most common pathogen associated with brown spot and is found across the entire range from Three Lakes to Seul L. Apparently, H. sativum is present wherever there is wild rice.

Table 1

The number of lesions damp-chambered from each sample along with the types and numbers of pathogens isolated. 1979.

Legend:

H.S. Helminthosporium sativum
S.H. Sclerotium hydrophilum
D. Dichotomophthoropsis sp.

	#LESIONS	H.S.	S.H.	D.
Three Lakes Flowering	13	-	-	-
Harrop L. Floating	10	1	-	-
Harrop L. Flowering	16	2	-	-
Harrop L. Harvest	22	8	-	-
Assinika R. Harvest	12	-	-	-
Beren's R. Harvest	8	-	-	-
Shallow L. Floating	28	7	-	4
Shallow L. Flowering	10	3	-	-
Shallow L. Harvest	15	1	-	-
Wallace L. Floating	34	-	-	16
Wallace L. Harvest	23	7	-	-
Long L. Floating	31	2	2	5
Long L. Harvest	18	6	-	-
Shoe L. Floating	29	1	-	20
Saddle L. Harvest	22	11	-	-
Lone Is. Floating			-	-
Lone Is. Flowering	22	-	-	-
Lone Is. Harvest	41	14	-	-
White L. Floating	36	-	7	-
White L. Flowering	21	2	1	-
White L. Harvest	67	20	-	-
Mud L. Harvest	37	8	-	-
Paddies. Harvest	126	93	-	-
Hazel Cr. Floating	16	11	1	-
Hazel Cr. Harvest	11	33	-	-
LaSalle R. Floating		-		
LaSalle R. Flowering	36	10	-	-
LaSalle R. Harvest	46	26	3	-
Seul L. Flowering	25	8	-	-

Table 2

The number of lesions damp-chambered from each sample
along with the types and numbers of pathogens isolated.
1980.

Legend:

H.S. Helminthosporium sativum
S.H. Sclerotium hydrophilum
D. Dichotomophthoropsis sp.

	#LESIONS	H.S.	S.H.	D.
Three Lakes Flowering	6	2	-	2
Three Lakes Harvest	5	1	-	-
MacPhail R. Harvest	8	-	-	-
Harrop L. Flowering	3	-	-	-
Harrop L. Harvest	2	-	-	1
Assinika R. Floating	17	-	-	-
Assinika R. Flowering	1	-	-	-
Assinika R. Harvest	1	-	-	-
Beren's L. Harvest	12	1	-	-
Shallow L. Floating #1	4	-	-	-
Shallow L. Floating #2	6	-	-	-
Shallow L. Flowering	5	2	-	-
Wallace L. Floating #1	13	-	-	-
Wallace L. Floating #2		-		
Wallace L. Floating #3	7	-	-	-
Wallace L. Flowering	6	-	-	-
Wallace L. Harvest	8	-	-	-
Long L. Floating #1	14	-	-	-
Long L. Floating #2	15	-	-	-
Long L. Floating #3	7	-	-	4
Long L. Flowering	15	-	-	-
Long L. Harvest	8	-	-	-
Maskwa L. Flowering	2	-	-	-
Moose L. Floating	9	-	-	-
Saddle L. Floating	17	1	9	-
Saddle L. Harvest	7	-	-	-
Lone Is. Floating	28	-	-	-
Lone Is. Harvest	3	1	-	-
Mud L. Floating	24	8	1	2
LaSalle R. Floating	15	1	-	-
LaSalle R. Harvest	12	5	-	-

Surprisingly, although Helminthosporium oryzae is the main cause of brown spot in Minnesota (Bean and Schwartz 1961), it was not found in either 1979 or 1980. The elongate spores of H. oryzae are easily distinguishable from those of H. sativum.

Table 1 also lists several other fungi isolated from necrotic lesions. Sclerotium hydrophilum was frequently found on damp-chambered lesions taken from floating leaves collected in 1979. S. hydrophilum is a known pathogen of wild rice causing a stem and sheath rot. The presence of this fungus on floating leaves is of interest because it could provide a source of inoculum from which infections of aerial leaf sheaths could be initiated early in the season.

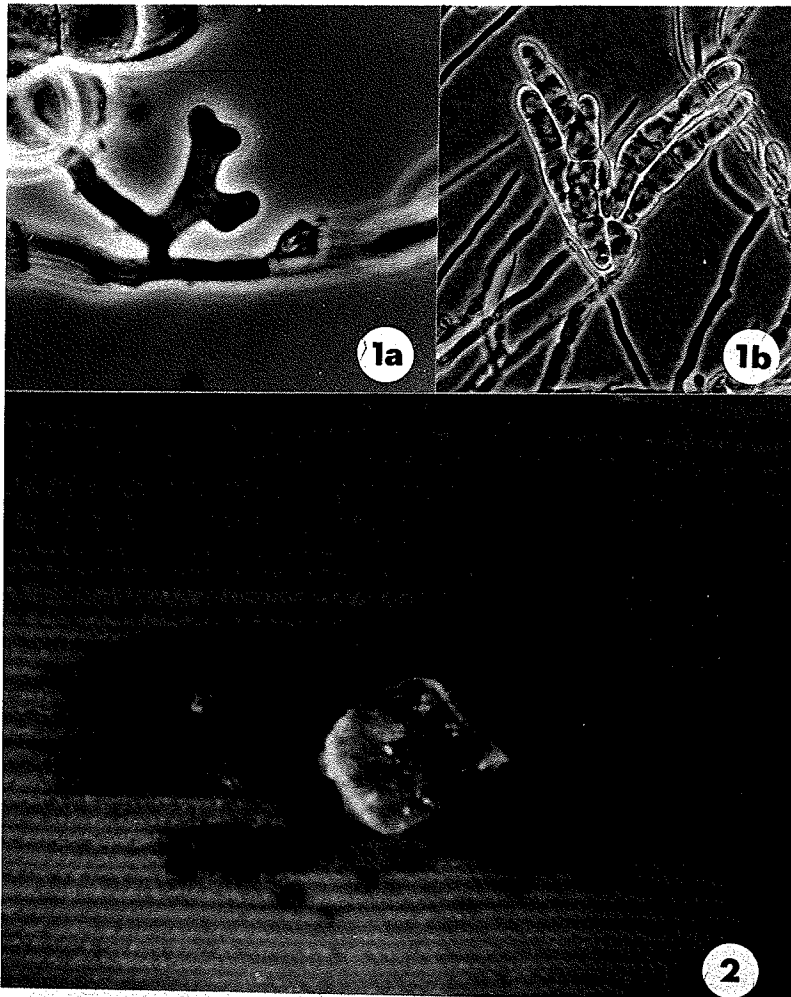
Another pathogen not previously reported to infect wild rice was found associated with the brown spot syndrome on floating leaves. The first evidence of this organism was the appearance of erumpent, irregular sclerotia on damp-chambered leaf segments. Single spore isolates of this fungus sporulated well when grown on WPCA with 12 hour days of near UV light. It was assigned to the genus Dichotomophthoropsis on the basis of spore shape and the morphology of the conidiogenous apparatus (Plate 2.1). To test pathogenicity, single spores were placed on leaves grown in soil-less culture. Inoculated leaves developed typical necrosis around the inoculum (Plate 2.2). Dichotomophthoropsis was

successfully reisolated when these lesions were damp-chambered.

The lesions from which Dichotomophthoropsis was isolated could not be distinguished from lesions that yielded H. sativum or S. hydrophilum. The extent, therefore of this fungus could not be readily determined by visual examination.

Plate 2: Dichotomophthoropsis

- 1(a). Dichotomophthoropsis conidiogenous apparatus. 720X
- (b). Dichotomophthoropsis spores. 300X
2. Necrotic lesion resulting from the placing of a single spore on a floating leaf.



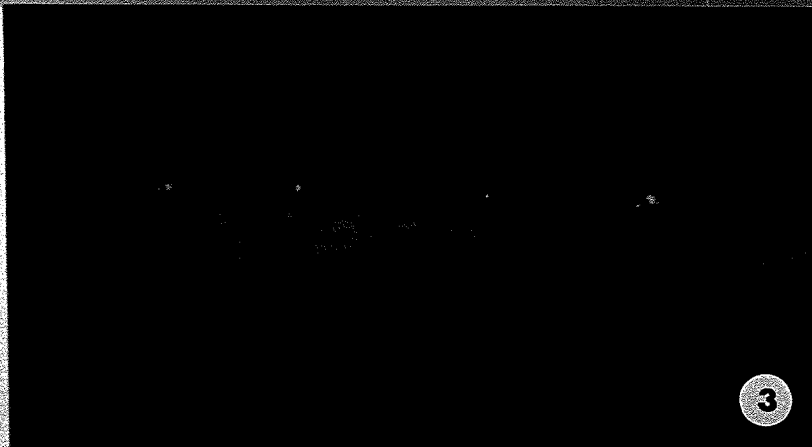
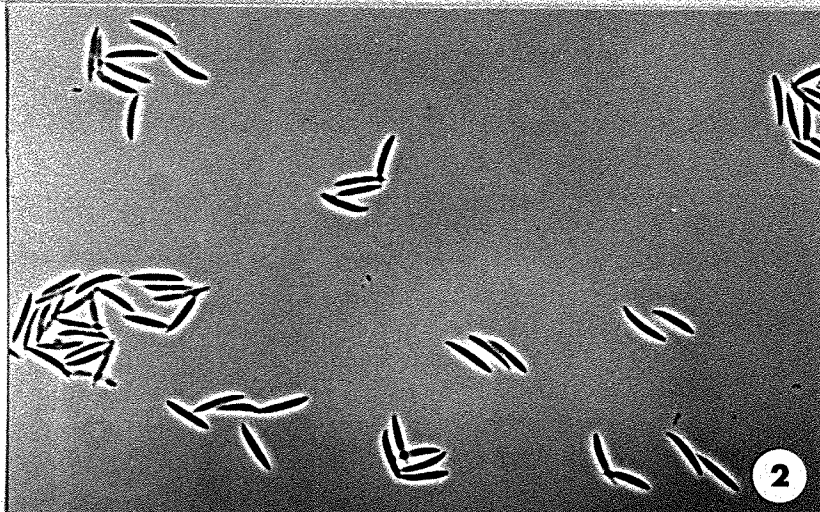
3.3.3 Anthracnose

A syndrome distinct from brown spotting was first observed on floating leaves and later on aerial leaves. The typical lesion, illustrated in Plate 3.1, is large and elliptical with no necrosis within the lesion. There is a dark blue reflectance from small eruptions within the lesion. At 50X magnification the small eruptions are seen to be typical acervuli with numerous setae on their perimeter and orange spore masses in the centre. The fungus was assigned to the genus Colletotrichum on the basis of the acervulus and the curved spores (Plate 3.2).

Single spore isolations of this fungus sporulated well when grown on oatmeal agar and incubated under near UV light at 25° C. Within one week most floating leaves inoculated with this organism were showing typical symptoms (Plate 3.3). Colletotrichum was successfully reisolated from damp chambered lesions. After two weeks most of the inoculated leaves had senesced. One plant showed symptoms after two days suggesting that there is some difference between plants with respect to susceptibility. Whether or not the variation in susceptibility is due to genetic differences must be considered along with certain weaknesses in the experimental design. Although cultural conditions were similar for all plants, differences in the orientation of inoculated

Plate 3: Anthracnose

1. Leaves taken from Wallace L. late floating leaf sample infected with Colletotrichum. The necrosis associated with some of the lesions could be due to Microdochium which was commonly isolated from these leaves.1X
2. Colletotrichum spores.300X
3. Close up of an artificially inoculated leaf. The elliptical shape of the lesion is similar to the type of lesion observed in nature. Spore masses are being exuded by acervuli.17X

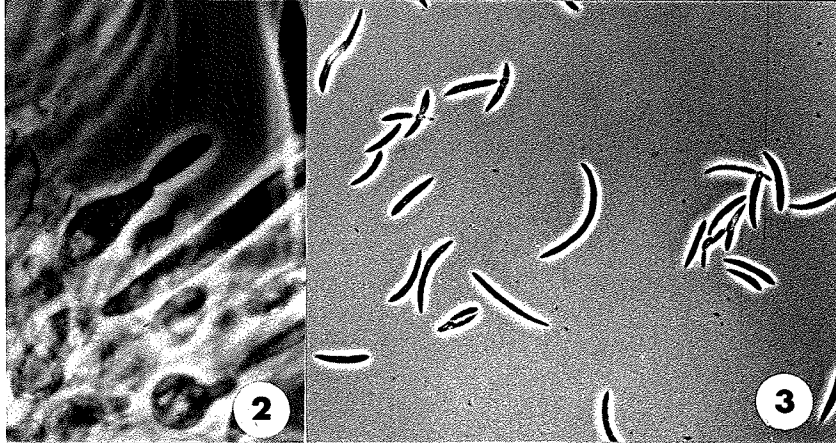
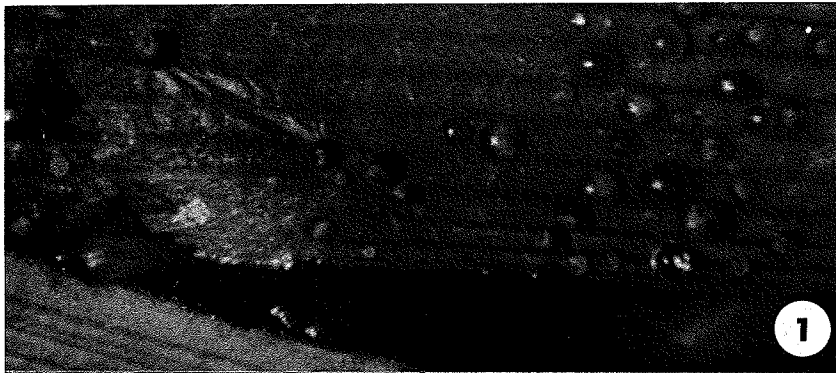


leaves may have affected the amount of inoculum that a leaf received. On some plants, the floating leaves were orientated so that they laid flat on the water. On others, the floating leaves could ride up on the glass so that they were perpendicular to the surface of the water. Because the spore suspension tended to run off a vertical surface, these leaves may have received less inoculum than those lying flat on the water. Secondly, some differences between plants may have arisen as a result of the methods used to apply the inoculum. The nature of a manually operated atomizer makes it difficult to ensure that each plant is receiving a consistent amount of the spore suspension.

Colletotrichum was not the only fungus to sporulate when lesions of this type were damp chambered. Pink, slimy spore masses were found near the lesions (Plate 4.1). The morphology of conidigenous cells and conidia produced on colonies derived from single spore isolates on PCA suggests that this organism should be placed in the genus Microdochium (Plate 4.2 and 4.3). Leaves inoculated with Microdochium showed necrosis on both the stem and leaves. Plate 4.4 shows a typical foliar lesion. The necrosis following inoculation suggests that a similar necrosis sometimes associated with Colletotrichum lesions in the field may be due to Microdochium.

Plate 4: Microdochium sp.

1. A senesced leaf showing the light orange sporodochia that were commonly observed on infected leaves.
2. Conidiogenous cell. 1800X
3. Spores. The number of septa varies from none to several. 300X
4. A plant artificially inoculated with Microdochium. There is only one small necrotic lesion but there is extensive chlorosis radiating from it.



Colletotrichum was not always found together with Microdochium but often enough to raise the suspicion that the causes of this disease may not be as straightforward as originally thought. There are several possible explanations for the role of Microdochium. It may be a saprophyte utilizing the tissue destroyed by Colletotrichum. This is unlikely when the positive pathogenicity tests for Microdochium are considered. It may be the major cause of the lesion and Colletotrichum only a secondary invader. However, the similarity in the lesion type between leaves inoculated with Colletotrichum and lesions found in the field suggest that Colletotrichum is quite capable of causing most of the syndrome on its own. The Microdochium may facilitate infection by Colletotrichum by initiating a small lesion through which the latter can enter more readily. However, the rapid infection of some leaves by Colletotrichum in the absence of Microdochium indicates that this is not an essential role. Finally, the Microdochium may work in conjunction with the Colletotrichum, remaining on the perimeter and breaking down the resistance of the healthy tissue so that Colletotrichum can spread more rapidly. The presence of Microdochium in one or more of these suggested roles complicates the etiology of this syndrome. Further study is needed to clarify the situation. Nevertheless, because the pathogenicity tests have shown that Colletotrichum is able to cause typical lesions, the term anthracnose has been adopted to describe

this syndrome in conformity with similar diseases caused by Colletotrichum spp.

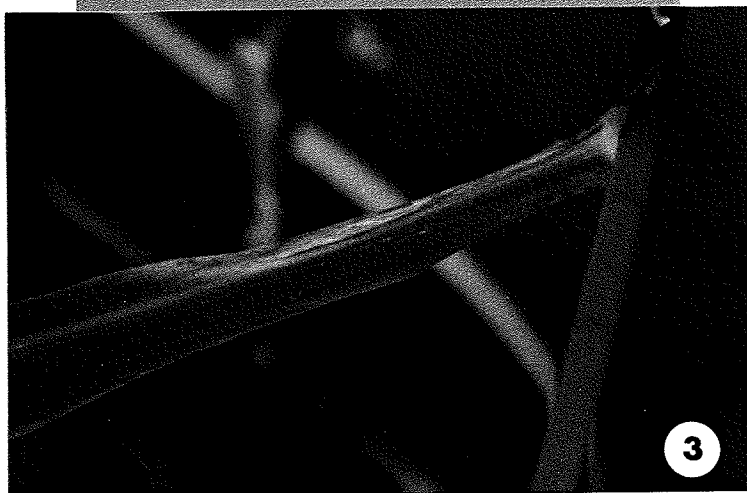
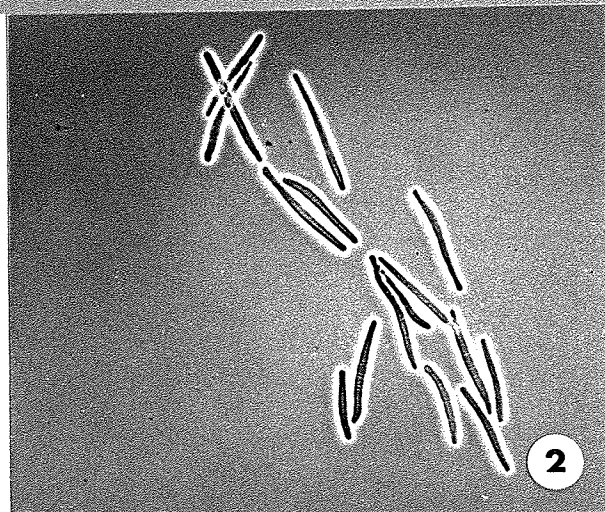
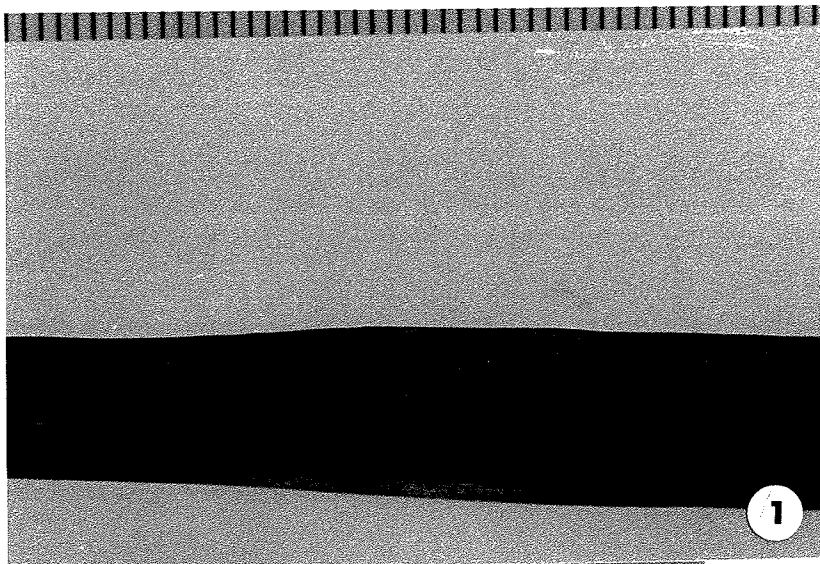
3.3.4 Leaf Blotch

A third syndrome distinct from brown spot and anthracnose was observed to occur on aerial leaves. The fungus was first seen on the senesced tissue of floating leaves readily evident as dark spherical pycnidia. Single spore isolates sporulated well on autoclaved leaf pieces resting on SDWA. The possibility of this organism being pathogenic was not apparent until lesions with pycnidia were observed on aerial leaves. Plate 5.1 shows a typical lesion of with the pale interior surrounded by a coal black perimeter and clearly distinct from any other symptoms known to occur on this plant. Single spore isolates taken from lesions on aerial and floating leaves were found to be identical in pycnidial morphology, as well as in spore size and shape.

Cultures of this fungus were sent to the Commonwealth Mycological Institute for identification. They were unable to give a positive identification, but suggested that it should be referred to as either Sclerostagonospora or Scolecosporella. However, the shape of the spores (Plate 5.2) seems to suggest that this fungus would be better placed in Phaeoseptoria. Greenhouse inoculations were successful with the appearance of lesions on artificially inoculated leaves simi-

Plate 5: Leaf Blotch

1. Leaf blotch lesion taken from the field. Pycnidia are easily visible in the chlorotic centre of the lesion. The very dark perimeter is characteristic of this disease.(Scale in mm)
2. Pycnospores of the causative fungus.300X
3. Leaf blotch lesion on an artificially inoculated leaf.



lar to those found in the field (Plate 5.3). The presence of pycnidia and successful reisolation confirmed that this fungus was the causative agent. This disease has been provisionally called leaf blotch because the causal fungus and the appearance of the syndrome are reminiscent of *Septoria* leaf blotch of wheat.

3.3.5 Leaf and Stem Smut

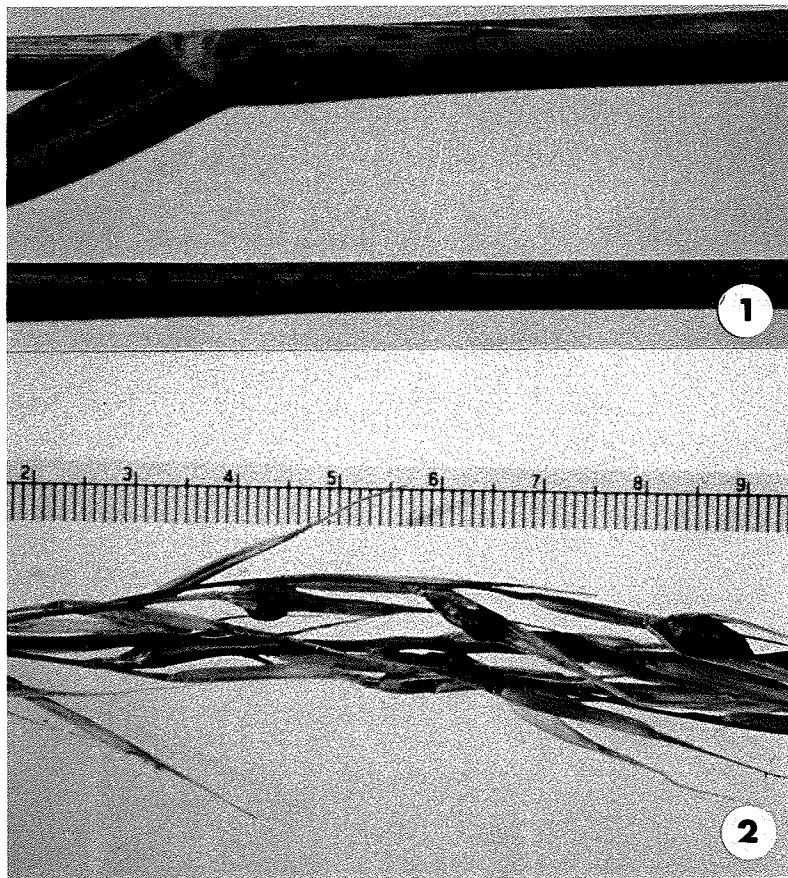
Very much an enigma in this study was Entyloma lineatum, the cause of leaf and stem smut of wild rice (Plate 6.1). Because both Dore (1969) and Gilbert (1974) considered it an organism of low pathogenic ability, little attention was given to it in this study. However, they may have underestimated the virulence of this pathogen.

3.3.6 Ergot

Claviceps zizaniae (plate 6.2) was the only non-foliar disease found during the coarse of the survey.

Plate 6: Leaf Smut and Ergot

1. Entyloma lineatum on a leaf.
2. Ergot bodies of Claviceps zizaniae found in Three lakes (Scale in mm).



Chapter IV

DISTRIBUTION AND SEVERITY OF DISEASE

4.1 INTRODUCTION

Because natural wild rice stands are not subject to the continual supervision characteristic of cultivated crops, the only acceptable method of determining the amount of disease in wild rice is through an organized regimen of sampling incorporating accepted methods of disease measurement. If the impact of the diseases described in the previous chapter is to be determined, it is first necessary that the amount of disease in each stand be quantified.

One of the earliest comprehensive treatments of disease loss assesement was by Chester(1950). He considered that loss is the final measurement of the effect of disease on a crop and provided an intensive review of the methods used to determine the loss resulting from a particular level of disease. In the case of foliar disease, loss of grain cannot be calculated directly, but must be derived through application of a known disease-loss relationship. Once the disease-loss relationship has been elucidated, loss can be estimated by measuring the amount of disease present in the population.

Chester suggested that the amount of disease present be measured quantitatively using either the frequency of plants infected (incidence) or the percentage of leaf area destroyed (intensity). He recommended that incidence be used when the amount of disease on different plants is uniform. He derived intensity by sampling a population, measuring the percentage leaf area destroyed by disease and then calculating a sample mean. This mean is a measure of the intensity of disease in that population.

Although more information is contained in the intensity statistic, incidence is particularly useful in providing a straightforward method of presenting the distribution of a pathogen. As a means of comparing stands incidence is of more limited value. Usually, it is assumed that before incidence can be used in a study, the amount of disease between plants should be similar. However, even when this is not the case, there are relationships between incidence and intensity that make the former a useful statistic.

For example, under normal circumstances one would not expect to find a low incidence in conjunction with a high intensity. A high intensity of disease implies the possibility of an elevated inoculum load. The high inoculum level decreases the likelihood of any given plant escaping infection. Consequently, if intensity is high it is likely that incidence will be high as well. The reverse is not neces-

sarily true because incidence does not provide any information on the amount of disease on individual plants. A high incidence could mean that there are many plants with only a little disease or that there are many plants with much disease: two very different situations.

4.2 METHODS

The samples used in this phase of the study were the ones described in the previous chapter.

Incidence values were derived by noting the number of plants in the sample which harbored a particular disease and expressing this as a percentage of the total.

Intensity was measured using a technique developed by Cobb(1890) . It involves the use of disease assessment keys consisting of a series of graphic representations of diseased leaves (Plate 7). There is a separate key for each level of disease. To determine this level, the leaf in question is matched to a key that resembles the amount of disease on that leaf. As the area taken up by the graphically represented lesions is known, an estimate of the percentage leaf area damaged can be made.

Traditionally, intensity has been expressed by an average value obtained by adding together the percent lesion cover values found on all plants, and dividing by the sample size.



Plate 7: Disease Assessment Keys

1. The keys used to assess the percentage lamina affected by anthracnose. The areas for each key are approximate, but are within 10%.

1%



10%



20%



30%



However, certain characteristics of the wild rice plant and its diseases invalidate the use of an average for each stand. Being an average, it ignores the extremes of disease so that the presence of severely infected plants may be overlooked. This may be acceptable in crops where the disease-loss relationship is better known and understood. For example, there may be a linear relationship between the amount of disease and the amount of loss. If the relationship is indeed linear, finding a mean disease level will be satisfactory but until this relationship is known the use of a mean intensity value is inadvisable. To avoid using means another method of presenting intensity information has been used. Rather than averaging the sample as a whole, it was broken into groups based on the percentage of leaf area damaged. Five classes were created for anthracnose:

1. No disease
2. 0.1-5%
3. 5.1-10%
4. 10.1-20%
5. 20.1-40%

One extra class - 40% to 80% - was created for leaf blotch because the intensity of this disease sometimes exceeded the maximum found for anthracnose.

The classes used for brown spot were based on the leaf rust key of James (1971).

1. No disease
2. 0.1-5%
3. 5.1-15%
4. 15.1-25%

Except for class 2, each class in both series represents double the amount of disease found in the preceding class. anthracnose the first class of 0.1 to 5% disease was derived more or less arbitrarily because a 5% difference was thought to be the maximum accuracy achievable with the Cobb system of disease measurement. Once the boundaries of the classes had been determined, it was possible to create a series of histograms with the five classes along the x-axis and the percentage of leaves per class on the y-axis.

The measurement of disease was based on the percentage of injury to the individual leaves. Because most plants had more than one leaf some method was initially sought which would provide an overall intensity for the plant combining all its leaves. Upon further consideration it became clear that deriving a single intensity value for each plant would be inadvisable because all the leaves within a stand do not constitute a homogeneous population; they are responding to the pathogen in different ways depending upon their age or position on the plant. Leaf age is significant for two reasons. First, the susceptibility of a leaf may change with

age and an older leaf could be more susceptible than a younger, more vigorous leaf. Secondly, the longer a leaf is exposed to the environment the greater the probability of inoculum and leaf coming in contact. To compensate for this the data has been arranged so that leaves of similar age in each sample are grouped together. The newest or top-most leaves were grouped together as ultimate leaves. The next leaves were grouped together as ultimate-1 and ultimate-2 leaves. Ultimate-3 leaves were also found to occur in some samples. Consequently, in the presentation of intensity data, intensity histograms for each group of leaves present in a stand have been derived.

4.3 RESULTS AND DISCUSSION

4.3.1 Introduction

Each of the major types of disease is discussed in turn with respect to both incidence and intensity. Each disease except for stem and leaf smut is described using 6 figures. The incidences of disease found in 1979 and 1980 are presented on the first two figures as pie charts for each sample. The three columns in the 1979 incidence maps represent floating, flowering, and harvest samples respectively. The first three columns of the 1980 incidence map are floating leaf samples taken at two-weekly intervals. These have been designated as FL#1, FL#2, and FL#3. The fourth and fifth columns are the flowering and harvest samples respectively.

The last four figures illustrate the intensity of disease with the most northerly sites first progressing towards the more southerly.

4.3.2 Anthraconose

Anthraco

nose occurred in both 1979 (Figure 7) and 1980 (Figure 8). It was first observed in 1979 on floating leaves collected from the Nopiming Zone stands. At this time the identification of Colletotrichum was in its initial stages and, as a result, incidence values were not obtained until the aerial leaf stage.

It soon became apparent that amongst the stands sampled, there were two distinct types of intensity distributions (Figures 9a-d). The first group, or Type I stands assume a highly skewed distribution and includes those samples with no disease, or samples with leaves infected at or below the 5% level. The second group includes those samples with leaves that are infected above the 5% level and whose intensity distribution is approaching the normal. These have been designated as Type II stands. However, there is an exception. Long L. (Figure 9c) did have leaves with greater than 5% infection, but the intensity distribution was closer to a Type I stand than a Type II. Therefore, there appears to be a threshold level of 5%. If there are leaves with greater than 5% infection, then disease development acceler-

FIGURE 7
Incidence of Anthracnose 1979

The shaded portion of each pie chart represents the incidence of anthracnose in the sample. There are three columns. The first column is comprised of floating leaf samples, the second flowering samples and the third harvest samples.

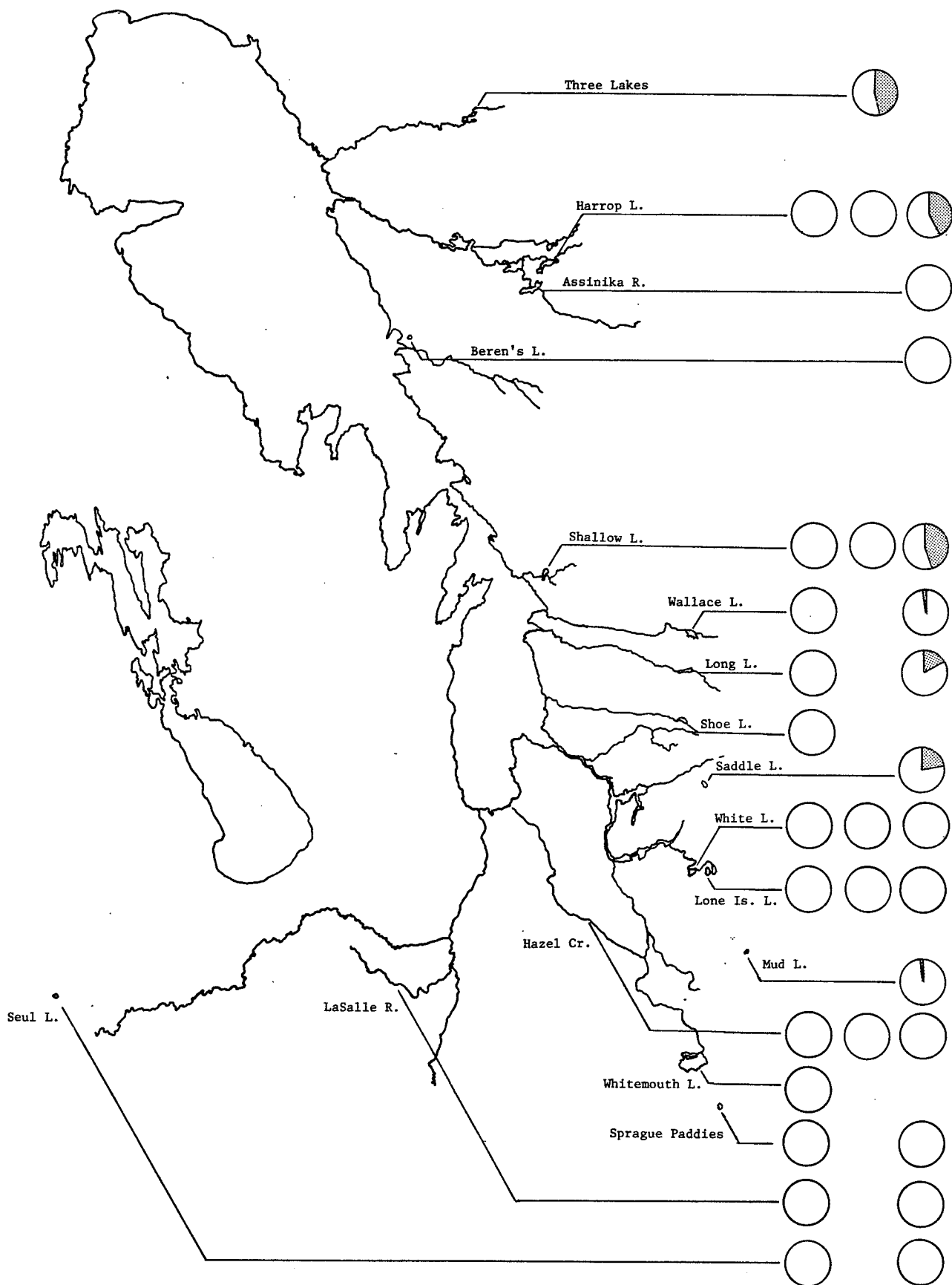


FIGURE 8
Incidence of Anthracnose 1980

The shaded portion of each pie chart represents the incidence of anthracnose in the sample. There are five columns based on the five samples taken from Long and Wallace lakes. Column I represents samples taken at two weeks, Column II four weeks, and Column III six weeks after the emergence of floating leaves. Stands in the flowering stage are in Column IV and stands in the harvest stage are in Column V.

Legend:

* Type II Stands

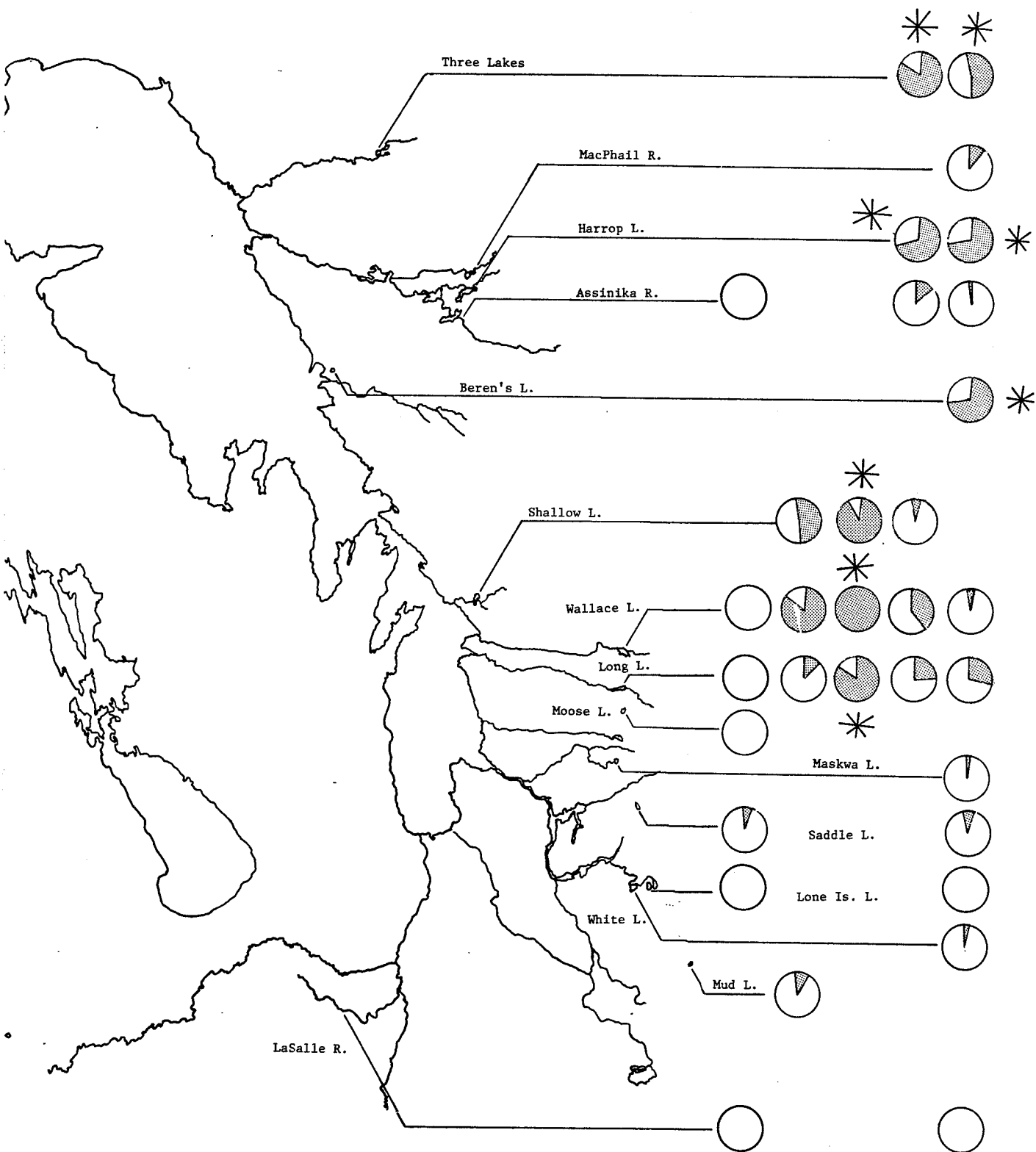
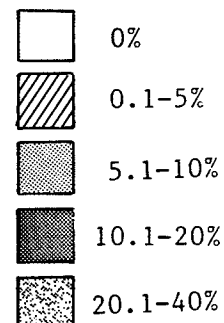


FIGURE 9a
Intensity of Anthracnose 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



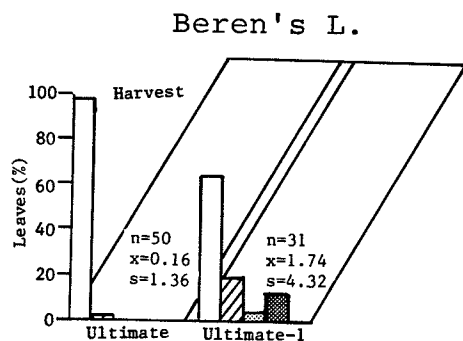
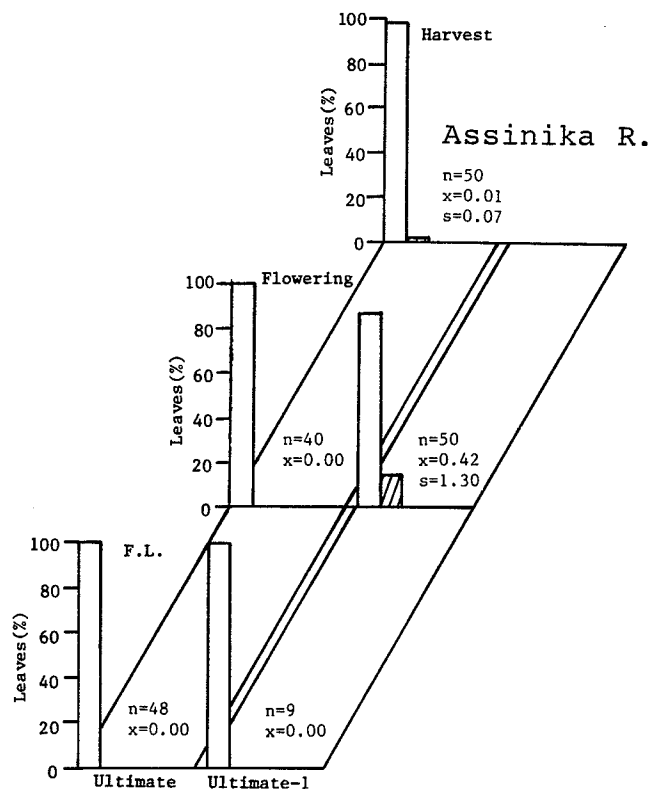
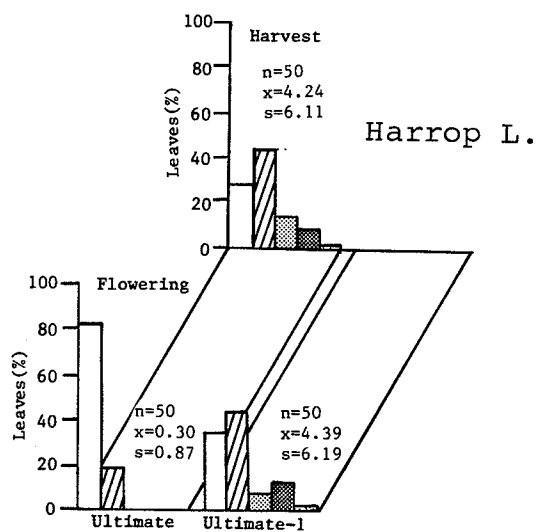
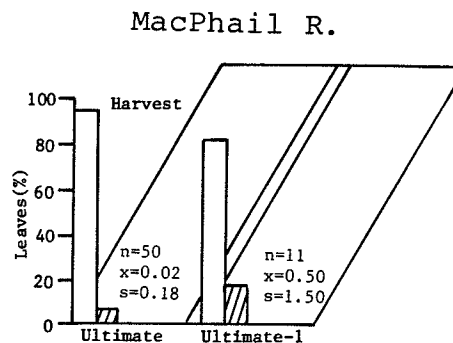
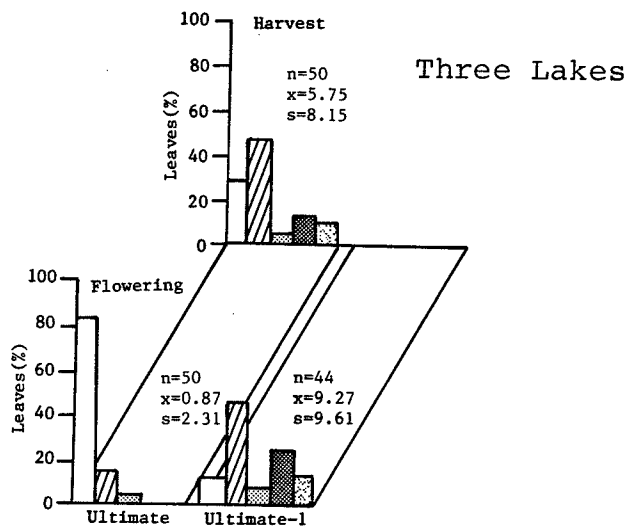
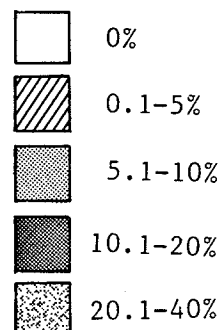


FIGURE 9b

Intensity of Anthracnose 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



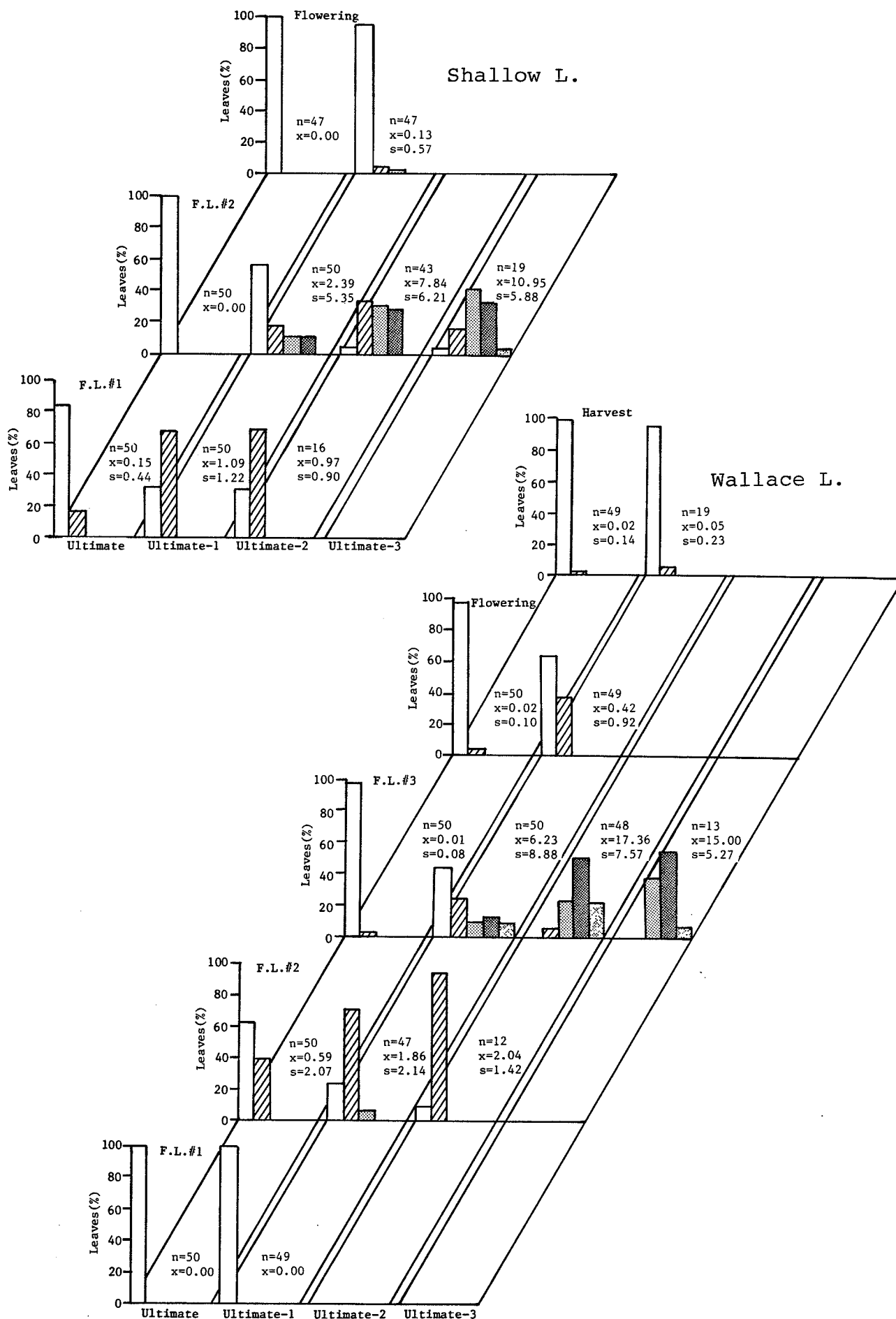
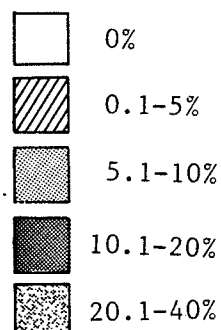


FIGURE 9c
Intensity of Anthracnose 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



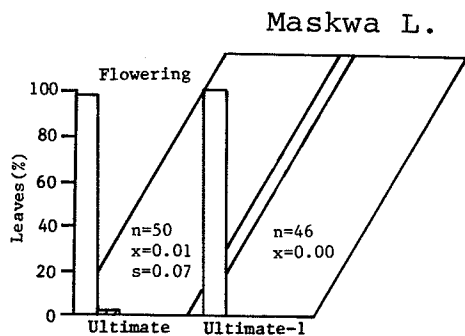
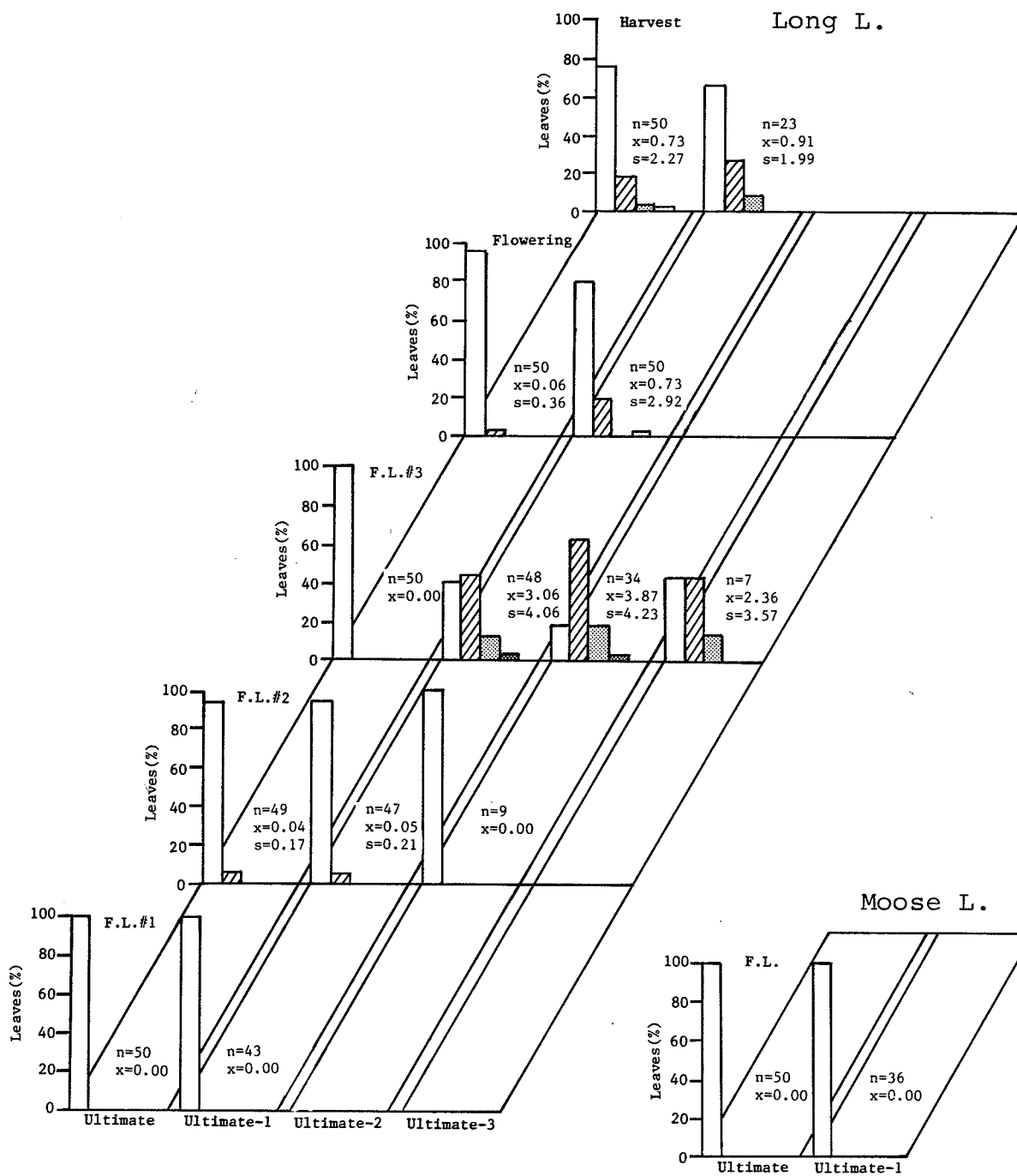
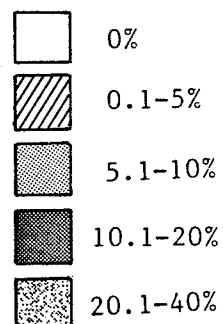


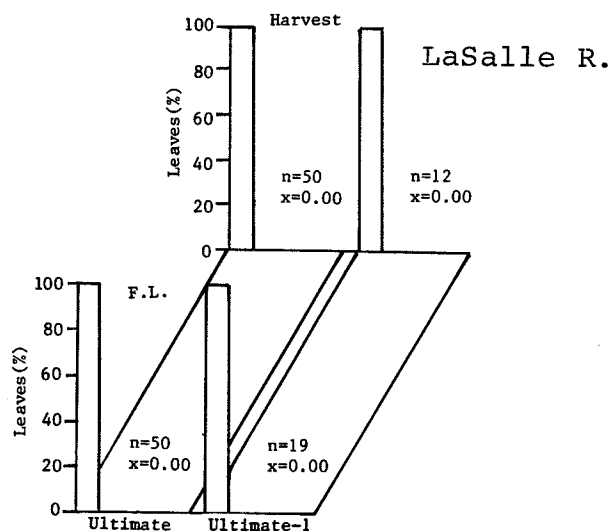
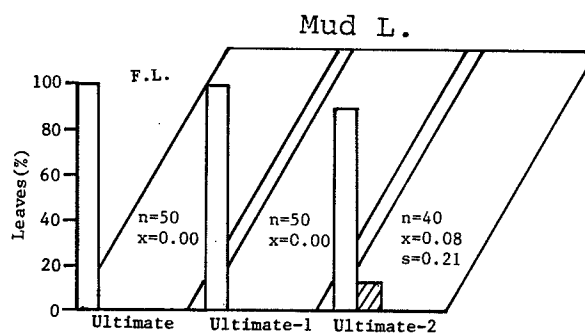
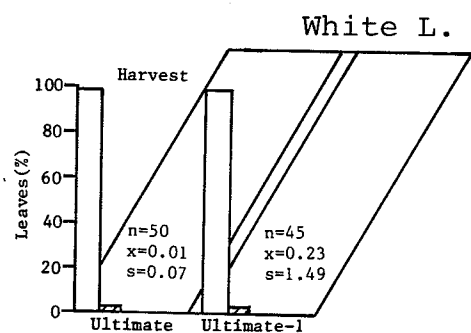
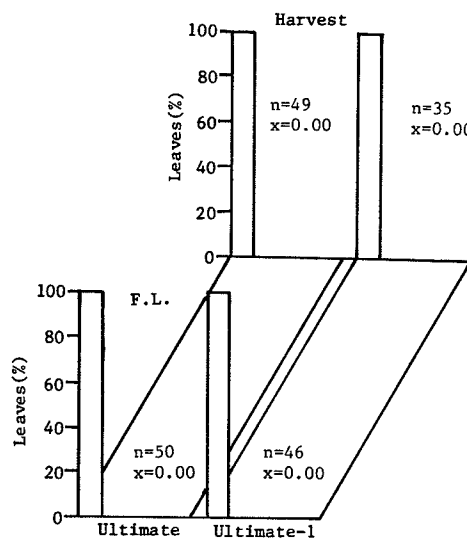
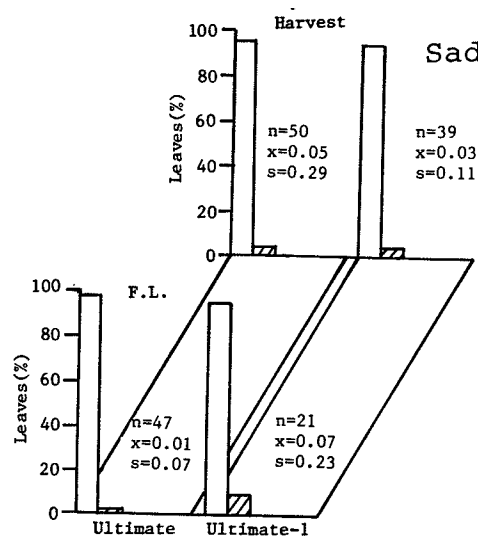
FIGURE 9d
Intensity of Anthracnose 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



Lone Is. L.



ates causing many of the leaves in a stand to have greater than 5% lesion cover.

When the incidence and intensity of 1980 samples are compared it can be seen that most Type II stands have an incidence of greater than 75%. To clarify this, the stands on the incidence maps with a Type II intensity distribution have been labelled with an asterisk (Figure 7). This relationship between high intensity and high incidence validates the use of incidence maps when considering the geographical distribution of this disease.

The incidence maps and intensity histograms all suggest that anthracnose is most severe in the Nopiming zone. However, the geographical distribution of anthracnose on floating leaves must be considered in conjunction with the timing of samples. Timing is particularly important in the examination of floating leaves because of their tendency to break off after senescence with consequent loss of any evidence of disease. Before northern and southern samples can be compared, it is essential that early, intermediate and late samples be taken from both areas. On Figure 8 the first column represents those stands sampled in the early floating leaf stage. The first floating leaf samples taken from Shallow L., Wallace L., Long L., and Lone Is. L. are all known to consist of plants less than two weeks in that stage. Two weeks previously, these same stands had been

visited and the only evidence of wild rice was uprooted seedlings in the 2-3 submerged leaf stage. It is assumed then that all samples in the first column, are of plants with less than two weeks in the floating leaf stage. In all but the Nopiming zone stands the samples were taken after the floating leaf stage had passed. Consequently, it is conceivable that there was a high incidence of anthracnose in stands other than those in the Nopiming zone, but this was not detected because of inadequate sampling. The floating leaf sample from Mud L. in 1980 adds further evidence to this suggestion. This sample (Figure 9d) had a disease intensity similar to that of the Nopiming stands sampled at the same time. If Mud L. had been sampled two weeks later, it is possible that the sample may have shown that disease in this lake had, in fact increased to levels similar to those found in the Nopiming Zone.

The stands within the Nopiming Zone did not have equal amounts of anthracnose on the floating leaves. The plants from the Wallace L. stand showed (Figure 9b) considerably more disease than those taken from Shallow and Long Lakes (Figures 9b and 9c). This conclusion can be drawn from the intensity distributions where the Wallace L. floating leaf samples had no ultimate-2 or ultimate-3 leaves free of disease. In addition, the Wallace L. F.L.#3 sample had leaves with greater than 20% lesion cover which is in contrast to the corresponding sample taken from Long and Shallow Lakes

where there were no leaves with more than 20% lesion cover. Thirdly, all Ultimate-3 leaves, in the Wallace L. F.L.#3 sample had infections of greater than 5%. This was not found in either the Long L. or Shallow L. samples.

It is difficult to explain this disparity on the basis of environmental differences between stands. Certain characteristics of the distribution of anthracnose between the Ultimate-1, Ultimate-2 and Ultimate-3 leaves suggest that it is a difference in stand development and not the result of environmental differences. The amount of anthracnose increases between the Ultimate-1, Ultimate-2, and Ultimate-3 leaves. Senescing leaves appear to become more susceptible to anthracnose. It may be that the plants at Long and Shallow lakes are at a different stage of development from the plants in Wallace lake and their leaves are reflecting this difference.

The interpretation of disease measurement data on wild rice is more straightforward when disease of the aerial leaves is measured. Because the aerial leaves do not break off, timing of the sample is not as crucial if comparisons between stands are desired.

Again, there seems to be more anthracnose affecting aerial leaves in the north than in the south. The apparent increase in incidence and intensity in a northerly direction is mostly the result of the high levels of disease in Harrop

and Three Lakes (Figures 7 and 8). If, for the moment, these high levels of anthracnose are disregarded, then the distribution of the disease assumes a more homogeneous nature along the north-south axis.

The high incidence of disease at Three Lakes (Figure 8) suggests that inoculum levels during the floating leaf stage may have been that much higher than in the Nopiming Zone and resulted in a proportionately higher level of disease on the aerial leaves. Alternatively, the increased disease may be the result of environmental factors that have predisposed the aerial leaves to disease. Even though floating leaves in the Nopiming Zone are known to have been severely infected, the low incidence of anthracnose on aerial leaves suggests that inoculum transfer from the floating to aerial leaves is not easily accomplished. On the other hand, the lack of anthracnose on aerial leaves in other stands in the Northern Zone does suggest that environmental factors are not the cause of the increased incidence at Harrop and Three Lakes. If it was due to climatic differences along the north-south axis a high incidence would also be expected in the stands of MacPhail R. and Assinika R. In addition, the developmental stage of the stands in the Northern Zone would be similar even if water depth were causing differences in the development of the stand. Although the MacPhail R. and Beren's R. stands had not yet begun to shatter, the leaves were in the early stage of senescence with yellowing at the

edges, as were the plants in Harrop and Three Lakes. A possible explanation for this apparent scattering of high incidence stands is that some local environmental condition is required either to provide inoculum transfer from the floating leaves, or to predispose the aerial leaves to infection by Colletotrichum. One such condition may be scattered local rain showers that would provide a mechanism for inoculum transfer and also explain why some stands had a higher incidence than others.

The flowering and harvest samples from Three Lakes and Harrop L., like those from the Nopiming Zone, are directly comparable. Both types of samples from these lakes were collected on the same day. Examination of the fruiting panicles confirmed that both samples were in the post harvest stage. Of particular interest in the Harrop and Three Lakes samples is the similarity of the two with respect to the intensity distributions found on the flag-1 (ultimate-1) leaf at the flowering stage (Figure 9a). This similarity suggests two things. First, the accuracy of the sampling and measurement systems is supported. If there was some discrepancy in the survey, one would not expect to find this degree of correspondence between individual samples. Secondly, anthracnose appears to be progressing up the stem axis. This, if anything, adds support to the assumption that anthracnose on wild rice is a disease of senescence. The severity of anthracnose on flag-1 leaves at the flowering stage

suggests that there is plentiful inoculum available. However, the low intensity of disease on the flag leaves does not reflect the presence of high concentrations of inoculum. It was not until the flag leaves were beginning to senesce, as they were at harvest, that anthracnose increased its intensity on the flag leaves. This implies that infection of the flag leaves was not occurring until senescence had begun. It is important that the time of infection be determined. Whether the flag leaf is becoming infected early or late in the flowering stage is likely to have important consequences when the impact of the disease on the plant is considered.

4.3.3 Brown Spot

This disease was present in all stands sampled in 1979 and 1980 (Figures 10 and 11). Like anthracnose, it shows a north-south distribution pattern, but trending in the opposite direction with higher levels of disease in the south than in the north. The intensity of brown spot rarely exceeded 5% (Figures 12a-d).

The uniformity of disease intensity between plants makes incidence a more reliable statistic in this case so that the distributions apparent on Figures 10 and 11. are probably more indicative of the actual levels of disease than those be for anthracnose (Figures 7 and 8).

FIGURE 10
Incidence of Brown Spot 1979

The shaded portion of each pie chart represents the incidence of anthracnose in the sample. There are three columns. The first column is comprised of floating leaf samples, the second flowering samples and the third harvest samples.

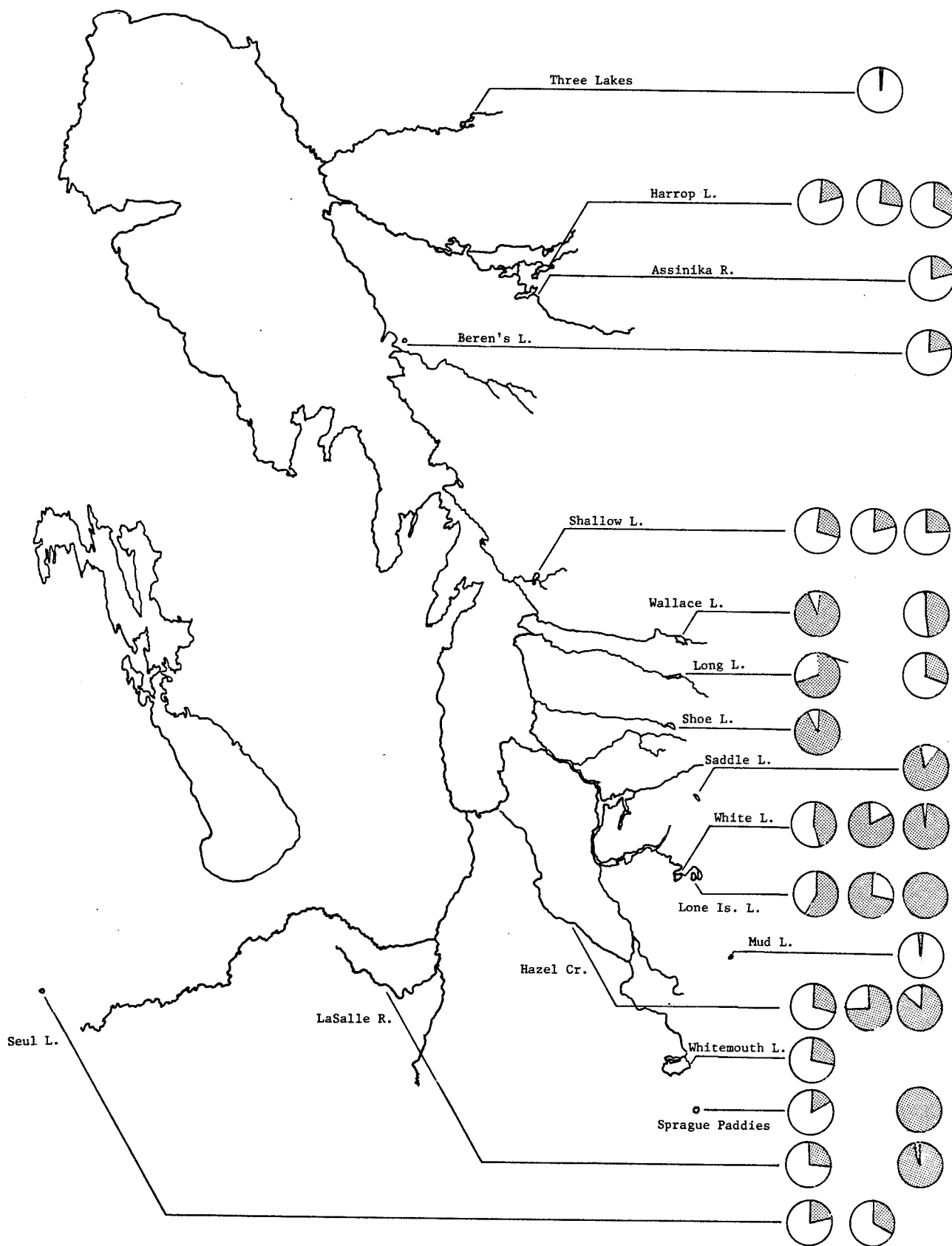


FIGURE 11

Incidence of Brown Spot 1980

The shaded portion of each pie chart represents the incidence of brown spot in the sample. There are five columns based on the five samples taken from Long and Wallace lakes. Column I represents samples taken at two weeks, Column II four weeks, and Column III six weeks after the emergence of floating leaves. Stands in the flowering stage are in Column IV and stands in the harvest stage are in Column V.

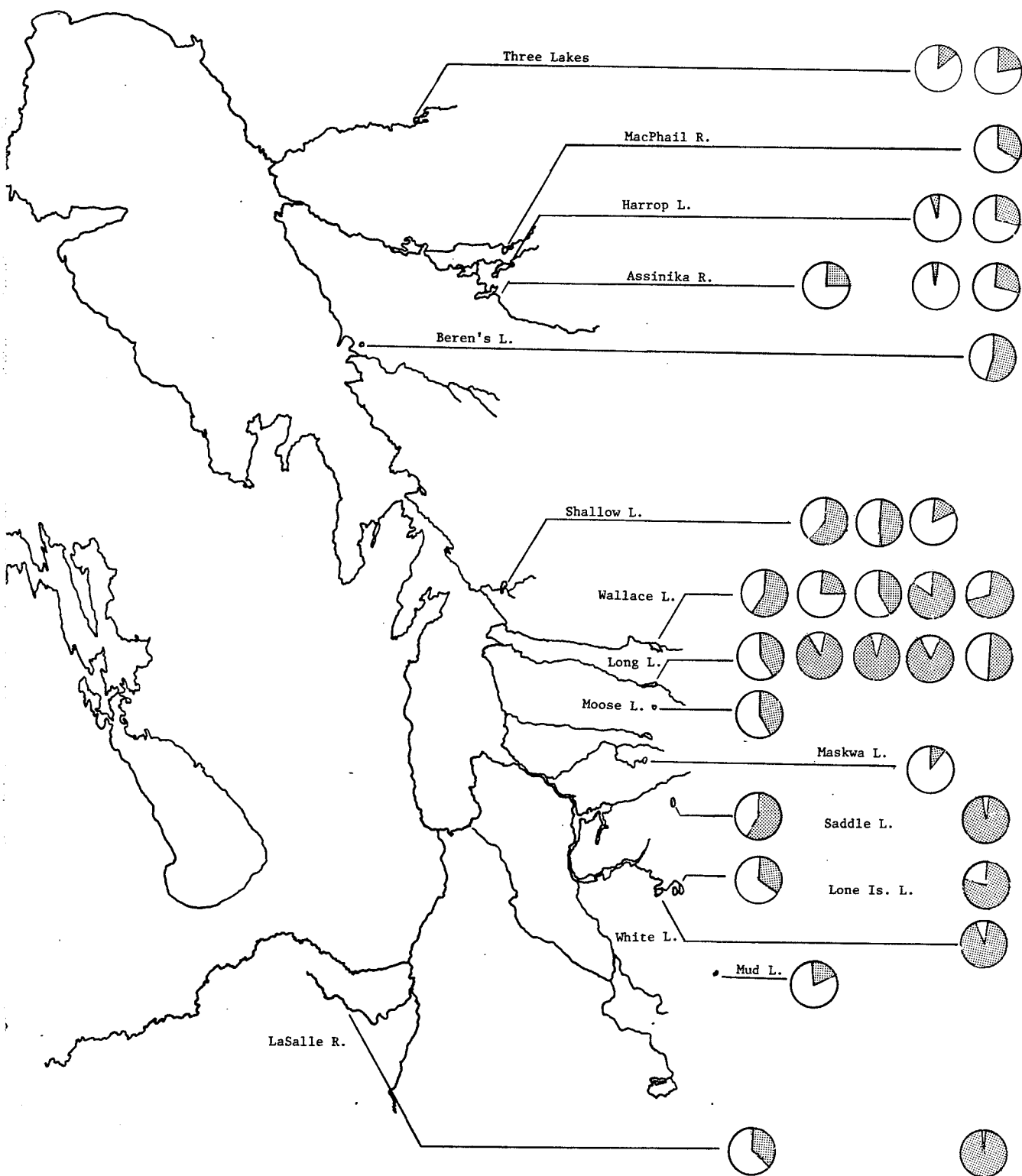
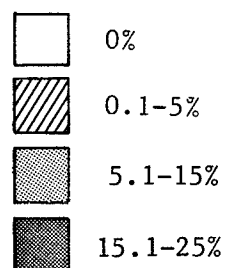


FIGURE 12a
Intensity of Brown Spot 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



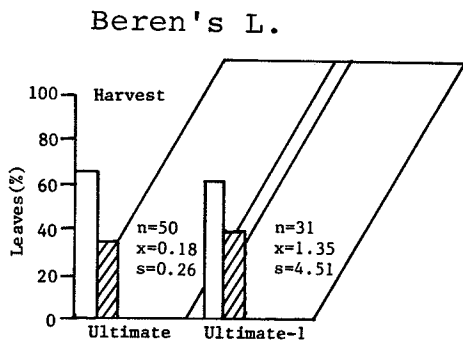
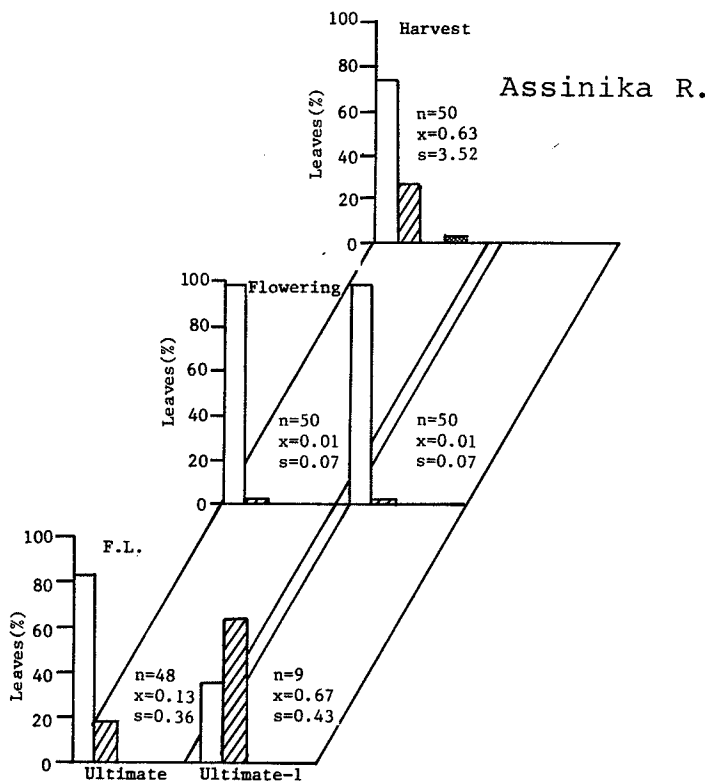
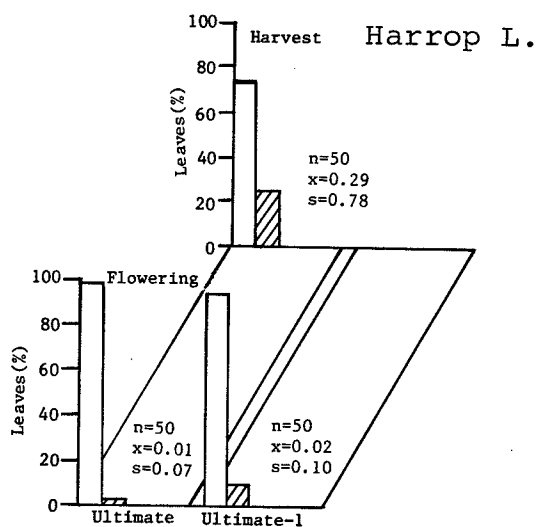
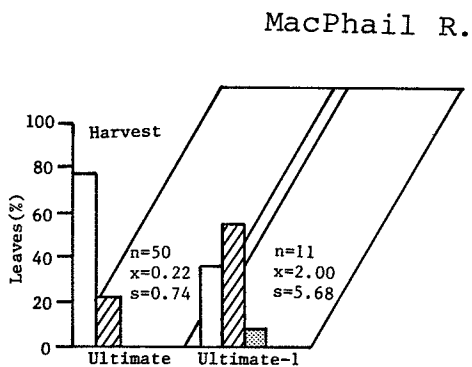
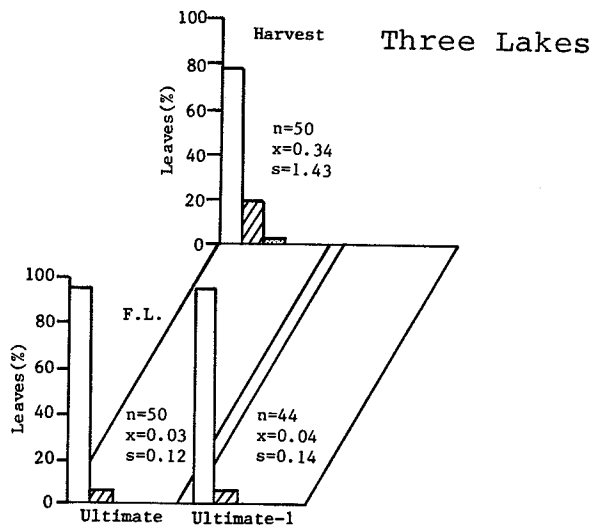
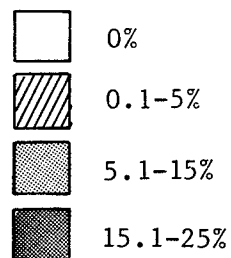


FIGURE 12b
Intensity of Brown Spot 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



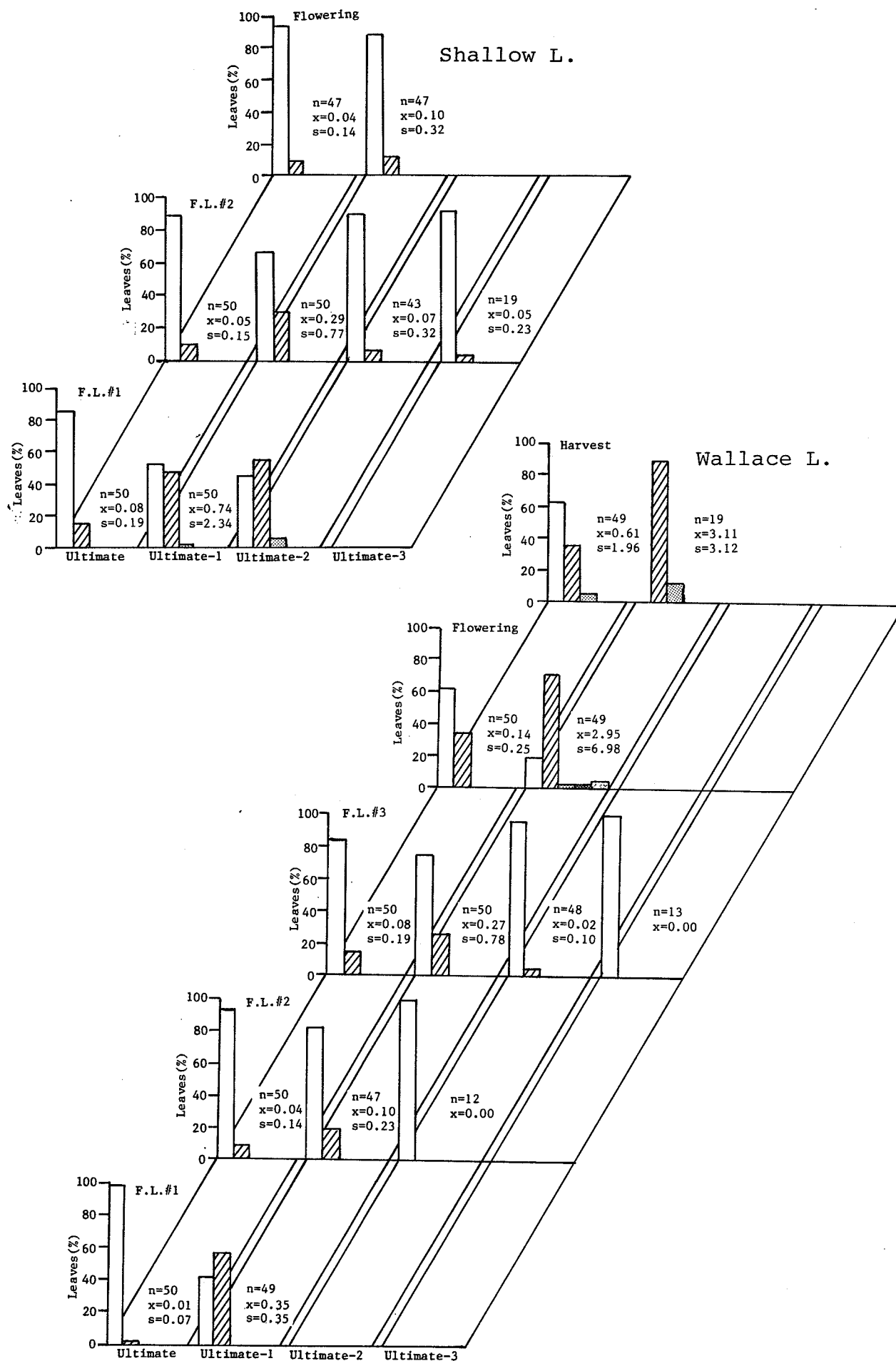
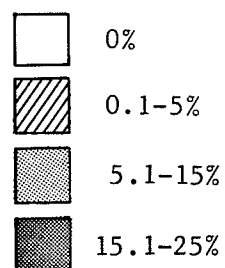


FIGURE 12c

Intensity of Brown spot 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



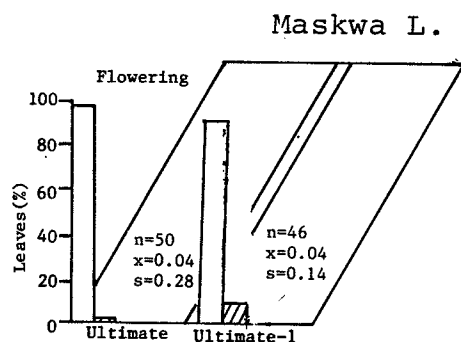
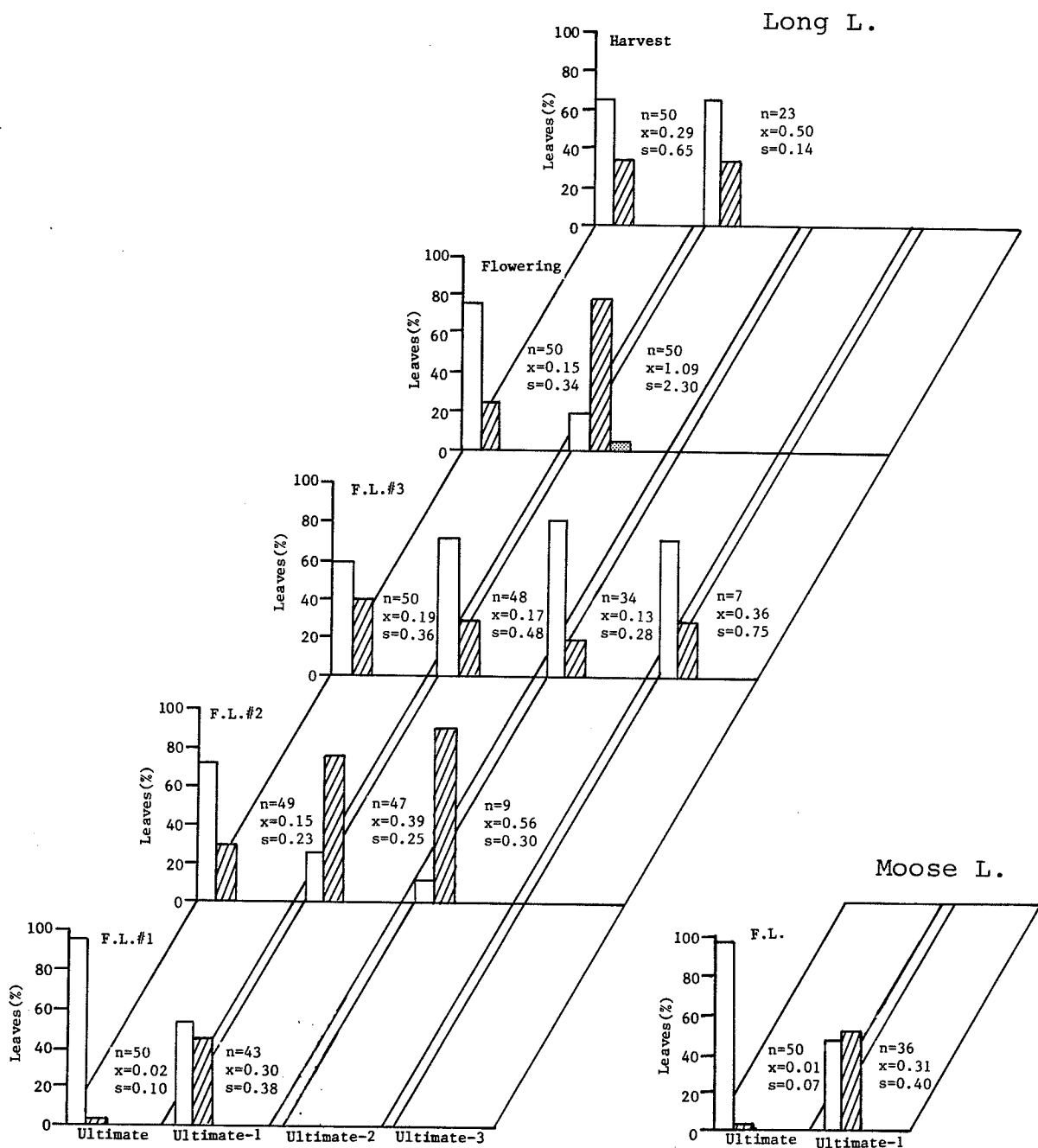
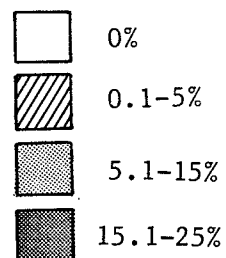


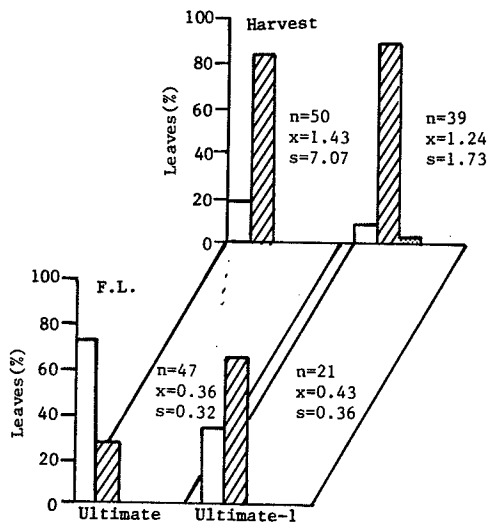
FIGURE 12d
Intensity of Brown Spot 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

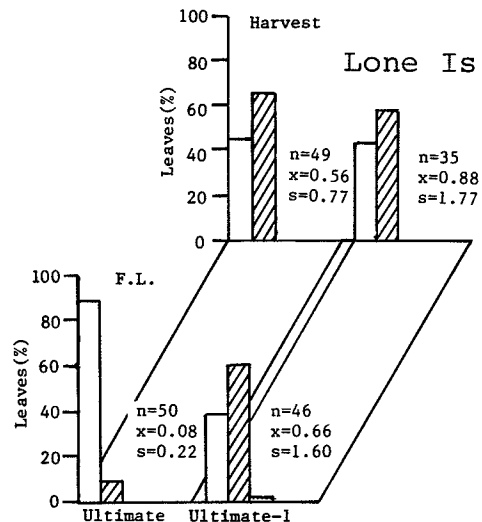
Legend:



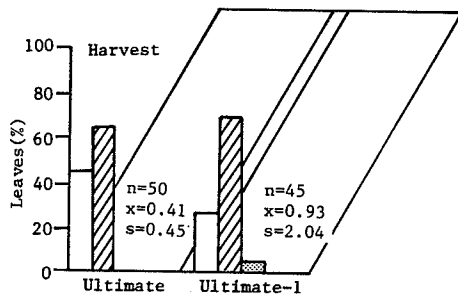
Saddle L.



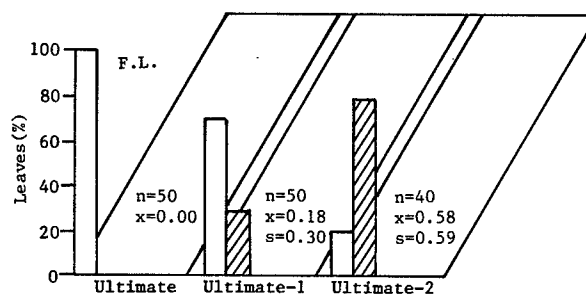
Lone Is L.



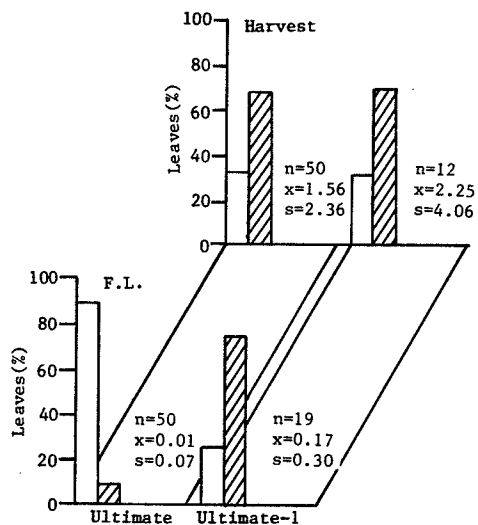
White L.



Mud L.



LaSalle R.



In view of the north-south trend occurring in both 1979 and 1980 it is fairly certain that the conditions in the south are more favorable to disease development than conditions in the north. The reasons may be climatical. Temperature and moisture may result in less favorable conditions for initiating infection in the north. Differences in the flora associated with the stands along the north-south axis may also be of significance. The grasses, which are more varied and abundant in the south could be increasing the probability of infection.

The 1979 floating leaf samples (Figure 10), at first sight, seem to contradict the trend. The highest incidences of brown spot were found in those samples collected in the Nopiming Zone from Wallace, Shoe and Long lakes. One possible reason for this discrepancy is the presence of Dichotomophthoropsis in the Nopiming Zone stands. Although it is not possible to determine what percentage of the lesions in these stands were caused by Dichotomophthoropsis, the fact that a large proportion of the lesions damp-chambered did produce this fungus, does suggest that a high proportion of the lesions were indeed caused by it. Consequently, a higher incidence of brown spot would be expected when Dichotomophthoropsis is present. The involvement of Dichotomophthoropsis in the brown spot syndrome only in this central portion of the survey range probably accounts for this distortion of the overall trend.

Also of interest in 1979 and 1980 is the high degree of similarity within individual stands between the various samples. In 1979 it was especially noticeable at Harrop L., Shallow L. and Seul L. It suggests that there may have been a relatively constant amount of inoculum available throughout the season. Because brown spot occurs on both floating and aerial leaves, inoculum must be available during these portions of the life cycle.

4.3.4 Leaf Blotch

The incidence of leaf blotch in both 1979 (Figure 13) and 1980 (Figure 14) suggests there is no obvious north-south trend. There are stands in the north (Assinika R. and Beren's L.) and in the south (Lone Isle L.) that have a significant incidence of this disease. Consequently, at this time any speculation on a north-south distribution of this disease would be tenuous.

Of more interest is the apparent absence of the pycnidial organism from the Alluvial Zone stands. Leaf blotch was not found in any stands in this zone in either year. On the other hand, it was observed in all other stands in the Whiteshell, Nopiming, and Northern Zones in 1979 and in all but Mud L., and Beren's L. in 1980.

FIGURE 13
Incidence of Leaf Blotch 1979

The shaded portion of each pie chart represents the incidence of anthracnose in the sample. There are three columns. The first column is comprised of floating leaf samples, the second flowering samples and the third harvest samples.

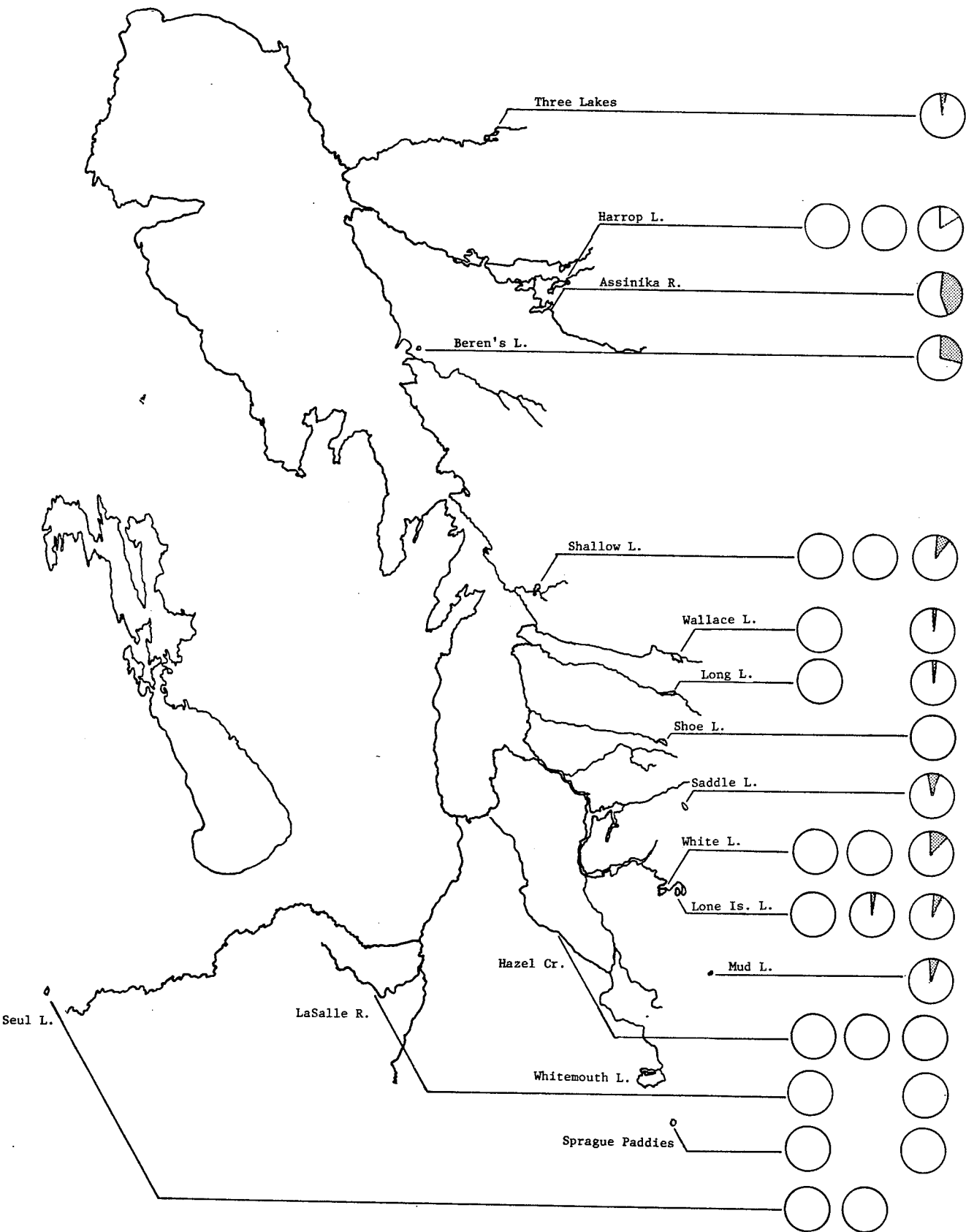


FIGURE 14

Incidence of Leaf Blotch 1980

The shaded portion of each pie chart represents the incidence of leaf blotch in the sample. There are five columns based on the five samples taken from Long and Wallace lakes. Column I represents samples taken at two weeks, Column II four weeks, and Column III six weeks after the emergence of floating leaves. Stands in the flowering stage are in Column IV and stands in the harvest stage are in Column V.

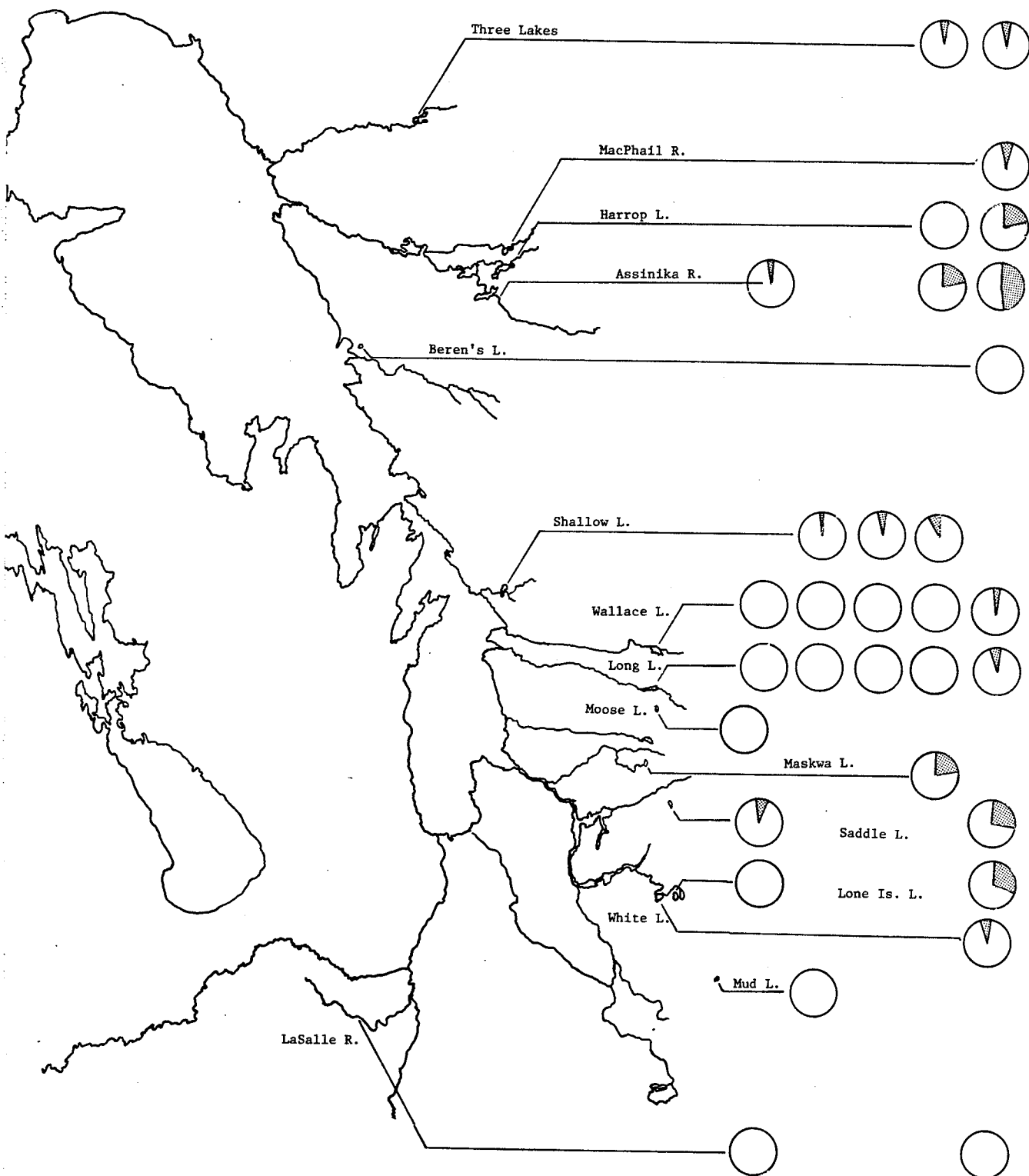
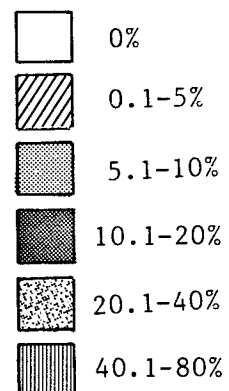


FIGURE 15a
Intensity of Leaf Blotch 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



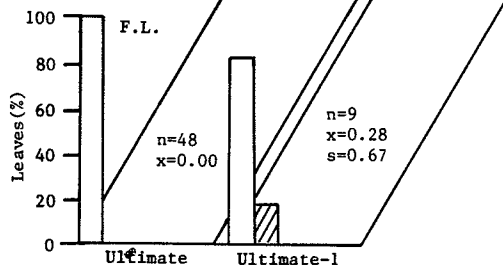
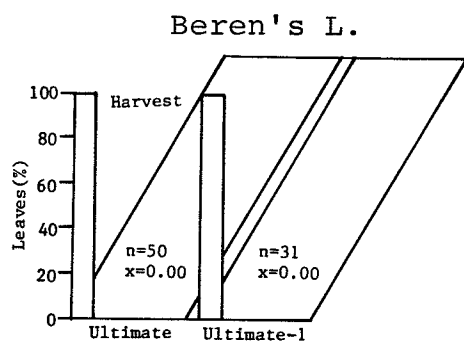
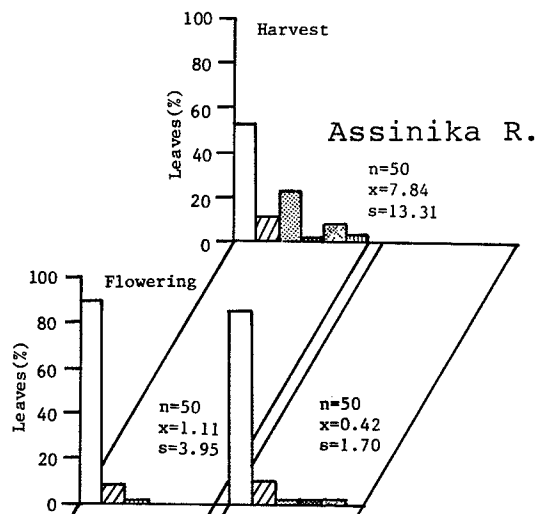
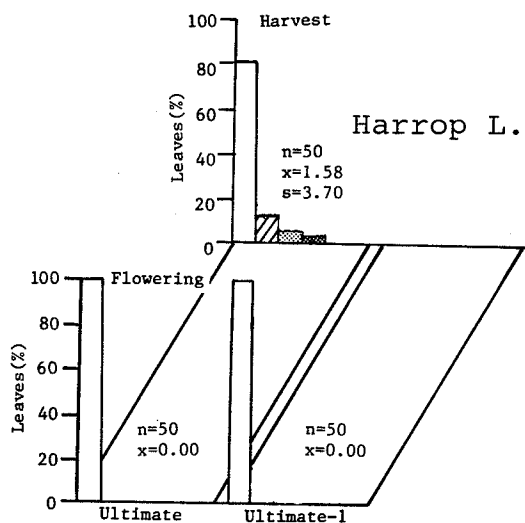
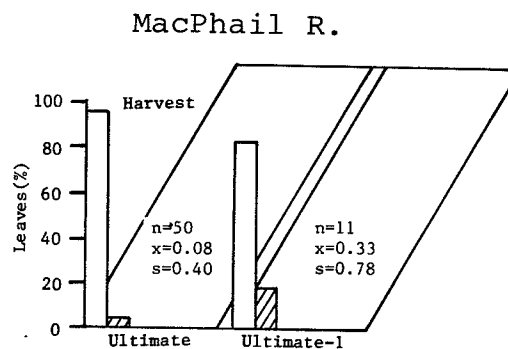
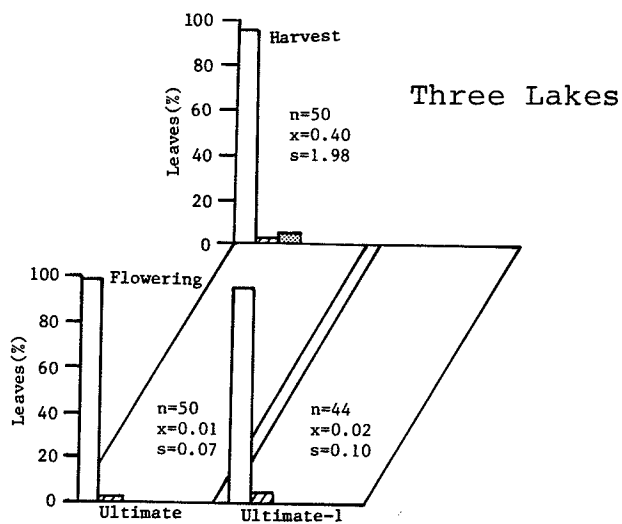
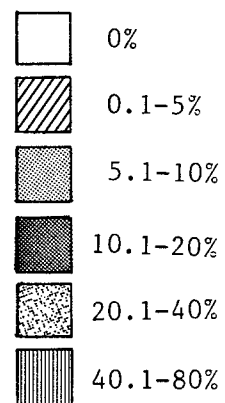


FIGURE 15b
Intensity of Leaf Blotch 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



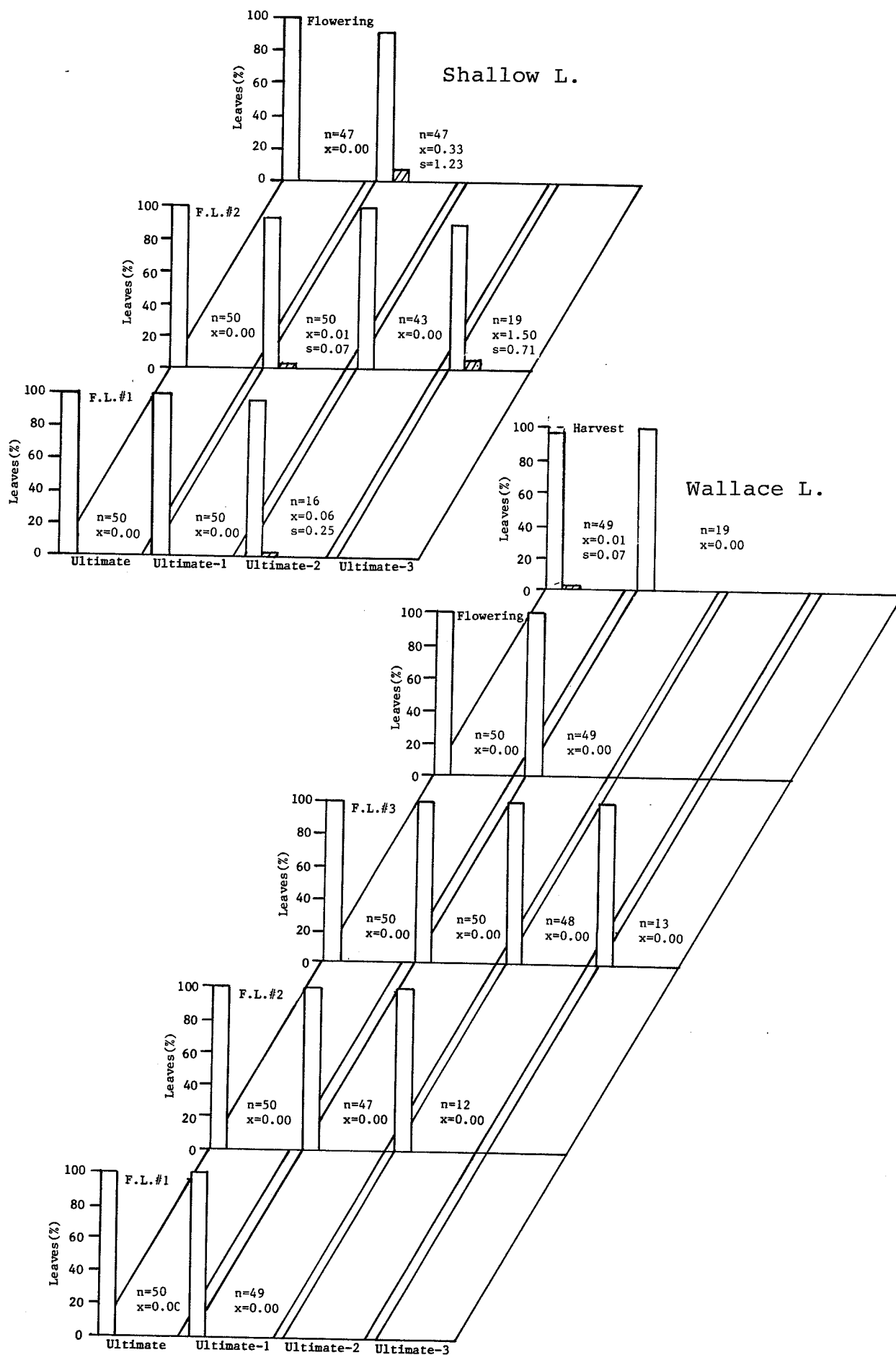
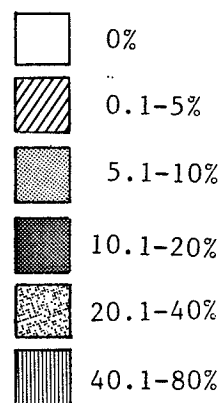


FIGURE 15 c
Intensity of Leaf Blotch 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:



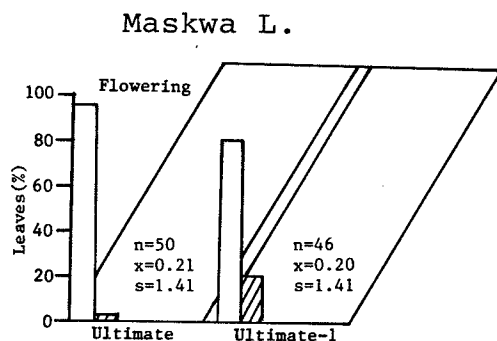
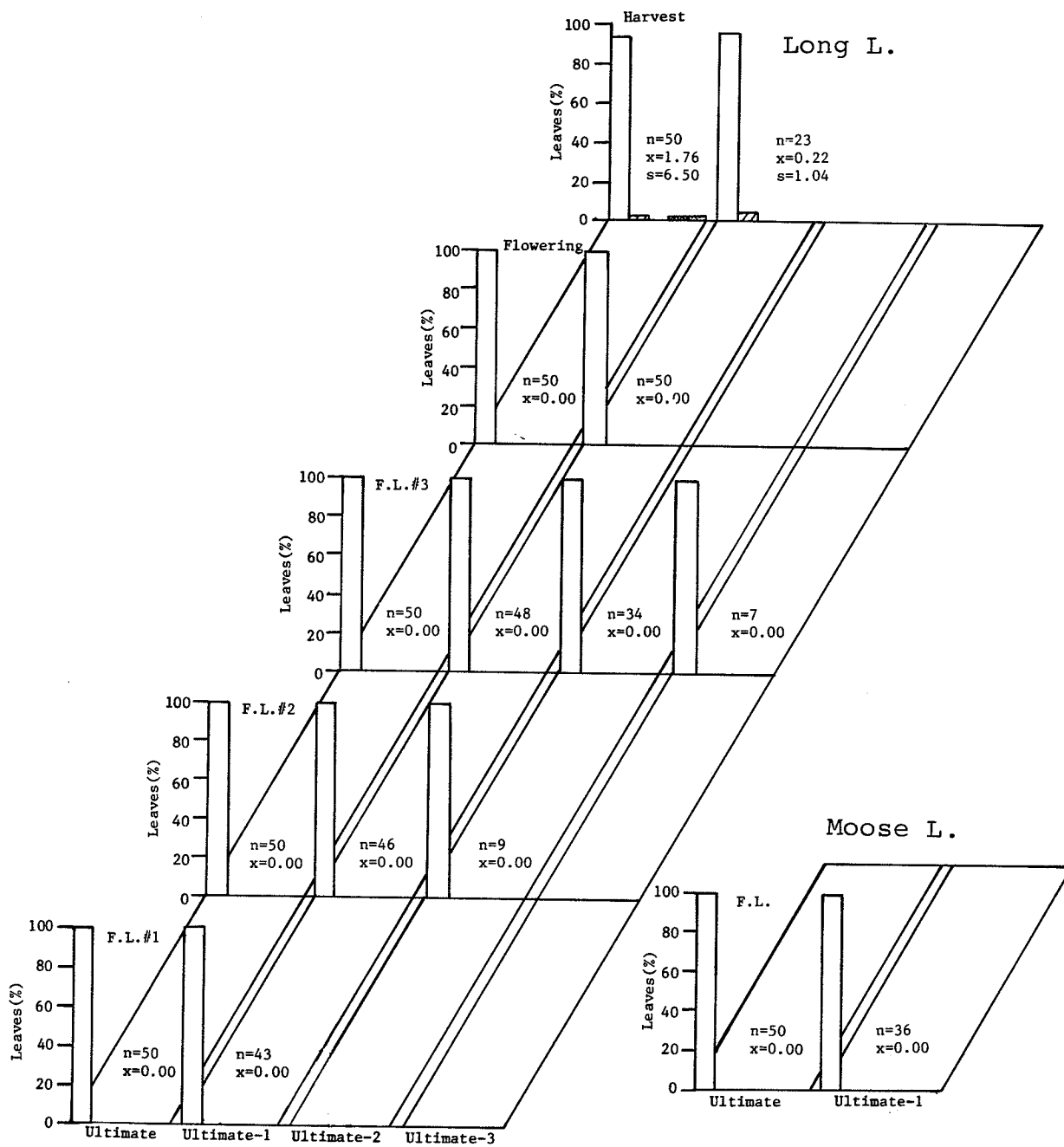
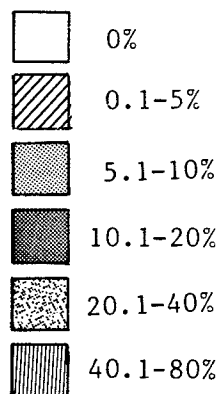
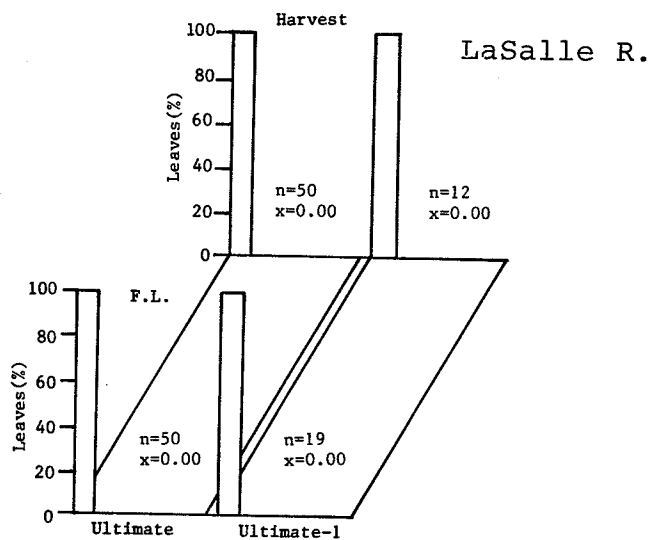
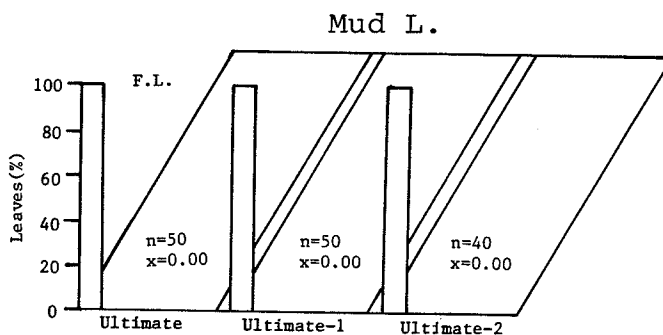
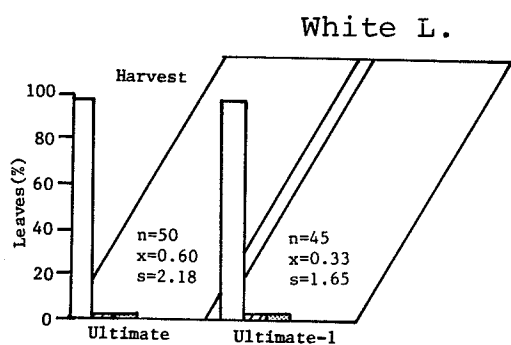
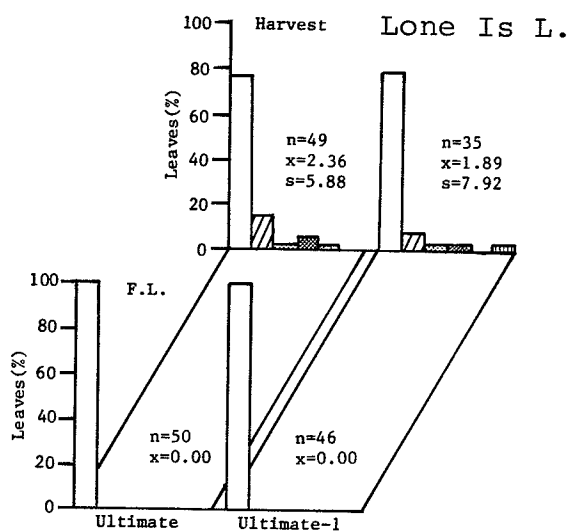
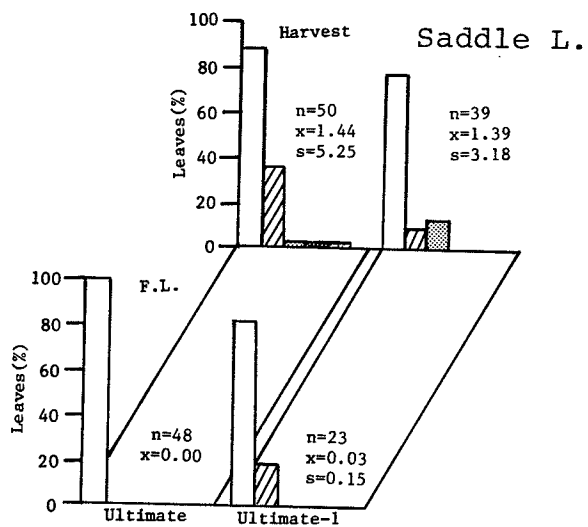


FIGURE 15d
Intensity of Leaf Blotch 1980

Each histogram describes the distribution of disease within a sample. The leaves were separated into classes based on the percentage area of lamina damaged by lesions. Each bar represents the percentage of leaves in each disease class.

Legend:





The absence of the pycnidial organism from Beren's L. in 1980 was peculiar because in 1979 there had been an incidence of greater than 25% in this lake. Such a dramatic reduction could be the result of winter conditions which prevented survival of inoculum, or summer conditions which were unfavorable for infection compared to those in 1979. The differences between 1979 and 1980 in the incidence of leaf blotch is suggestive of wide local fluctuations.

The histograms representing the intensity of leaf blotch (Figures 15a-d) do not show the same pattern exhibited by anthracnose. In this case there are no histograms approaching a normal distribution and all are clearly skewed. There are, however, seven samples that are distinct from the rest. The harvest and flowering samples from Assinika R. (Figure 15a) and the harvest samples from Three Lakes (Figure 15a), Long L. (Figure 15b), Saddle L. (Figure 15d), Lone Is. L. (Figure 15d), and White L. (Figure 15d) are all unique because they had leaves with leaf blotch lesions covering more than 5% of their surface.

The large size of lesion that is characteristic of this disease leads one to speculate that it could be a fairly serious cause of loss. There were some leaves in the Assinika R. and Lone Island Lake harvest samples that had single lesions covering more than 40% of the leaf surface. However, until there is more information as to the virulence of the pathogen little can be said with certainty.

4.3.5 Leaf and Stem Smut

Leaf and stem smut was considered by Dore (1969) and Gilbert (1974) to be of little significance. However, in this study some samples showed what could be significant amounts of disease. It was particularly noticeable at Lone Island L. where incidences exceeded 50% in both 1979 and 1980 (Figures 16 and 17). Again, the significance of this disease cannot be determined until the virulence of the pathogen has been more clearly elucidated.

FIGURE 16

Incidence of Leaf and Stem Smut 1979

The shaded portion of each pie chart represents the incidence of leaf and stem smut in the sample. There are three columns. The first column is comprised of floating leaf samples, the second flowering samples and the third harvest samples.

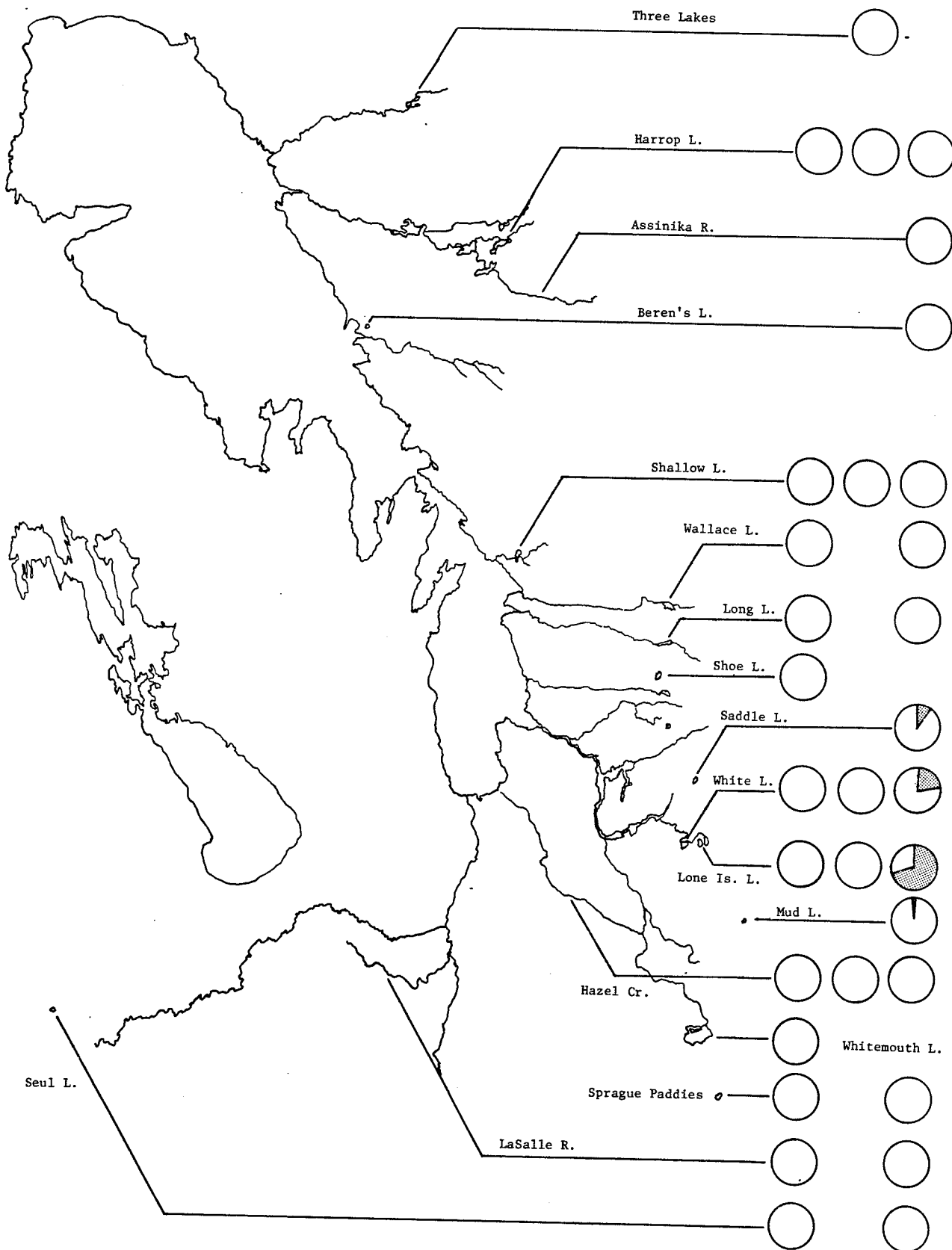
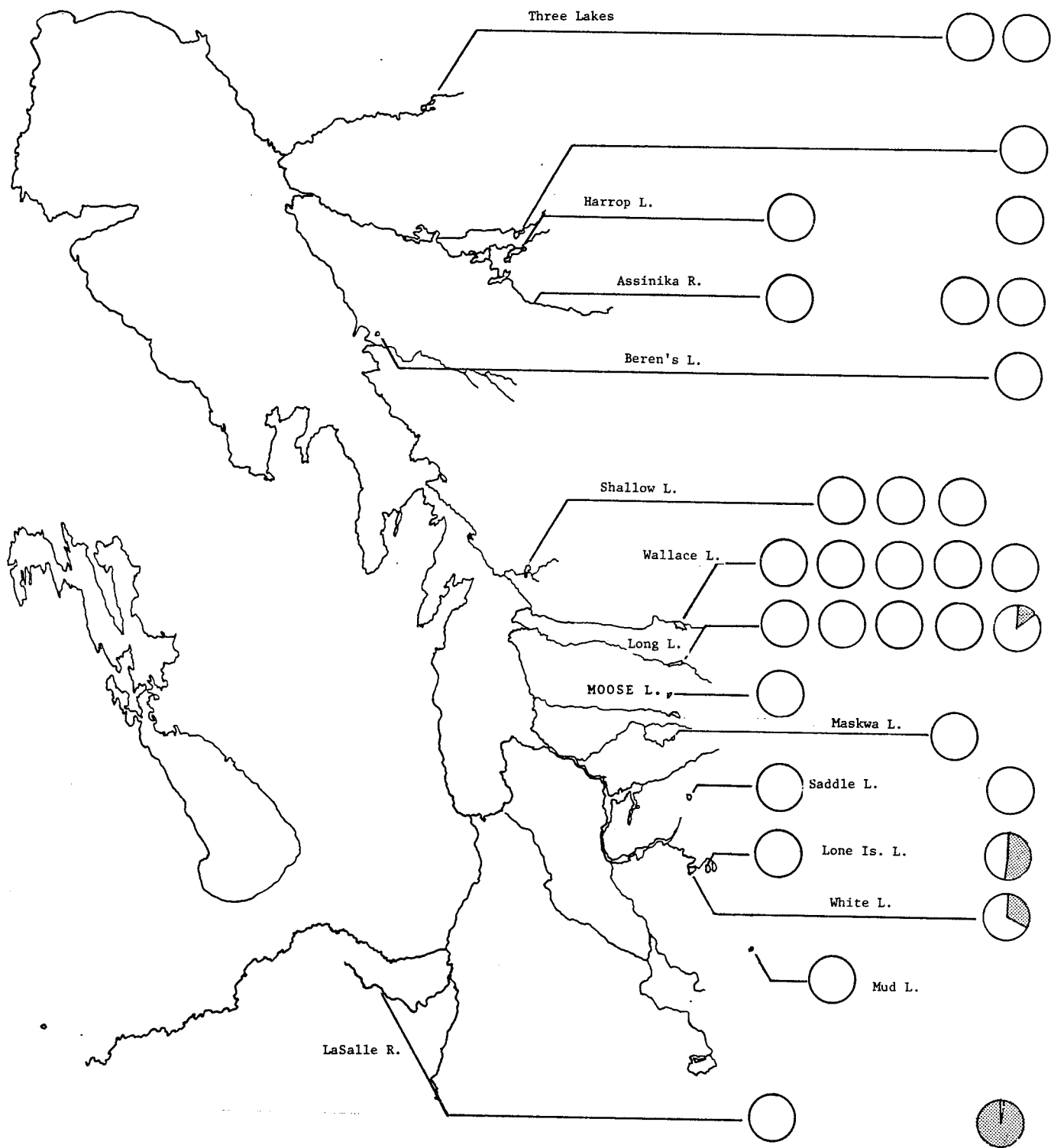


FIGURE 17

Incidence of Leaf and Stem Smut 1980

The shaded portion of each pie chart represents the incidence of leaf and stem smut in the sample. There are five columns based on the five samples taken from Long and Wallace lakes. Column I represents samples taken at two weeks, Column II four weeks, and Column III six weeks after the emergence of floating leaves. Stands in the flowering stage are in Column IV and stands in the harvest stage are in Column V.



4.3.6 Ergot

With respect to yield, probably the most important pathogen of wild rice is ergot caused by Claviceps zizaniae. Visual observations found this disease in most stands. Timing of samples is of particular importance with this disease. The sampling must be done before harvest so that the ergot bodies are still on the seed spike. One method of measurement would be to take samples from the bins as the wild rice is being harvested and determine what percentage of the seeds have been parasitized. This then could be applied to the overall effect of the disease in the stand.

Chapter V

GENERAL DISCUSSION

The information presented in this thesis falls into two main areas. First, there was the search for a more thorough and complete list of those fungi that parasitize wild rice. Four new organisms have been added to this list:

1. Colletotrichum
2. Dichotomophthoropsis
3. The pycnidial organism
4. Microdochium

Secondly, there was the quantification of disease in natural stands. This is something that has not previously been done, because many thought that the wide genetic base of wild rice might protect it from significant losses due to disease. However, this study has shown that even the floating leaves of wild rice are subject to disease and because of the plant's vulnerability at this stage there may be potential for considerable loss. Also, the pycnidial organism, with its large lesions may have the potential for causing serious loss, although at this stage one cannot be certain that it is not a disease of senescence.

Two major benefits arise from a study of this type. Those responsible for the management of wild rice stands will be enabled to recognize disease problems as they occur and plant breeders will be aware of the types of diseases that must be taken into account when developing new varieties. The identification and quantification of disease are, however, only the initial steps toward divulging the relationship between wild rice and its diseases. A much fuller understanding of the interactions between the plant and its pathogens is essential to allow translations of disease measurements into estimates or predictions of crop loss.

The overall result of the host-pathogen interaction can be expressed in the form of visible symptoms or in more subtle ways such as loss in productivity. The symptoms of a disease are usually closely connected with the amount of productivity lost. Horsfall and Dimond(1959) devoted several chapters in their treatise to the relationship between symptoms and the type of damage caused. The growth of the plant may be affected(Braun 1959); this may involve stunting or exaggerated growth such as witches brooms. The host may be starved of nutrients or water as in root rots or in stem galls that block the vascular stream (Subramanian and Saraswathi-Devi 1959). Reproduction may be affected as in the case of pathogens of the reproductive parts such as Claviceps spp. (Ciccarone 1959). Finally the plant may be affected by the disintegration of tissue (Husain and Kelman

1959). Most plant diseases involve one of the above forms of damage.

The diseases found on wild rice are characteristic of the latter two categories. Disease of the reproductive parts occurred in the form of ergot. Disintegration of leaf tissue was associated with a variety of symptoms and pathogens. The effect of ergot on the productivity of the plant is fairly clear cut. One ergot infection means the loss of at least one seed. On the other hand, the effect of disintegration of leaf tissue on the productivity of the plant is vague at best. Chester(1959) documented some of the difficulties. He recognized that greenhouse and plot studies were not sufficient to correlate the percentage of tissue damaged to the percentage of reduction in yield. He suggested that the effect of the environment on the interaction between the plant and the pathogen requires that field experimentation take place in a variety of locations over a number of years before any estimate of the effect of lesion damage on the plant can be made.

In this study, it has been possible to measure only the amount of disease present. The effect of disease on the productivity of wild rice crop has not been estimated because of the lack of information in two important areas. First, little is known of the effects of individual diseases on wild rice. Experimental studies must be conducted so

that the intensity of the disease can be correlated with the amount of loss expected. Greenhouse and plot studies must be conducted to correlate the intensity of disease with the amount of loss expected. Before these studies are feasible it must be possible to obtain a predictable level of disease on artificially inoculated plants. In addition, because of the excessive differences in yield experienced when plants are compared under normal circumstances, very large sample sizes are needed before differences due to the effect of disease can be measured. Secondly, the times at which infection is occurring are only known within broad limits. The time of sampling is important because the stage at which the the plant is infected has important consequences on the overall effect of the disease. The effect of a lesion is minimized if the leaf only becomes infected during the later portion of its productive phase. Obviously, damage to the lamina of a senescing leaf has less effect on the overall development of a plant than a lesion on a healthy photosynthesizing leaf. Therefore, the impact of a lesion is partially determined by the age of the leaf at the time of infection.

Because the samples in the survey were collected at fairly wide intervals it is not possible to determine the exact point at which infection was occurring on the leaves. The rapidity at which the floating leaf stage develops was attested to by the biweekly samples taken from the Nopiming

Zone, where the nature of the stand with respect to plant morphology and disease was significantly different between each of these samples. Consequently, weekly samples would probably be required to provide sufficient information to determine the impact of a particular disease on a plant. Some conclusions, however, can be drawn from what is already known about wild rice as well as information that has been gathered about the effect of disease on other crops.

Corn is a crop in which the relationship between stress and yield is well known. Arnon (1974) stated that "the most critical stage in the life of a plant" is during tasseling. It is at this time that the requirements for moisture and nutrients are at their greatest, and any deficiencies could have a serious effect on yield because of a delay in silking. During leaf development Hanway(1962) believed that the most crucial stage was when the leaves had reached 85% of their weight, and any stress during this period may seriously reduce the final weight of leaves. Therefore, in corn the especially critical times are during the later portion of leaf development and during tasseling. Wild rice may be equally sensitive to disease at corresponding stages and this suggests that samples must be obtained during these phases of the plant's life cycle.

During the course of the study it became apparent that the precise designation of floating leaves was not always

easy. For example, in the Nopiming Zone late floating leaf samples, although the ultimate-1, ultimate-2 and ultimate-3 leaves were found floating on the water, they had the breadth and protruding mid-rib of an aerial leaf. Even though these leaves could have been, in an anatomical sense, aerial leaves, they have been designated as floating leaves because their relationship with the environment was closer to that of floating leaves than aerial leaves. The fungal inoculum produced on true floating leaves would be more accessible to a leaf lying flat on the water than a leaf standing above it, if only from the point of view of distance. Possibly just as important, is the drier microenvironment of the aerial leaves that would be less conducive to infection by most fungi than the moist microenvironment of a leaf lying flat on the water.

Although little is known of the relationship between the floating leaves and the overall development of the plant, it is possible that fungal lesions on the floating leaves may have a more profound effect than they do on aerial leaves. The plant, during the floating leaf stage, is at a particularly vulnerable point in its life cycle. Floating leaves are long, thin leaves extending from the bottom of the water body to its surface. High winds or insects could easily sever these leaves from their anchor. A great deal of carbohydrate must be produced by them to allow development of the flowering culm. Anything which interferes with the opera-

tion of the floating leaf at maximum photosynthetic capacity, may prevent or delay flowering to the point where seed will not ripen, especially in northern areas where the growing season is already short. The large typical lesion of anthracnose must cause a significant loss of photosynthetic area on the thin floating leaf. Even a single lesion may have a significant effect if the infection occurs early enough. If the lesion is in a critical location such as at the base of the lamina, the whole leaf may be lost. In the aerial stage, if a leaf is lost there is usually enough reserve photosynthetic area with other leaves on the culm and tillers that although there may be some loss, the inflorescence will still develop. However, if a floating leaf is severely damaged there is no reserve of photosynthetic area and it is possible that the whole plant will succumb. Because of the particular vulnerability of the floating leaf stage to total loss through pathogenesis, it is important that these stages be clearly distinguished in any disease survey.

Chapter VI

SUMMARY AND CONCLUSIONS

1. Representative stands of wild rice were sampled during 1979 and 1980 in order to extend the knowledge of the pathology of the crop.
2. Five distinct disease syndromes were observed. Three of these: brown spot, leaf and stem smut, and ergot were known diseases of wild rice; anthracnose and leaf blotch have not previously been reported on this host.
3. In addition to Helminthosporium sativum and a Fusarium sp. Dichotomophthoropsis and Sclerotium hydrophilum were isolated from brown spot lesions.
4. Colletotrichum sp. and an unidentified pycnidial form were shown to be the causes of anthracnose and leaf blotch respectively.
5. Microdochium sp. which was frequently associated with the anthracnose syndrome proved to be a minor pathogen in its own right.
6. Brown spot was found to be more severe in the south. Anthracnose, on the other hand, increased in intensity and incidence in the north.

7. Although the phenology of wild rice is understood in general terms, there is at the present time no satisfactory method of determining the substages of the floating leaf stage (the floating leaf stage may even include aerial leaves that are floating). This deficiency complicated attempts to compare the development of disease between stands at corresponding physiological ages.
8. Before the effect of disease on the yields of wild rice can be determine, the relationship between loss in yield and severity must be known. This will require extensive greenhouse and field studies where the intensity of disease can be correlated to loss in yield.
9. The qualitative and quantitative results of this study negate the hypothesis that disease is unimportant in the natural stands of of wild rice.

Appendix A

THE PREPARATION OF NUTRIENT AMENDED AGAR

1.	1M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	5 ml
2.	1M KNO_3	5 ml
3.	1M KH_2PO_4	1 ml
4.	1M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2 ml
5.	FeEDTA	3 ml
6.	Micronutrients	1 ml
	a) H_3BO_3	2.86 g
	b) $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81 g
	c) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.05 g
	d) ZnCl_2	0.11 g
	e) $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.025 g
7.	Agar	15 g
8.	Water to Make	1 l

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