UNIVERSITY OF WATERLOO
Faculty of Environmental Studies

AN EVALUATION OF METHODS REQUIRED TO DETERMINE OVERWINTERING PLANT HEALTH IN CATTAILS GROWING ON ACIDIC MINE TAILINGS

Boojum Research Ltd.
Toronto, Ontario

Prepared by:

M. W. English
ID 87001473
3B Environment & Resource Studies
20 August 1991

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ABSTRACT

As part of an overall Ecological Engineering strategy to ameliorate acid mine drainage - a water pollution problem associated with the mining industry - by enhancing natural water treatment processes, this report has evaluated (and discussed data obtained from) methods and analyses used to determine below-ground cattail starch quantities and distribution, metal uptake, and general morphology. With the vision of increasing plant biomass and biological polishing capacity through fertilization, results from this report will be used to formulate a sampling and analytical strategy for determining the effects of foliar fertilization on below-ground biomass of cattails growing on acidic tailings at a uranium mine in Elliot Lake, Ont. Preliminary cattail samples were collected locally and subjected to the procedures deemed appropriate for determining whether applications of foliar fertilizer on tailings-grown cattails would have an effect on plant health. The methods used during the preliminary trial were appropriate for providing data on a number of growth-related parameters and required only minor sample-specific adjustment to obtain accurate results. Data obtained while evaluating the methods, along with findings from other studies, indicated that pith size may be an important below-ground morphological indicator of plant health.

CONCLUSIONS

Cattails can be extracted in the winter with a spade, provided there is minimal ice build-up over the substrate. Plant damage due to extraction was kept to a minimum by digging approximately 0.5m away from the chosen senescent shoot in a circular fashion and periodically checking for submerged new shoots in the substrate.

Minimal disfiguring of rhizome hand sections occurred when samples, before sectioning, were placed back in the freezer for one-half hour after laboratory washing.

Storing hand sections in 95% denatured ethyl alcohol did not change the distribution of starch in the rhizome. Changes in actual starch quantity as a result of alcohol storage were not confirmed.

Removing roots at the exodermis was not microscopically precise. It could not be determined whether any remaining root tissue affected rhizome weight measurements.

Iodine/potassium iodide staining for starch distribution was an effective method for elucidating the presence and location of starch within a rhizome section. Colouration of tissue resulting from stain application was consistent throughout the samples.

Hematoxylin staining for metal uptake in tissue was very time-specific, with the 2-4 hr. retention interval producing the best colouration. Colours produced by stock hematoxylin application were too intense for effective examination; those produced from fresh solutions were adequate.

Photographing rhizome sections produced the best results when the camera shutter speed was slowed down from the suggested setting. Measuring sections was made effective and easy using a movable, graduated (mm^2 grid) cover slip.

Blending fresh cattail tissue produced considerable variability in final quantitative starch determinations. Because the tissue could not be blended into a consistent slurry, accuracy of the starch data was questionable.

Twenty-seven of 29 rhizome sections had piths whose diamaters were greater than 50% of their respective rhizome's overall diameter. Ninety percent of the rhizomes had piths that became proportionately larger toward the new shoot. Pith diameter may be an important parameter in determining plant health.

RECOMMENDATIONS

Once extracted from the substrate, cattails should be kept frozen until analyses are undertaken. Ice-filled coolers should be used to transport samples from the field to the laboratory.

Below-ground biomass claculations should be performed in the summer months when the conditions are suitable to do so.

Random sampling of cattails may be done by pacing out the experimental plots and using a table of random numbers to locate samples.

Total soluble carbohydrate tests should be carried out to determine what percentage leaves the plant cells and passes into solution.

Fresh hematoxylin solution should be prepared daily and allowed to develop colour for at least two hours, but no longer than four.

To ensure proper exposure when taking pictures of rhizome sections, small samples should be photographed at a ¼s slower exposure setting than suggested by the camera's light meter. Exposure times for large samples need only be moved to the maximum setting on the fine adjustment control while keeping the macro-adjustment as suggested.

A micro-ruler with 0.1mm graduations should be useful and more accurate for rhizome dimensioning under the microscope.

To decrease data variability between replicates of a single sample, tissue for quantitative starch analysis should be dried at 110°C to constant weight and ground into a powder.

Graduated 5ml pipettes should be used to improve accuracy and consistency when taking

aliquots.

Attention should be paid to measuring pith and rhizome diameters of the Denison cattails since a relationship between proportional pith size and plant health has been suggested.

1. INTRODUCTION

1.1 Problem Areas

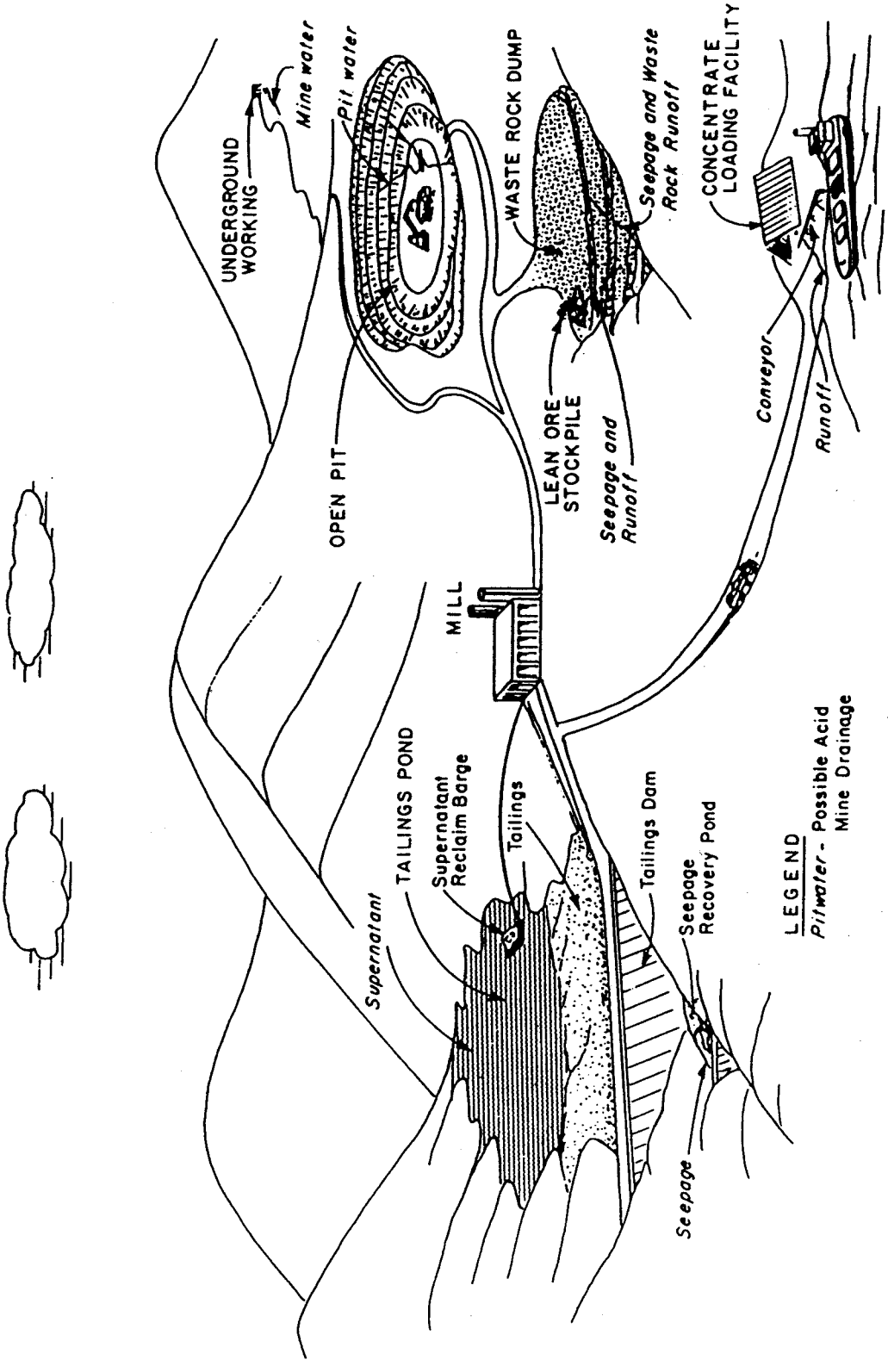
Conventional approaches to wastewater treatment at acid-generating waste sites focus on collecting and neutralizing contaminated water, typically with the use of costly treatment plants and liming programs. Unfortunately for the operators, these costs are perpetual as wastes continue to produce acid long after revenue operations have ceased. To ensure environmental integrity, government abatement regulations are now mandating operators of base metal and precious metal mines, for example, to set aside money for environmental initiatives like wastewater treatment. Because, from a business standpoint, the environment represents a long-term liability, novel and cost-effective treatment methods need to be developed so that honest and effective efforts can be made to protect the natural environment.

Ecological Engineering addresses these concerns in its development of a conceptual framework for a self-sustaining treatment system. Cattail studies are only part of a broader scheme whose aim is to produce close-out conditions which are environmentally acceptable, self-sustaining, and cost-effective; other areas of investigation include microbiological sulphate-reducing processes, biological filtration systems, and solid waste research.

In recent years it has been acknowledged that cattail wetlands possess the ability to treat wastewater (Lakshman, 1987; Kadlec, 1987; Watson et al, 1987), and attempts have been made to use this quality for ameliorating acid mine drainage (Kalin, 1986). Recognizing that small but persistent stands of naturally-colonized cattails do grow on acidic tailings sites, ecological studies have been carried out on the growth of these emergent macrophytes (Kalin, 1984).

Resulting from the oxidation and hydrolysis of sulphides in waste rock and tailings, acid mine drainage (AMD) is a water quality problem commonly associated with metal mining activities (Tucker at al, 1987) - see Schematic 1. Acid is produced, in the presence

Schematic 1. Major Components of a Metal Mine and Sources of Acid Mine Drainage



of water and oxygen, when Fe and other metallic sulphides oxidize to form soluble hydrous Fe sulphates:

$$(1)\quad 2FeS_2 + 7O_2 + 2H_2O \leftrightarrow 2Fe^{+2} + 4SO_4^{-2} + 4H^+$$

In contact with water, ferrous Fe oxidizes to the ferric state where it further complexes with ferrous and ferric oxyhydroxides to form a precipitate and more acid:

$$(2)\quad Fe^{+2} + 1/4O_2 + H^+ \leftrightarrow Fe^{+3} + 1/2H_2O$$
$$(3)\quad Fe^{+3} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$$

In addition, *Thiobacillus ferrooxidans*, an Fe bacteria which occurs in aqueous environments with a pH range of 2.8 to 3.2, can bring about reactions 2 & 3 and produce more H^+ ions, thereby further increasing the acidity of the system (Tucker et al, 1987).

Cattails play a role in reducing acid generated in the above processes by providing an organic source for sulphate reducing bacteria (SRBs); these microorganisms reduce acid in wastewater back to hydrogen sulphide:

$$(4)\quad 2CH_2O + SO_4^{-2} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$$

With this reduction of acidity comes the precipitation of ferric hydroxide and other metals. In addition, cattails require oxygen in the rhizosphere for respiration; under circumneutral conditions, CO_2 released by respiration around cattail rhizomes remains in solution thus making surrounding water more resistant to changes in pH. This buffering capacity, however, is greatly reduced in acidic conditions where CO_2 does not remain in solution and quickly bubbles away as gas (Wetzel, 1983).

Cattails also help ameliorate AMD by providing, over waterlogged areas, an

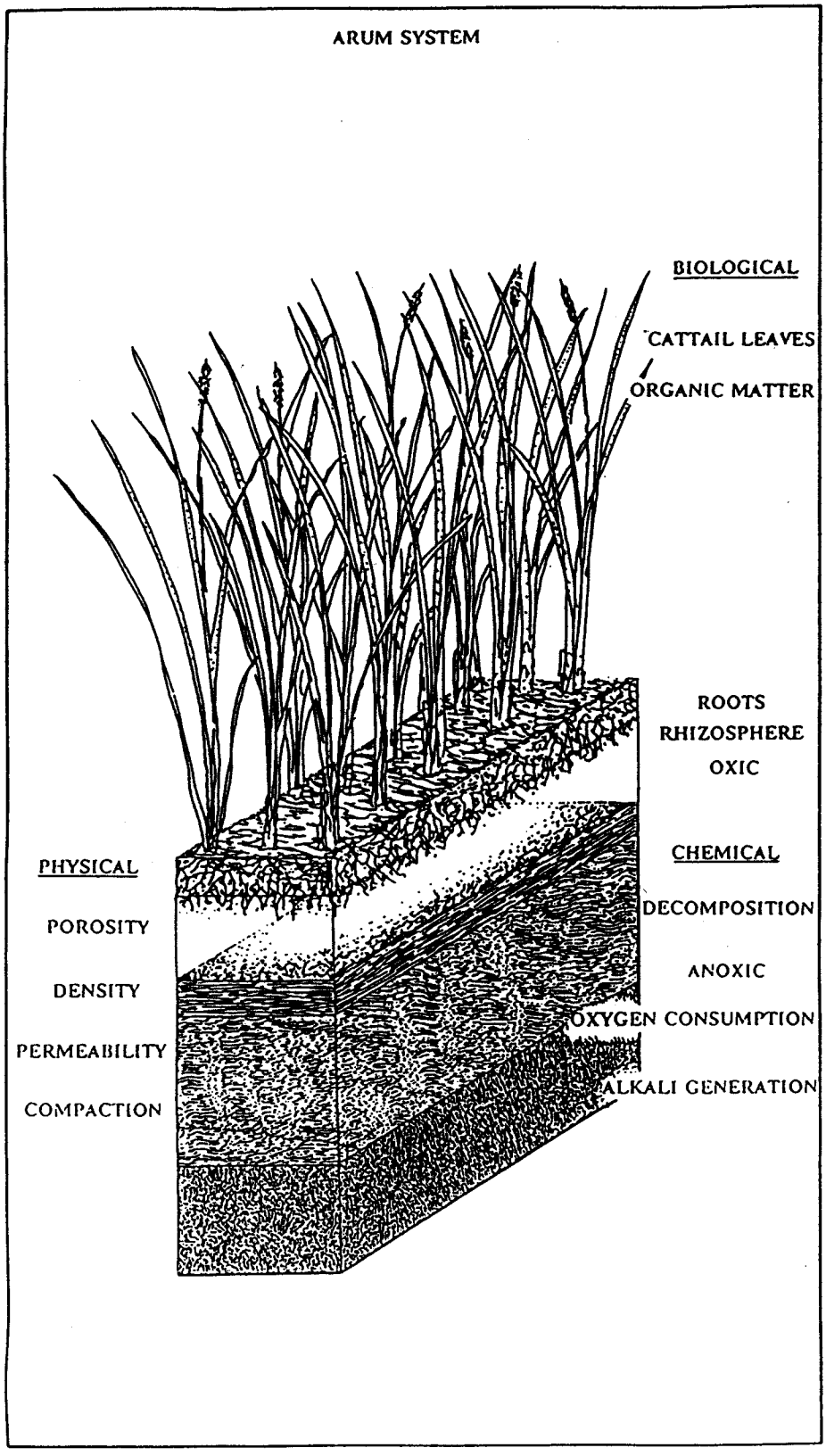
intercepting cover that prevents the mixing of oxygen (an essential component in the acid-generating process) with water - see oxygen consumption and alkalinity generation in the anoxic layer in Schematic 2. Furthermore, emergent wetland biota like the cattail prevent the accumulation of water in these areas by intercepting and transpiring much of the precipitation incident on a wetland (Larcher, 1987).

In light of these phenomena it becomes clear that cattails play an important role in AMD treatment. In response, then, to their significance in natural wastewater treatment and industry's growing need for cost-effective solutions to its waste problems, studies commenced in 1988 to determine the growth and development patterns of cattails in acidic waters. Investigations focused on comparing cattail development among sites of increasing environmental severity in relation to pH and associated increasing levels of metal toxicity (Kalin & Scribailo, 1989; Kalin, 1990). In addition, and of particular relevance to this study, several different fertilizer treatments were applied to cattail plots at Denison Mines (Elliot Lake, Ont.) to determine their effectiveness in enhancing growth and survivorship.

Results of experiments on acidic tailings at a uranium mine in Elliot Lake, Ontario indicated that roots of cattails showed large accumulations of metals on their exodermal and hypodermal layers (for a biological description of cattails see Section 2.1). Scanning Electron Microscopy (SEM) analyses, employing X-ray spectral determinations of metal concentrations, showed that high levels of iron were associated with high concentrations of sulphur and greatly reduced levels of calcium; crystal formation was also noted in the iron-sulphate plaque accumulation on the roots. These findings suggested that the cattail rhizosphere may have been active in ameliorating AMD (Kalin & Scribailo, 1989).

The latter part of this study attempted to determine the morphological and growth effects of foliar fertilizers on cattails with the rationale that if these macrophytes have an ameliorative effect on AMD, then by increasing plant biomass through fertilization in areas where growing conditions are harsh, their treating effect should also be increased (i.e. the more biomass, the greater the treatment capacity). Foliar fertilizers, as the name implies,

Schematic 2. The ARUM (Acid Reduction Using Microbiology) System

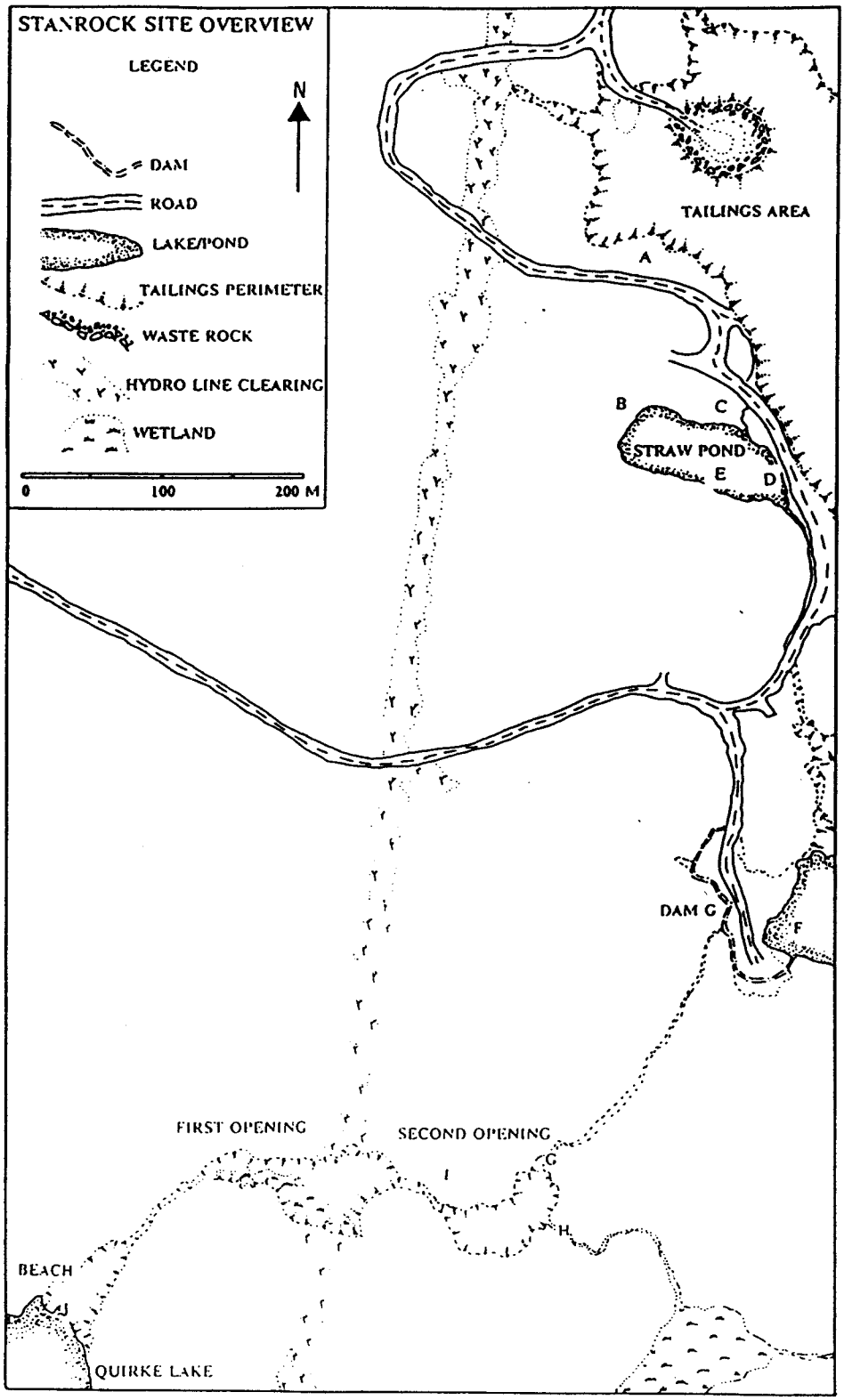


are applied to the above-ground portion - the shoots and leaves - of plants; this method is deemed more effective, in terms of nutrient uptake, than ground-applied fertilizers in areas where the potential of moving surface water and resultant transportation of nutrients away from the plant exists (as is the case at Denison). From a chemical perspective, foliar fertilizers are appropriate because, under acidic conditions (in this case, acid is abundant in the tailings where the cattails have taken root), the plant availability of nitrogen and phosphorus is greatly reduced due to a lower rate of organic matter mineralization. As acidity increases the solubility of P in Fe phosphates decreases, as does the activity of N-fixing bacteria (Tucker et al, 1987); the implication here is that ground-applied fertilizers will result in minimal N and P uptake because of the acidic conditions.

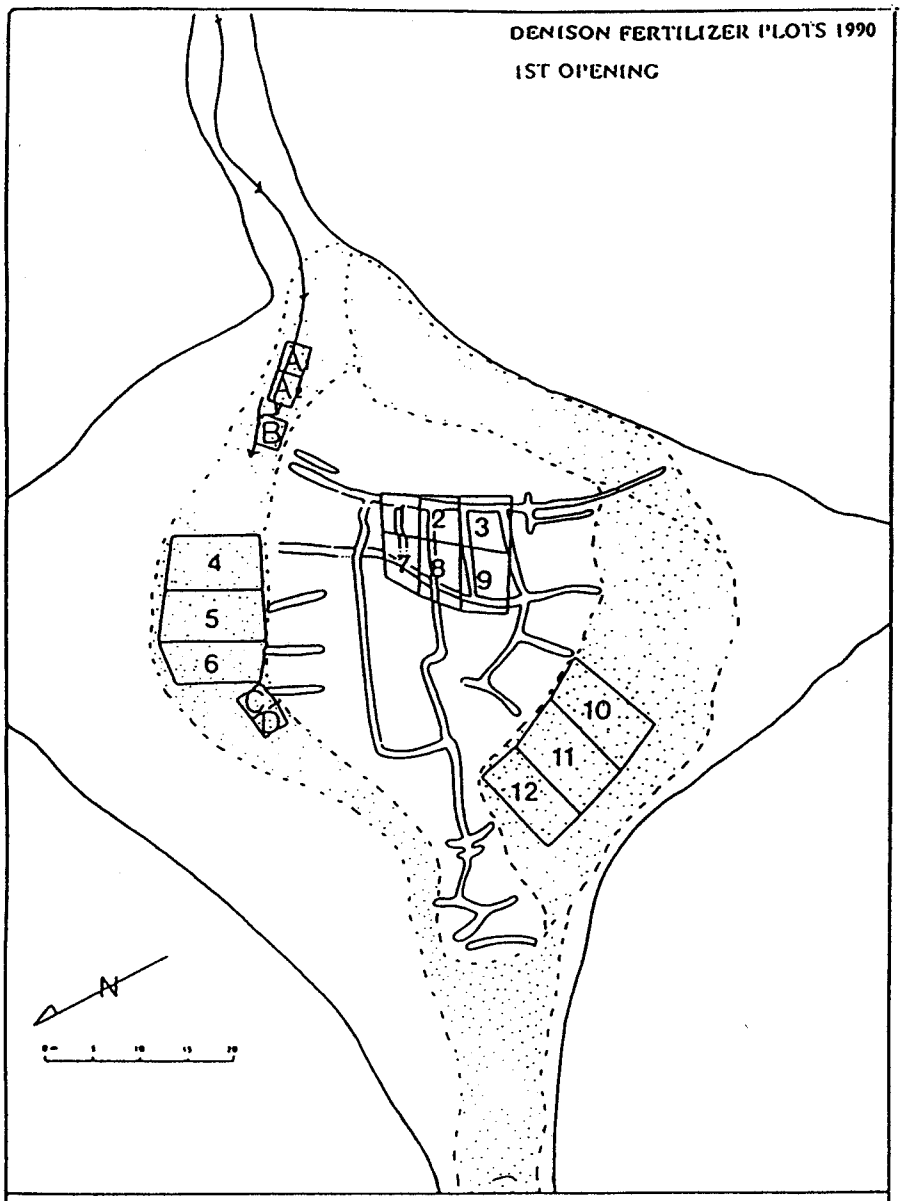
To maximize fertilizer-induced nutrient uptake in cattails, preliminary foliar applications began in 1989 at Denison in the First Opening of the Stanrock Dam G site (see Map 1.) to determine concentrations of fertilizer that would not produce leaf burn and, at the same time of course, enhance growth. Separate applications of 14-4-6 and 4-18-6 NPK (Nitrogen-Phosphorus-Potassium) fertilizers in 1/10 and 1/20 dilutions were applied in the summer of 1989, and results indicated some leaf burn using all four combinations. Greatly increased root growth was observed with high phosphorus and potassium fertilizer relative to unfertilized control plots, while high nitrogen fertilizer increased above-ground leaf development (Kalin, 1990).

Based on these observations, the 1990 fertilizer campaign saw the use of 14-4-6 NPK applications at a dilution of 1/10 with the addition of 1/50 diluted calcium fertilizer - see Map 2. As indicated in Kalin (1990), results to this point are too preliminary to determine whether foliar fertilizer applications will increase survivorship and clonal growth in cattails. Comparative studies need to be undertaken to find out what the effects of applying foliar fertilizer are on root and rhizome development relative to unfertilized controls in order to determine if, in fact, nutrient addition of this nature will help to maximize polishing capacity in cattail stands.

Map 1. Stanrock Site Overview



Map 2. Denison Fertilizer Plots in the First Opening



PLOT #	DESCRIPTION	PLOT #	DESCRIPTION
1	NPK LEAVES	11	CONTROL
2	NPK LEAVES	12	NPK LEAVES
3	NPK LEAVES	A1	Ca LEAVES
4	NPK LEAVES	A2	NPK LEAVES, Ca GROUND
5	CONTROL	B	NPK LEAVES, Ca LEAVES
6	NPK LEAVES	C	NPK GROUND
7	NPK LEAVES	D	Ca GROUND
8	NPK LEAVES		
9	NPK LEAVES		
10	NPK LEAVES		

1.2 Purpose and Objectives of Study

This study is, in a sense, a "stepping stone" to achieving the ultimate goal of determining whether foliar fertilizer applications on tailings-grown cattails is a viable method for increasing biomass production and, hence, biological polishing capacity. By comparing root and rhizome development between fertilized and non-fertilized control plots, it should be feasible to determine the morphological and biochemical (i.e. starch reserves) effects of NPK and Ca-fertilized cattails.

An investigation of below-ground tissues is appropriate because they act as regions of material storage for future growth and development. In particular, the pith region of the rhizome is relevant to the study because it is there that sugars produced during photosynthesis are stored as starch. And when there is an unlimited availability of nutrients the pith sees a reduction in sugar and starch accumulations (Lakshman, 1987). Based on this point, all parameters otherwise being equal, cattails in control plots should see greater levels of starch in their piths than those receiving additional fertilizer-induced nutrients.

During winter months, cattails store sugars and starch at relatively stable levels; carbohydrate use during this period is minimal with only small amounts utilized for suppressed respiration. Winter sampling and experimentation, then, is useful under this "steady state" condition because it eliminates sampling problems arising from daily and growing-season fluctuations in starch levels. Starch content in rhizomes of fertilized cattails relative to that in the unfertilized ones should indicate to some degree the relative availability of fertilizer-induced nutrients to the plants. Analyses will focus on pith diameter relative to overall rhizome diameter, starch distribution, and starch concentration. Since Kalin and Scribailo (1989) observed tissue damage from metal uptake in cattail rhizomes and roots, metal distribution will also be looked at.

Before commencing field work at Denison Stanrock, however, samples for preliminary testing will be collected locally to first determine if it is at all possible to extract cattails

during winter, and then to work out appropriate techniques for starch analysis. In the latter case, it will need to be determined how to sample cattail populations representatively, while doing the same for individual plants before morphological and starch investigations. This report, then, presents findings from a preliminary study on overwintering, below-ground cattail morphology, starch storage, and metal uptake using locally-collected samples. An evaluation of the methods used to examine these parameters is provided along with a discussion of actual data obtained. Conclusions drawn, and recommendations made from this preliminary study will be used to develop an appropriate strategy for extracting and analysing the Denison cattails.

2. BACKGROUND INFORMATION

2.1 Cattail Anatomy, Morphology, and Development

The following description is based on that found in Kalin & Scribailo (1989).

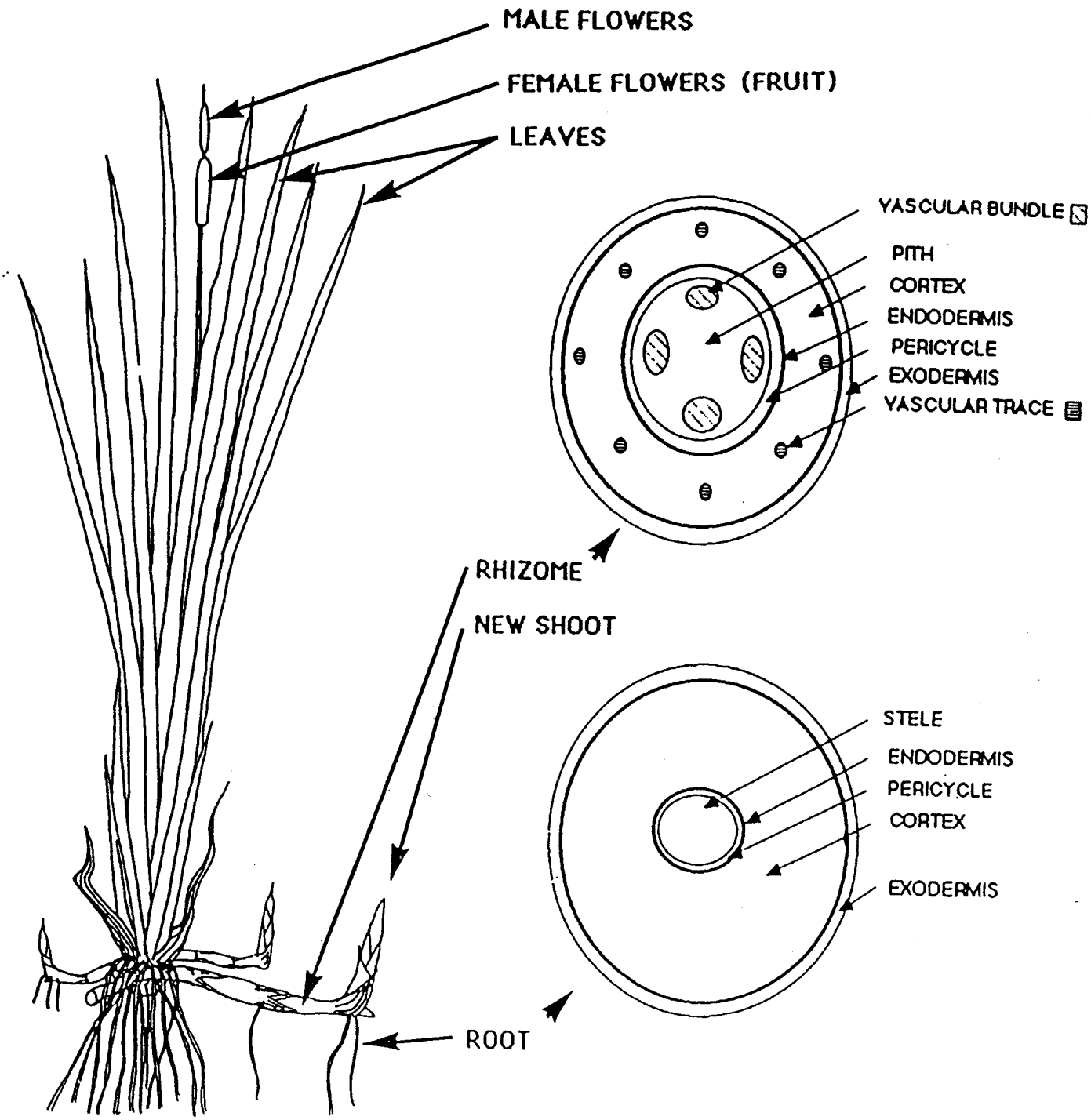
Cattails reproduce vegetatively, with the production of rhizomes (underground stems), to produce individual plants consisting of a rosette of opposite leaves, typically six per side - see Schematic 3. By developing small vegetative buds at the base of each leaf, referred to as the axil, these emergent macrophytes possess the ability to develop vertical, upright shoots. Rhizomes characteristically produce roots at regular intervals along their length, although the majority of roots tend to develop at the bases of upright shoots.

In cross section, rhizomes consist of two distinct zones - an outer cortex and an inner pith. The pith is mainly composed of tightly-packed storage cells used for holding starch for future growth and development. To inhibit lateral movement of substances from the cortex into the inner zone, an endodermis borders the pith's periphery.

The cortex is largely composed of star-shaped cells, collectively referred to as stellate, with significant quantities of air between them. This outer region of the cattail rhizome is bordered on the outside by an exodermis which acts as a barrier to the transportation of materials from the external environment into the rhizome. Vascular bundles are scattered throughout the cortex, but are more concentrated in the pith. Their primary function is to transport water from the roots to the shoots, and to transport sugars produced during photosynthesis from the leaves to the rhizome for storage as starch in the pith.

Root anatomy is similar to that of the rhizome with the exception of the pith region. In cattail roots, rather than many individual bundles, the pith is replaced by a solid cylinder of vascular tissue called a stele. Roots are produced laterally from a thin layer of tissue just beneath the rhizome's endodermis called the pericycle; these roots may also produce lateral roots from their pericycle.

Schematic 3. Cattail Anatomy and Morphology



2.2 The Cattail Rhizosphere

The significance of cattails, and particularly the rhizosphere, in ameliorating AMD has been suggested by Kalin and Scribailo (1989) and is one reason why this study focuses on the below-ground tissue of these emergent macrophytes. There are other reasons, as well, for concentrating on the rhizome, yet before effective data interpretation can take place these reasons need to be elucidated in light of an understanding of the phenology and development of the cattail rhizome.

Cattail plants are connected together by an underground network of stems, or rhizomes, growing parallel to the surface of the bottom (Linde et al, 1976). By reproducing vegetatively with aerial shoots arising from rhizomes, cattails produce colonies that migrate outward to invade new areas. Because each shoot originates from a common ancestor, all are genetically identical - thousands of aerial shoots, composed of leaves and occasional fruiting stalks, can be produced by a single parent.

Rhizomes have a number of functions which are important to the survival and propagation of the cattail. During the growing season, reserves of carbohydrates are built up in the pith region of the rhizome and stored through winter to provide food for the pre-photosynthetic (i.e. submerged) development of the new aerial shoot in the spring. In the summer months, migration of the cattail beyond its periphery is brought about by the growth of rhizomes, thereby maintaining established populations while also expanding them into new, suitable habitats. During the winter, rhizomes also serve to carry the plant through this dormant period by being able to obtain oxygen for survivability. Even though the aerial shoot is dead at this time, aerenchyma cells in the rhizome, which are connected to those in the shoot, receive conducted oxygen from the shoot and ensure the cattail's survival during winter-induced anaerobic conditions.

Growth and development studies of cattails in a shallow marsh in Wisconsin, U.S.A. receiving heavy nutrient loading were undertaken by Linde et al (1976) during a three-year

period to gain an understanding of how to control and manage the plant for associated wildlife habitat. Growth and development of leaves, fruiting heads, and rhizomes were measured weekly throughout each growing season. Of particular relevance to this study was the weekly collection and analysis of total nonstructural carbohydrates (TNC); this was seen as a means for determining stored energy levels on which the plant depended for initial growth in the spring (Linde et al, 1976).

Findings from that study showed that old rhizomes - those produced during a previous growing season - were the principal storage organ with maximum levels of TNC occurring during the early winter period (Linde et al, 1976). Levels of TNC gradually declined with the onset of growth in the spring to a minimum in mid-summer during fruiting head development. Beyond this point, carbohydrates were produced in excess of the plant's needs so they were translocated to the rhizomes for storage. Conclusions from the study indicated that the stored carbohydrates were available to help the plant recover from severe injuries and to develop new shoots the following spring before it is capable of photosynthesizing sufficient carbohydrates for its needs (Linde et al, 1976).

Other findings on cattail rhizomes indicated that adventitious buds at the base of growing aerial shoots developed into new rhizomes in late spring, and maximum rhizome growth of one inch per day. Sprouts were observed forming on the new rhizomes during mid summer and were later found to have produced the following summer's crop of aerial shoots; the sprouts went into dormancy in late fall and resumed growth in early spring (Linde et al, 1976).

2.3 Overwintering Starch Distribution in Cattail Rhizomes

Although the literature is scant in dealing with the exact subject, one paper was found which might be of significance to this examination. To determine the amount of starch storage during the overwintering period in different tissues of the cattail, a study was

conducted in 1977-78 at a single clonal cattail community near the southeast shore of Lake Ontario in New York State (Kausch et al, 1981).

In keeping with previous studies of the same nature, results from analyses of starch distribution in cattail leaves, stems, and rhizomes showed the rhizome as the major storage organ for starch. The central core region of the rhizome - the pith - showed extensive accumulations of globose starch grains during late fall and early winter with significant reductions from late winter through to early spring (Kausch et al, 1981). Small amounts of starch were also noted in the rhizome endodermis and adjacent cortical parenchyma in late fall and winter, with almost none in spring. Beneath the outer cortex, large accumulations of starch were found in scattered parenchyma cells; by spring, this layer lost most of its starch.

The outer cortex of intact roots contained starch in late fall, while the middle cortex remained starch-free all year. Starch was also noted in a number of cells in the inner cortex, but very little was seen in the endodermis. Overall, cattail roots saw a gradual decrease in starch levels in midwinter followed by a sharp drop in late winter with almost zero deposits by spring.

Coupled with findings from investigations of cattail buds and young leaves it was concluded that a decrease in starch in storage tissues of rhizomes and roots by late winter and early spring coincided with increasing bud growth and starch accumulation in or near zones of rapid development (Kausch et al, 1981). This strongly supported the idea that most of the stored starch was transferred from the storage organs to tissues of the developing shoot until the new shoots emerged from the water and became photosynthetic. It was apparent from the study that cattails are extremely productive plants with as much as 45.03% starch, dry weight, accumulating in rhizomes in early winter and 22.80% in roots; interestingly, not all stored starch was used for productivity as rhizomes retained a significant amount (27.40% dry weight) into the start of their second year (Kausch et al, 1981).

3. METHODS

3.1 Field Sampling and Commentary

On January 29, 1991 cattails for preliminary testing were extracted from a seepage/seasonal surface flow area at the base of the Scarborough Bluffs in Metropolitan Toronto. Because the daytime temperature was below 0°C, and had been that way for two days prior, most of the cattail population was "locked-up" in frozen water. This state made it impossible to successfully extract any plants with a spade; efforts were made to chip ice away from around the cattail, but after considerable time and effort without substantive results the strategy was deemed ineffective under conditions present at that time.

After testing various locations for ice build-up, a small ditch was discovered adjacent to the parking lot where a number of cattails existed. Upon testing the ice cover with the spade it was found that the ice was softer here than the areas previously tried. The ice chipped away easily to reveal a soft, unfrozen substrate layer that accepted the spade to the depth of the rhizome layer without any problem.

Due to time limitations, only a small area with approximately five plants was excavated. Any upright shoot with intact rhizomes was kept for testing along with detached sections of rhizomes found after dredging the water-filled, excavated hole with the spade. As much substrate as possible was then removed from around the cattail rhizomes by hand in the field; some blackening of rhizome and root tissue was noticed at this time. To keep the cattails in a frozen state, samples were transferred to an ice-packed cooler for transportation to the laboratory freezer.

Because of the conditions previously described, sampling was restricted to one small area of the population. Techniques, therefore, for representatively sampling a population were not used. The same applied to extracting individual plants - the combination of ice and spade made it difficult to accurately cut and excavate a prescribed plot around each cattail.

During the second week of February, 1991 a warm spell presided over the Toronto area so a second trip was made to the Scarborough Bluffs to excavate additional cattails. Much of the seepage area that was frozen solid during the previous sampling trial was free of ice and, as such, made extraction easy. A location within the overall stand was found where a number of cattails were rooted under free-flowing water. Digging commenced with great success - the substrate in which the cattails were rooted was soft, thereby making extraction simple and fast.

After a number of plants were excavated by locating emergent, senescent shoots and digging around them to obtain their intact rhizomes, it was found that this approach often damaged the year-old underground stems. Because the year-old rhizomes were underground there was no way of determining how far out from the parent shoot one needed to dig in order to keep the rhizome intact. As a result, many of the initial samples had broken rhizomes where the spade had cut through the tissue when digging.

In order to alleviate the problem, an attempt was made to locate emergent shoots and work "backwards" by digging approximately 0.5m away from the parent in a circular fashion around the plant. This method proved highly successful, and within an hour ten plants with intact rhizomes were extracted. The samples underwent an initial field washing in adjacent Lake Ontario and were placed in an ice-filled cooler for transportation to the laboratory. Once at the lab, the samples were placed in a freezer to await examinations.

3.2 Sample Preparation

To prepare the cattails for morphological and chemical investigations, individual plants were taken from the freezer and quickly washed under hot tap water to remove any excess substrate remaining after field washing. Year-old rhizomes with yet-to-emerge new shoots were then located on each plant, measured for their length, and described as to their appearance. The central 12cm portion of each rhizome was kept for further investigations,

as per Kausch et al (1981). Because sectioning problems arose from thawing plant material during this initial preparation period, an additional step was later incorporated into the protocol; to ensure that the rhizome was frozen to such an extent that hand sectioning had no damaging or mishaping effects on the tissue, individual plants were placed back in the freezer for half an hour immediately after laboratory washing.

All intact root material on each 12cm length was removed with a razor blade at the outer surface of the rhizome exodermis and placed in individually-labelled sample jars containing 95% denatured ethyl alcohol. Two immediately-adjacent hand sections - one for IKI staining for starch and the other for hematoxylin staining of metal uptake -were made at both ends and in the middle of the rhizome sample, for a total of six. The two sections at each location were placed together in a scintillation vial containing the 95% alcohol and labelled according to rhizome number and position within the 12cm portion. Sections at the parent-shoot end of the rhizome were labelled "A", those in the middle "B", and the sections closest to the new shoot "C." The remaining tissue was placed in appropriately-labelled sample jars (containing alcohol) for quantitative starch analysis.

3.3 Iodine/Potassium Iodide (IKI) Staining for Starch

Before staining took place, the alcohol inside the cells of the rhizome sections had to be washed out. To accomplish this, one hand section from each grouping of two was removed from its respective scintillation vial and immersed in each of a series of four beakers containing distilled water. After the final immersion, the section was placed on a microscope slide ready to accept the iodine/potassium iodide (IKI) solution. Two to three drops of pre-prepared IKI were placed on the section and allowed to develop into the stain's characteristic bluish-purple colour when in contact with zones of starch accumulation.

Once sufficient colour had developed, excess stain was washed off and the section was put in a petri dish modified with a movable glass cover slip. The dish was filled with distilled

water and the section was placed under the cover slip to prevent it from moving around while under microscopic examination. Sections were viewed under a Ziess stereomicroscope, model SV 8, fitted with a MC 63 M 35 photomicrographic camera. Visual observations were recorded and, where appropriate, slides were taken using Kodak Ektachrome 160T professional slide film.

3.4 Measuring Rhizome Section Dimensions

After recording observations and photographing the IKI-stained rhizome tissue, each section was removed from the viewing dish and placed in another petri dish fitted with a mm^2 grid superimposed on a movable, clear acetate cover. Each section was placed under the acetate cover, making sure the cover was in contact with the entire surface of the section, and centred about a prescribed grid intersection point. Pith diameters as well as overall rhizome diameters were recorded in both the X and Y directions by counting the number of millimetre squares each feature took up. Sections were then discarded.

3.5 Hematoxylin Staining for Metals Uptake

In order to elucidate the presence and distribution of metals in cattail rhizomes, sections were stained with 1: 10 000 dilution of unoxidized hematoxylin in 0.01M phosphate buffer, as per Pizzolato and Lillie (1967). Hematoxylin staining for metals in tissue produces the following colours: Al, Cr, Ga, Hf, In, Fe, and Zr - dark blue granules; Be, Dy, Ho, Ir, Pb, Mn, Mo, Nd, Ni, Pt, Rh, Tb, U, Yb, and Zn - lighter blue granules; Bi - purple; Ta - brown-red; Nb and Ti - brown; Cu - greenish blue; Sn and Th - purplish red; and, Os -blue- or green-brown (Pizzolato & Lillie, 1967).

A stock solution was prepared by dissolving 1g hematoxylin in 100ml posphate buffer, 0.01M and pH 7.0. Fresh solutions were made by diluting 2ml stock solution with 200ml

phosphate buffer. The remaining rhizome section from each scintillation vial was prepared, viewed, and photographed for hematoxylin staining in the same manner as that for IKI. Colour development was compared between stock and fresh solutions and between different stain retention times. Linear measurements were not taken since those obtained from immediately adjacent sections during IKI-starch viewing were deemed applicable. Again, sections were discarded after examination.

3.6 Quantitative Determination of Starch

A modified version of the Nielson method (1943) was used. Leftover material from each 12cm rhizome portion was taken out of the storage alcohol, wrapped in absorbant towel to remove excess liquid, and weighed to two decimal places of a gram on a Sauter electronic balance. The weighed material was placed in a Waring Blendor and ten times that weight of water was added to make a 10% tissue slurry. After the blendor was allowed to run at the high-speed position for 4-5 minutes, 2ml of the resultant slurry was measured into a graduated cylinder and then placed in a 50ml beaker.

Exactly 2.7ml of 72% perchloric acid was added to the aliquot while stirring thoroughly to ensure no momentary high concentrations of acid in any portion of the sample. The mixture was allowed to stand with occasional stirring for approximately ten minutes. A 1ml aliquot of the solution was then pipetted into another 50 ml beaker and 6ml of distilled water was added. The solution was brought to a pH of 8.3 with a few drops of 6N sodium hydroxide and then to a pH of 4.5 with 2N acetic acid.

Two and a half millilitres of 2N acetic acid was then added in excess, followed by 0.5ml 10% potassium iodide, and 5ml of 0.01N potassium iodate. The solution was allowed to stand for 5 minutes so that effective colour development could take place. Using a graduated cylinder, the coloured solution was made up to 50ml with distilled water and transferred to a suitable cuvette for colour estimation in a photoelectric colourimeter.

Colourimetric readings were made on the final solutions using a Coleman Junior II Spectrophotometer set at 680 nm with a red filter. The spectrophotometer was calibrated to zero absorption prior to each reading using a reference blank containing all reagents except for actual starch material. To provide a control against turbidity in the final solutions, a blank was also made up with the slurried rhizome material and all reagents except for the potassium iodide and iodate, and checked against distilled water.

3.6.1 Standardization

Starch content in cattail rhizome and root tissue was calculated on a fresh weight basis (mg starch/g of tissue) from a standard curve prepared from the colourimetric readings of a known range of starch concentrations. Various amounts of corn starch were accurately weighed to the nearest 10μg on a Sartorius analytical balance and made to final 50ml solutions in the same manner as the cattail tissue. The amount of starch in the final solutions was calculated, and colourimetric readings were taken on the spectrophotometer. A curve was developed by plotting starch in 50ml solution against spectrophotometer absorbance.

Absorbance readings of cattail tissue solutions were then read from the curve to obtain starch concentrations in 50ml. Starch content in fresh weight of cattail material was then calculated by relating the observed concentration to the amount of tissue in the 50ml solution (while incorporating the turbidity blank result). Leftover material from the blending operation was air dried to constant weight for dry weight biomass calculations.

4. DATA RESULTS

4.1 Staining

Results from the staining of cattail rhizome sections are presented in Tables 1 and 2. Iodine/potassium iodide staining for starch yielded consistent results with the pith showing extensive bluish-purple colouration (indicative of starch) as well as a thin band beneath the epidermal layer. Starch was detected at the base of roots in some sections but not in others. A number of sections experienced damaged piths in the form of lost material.

Hematoxylin staining of the entire sample run for metal uptake produced no apparent colouration with a >18 hr. stain retention time. A test of fresh hematoxylin on a rhizome section showed excellent colouration after only three hours of retention with a pale orangy-brown pith and a dark blue epidermis fading to a lighter blue through the cortex.

4.2 Rhizome Dimensioning

Results of cattail rhizome measurements are presented in Table 3 and graphically in Figures 1 and 2. Figure 1 is a general overview of results obtained from rhizome dimensioning showing a relatively steady ratio, from location to location within each rhizome, between pith diameter and overall rhizome diameter. On closer inspection, the data reveal that in 90% of the rhizome samples the pith becomes proportionately larger toward the new shoot, as revealed in Figure 2.

Table 1. VISUAL OBSERVATIONS OF IKI STAINING FOR STARCH

Section #	Description
1A	- starch concentrated in pith, purple-blue colouration (colour consistent for all remaining observati - starch also located in a thin band beneath epidermal layer - some starch concentrated around roots in cortex
1B	- cortex retained blue colouration, but no starch granules present - starch distribution the same as above (no roots present) - some white areas in pith (vascular bundles)
1C	- same as 1A (no roots)
2A	- same as 1B
2B	- expected starch distribution - pith damaged from sectioning
2C	- same occurence as 2b
3A	- characteristic starch pattern - stray starch granules from unstable pith present in cortex
3B	- expected starch distribution - concentration of starch around roots in cortex
3C	- pith destroyed after stain rinsing - material left showed expecected pattern
4A	- expected starch distribution, but decreased amounts relative to the above sections - no starch around root
4B	- characteristic starch pattern
4C	- reduced amount of starch in pith - concentration of starch between two roots and damaged epidermis - overall, expected distribution present
5A	- loose pith with subsequent scattering of its tissue - basic starch pattern
5B	- normal distribution
5C	- normal distribution
6A	- characteristic starch pattern - vascular bundles in pith very definite
6B	- normal starch distribution - good accummulation of starch around root
6C	- expected starch pattern
7A	- section damaged - characteristic pattern in intact material
7B	- section damaged - little starch under epidermis - starch accummulatio around root
7C	- blue tinge, but no starch granules, in cortex - otherwise, normal starch pattern
8A	- darker colouration to one side of pith in cortex - expected distribution otherwise
8B	- badly damaged section - material left shows expected starch pattern
8C	- irregularly-shaped rhizome, jagged on one side - pith butts up against jagged edge - pith filled with starch - more starch under jagged epidermis than under smooth portion
9A	- expected distribution - pericycle pronounced with blue colouration
9B	- expected distribution
9C	- characteristic pattern
10A	- expected distribution
10B	- normal starch pattern
10C	- expected distribution

Table 2. VISUAL OBSERVATIONS OF HEMATOXYLIN STAINING FOR METALS UPTAKE

Section #	Description
Test Stock Sol. (<15 min.)	- bright red pith - dark blue pockets in cortex
Test Stock Sol. (>3 hr.)	- purple & pink cortex - strong pink/red in pith - epidermis black
Test Final Sol. (<15 min.)	- no apparent colouration
Test Final Sol. (>3 hr.)	- dark blue epidermis fading to lighter blue towards pith - pith pale orange/brown
After 18 hrs.:	**Description**
1B	- some of pith washed out - pale purple beneath epidermis - pale purple in pith with scattered red spots (vascular bundles)
1C	- same as 1B - pale orange at root base
2A	- pith washed out - pale purple cortex
2B	- same pattern as above - darker blue towards one side of rhizome
2C	- stain concentrated around vascular traces in cortex
3A	- concentrated stain around root
3B	- expected pattern (as in 1B)
3C	- no cortical colouration
4A	- pale pink pith - no blue tinge in cortex
4B	- same as 4A
4C	- same as 4A
5A	- dark blue where pre-stain blackened conditions existed
5B	- same as 5A
5C	- more blue colouration in one side of cortex
6A	- no blue tinge in cortex - pale pink pith
6B	- no cortical colouration - pale pink pith
6C	- same as 6A&B
7A	- light brown cortex - some blue colouration under epidermis
7B	- slight blue tinge in cortex
7C	- even colouration throughout rhizome
8A - 10C	- evident that hematoxylin stain lost its colouring effects from 1A onward

Table 3.

CATTAIL RHIZOME MEASUREMENTS

Sample	Rhizome Length (cm)	Axis	Pith Width (mm)	Average	Cortex & Epidermal Layer (mm)	Axis	Rhizome Width (mm)	Average	Ratio Pith: Rhizome Diameter
1A	14.0	x	7.0	7.40	5.60	x	12.50	13.00	1: 1.69
		y	7.8			y	13.50		
1B		x	4.7	4.10	3.95	x	9.00	8.05	1: 2.20
		y	3.5			y	7.10		
1C		x	4.3	4.10	4.05	x	8.60	8.15	1: 2.10
		y	3.9			y	7.70		
2A	25.0	x	8.2	8.25	6.20	x	14.10	14.45	1: 1.71
		y	8.3			y	14.80		
2B		x	7.9	7.75	4.95	x	11.90	12.70	1: 1.54
		y	7.6			y	13.50		
2C		x	7.5	7.15	4.55	x	11.50	11.70	1: 1.61
		y	6.8			y	11.90		
3A	25.0	x	5.8	5.85	4.30	x	10.60	10.15	1: 1.81
		y	5.9			y	9.70		
3B		x	6.7	6.35	4.05	x	12.10	10.40	1: 1.91
		y	6.0			y	8.70		
3C		x	5.4	6.50	4.30	x	9.50	10.80	1: 1.46
		y	7.6			y	12.10		
4A	13.0	x	5.9	5.90	3.60	x	9.80	9.50	1: 1.66
		y	5.9			y	9.20		
4B		x	7.3	7.10	3.80	x	10.10	10.90	1: 1.42
		y	6.9			y	11.70		
4C		x	8.2	7.75	3.25	x	11.40	11.00	1: 1.47
		y	7.3			y	10.60		
5A	21.5	x	6.8	5.45	3.75	x	11.10	9.20	1: 2.04
		y	4.1			y	7.30		
5B		x	5.7	4.90	3.95	x	9.80	8.85	1: 2.00
		y	4.1			y	7.90		
5C		x	5.9	6.05	3.50	x	9.60	9.55	1: 1.59
		y	6.2			y	9.50		
6A	14.0	x	6.6	5.95	3.70	x	10.90	9.65	1: 1.83
		y	5.3			y	8.40		
6B		x	5.7	6.25	3.20	x	9.50	9.45	1: 1.52
		y	6.8			y	9.40		
6C		x	6.1	6.30	3.85	x	9.90	10.15	1: 1.57
		y	6.5			y	10.40		
7A	9.5	x	6.9	5.75	3.20	x	9.10	8.95	1: 1.58
		y	4.6			y	8.80		
7B		x	7.1	6.55	3.65	x	11.70	10.20	1: 1.79
		y	6.0			y	8.70		
7C		x	10.3	10.10	3.80	x	14.20	13.90	1: 1.41
		y	9.9			y	13.60		
8A	16.5	x	7.4	7.00	3.55	x	11.30	10.55	1: 1.61
		y	6.6			y	9.80		
8B	Section Too Damaged for Measuring								
8C		x	7.9	7.05	2.05	x	9.50	9.10	1: 1.35
		y	6.2			y	8.70		
9A	24.0	x	5.3	5.70	6.50	x	11.60	12.20	1: 2.04
		y	6.1			y	12.80		
9B		x	7.1	7.10	7.90	x	13.10	15.00	1: 1.85
		y	7.1			y	16.90		
9C		x	4.4	4.85	3.80	x	8.50	8.65	1: 1.75
		y	5.3			y	8.80		
10A	15.5	x	8.5	8.15	2.65	x	13.00	10.80	1: 1.60
		y	7.8			y	8.60		
10B		x	8.8	9.15	4.30	x	13.70	13.45	1: 1.50
		y	9.5			y	13.20		
10C		x	10.1	9.85	2.90	x	13.20	12.75	1: 1.34
		y	9.6			y	12.30		

Figure 1.

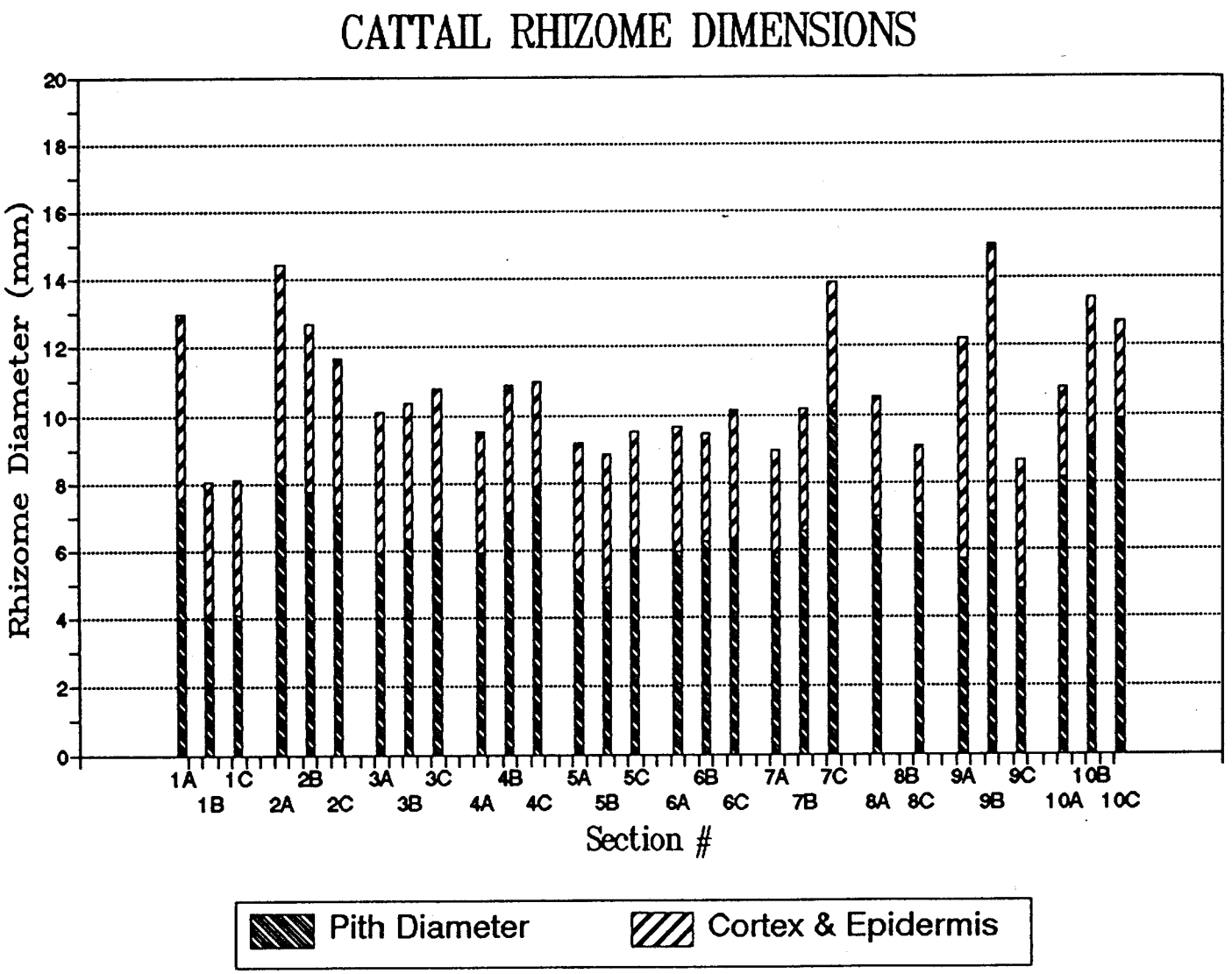
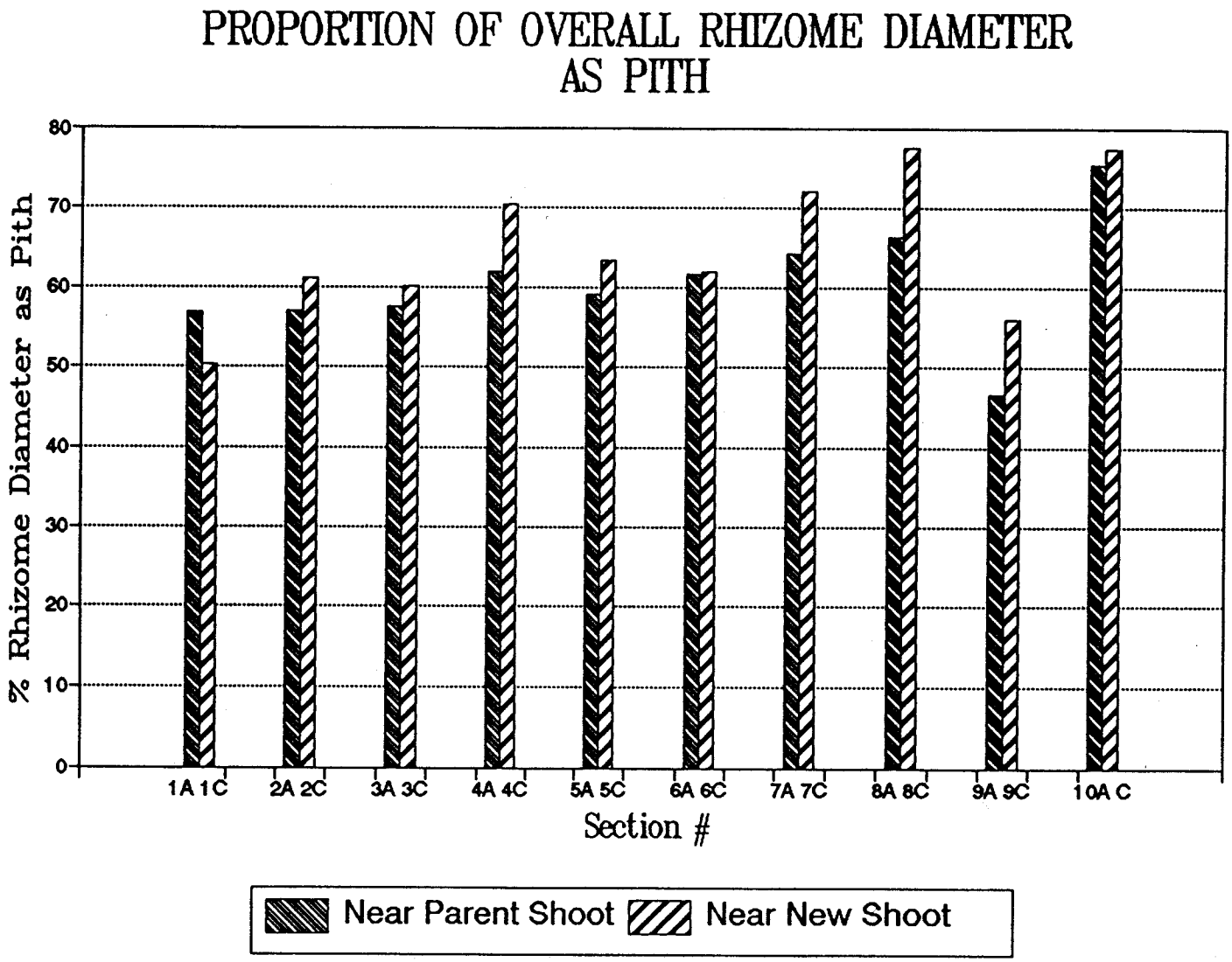

CATTAIL RHIZOME DIMENSIONS
Rhizome Diameter (mm)
0
2
4
6
8
10
12
14
16
18
20
1A 1B 1C 2A 2B 2C 3A 3B 3C 4A 4B 4C 5A 5B 5C 6A 6B 6C 7A 7B 7C 8A 8B 8C 9A 9B 9C 10A 10B 10C
Section #
Pith Diameter
Cortex & Epidermis

Figure 2.


PROPORTION OF OVERALL RHIZOME DIAMETER AS PITH
% Rhizome Diameter as Pith
0
10
20
30
40
50
60
70
80
1A 1C
2A 2C
3A 3C
4A 4C
5A 5C
6A 6C
7A 7C
8A 8C
9A 9C
10A C
Section #
Near Parent Shoot
Near New Shoot

4.3 Quantitative Determination of Starch

A standard curve is presented in Figure 3 with supporting data in Table 4 showing the absorption of light in a spectrophotometer by accurately known amounts of starch. With an R Squared of 0.950 in Figure 3, the amount of starch in solution almost perfectly predicts absorption readings on the spectrophotometer.

Table 5 shows the results of cattail rhizome and root tissue determinations. Absorption readings were obtained from the spectrophotometer, read from the standard curve to determine starch quantity in 50ml solution, and a ratio of starch weight to tissue weight calculated. For calculations on a dry weight basis, rhizome and root dry weight/fresh weight ratios of 17.66% and 14.35% respectively were obtained.

Both pooled samples of rhizomes and roots showed significant variability in final starch determinations with coefficients of variation (standard deviation expressed as a percentage of the mean) of 23% and 41% respectively, as indicated in Figures 4 and 5.

Figure 3. Standard Curve for Starch Concentrations

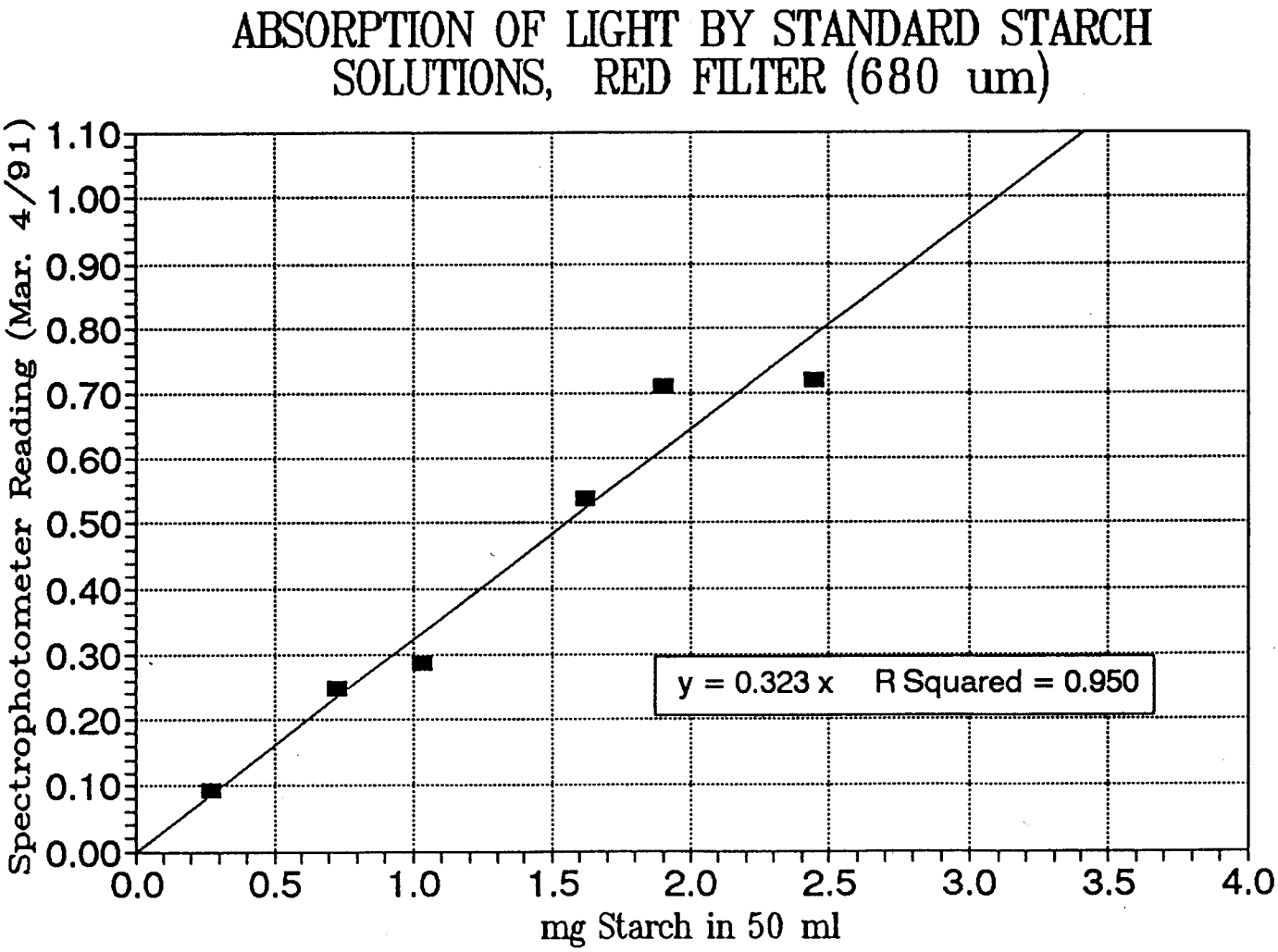


Table 4.

RESULTS OF STANDARD STARCH SOLUTION PREPARATIONS - MAR. 4/91

Standard #	Weighing Paper Weight (mg)	Weight of Paper + Starch (mg)	Starch Weight (mg)	Calc. Starch in 50 ml (mg)	Spectrophotometer Absorbance	% Transmittance
1	403.37	404.65	1.28	0.27	0.094	80.5
2	397.02	400.46	3.44	0.73	0.248	56.4
3	416.90	421.73	4.83	1.03	0.286	51.9
4	391.11	398.74	7.63	1.62	0.538	29.0
5	397.53	406.46	8.93	1.90	0.710	19.3
6	397.88	409.38	11.50	2.45	0.720	19.0

Table 5.

STARCH CONTENT IN FRESH TISSUE OF CATTAIL RHIZOMES & ROOTS

Sample		Spectrophotometer % Transmittance	Absorbance	mg Starch in 50 ml	Fresh Weight (g)	Starch Content (mg/g)
Turbidity Blank		93.0	0.030	-	-	-
Rhizome #	(i)	37.0	0.430	1.37	4.57	10.06
	(ii)	32.5	0.485	1.55		11.35
Rhizome #	(i)	12.3	0.900	2.87	11.6	8.30
	(ii)	15.8	0.800	2.55		7.38
Rhizome #	(i)	9.1	1.000	3.19	7.12	15.02
	(ii)	8.1	1.000	3.19		15.02
Rhizome #4		not analysed			6.89	
Rhizome #	(i)	20.3	0.690	2.20	5.31	13.90
	(ii)	32.0	0.490	1.56		9.87
Pooled Sample Rhizome #	(i)	47.3	0.325	1.04	49.2	3.53
	(ii)	58.3	0.233	0.74		2.53
	(iii)	65.8	0.181	0.58		1.97
	(iv)	60.9	0.216	0.69		2.35
	(v)	59.6	0.224	0.72		2.43
Pooled Sample Roots #1-1	(i)	73.0	0.160	0.26	5.32	16.08
	(ii)	64.0	0.194	0.31		19.50
	(iii)	78.0	0.107	0.17		10.76
	(iv)	88.5	0.053	0.08		5.33
	(v)	67.8	0.170	0.27		17.09

Variability:

Pooled Rhizomes #6-10		Pooled Roots #1-10	
Mean	2.563	Mean	13.751
Stand. Dev.	0.583	Stand. Dev.	5.691
Coefficient of Variance (%)	22.747	Coefficient of Variance (%)	41.386

Figure 4.

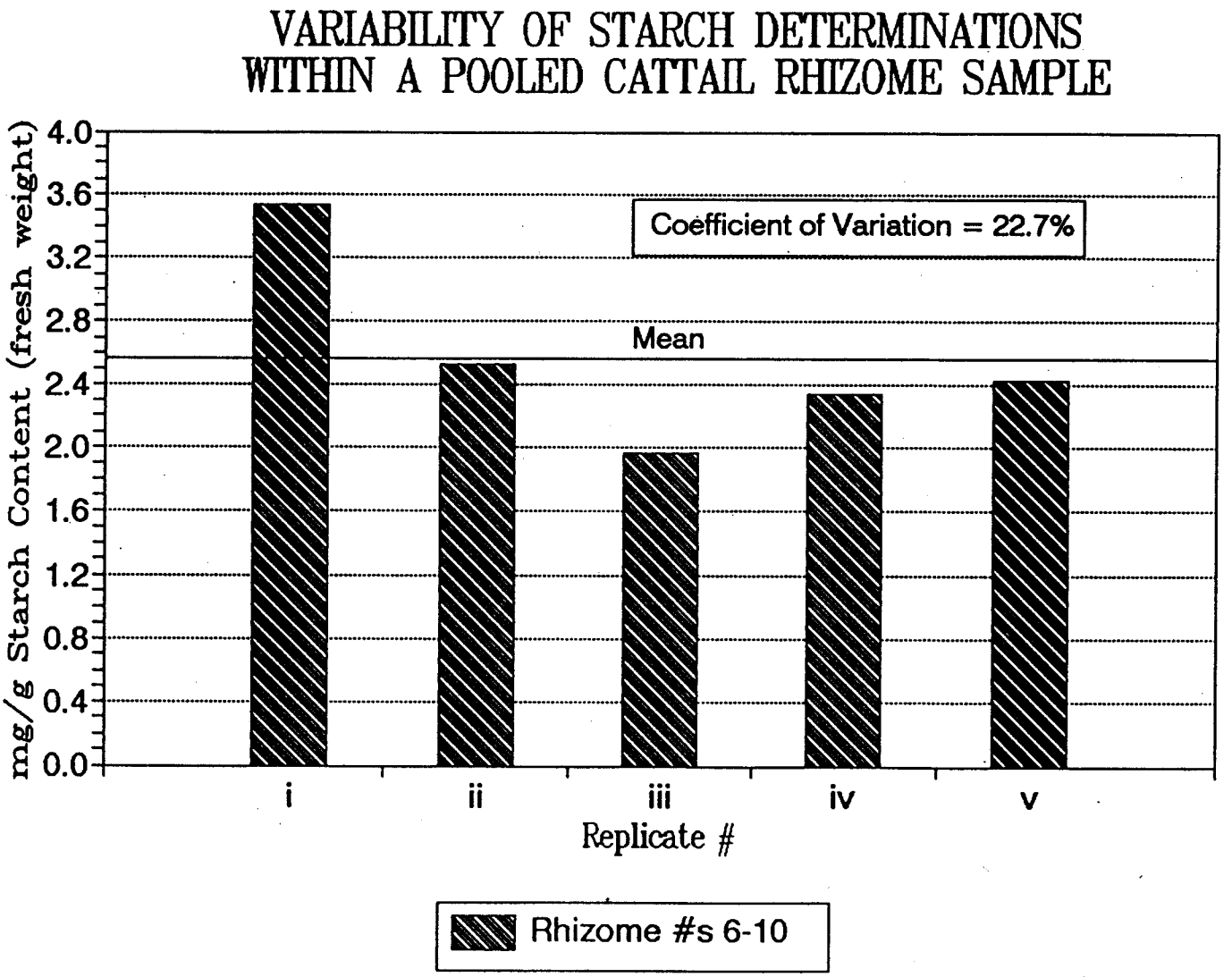
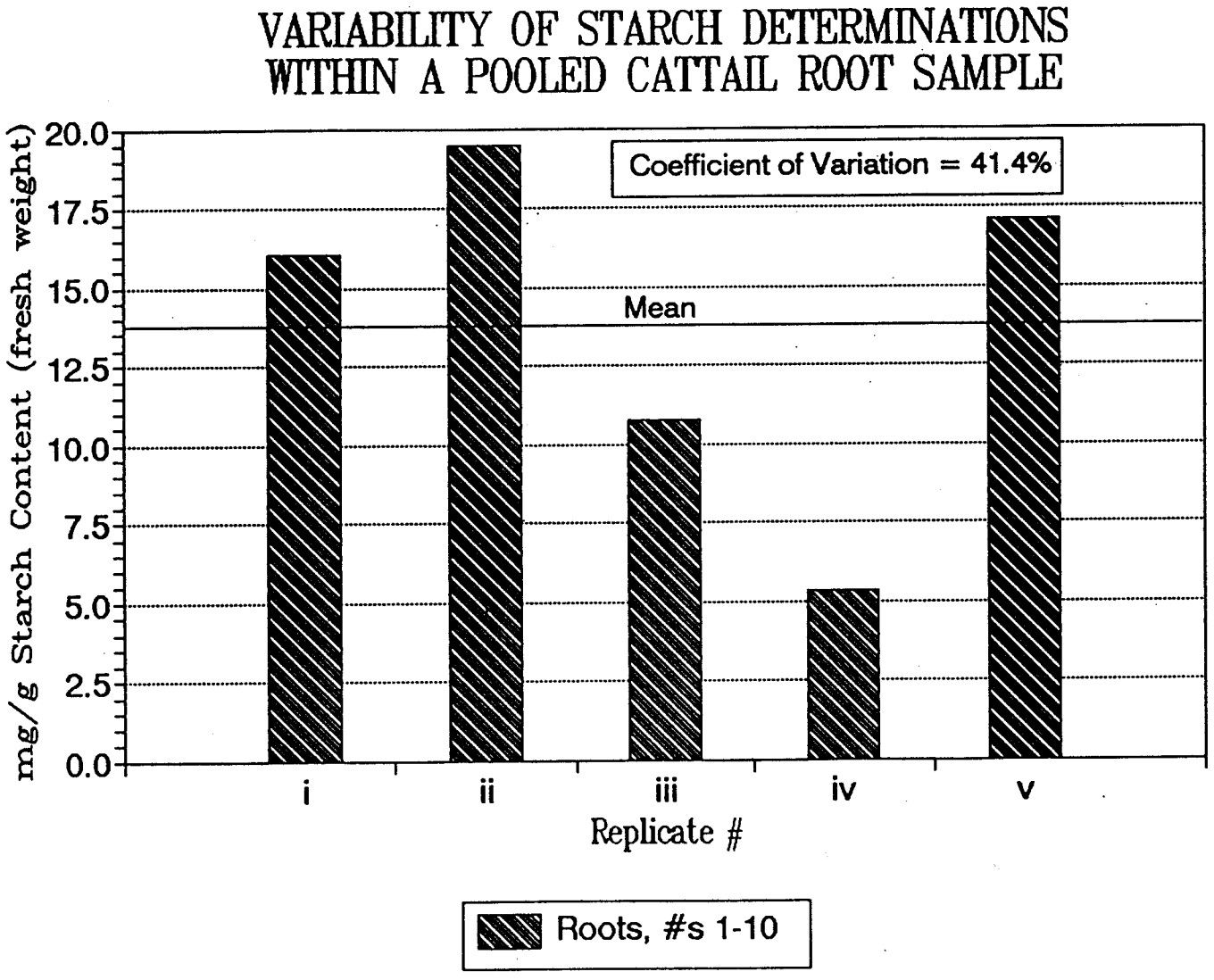

VARIABILITY OF STARCH DETERMINATIONS
WITHIN A POOLED CATTAIL RHIZOME SAMPLE
mg/g Starch Content (fresh weight)
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Coefficient of Variation = 22.7%
Mean
i
ii
iii
iv
v
Replicate #
Rhizome #s 6-10

Figure 5.


VARIABILITY OF STARCH DETERMINATIONS
WITHIN A POOLED CATTAIL ROOT SAMPLE
mg/g Starch Content (fresh weight)
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Coefficient of Variation = 41.4%
Mean
i
ii
iii
iv
v
Replicate #
Roots, #s 1-10

5. DISCUSSION

5.1 Cattail Extraction

Evidence from both sampling trials at the Scarborough Bluffs indicates that, provided there is no solid ice cover nor frozen substrate, cattails can be extracted quite easily with a spade. The winter season, at least in the Toronto area, sees great fluctuations in daily and weekly temperatures so attention needs to be payed to weekly forecasts for the coming of winter thaws. This is extremely applicable to the Scarborough Bluffs cattail stand because there is not enough flow through the area to keep water moving and, hence, unfrozen during cold spells. By extracting two to three days into a warm spell, sufficient time is allowed for accumulated ice to melt thereby making cattail sampling quick and easy.

Elliot Lake, however, is a different matter. Because it is farther north, daytime temperatures are not as warm as they are in Toronto, and winter thaws are less frequent. Fortunately, the cattails of concern at the Denison Stanrock site are located in a flow area so it is hoped that there will not be a buildup of ice in the fertilizer plots. Because the 1st Opening site is not easily accessed in the winter it is not likely that on-site staff will be able to report on conditions at the stand. Should ice buildup be a problem, though, when Boojum staff arrive, the use of an ice pick to extract the cattails may be a necessity.

The most effective way of extracting cattails with intact rhizomes, with a spade at least, is to locate a senescent, emergent shoot and dig at least 0.5m away from it in an encompassing fashion. Periodic checks should be made while digging to locate any year-old rhizomes with new shoots so as not to damage them. To save transportation weight it is advisable to wash as much substrate as possible from the extracted cattails in the field. As well, plants should be kept as frozen as possible in the field, and during transportation, so that starch levels do not start changing as a result of tissue thawing.

If below-ground biomass results are required, individual cattails should be stored in separate plastic bags so that loose material associated with a particular plant does not get

mixed up with that of others; otherwise, several plants can be transported and stored in a single bag. This, however, should not be an issue during the winter season since snow and ice conditions make it extremely difficult to cut and extract a prescribed plot of substrate around an individual cattail. On that point, below-ground biomass estimations should be performed in the summer months when conditions are more suitable to do so.

Random sampling of cattails could not be carried out at the Scarborough Bluffs because ice conditions dictated exactly where it was feasible to extract samples. Even during the second trial only small pockets of spade-acceptable cattails were found. If conditions allow for effective random sampling at Denison it is suggested that the width and depth of the cattail plots be paced out and a table of random digits used to locate plants. Starting at the bottom right hand corner of each plot, the first number in the table will dictate how many paces left, and the second how many forward. Any number encountered in the table greater than either the width or depth should be discarded and the following digit used. Once the destination is reached, the nearest cattail will be the one to extract.

5.2 Sample Preparation

Again, it is critical that the sample stay frozen until absolutely needed - tissue starch levels may change if the plant is allowed to thaw. It became evident while making hand sections through the rhizomes that the tissue was thawing and, as a result, softening up. This created problems because pressure had to be applied to the razor blade to make it cut, thereby mishaping the rhizome section and often squeezing out the contents of the pith. Fortunately, the samples were relatively homogenous in terms of starch distribution so lost material was not critical. However, the case may be extremely different in the Denison samples where the difference between fertilized and non-fertilized plants could be great. For this reason it is important to do as little damage to the tissue as possible.

By slightly altering the preparation protocol order, the problem was solved. After

washing the samples in the laboratory, instead of immediately commencing with hand sectioning, the cattails were placed back in the freezer for at least a half hour to allow them to harden again. With this change, no compression of the rhizome, or loss of material was encountered when sectioning.

To see if storing hand sections in 95% denatured ethyl alcohol had any effects on starch distribution, two test sections were cut from an unused portion of a rhizome. One was immediately stained with IKI and examined under the microscope while the other was stored in alcohol overnight, washed, stained, and examined in the same manner. There was no appreciable difference between the two in terms of distribution, but in terms of actual quantity nothing was confirmed. During the Denison work it may be useful to do a total soluble carbohydrate test on the storage alcohol to determine the amount of carbohydrates that leave the tissue cells and pass into solution. The Dubois et al (1956) method also makes use of colourimetric estimations to obtain results and may, therefore, be appropriate.

Removing roots at the surface of a rhizome is a lengthy and tedious process. Determining the percentage of rhizome weight (including roots) as roots must be looked at with the realization that the precision with which roots are removed accurately at the exodermis surface is questionable. Macroscopically speaking, it may appear that all roots have been accurately removed at the exodermis, but under the microscope the case is different. Microscopic examinations show that no matter how much time is spent removing root material, there will always be some amount still attached to the rhizome. Though it cannot be concluded from this report that those "leftovers" are neglible, it is worth highlighting the point before future data interpretation takes place.

5.3 Iodine/Potassium Iodide Staining

IKI staining for starch distribution proved highly successful with the production of a dark, bluish-purple colour in areas of starch accumulation in plant tissue. Results were

consistent throughout the samples with the pith showing strong colouration as well as a thin band underneath the epidermal layer. Some samples showed an uncharacteristic blue tinge in the cortical layers, indicative of starch, but on closer inspection no granules were seen. The blue colouration in the cortex may be a result of ineffective post-stain rinsing, or the movement/smearing of residual stain-retaining alcohol from the pith across the cortex. Evidence from the microphotographs depicting some smearing, and the fact that post-stain rinsing was thorough in all cases suggests that the latter reason may be the cause.

5.4 Hematoxylin Staining

Hematoxylin staining of cattail rhizome sections for metals uptake proved to be a very exacting task. Initial staining with fresh hematoxylin showed little tissue colouration immediately following application (unlike that for starch which had fast results) so it was thought that the fresh mixture was too dilute. As a means of comparison, a few drops of stock hematoxylin were added to another section - colour development was immediate with a bright red pith and dark blue pockets in the cortex. After three hours the colours were more intense with much of the exodermis and cortex black. The section stained with the fresh solution at that time had developed good colouration with a dark blue exodermis fading to lighter blue in the cortex, and a pale orangy-brown pith.

Some of the metals known to produce the above colouration, and which may be of significance at Denison, include: iron and aluminum - dark blue; nickel, manganese, and zinc - light blue: and, copper - greenish blue (Pizzolato and Lillie, 1967).

The test indicated that the stock solution was too concentrated and that, given time to develop, the final solution was appropriate. Too much time, however, meant a loss of stain effectiveness as evidenced by the lack of any real colouration in the entire run of samples, from #1B-10C, left sitting in the hematoxylin overnight. Though the fresh hematoxylin solution is effective in elucidating metal uptake in plant tissue, it is very time-

specific. As reported in Pizzolato and Lillie (1967), the 2-4 hour interval for stain retention appears to be the best.

Furthermore, the fresh solution does not last more than one day. After attempting to stain a section with day-old 'fresh' hematoxylin, and with no appreciable colour development after three hours it was decided that fresh solutions needed to be prepared daily (not clear from the Pizzolato and Lillie paper). To further strengthen this argument, a sample of the day-old solution was placed in a vial alongside one containing fresh hematoxylin; that in the first vial was much paler than that in the second. Because of this rapid change (i.e. within 24 hours) in solution colour, it is recommended that the 2-4 hour stain retention time reported in Pizzolato and Lillie be strictly adhered to.

5.5 Microphotography and Rhizome Dimensioning

Photographing rhizome sections under the stereomicroscope has the obvious benefit of providing an everlasting record of tissue conditions; notes made at the time of examination can be cross-checked with results from permanent pictures for accuracy. A number of observations with respect to the process of photographing sections need to be highlighted to ensure the success of future attempts in recording material for permanency.

First of all, cameras are sensitive to subject movement, especially at slower shutter speeds. It is therefore essential to keep, in this case, the tissue section as still as possible when taking a photograph so that no blurring of the image occurs. To prevent glare from the material (the Ziess scope used in this study shines light onto the sample from above) caused by reflected light, samples must be immersed in a sufficient depth of water (approx. 1cm). Because a tissue sample in water has a tendency to move, it must be secured in a manner that does not alter the overall effect of the image. By installing a lifting clear glass cover plate in a petri dish it is possible to do just so; a sample can be secured in water by placing it underneath such a lifting cover (anchored to the dish, by silicone, with a flexible

plastic arm) without harming the photographic effect. As a further check against sample movement, the area around the scope should be clear of people moving back and forth.

The MC 63 M 35 camera on the Ziess scope is equipped with an automatic light meter that reads the amount of light coming into the camera from the image surface and tells the photographer what exposure setting to use. It was found during the study, however, that a number of rhizome sections which did not fill the entire image plane were underexposed; those sections that did were just slightly underexposed. This occurs because the light meter obtains its reading from the entire image plane, so when there is a lot of white background the meter suggests a fast exposure - the resultant image has a very nice white background but a dark subject. It was found that by setting the exposure control ¼ of a second slower than the suggested setting, small subjects could be properly exposed. Subjects that took up the entire image plane gave more accurate exposure readings, but it was found that adjusting the fine exposure control to the maximum level (rather than the suggested mid-point) for any particular macro-setting gave the best results.

Other things that need to be watched for in order to obtain good photographs are air bubbles and floating particles in the petri dish. Air bubbles that get trapped in the tissue or between the material and the cover plate can cause incoming light to reflect unevenly producing unwanted glare; one way to get rid of air bubbles is to vaccuum them out of the water with a syringe. The best way to keep solid particles out of the water is to thouroughly rinse the petri dish between samples.

Dimensioning rhizome sections was made quick and easy by replacing the clear glass cover slip previously mentioned with a grid superimposed on a clear acetate cover. It is important to note that once magnified, grids that appear precise to the naked eye are quite rough; for the purposes of this preliminary study, though, the grid used was sufficient. To increase accuracy in the future, a micro-ruler with one-tenth of a millimeter graduations may be useful.

5.6 Quantitative Determination of Starch

The Nielson method (1943) proved to be an effective and rapid way of quantifying the amount of starch stored in the rhizome and root of a cattail. Results from replicate determinations of the same sample, however, do indicate some variability in the process; this variability is either due to the sampling technique, the instrumention, or the random turbidity of the tissue slurry (or a combination thereof).

Variability due to instrumention (i.e. the spectrophotometer) was shown to be negligible since the prepared reference blank continually gave consistent readings, with only minor fine adjustment, before each tissue sample was estimated. An important point to note here is that the spectrophotometer sample cuvettes must be thoroughly rinsed between samples and wiped clean of any solid particles if accurate data are required. Because this method is a colourimetric determination (i.e. the amount of light a given sample absorbs), it is paramount that particles foreign to the actual sample be removed so as not to interfere with light penetration and give misleading results.

Sampling variability, in the case of starch standard determinations, was also neglible as indicated by the standard curve prepared using very precise amounts of starch. With an R Squared value of 0.950, the graph shows that the predictability of spectrophotometer readings from the amount of starch in solution is close to perfect. In this case, 95% of the variance in spectrophotometer readings is accounted for. Not only does this strengthen the argument that the spectrophotometer is consistent, but it also suggests that, given the accuracy of the sampling during this stage, variability due to sampling was negligible as well. The key to minimizing sampling variability here was the use of 1ml and 3ml syringes to very consistently obtain, from standard to standard, the appropriate aliquots at the various stages in the process. Furthermore, the absence of interfering material (like the strands of tissue in the rhizome/root samples) of different shapes and sizes moving through the final solution in a random fashion makes for very consistent light transmission in the spectrophotometer.

The latter point, in fact, appears to be the primary reason why there is variability in the starch concentrations between replicates of the same sample, especially the roots. Because rhizomes and roots contain large amounts of cellulose, blending the tough material results in an unsatisfactory haphazard mixture of liquid, strands of tissue, and other chopped up particles. Much of the material does not get ground up to anything near the fineness required for homogeniety, and as such the resultant random slurry offers little consistency from one replicate sample to another. Because a substantial amount of material remains intact throughout the blending operation it cannot even be assured that the results obtained are indicative of conditions in the plant tissue; because the blended slurry is not homogenous there is no way of knowing whether or not an aliquot taken from it has the correct percentage of starch weight to tissue weight.

This variability in the slurry contents and, hence, turbidity causes problems for sampling and spectrophotometer operation. Because of the large particles in the slurry, the only way to take a 2ml aliquot was to measure that amount into a graduated cylinder - pipettes and syringes were tried but were instantly blocked up with large particles thereby rendering them useless. Trying to actually detect the miniscus of such a rough mixture in a graduated cylinder was anything but consistent, and further added to the variability of the process. Though there is no data to support the claim, it is likely that the apparent randomness of the slurry also created inconsistent turbidity results from sample to sample thereby making comparative spectrophotometer estimations inaccurate. Nevertheless, a single turbidity blank was produced and its results incorporated into the calculation of starch content in all tissue samples.

It is clear from the above that attempting to blend belowground cattail tissue into a homogenous, workable solution does not work and leads to variability and inaccuracy in the final data. To solve the problem it is suggested that, in the future, samples for starch determination be oven dried to constant weight at 110°C, weighed, and ground into a powder with a mill using a 60- to 80- mesh screen [as recommended by McCready et al (1950)]. This method should allow for a much more workable and consistent solution. Another

improvement to minimize sampling variability is the use of precision graduated 5ml pipettes (in 0.1ml graduations) rather than syringes or minimum capacity pipettes for taking aliquots - attempting to measure 2.7ml of perchloric acid with only a 1ml graduated pipette meant drawing up liquid an extra two times for one sample and increasing the possibility for error by three.

5.7 Rhizome Pith Width and Starch Content

Though this report focusses on evaluating the methods for collecting cattails, describing their morphology, and examining tissue for starch content and metal uptake, the data do point to some interesting ideas. These ideas will undoubtedly guide the investigation of future work at Denison Mines to determine the effects of foliar fertilizer on cattail below-ground biomass (growing on tailings).

In this study, roots showed significant amounts of starch to be present in the tissue. Though the variability between five replicates from the same sample was high, starch had definitely accumulated. A study done at a community near Lake Ontario on overwintering starch distribution in cattails showed that roots contained as much as half the starch that rhizomes did. It appears, then, that roots play a significant role in starch storage during the winter month so it is suggested that sections be made to examine starch distribution. The argument for making hand sections of cattail roots is further strengthened by Kalin and Scribailo's (1988) observations of metal damage to transplanted cattail rhizome and roots at the Denison site. Provision, then, should be made for hematoxylin staining of root sections.

The most interesting data to come of this preliminary study are the diameters of the pith in the rhizome. Of the 29 sections measured, only two had piths whose diameter was less than 50% of the overall rhizome diameter. In addition, nine out of ten times the pith got proportionately larger toward the new shoot-end of the rhizome (it is suspected, but not

proved, that the one time it did not was a result of unknowingly reversing the rhizome, thereby doing the same to the measurements). If rhizome biomass, then, is related to productivity, as implicated by Fiala (1971), and the pith is the major overwintering storage organ of the cattail (Kausch et al, 1981), then the percentage of the rhizome diameter as pith may be an important morphological indicator of plant health.

If this is the case then particular attention must be paid to measuring pith and rhizome diameters so that comparisons can be made between fertilized and non-fertilized tailings-grown cattails, natural ones, and even those on floating mats. Should pith size be an overwintering indicator of plant health and future growth (i.e. the coming growing season) capability, those plants who are healthy should see larger piths than those that are not. To reinforce this statement, quantitative starch measurements should be made to determine the actual amount of overwintering growth capacity cattails have.

The relationship between pith size and starch content can be determined by considering the dimensioning results of this study. If the pith becomes proportionately larger toward the new shoot it would make sense to do separate starch determinations at each end of the rhizome and relate those results to corresponding pith diameters. The sample preparation method used in the preliminary study could be altered to test the pith size/starch content hypothesis. Rhizomes from future cattail samples could be measured for length, and a constant central percentage of that length used for morphological and chemical investigations. Portions sufficient enough for quantitative starch determination can be cut at each end of the rhizome sample (i.e. parent shoot and new shoot ends) and bracketed by two hand sections for dimensioning. This should provide the means for relating pith size to starch content.

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