A Survey of the Diseases of Wild Rice in Manitoba

by

David Andrew Ross McQueen

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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 entepreneur. 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 specimems 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 (Fyles,1915;Pantidou,1959). Conners (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 essentialy 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

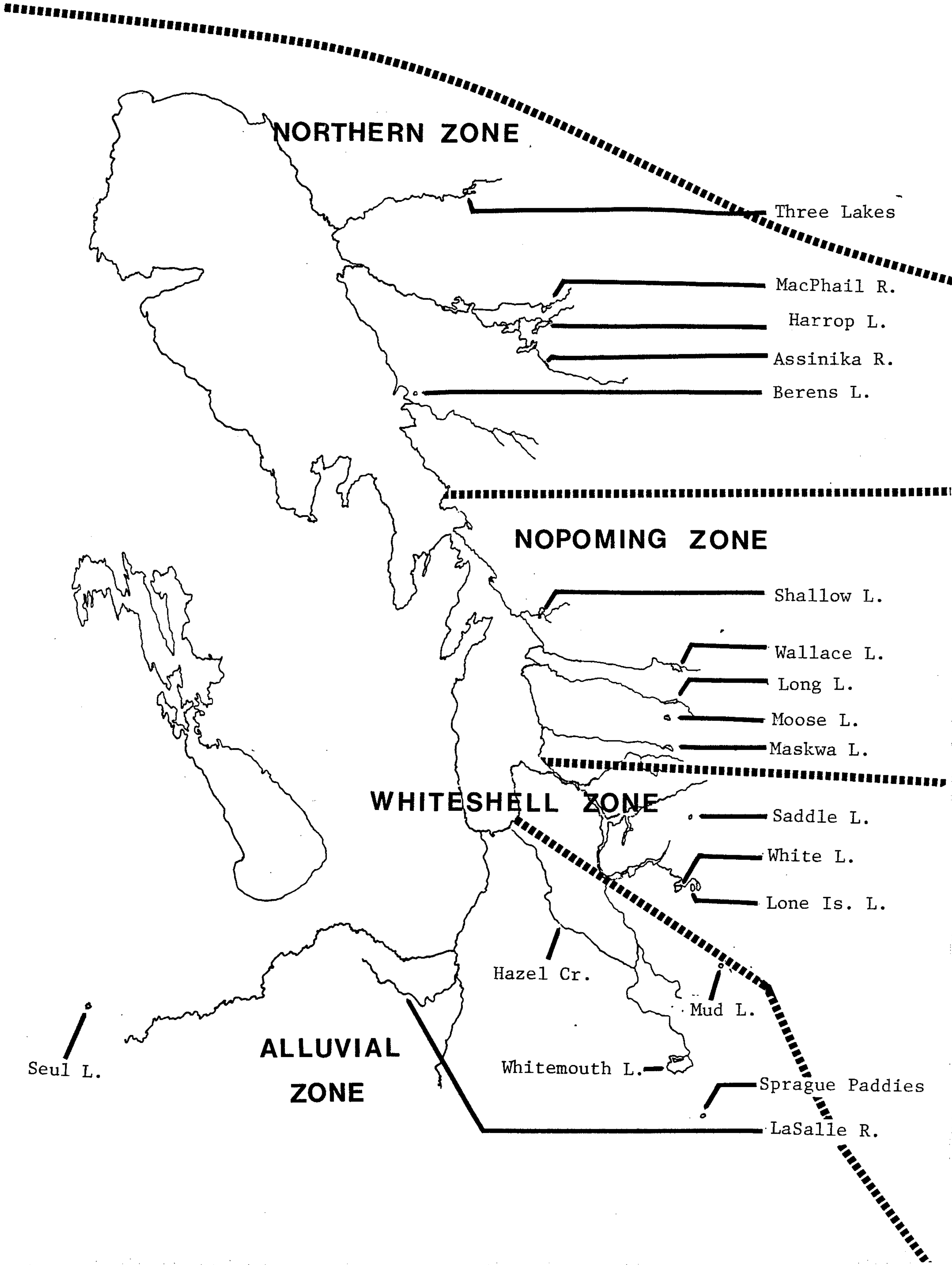
The sampling range extended from 49° N to 53° N and from 96° W to 100° W. The sample sites used in this study are presented on Figure 1.

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



NORTHERN ZONE
Three Lakes
MacPhail R.
Harrop L.
Assinika R.
Berens L.
NOPOMING ZONE
Shallow L.
Wallace L.
Long L.
Moose L.
Maskwa L.
WHITESHELL ZONE
Saddle L.
White L.
Lone Is. L.
Hazel Cr.
Mud L.
Whitemouth L.
Sprague Paddies
LaSalle R.
Seul L.
ALLUVIAL
ZONE

FIGURE 2

Surface Deposits

Redrawn from the Manitoba Economic Atlas.

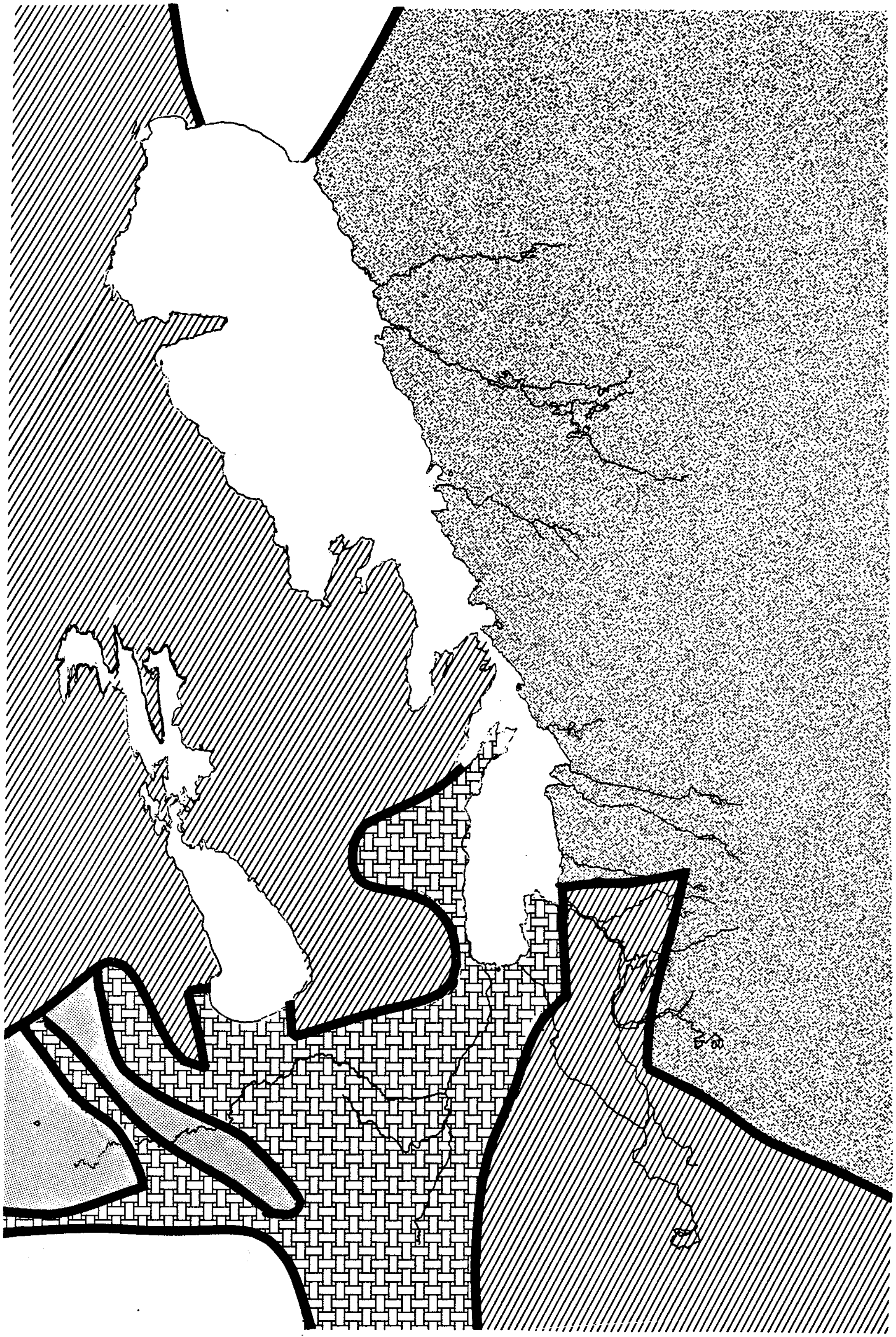
Legend:

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

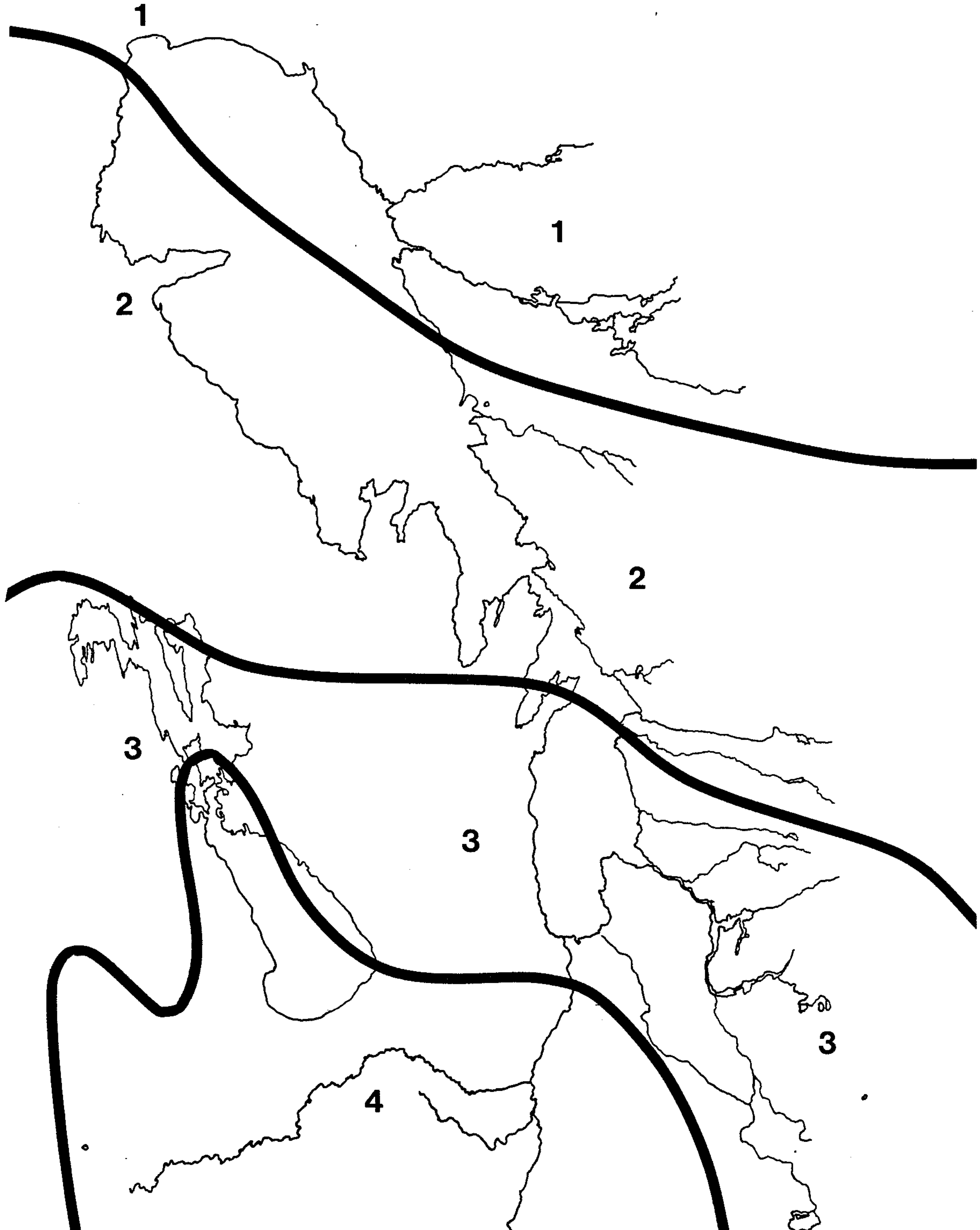
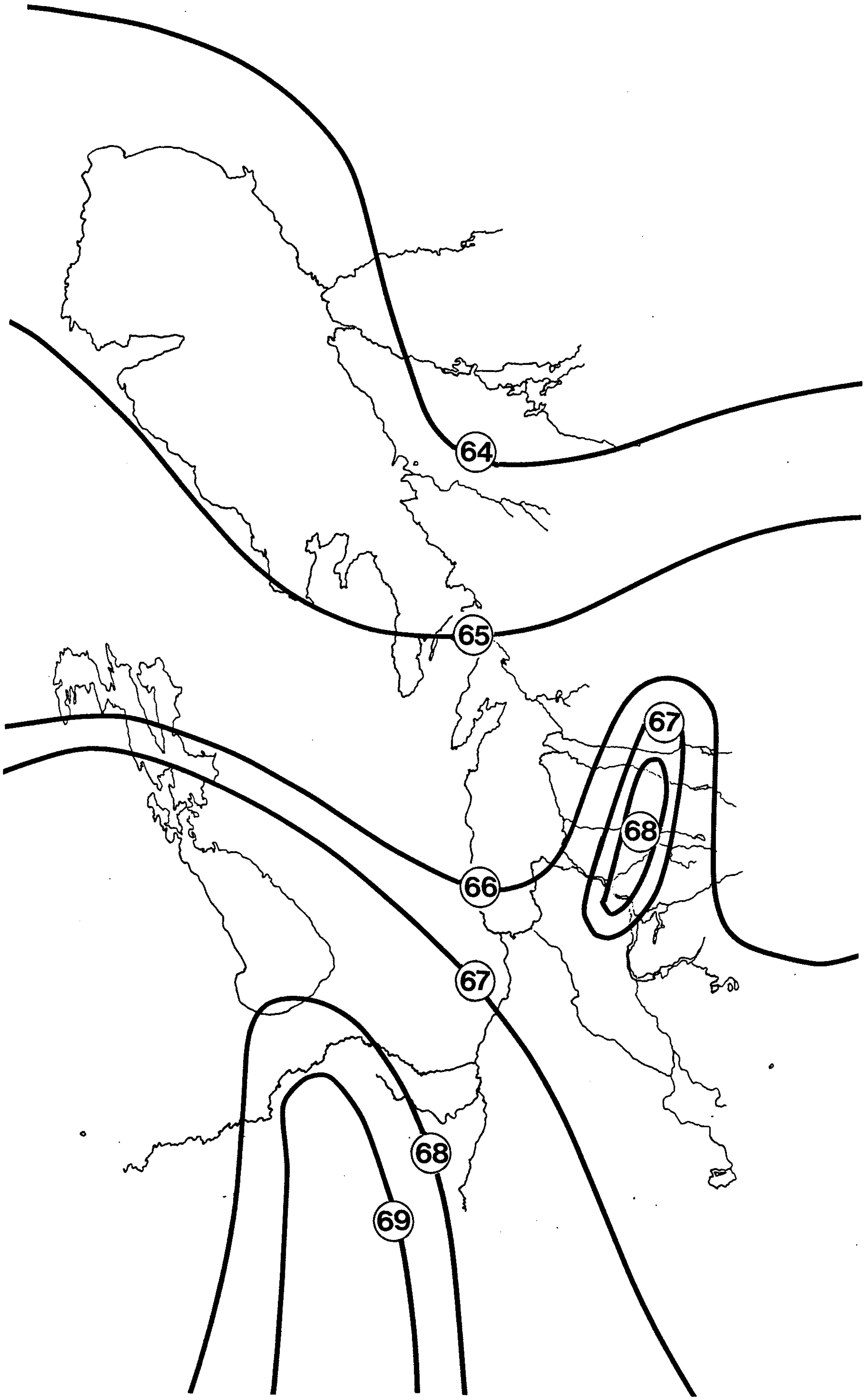

1
1
2
2
3
3
3
4
5

FIGURE 4

Average July Temperature

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



64
65
67
68
66
67
68
69

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)	13/8/80 (47)		
Wallace L.	11/6/80 (50)	26/6/80 (50)	10/7/80 (50)	20/8/80 (50)		3/9/80 (49)
Long L.	11/6/80 (50)	28/6/80 (49)	10/7/80 (50)	20/8/80 (50)		3/9/80 (50)
Moose L.	11/6/80 (50)					
Maskwa L.				11/8/80 (50)		
Saddle L.	11/6/80 (47)					3/9/80 (50)
Lone Is. L.	11/6/80 (50)				28/8/80 (49)	
White L.					20/8/80 (50)	
Mud L.		26/6/80 (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