MONTANA STATE UNIVERSITY

Acid tolerance of several plant species indigenous to hardrock mines in western Montana

by Kathleen Wheeler Johnson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University
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Abstract:
This study identified the vegetation on two inactive hardrock mines, Champion Mine and Porphyry Dike. It also characterized the acid tolerance of vegetation from those mines as well as vegetation from the Anaconda smelter tailings.

A greenhouse trial was used to determine acid tolerance. Before acid tolerance could be evaluated, however, 19 plant populations selected from the two tailings sites and the Anaconda tailings were screened for acid tolerance. Seven clones representing five species showed tolerance to acid tailings materials and were selected for the experiment. . This study utilized a sand/drip culture apparatus to determine acid tolerance of the selected native populations and corresponding commercial varieties. An "in parallel" technigue used three acid pH levels. Statistical analysis was used to identify differences in acid tolerance between tailings and commercial populations.

Results indicated that although there were no significant differences between tailings and commercial populations of all species at pH 3, there were significant differences at pH 4 and/or pH 5 in four out of five species tested. The Agrostis tenuis and Deschampsia cespitosa tailings populations from the Anaconda smelter tailings were significantly more acid tolerant than the commercial populations. The commercial population of Agropyron trachycaulum proved to be statistically more tolerant than the two tailings populations. A similar response was found for Fragaria vesca. No definite difference in acid tolerance was found between the commercial and tailings populations of Trifolium repens.

The use of acid tolerant vegetation is a reclamation technique that has great potential in the Rocky Mountains. The confirmed acid tolerance of several species from this study indicates that they may be suitable for use in revegetating acid metalliferous mine wastes.

ACID TOLERANCE OF SEVERAL PLANT SPECIES INDIGENOUS TO HARDROCK MINES IN WESTERN MONTANA

by

Kathleen Wheeler Johnson

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ABSTRACT

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Results indicated that although there were no significant differences between tailings and commercial populations of all species at pH 3, there were significant differences at pH 4 and/or pH 5 in four out of five species tested. The Agrostis tenuis and Deschampsia cespitosa tailings populations from the Anaconda smelter tailings were significantly more acid tolerant than the commercial populations. The commercial population of Agropyron trachycaulum proved to be statistically more tolerant than the two tailings populations. A similar response was found for Fragaria vesca. No definite difference in acid tolerance was found between the commercial and tailings populations of Trifolium repens.

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INTRODUCTION

Discoveries of gold between 1857 and 1863 resulted in a mining rush to southwestern Montana (Malone and Roeder 1976). Mining initially began as placer mines where the sand and gravels of the creek bottoms and the soils along the creeks were sieved for nuggets of gold. As the placers were worked out miners began looking for the mother lodes from which the placers had originated. Most of the underground lode mines around the Helena area produced gold although some worked silver or lead veins. Those near Butte and Phillipsburg generally produced silver. Most placer gold was produced before 1875. Lode gold production peaked between 1890 and 1900 while most of the silver and lead was produced between 1880 and 1900 (Pardee and Schrader 1933). Copper was discovered at Butte in the early 1880's (Malone and Roeder 1976). Copper production fluctuated since then and has tapered off recently. Other metals such as zinc, molybdenum, and chromium have been by-products of this copper mining industry.

Today hundreds of mines can be found in the mountains between and around Helena, Phillipsburg, and Butte. Most have been abandoned within the past 60 years either in the sense of relinquished ownership or in terms of active mining. There were several reasons for abandonment: the ore ran out or became uneconomical to transport to a mill; the ore became too difficult or too hazardous to obtain; or a metal's market value dropped (Wyman 1979). Sometimes the miner/operator or the mining company went bankrupt and closed a mine. Occasionally mines or mills were closed because tailings contaminated streams that provided drinking water for nearby cities (Pardee and Schrader 1933).

Generally when mines closed, operators took what they needed and could carry or pack out on horses or mules. The mine buildings were no longer of use as it was easier to build new buildings than to tear down, transport and rebuild the old buildings. Waste dumps, tailings piles and played-out mines were forgotten in the miner's haste to find new riches elsewhere in this vast region (Wolle 1963, Malone and Roeder 1976). Environmental impacts were seldom considered, so few attempts were made to clean up, stabilize or reclaim mining disturbances.

There is a growing concern about the hazardous conditions of these small mining disturbances which range from less than one hectare for a single mine to several hectares where numerous mines are located in the same area. Decaying buildings and open adits or shafts at these sites present serious hazards to the health and safety of human life, wildlife and livestock. The unprotected steep slopes of mine wastes and tailings dumps and the more level tailings ponds create soil and water pollution problems. These unstable slopes and materials are subject to severe erosion especially during spring runoff (Nielson and Peterson 1978) and heavy storms. They often contribute acid and heavy metal burdens as well as sediment to surface and ground water resources (Johnson et al. 1975). Reclamation of these small mines could reduce sedimentation and acid drainage, alleviate health and safety hazards and lessen the visual impacts of disturbed areas.

Rehabilitation of these disturbances will not be an easy task. There are many site factors which inhibit the establishment of vegetation, an essential part of reclamation. The rugged terrain can generate drastic microclimatic changes which result in very different soils and plant populations within short distances. Due to the high elevation of most mines, any plants used to revegetate these disturbances must be adapted to short growing seasons and extended periods of low temperatures. In addition to these general regional stresses, the plants must be capable of tolerating adverse characteristics of wastes and tailings materials left on the mine sites. Years after the cessation of mining activities, many derelict sites are either devoid of vegetation or at best sparsely colonized (Troug 1946, Bradshaw et al. 1978). There must, therefore, be some limiting factor(s) in the mine waste or tailings materials that inhibits or prevents nearby native plant populations from colonizing the disturbances. The limiting factor(s) may not be the same from one mine to another.

The variability of the mine waste materials at and between different mines is "related to the type of mining operation, ore composition, the amount of pyrite and other sulfides present, the type of milling process, ... and the age and degree of exposure of the tailings" (Peterson and Nielson 1978). Many hardrock tailings are acidic due to high pyrite concentrations. The pH of the materials affects the concentration of different ions in the soil solution and therefore their availability to the plant (Russell 1973, Mengel and Kirkby 1982). Such soils often have higher water soluble or plant available levels of metals such as aluminum, manganese and zinc and lower concentrations of calcium and other nutrients than calcareous or neutral soils (Brady 1974, Rorison 1980). Heavy metals are the major problems in many acid mine wastes. These chemical characteristics combined with undesireable physical properties (i.e. rocky or clayey materials, steep slopes, etc.) result in an environment that inhibits the establishment of many native species.

The more traditional methods of reclaiming these wastes have been by surface manipulation, burial, or addition of chemical or organic amendments to the spoils or tailings. However, these methods may not permanently remove or reduce a site's undesirable characteristics or they may be too costly to use on remote, inactive sites. The seeding of plant species tolerant to the plant growth inhibiting properties of the sites is a revegetation technique that has great potential.

Bradshaw et al. (1965 and 1977) found that plant populations growing on mining wastes and tailings are usually more tolerant of heavy metals than the same species found on nearby undisturbed sites. Several plant species have been found to be tolerant of elevated levels of various metals by several researchers in the United Kingdom (Gregory and Bradshaw 1965, McNeilly 1968, Bradshaw et al. 1978) and in Canada (Cox 1979, Hutchinson and Kuja 1979). Some of these same species, such as Agrostis tenuis, are found in western Montana. If these and other species are found growing on mine disturbances, they may be expressing tolerances to the chemical and physical properties of the mine wastes. These tolerant populations should be better adapted to the climate and site conditions than commercial varieties. It is not known if plants surviving on these materials are tolerating elevated hydrogen ion concentrations, high metal concentrations, nutrient deficiencies or all three.

Plant tolerances are related to a plant's ability to adapt to physical and chemical properties of the root zone as well as climatic and microclimatic conditions. The use of native populations may eliminate concerns about climate. If physical and chemical properties of mine waste materials on which vegetation is growing can be analyzed and related to plant growth, then one can begin to understand some of the various soils properties the plant populations are tolerating. Greenhouse trials on representatives of those populations could determine tolerance ranges for growth inhibiting parameters such as pH or heavy metal concentrations. If the plants cannot persist because of the mine waste's physical or chemical characteristics, then long term revegetation and thus rehabilitation cannot be achieved utilizing these plants without other reclamation activities.

Vegetation was found growing on portions of mine wastes and tailings of two inactive hardrock mines in west-central Montana (Figure 1): Champion Mine and Porphyry Dike. Soil analyses indicated that the vegetation might be tolerating acid and/or elevated heavy metal concentrations. The main objective of this research was to evaluate the acid tolerance of several species found growing on the acid mine tailings through greenhouse trials. To do this it was necessary to identify the plant species growing on and around the mine wastes and tailings, and select those species which appeared to have some tolerance to an acid root environment.

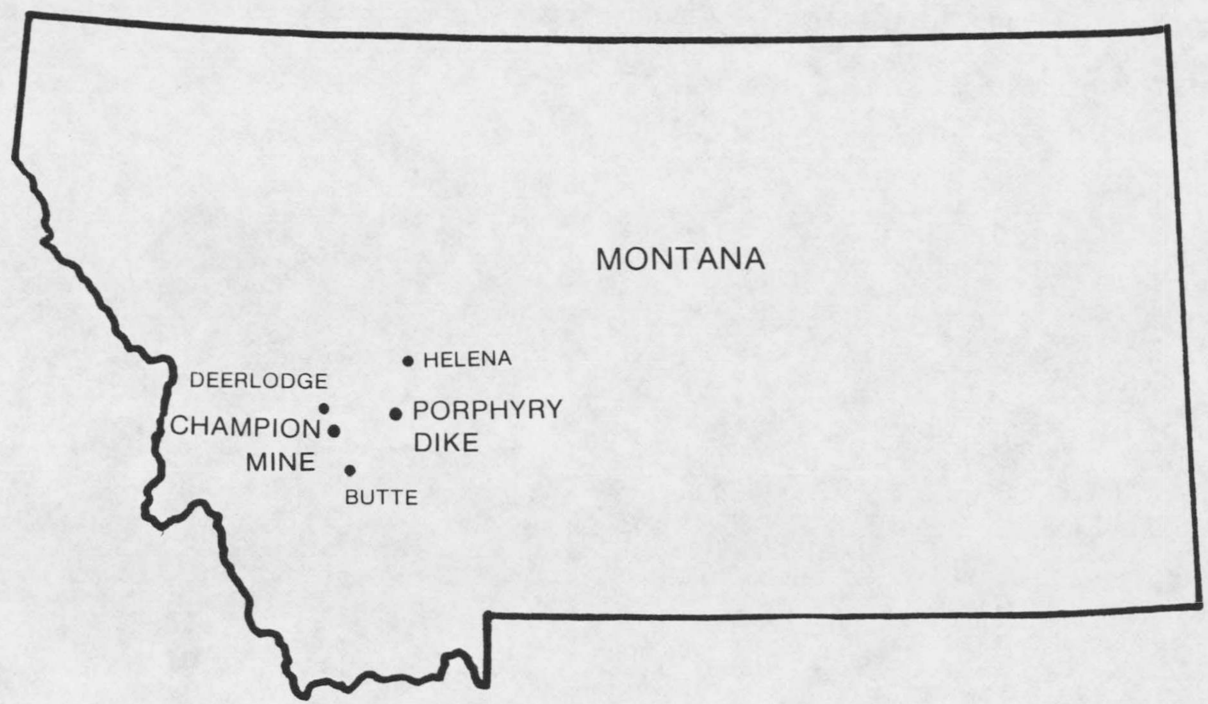

Figure 1. Location map for Champion Mine and Porphyry Dike.

MINE HISTORY AND DESCRIPTION

Two inactive hardrock mines owned by the Anaconda Minerals Company were selected because of their accessibility and because vegetation was growing on tailings at these sites. The Champion Mine (Fig. 2) is located about 10 kilometers east of Galen, Montana at an elevation of approximately 1800 meters. Its tailings are situated in a moist mountain meadow. A nearby stream, which is a tributary of Dry Cottonwood Creek, has been rerouted around the tailings pond. Porphyry Dike (Fig. 3) is located about 12-16 kilometers south of Rimini, Montana and is about 29 kilometers southwest of Helena, Montana. It is alongside Monitor Creek, a tributary of Ten Mile Creek. The mine extends from an open pit on a slope near the 2300 meter high ridge to a large tailings pond in the creek bottom several hundred meters below the pit. These two mines have interesting histories that have resulted in different types of tailings deposition. Both mines began after placer mines in nearby streams had been worked out.

Champion Mine

Champion Mine and several other mines such as The John, Last Resort, and Independence mines in the Oro-Fino District were worked mostly for silver ore from quartz lodes (Wolle 1963). Some gold, however, was also obtained, but it was usually a by-product not the major metal being mined (Malone and Roeder 1976).

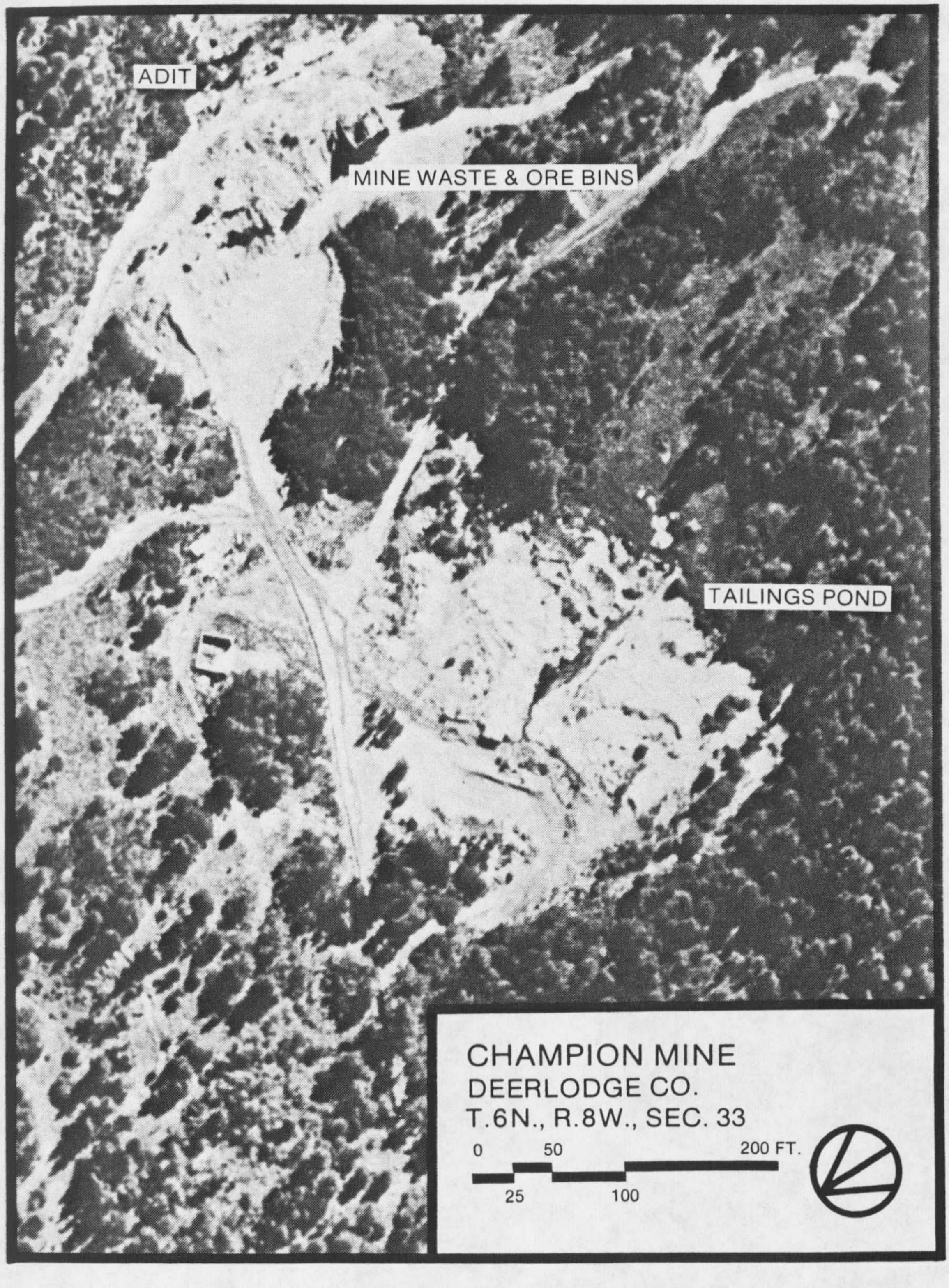

Figure 2. Champion Mine.

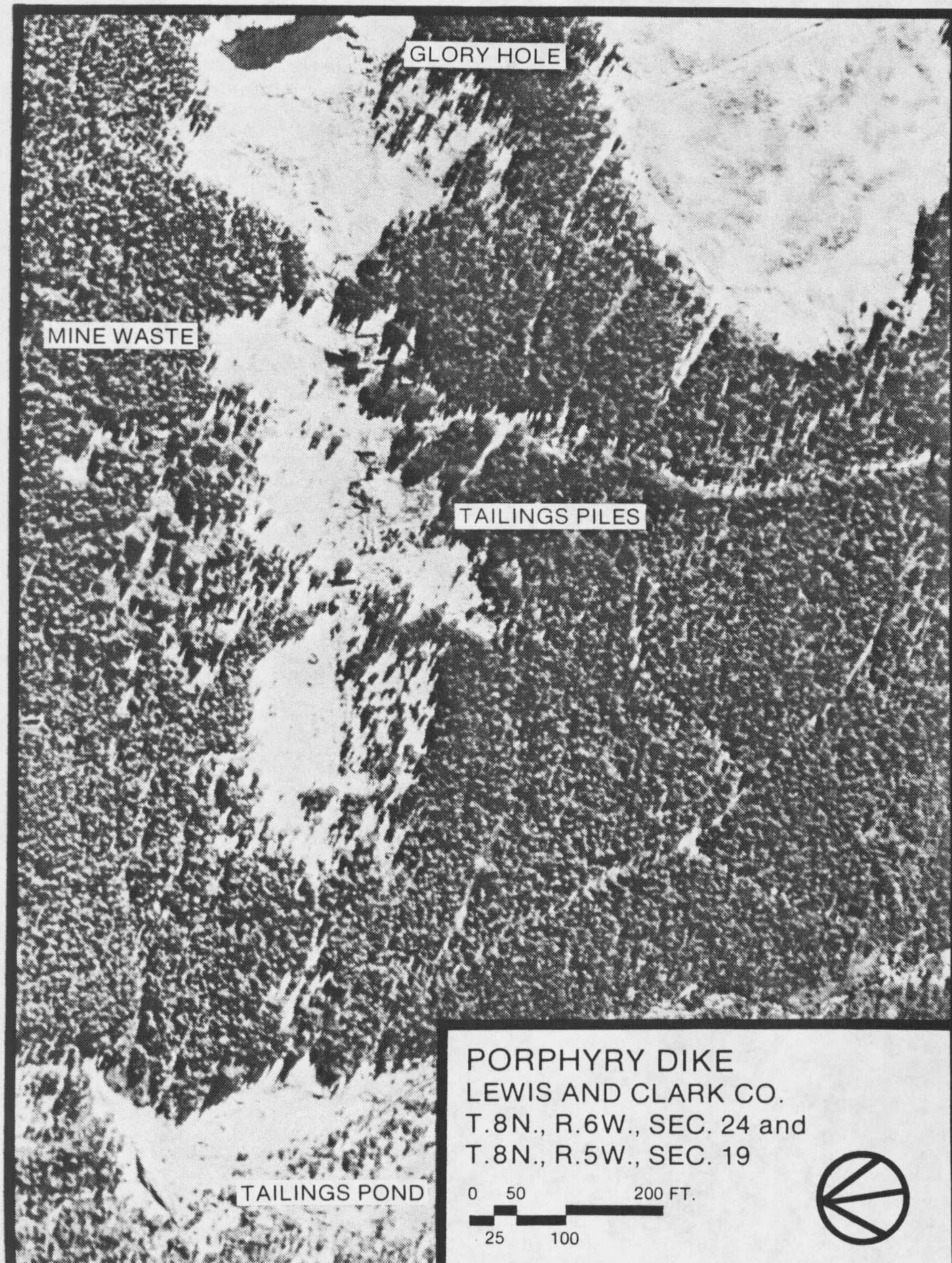

Figure 3. Porphyry Dike.

A fairly large community had lived in the town near the mine. It boasted 60 to 70 buildings along its main street and numerous cabins in nearby gulches and hillsides. It had several saloons, a lumber store, a grocery, a couple of restaurants, two barbershops and two butcher shops (Wolle 1963). The town of Champion quickly ceased to exist in the late 1920's with the closure of most of the neighboring mines. Very little except building foundations and deteriorating cabins remain as a reminder of this once prosperous mining town.

The Champion Mine was worked by the Champion Mining and Milling Company from about 1886 to 1893 (Hogan 1891, Pardee and Schrader 1933). The ore was processed at a company mill near Deerlodge. The high cost of transportation and the drop in silver prices resulting from the panic of 1893 closed the mine in the mid 1890's (Malone and Roeder 1976). In 1917 the Butte-Jardine Metals Company purchased the mine (Trauerman and Waldron 1940, Reyner and Trauerman 1949). The company borrowed $100,000 to build a 100 ton flotation mill (Reyner and Trauerman 1949). The Butte-Jardine Metals Company went bankrupt when silver prices dropped sharply and the company was forced to close the mine in 1926. One year later W. B. Daly purchased the mine but it was never reopened (Trauerman and Waldron 1940).

When this mine closed it was being worked at the 214 and 244 meter levels but the main shaft had reached the 274 meter level (Reyner and Trauerman 1949). The underground workings are now flooded and the water flows out of the mine through the collapsed adit. The exterior surface portion of the mine can be divided into two areas. The upper area consists of the adit, the mine waste dump and the ore bins. The lower area contains the mill tailings pond.

The tailings area reflects the flotation milling process. Pits dug into residual piles reveal distinct layering of tailings material. Wet tailings were probably dumped into the pond and flowed over the surface of the previous batch of tailings. The varying colors probably correlate to the fluctuating composition of the ore and country rock processed in each "batch" at the mill and the oxidation-reduction potential of the material. Much of the tailings has eroded away leaving barren ridges and piles of tailings materials separated by swales that are heavily vegetated with *Carex* and *Juncus* species, grasses and several shrubs.

The upper area is dominated by a large waste rock dump. Much of the rock material is dry and barren. Vegetation, mostly weedy species, is scattered over the dump but is concentrated where rain water collects on level areas around the ore bins. Along the outer edges of the dump near the forest, lodgepole pine (*Pinus contorta*) and aspen saplings (*Populus tremuloides*) are colonizing the waste rock.

Porphyry Dike

The Porphyry Dike mine occurs in an area that differs geologically from the surrounding mountain ranges. The gold ore occurs in quartz rhyolite, sometimes called rhyolite porphyry, that lies directly on top of granite intrusions. Andesite more commonly overlies the granite. This free milling ore occurs only in minute cracks and fissures in rhyolite porphyry and varies a great deal in form, color and texture (Sisley 1897). Several mines in the neighborhood such as the Ontario, Josephine, Lee Mountain and Valley Forge were located on rich silver or lead and silver veins in the granite. Paupers Dream and Porphyry Dike, however, mined the porphyry dike deposits which are chiefly low grade ores and should be concentrated at or near the mine (Sisley 1897, Schrader 1929, Wolle 1963).

The Columbia workings, a large group of mining claims, appears to have been in the same area as Porphyry Dike (Sisley 1897) and may have been the name of the site prior to the opening of the Porphyry Dike mine by the Gold Cap Mining Company around 1910. In 1911 Porphyry Dike included 82 claims (Pardee and Schrader 1933). The ore was mined from a tunnel network 38 meters below a large glory hole. The ore was processed in a 10-stamp mill near the creek. The 10-stamp mill was replaced by a 20-stamp mill in 1917 and later by a 300 ton electric ball mill (Pardee and Schrader 1933). The mine was closed in 1927 when the city of Helena complained that mill tailings were polluting the city water supply derived from Ten Mile Creek (Pardee and Schrader 1933).

The Porphyry Dike property contained offices, shops and housing for 40 men and their families (Wolle 1963). A boarding house was also located near the mine, but the nearest large community was Rimini which is almost a ghost town today. Numerous cabins and foundations can be found in the forest surrounding the mine, but all of the buildings are deteriorating and rotting away.

Porphyry Dike had three distinct areas: the mine pit and mine waste dumps, the tailings dumps, and the tailings pond. Some time around 1927, the glory hole was expanded into an open pit, probably to obtain more of the low grade ore unobtainable with tunnelling. Several tunnel openings can be seen in the north facing rock wall of the pit. There are several mine waste dumps of country rock between the pit and the tailings dumps. These are dry, barren areas exposed to the weather like the surrounding talus slopes. Only a few stunted pines (Pinus spp.), some raspberry plants (Rubus ideaus) and some forbs have taken root amidst the rocks.

Below the mine waste dumps, the mine and mill buildings are rotting away amid several tailings dumps. The tailings are eroding down toward the valley bottom in braided rivers of tailings material through the trees. Vegetation, mostly grasses and forbs, is concentrated along the gullies and beside the stream that cuts across the uppermost tailings. Lodgepole pine, limber pine (Pinus albicaulis) and sub-alpine fir (Abies lasiocarpa) saplings are taking root near the forest edge.

The third area contains the tailings pond that was dammed up over Monitor Creek. The stream had been channelled into a rock-lined, timber-covered tunnel that was buried beneath tailings materials. The timbers eventually rotted, collapsed and blocked the tunnel. The stream backed up and finally washed out part of the dam. Over the years a major portion of the tailings has been washed downstream during spring runnoff and flash floods.

This tailings material has virtually no vegetation growing on it. However, swales in the material, the lower slopes, and the creek bottom are covered with grasses, Carex and Juncus species, and several types of shrubs. The ridges of gravelly material disturbed by earlier placer mining are dominated by lodgepole pine and shrubs.

Reclamation Problems

Field pH readings indicate that some of the tailings materials from these two mines are fairly acid ($pH < 5$). Yet there are also some tailings that are neutral to slightly alkaline ($pH \geq 7$). The large amounts of erosion indicate that slope and/or material stabilization is also a variable to be considered. Because there are many plant growth problems associated with acid metalliferous wastes, it is the vegetation growing on the acid tailings that is of prime interest.

LITERATURE REVIEW

Characteristics of Acidic Metalliferous Mine Wastes

The mineral composition of the ore and country rock (rock surrounding an ore body) greatly affects the properties of the mine wastes and tailings (Nielson and Peterson 1978, Bradshaw and Chadwick 1980). Many metalliferous deposits are associated with igneous intrusions such as the Boulder Batholith in southwestern Montana (Frith 1978). Mining in these deposits might result in tailings high in silica but low in nutrients. Soils developing from granitic parent material are typically sandy and somewhat acid (Tisdale and Nelson 1975).

Ore composition and processing

Hardrock mining removes waste rock from tunnels or pits dug to reach the ore. The piles of country rock or mine wastes are very coarse, arid, and low in nutrients (Bradshaw and Chadwick 1980, Antonovics et al. 1971). Mine and mill tailings are much finer as a result of ore processing. Although these finer materials may have good water retention, they are susceptible to wind and water erosion and often contain toxic concentrations of heavy metals. Unlike natural soils that have developed from the interaction of parent material, climate, relief, biological activity and time (Brady 1974), the physical and chemical characteristics of mine and mill wastes are determined by the mineralogy of the ore and country rock, the mining and milling processes, the method of waste deposition and the degree of exposure and weathering. Man is the major factor responsible for the creation of these highly variable, inhospitable sites. The reclamation potential of acidic mine wastes depends upon the magnitude of the chemical and physical characteristics, their interactions with each other and their impacts on vegetation as well as vegetation tolerances to those characteristics.

Acid generation

The pH of tailings materials may vary greatly. Strip mine spoils in the Northern Great Plains may be somewhat alkaline with pH values between 7.2 and 8.5 (Packer and Aldon 1978). Tailings with a high proportion of sulfides such as iron pyrite (FeS_2) are especially susceptible to a rapid drop in pH under the right conditions. Pyritic wastes commonly have pH values between 7 and 10 when deposited (Mays and Bengtson 1978).

When the pyrite particles are at or near the surface of a waste pile, they come into contact with moisture and oxygen and the following reactions occur (Peterson and Nielson 1973):

$$FeS_2 + H_2O + 3\ 1/2\ O_2 \longrightarrow FeSO_4 + H_2SO_4$$

$$2FeSO_4 + H_2SO_4 + H_2O + 1/2\ O_2 \longrightarrow Fe_2(SO_4)_3 + 6H_2O$$

$$Fe_2(SO_4)_3 + 6\ H_2O \longrightarrow 2Fe_2(OH)_3 + 3H_2SO_4$$

The resultant sulfuric acid generation lowers the tailings pH. As long as there is iron pyrite in the tailings this process is self-perpetuating and the tailings remain acid.

Some mine tailings are acid as a result of the use of acid in the metal extraction process rather than from the oxidation of pyrites (Peterson and Nielson 1978). Leaching of these tailings materials may be a suitable and simple solution.

Nutrient content

Peterson and Nielson (1973) found that although the copper (Cu) and uranium (U) mine tailings they studied were very low in nitrogen (N) and most were deficient in phosphorous (P), very few were deficient in potassium (K). When Chadwick (1973) compared colliery spoil to nearby soils he found that K, calcium (Ca), magnesium (Mg) as well as N and P levels were lower in the spoil. The low nutrient status of metalliferous mine wastes is of major importance to revegetation success (Goodman et al. 1973).

Plants utilize inorganic N, commonly NH_4-N in acid soils and NO_3-N in more neutral soils (Russell 1973, Rorison 1980). The major portion of N in any soil, however, occurs as part of the soil organic matter complex (Tisdale and Nelson 1975, Russell 1973). Since many mine tailings are unvegetated, their organic matter content is minimal. Furthermore, only a small portion of readily available soil N results from N fixation by microorganisms (Brady 1974, Tisdale and Nelson 1975), and these microorganisms cannot survive in very acid soils ($pH < 4$) (Russell 1973). Therefore, the N content of tailings is dependent upon the amount of N in the waste materials. Unless ammonia was used in the metal extraction process, most tailings are low in N.

Phosphorous is another major element that is often deficient in acid mine spoils. The availability of soil phosphate ($H_2PO_4^-$ and $HPO_3^=$) is highly pH dependent (Brady 1974, Tisdale and Nelson 1975, Bohn et al. 1979). At low pH values P deficiency is exaggerated by the formation of relatively insoluble iron (Fe) and/or aluminum (Al) compounds (Tisdale and Nelson 1975). High concentrations of Fe or Al in the tailings could immobilize most of the P resulting in P deficiencies although total soil P may seem adequate.

Potassium is seldom inadequate in mine spoils (Berg 1973). It is usually contained in sufficient quantities in the bedrock minerals so that natural weathering releases enough K for plant growth (Mays and Bengtson 1978).

Acid soils have often been found to be low in Ca and Mg. In humid regions this is the result of leaching cations from the soil profile. In semi-arid regions it is because the soils or spoils developed in base-poor material (Bradshaw and Chadwick 1980).

Metal content

Numerous studies indicate that excess manganese (Mn), Al and other metals, which come into solution in increasing amounts as acidity increases, are major factors in acid spoil toxicity to plants (Berg and Vogel 1973, Palmer 1978). New milling and concentrating methods have greatly reduced metal contents in tailings materials but old tailings may contain as much as 10% of the metal(s) extracted and other metals present in the ore (Bradshaw and Chadwick 1980). Nickle (Ni), lead (Pb), zinc (Zn) and copper (Cu) are less common toxicants than Al and Mn in acid soils. All these metals may potentially cause problems on mine wastes since there has not been enough time to weather and leach away toxic metals and the scarcity of vegetation limits chelate formation.

Effects of Low Soil pH on Plant Growth

Table 1 lists the common factors associated with acid soils that affect plant growth. The main soil characteristic that causes or influences all factors is the soil pH. Spoil infertility is perhaps the

Table 1. Factors associated with acid soil (Hewitt and Smith 1975).

1. Direct effect: injury by hydrogen ions

2. Direct and indirect effects due to low pH:
   (a) Physiologically impared absorption of Ca, Mg, and P.
   (b) Increased solubility and/or toxicity of metals: Al, Mn, Fe, etc.
   (c) Reduced availability of P: P x Al interaction.
   (d) Reduced availability of Mo.

3. Low base status:
   (a) Ca deficiency.
   (b) Deficiencies of Mg, K and possibly Na.

4. Abnormal biotic factors:
   (a) Impaired nitrogen cycle and fixation.
   (b) Impaired mycorrhizal activity.
   (c) Increased attack by soil pathogens.

5. Accumulation of soil organic acids or other toxic compounds due to unfavorable oxidation-reduction conditions.

easiest problem to overcome with fertilization but the excess potential acidity of many tailings materials perpetuates other problems (Costigan et al. 1981).

Impaired ion transport and uptake.

Cation absorption is sharply reduced below pH 5 (Moore 1974, Jackson 1967, Jacobson et al. 1957, Arnon et al. 1942, Maas 1969). Anion absorption, on the other hand, decreased when pH levels increased from 5 to 10 (Jacobson et al. 1957, Maas 1969, Hagen and Hopkins 1955).

Although Ca is probably the most important cation in the regulation of nutrient uptake (Jacobson et al. 1961, Rains et al. 1964), other divalent and polyvalent cations are also important in maintaining and regulating the absorption process (Fawzy et al. 1954, Viets 1944, and Jacobson et al. 1960). In terms of plant growth Ca levels may be adequate in acid soils. However, there may not be enough of this element to prevent impairment of ion transport mechanisms by H^+. Calcium levels may also be inadequate to prevent the competition between hydrogen ions and cations, expecially monovalent cations, for transport sites.

Hydrogen ion injury

The most noticeable effect of low pH on plant growth is root injury. Nutrient solution experiments conducted by Hoagland (1917) determined that while a H^+ concentration of 0.7×10^{-5} M (pH 5.15) was favorable to root growth in barley seedlings (Hordeum vulgare), a concentration of 0.3×10^{-3} M (pH 3.52) was decidedly toxic. There was a decrease in plant weight and close examination revealed that the root tips were dead. A similar experiment by Arnon and Johnson (1942) using Bermuda grass (Cynodon dactylon), tomato (Lycopersicon esculentum), and lettuce (Latuca sativa) showed a complete lack of root growth at pH 3, although all plants had substantial root growth at pH 4.

Plant growth mediums with pH values below 4.0 have been shown to cause the loss of previously absorbed ions from the root tissue (Fawzy et al. 1954, Jacobson et al. 1950 and 1957, Nielsen and Overstreet 1955, Moore et al. 1961a and 1961b). These losses indicate that high H^+ concentrations increase cell membrane permeability.

Calcium appears to buffer the plant roots from H^+ injury (Fawzy et al. 1954, Arnon and Johnson 1942, Sutton and Hallsworth 1958, Rains et al. 1964). It is not, however, as effective in preventing H^+ damage in solution cultures as in agar or sand sultures (Sutton and Hallsworth 1958). Plants growing in acid soils create a zone of higher pH around their roots by greater anion uptake than cation uptake (Jackson 1967).

Nutrient solution circulating around the roots dissipates this layer and maintains a low pH adjacent to the roots.

Hydrogen ions adversely affect both the ion transport mechanism and the permeability of cell membranes. The strong interaction between Ca and H suggests a common site of action. Adequate Ca in acid soils and spoils is necessary to prevent serious injury to vegetation by maintaining the integrity of cell membranes and regulating selective ion transport.

Metal ion injury

Aluminum toxicity is an important growth limiting factor in acid soils. In some plants it may result in symptoms resembling those of P deficiency (Foy et al. 1978, Hewitt and Smith 1975). In other cases it appears to be an induced Ca deficiency (Foy 1974, Johnson and Jackson 1964). Excess Al also causes severe root damage (Bradshaw et al. 1965, Foy and Brown 1964). The damaged roots are unable to absorb sufficient water and nutrients. At low Fe levels, Al may induce an Fe deficiency chlorosis (Hewitt and Smith 1975). Aluminum has also been shown to interfere with cell division in the roots, decrease root respiration, and interfere with the uptake, transport and use of several nutrients and water by plants (Jackson 1967).

Unlike Al, excess Mn usually affects plant tops more severely than the roots (Foy et al. 1978, Stiles 1961). Manganese toxicities are expressed as brown spots on the leaves and occasional browning of the roots (Vlamis 1953, Williams and Vlamis 1957). An excess of Mn also has been found to result in chlorosis similar to Fe deficient chlorosis and to reduce root respiration (Hewitt and Smith 1975).

Elevated levels of other metals such as cadmium (Cd), Zn, Ni, Cu, Pb and possibly Fe create a variety of symptoms which may vary with the age of the plant, the nutrient status and the cultivar (Stiles 1961, Antonovics et al. 1971). Incorrect metal ratios may also result in unexpected toxicity or deficiency symptoms. Metal toxicities may be the result of complex interactions with other toxic ions, essential and nonessential ions and environmental factors. This makes it difficult to isolate a toxic element and generalize about symptoms. Generally stunting and chlorosis are the most common symptoms, and these may indicate an interaction with foliar Fe (Foy et al. 1978, Jackson 1967).

The Evolution and Genetics of Plant Tolerances

Numerous reseachers (as reviewed in Antonovics et al. 1971) have found that populations from metalliferous mine wastes were able to grow in metal contaminated soils while similar populations from uncontaminated soils were not. Research has also shown that certain species, although not innately tolerant, have the capacity to evolve heavy-metal tolerant ecotypes which can persist on heavy-metal contaminated soils (Humphries and Bradshaw 1977).

Metal tolerances have been found to be largely metal specific (Gregory and Bradshaw 1965). The level of tolerance developed is related to the amount of the metal in the soil or spoil material. Wilkins (1957) found increased Pb tolerance in *Festuca ovina* plants from soils with greater Pb contamination. Similar relationships have been demonstrated in *Agrostis tenuis* to Pb (Jowett 1964), to Cu (Gregory and Bradshaw 1965, McNeilly and Bradshaw 1968) and to Zn (Gregory and Bradshaw 1965, Turner 1969). Some species can develop tolerances to more than one metal, either in separate races or simultaneously (Jowett 1958, Gregory and Bradshaw 1965), but tolerance to one metal does not automatically confer tolerance to another metal. Results of several studies (Jowett 1958, Bradshaw 1977, Cox and Hutchinson 1980) tend to suggest, however, that metal tolerant plants may possess a slightly greater tolerance to a range of metals than non-tolerant plants.

The evolution of tolerant populations must ultimately depend upon the genetic variation within a species which would enable it to adapt to toxic soil conditions. If seed of a normal population of a species like _Agrostis tenuis_ is sown on metalliferous mine waste, most of the seedlings die. The surviving individuals are tolerant and produce tolerant offspring (Walley et al. 1974). Research by McNeilly and Bradshaw (1968) showed that although less than 1% of the adult individuals sampled from a normal non-tolerant _Agrostis tenuis_ population were Cu tolerant, some 3% of the seed samples were found to be Cu tolerant. In the mine populations, however, the adult samples contained a greater percentage of Cu tolerant individuals than the seed samples. On normal soils, selection pressures are weak because both tolerant and non-tolerant populations can survive, although non-tolerant populations tend to be favored (McNeilly 1968). Selection pressures on toxic soils are considerable and can overcome the genetic input because only those plants tolerant of the mine wastes can survive. Tolerant populations can be established on mine sites within a few generations (McNeilly and Bradshaw 1968, Gartside and McNeilly 1974).

Metal tolerant plants often differ morphologically and physiologically from normal plants (Antonovics et al. 1971, Bradshaw

1959 and 1960). Metal tolerant plants also tend to be more drought tolerant than normal plants. As these characteristics are found in the same species on dry, non-toxic but infertile sites it has been assumed that these adaptations are independent of the selection of metal tolerance (Antonovics et al. 1971). If a plant is pre-adapted to soil infertility and drought, the chances of it developing metal tolerance(s) are greater than if the population had to adapt to numerous site factors. Multiple adaptations to environmental features are very difficult to acquire simultaneously (Antonovics et al. 1971). The inability of a population to tolerate metallic mine wastes may not be the lack of a metal tolerance but the lack of the population's adaption to other site and environmental characteristics.

Mechanisms of Plant Tolerance

There are two basic ways by which plants can avoid toxic effects of mine wastes. The first involves the changing of the external environment to limit or prevent uptake. The second method would involve some internal mechanism(s) that binds or alters toxic ions and/or permits more efficient use of deficient ions.

Effects of root activity on the root environment

As roots grow and respire they absorb and excrete various ions and compounds. It is through these physiological activities that the plant can alter the root environment. These functions are highly dependent upon the species and the original pH of the root medium. Excreted organic compounds may form complexes with some heavy metals and other elements which may either enhance or inhibit their subsequent absorption

(Jackson 1967). Biological oxidation may also influence the solubility of metal oxides.

The pH of the root environment can be influenced through the selective absorption of cations and anions. Generally monovalent cations are more rapidly absorbed than divalent cations (Maas 1969, Moore et al. 1961a, 1961b, Jacobson et al. 1960). Similarly monovalent anions are absorbed more rapidly than polyvalent anions (Hagen and Hopkins 1955, Jacobson et al. 1957). Rapid cation uptake tends to lower the soil pH while rapid anion uptake has the opposite effect. As discussed earlier high H^+ concentrations reduce cation uptake resulting in the plant absorbing more anions which would tend to raise the pH around the roots.

The absorption of NH_4^+, the most common form of N in acid soils, has the effect of decreasing the pH in the surrounding medium (Jackson 1967, Rorison 1980, Moore 1974). Selective uptake of NO_3^- instead of NH_4^+ could raise the soil pH.

A plant's ability to change the root environment is greater in soil systems than in nutrient cultures. Through selective uptake, the plant can induce a pH gradient away from the root. This buffer around the root is dissipated in nutrient solutions cultures by continuous aeration and mixing of the solution (Asher et al. 1965, Edwards and Asher 1974, Islam et al. 1980). In the soil system, mass flow is usually toward the root of a transpiring plant and substantial pH changes can occur and persist adjacent to the root (Sutton and Hallsworth 1958). An increase in pH adjacent to the root may reduce metal solubility and increase nutrient availability and/or absorption.

Internal mechanisms

The movement of ions into and through the plant may be regulated in a number of ways. Control sites may be located in the root and/or the shoot, in the cell walls or within the cell (Rorison 1980, Ernst 1976). This regulation can affect ion uptake, accumulation and translocation.

There are three general patterns of ion uptake and accumulation (Baker 1974). Some plants avoid taking up toxic ions until the increasing external concentration disturbs plant metabolism such that the internal concentration increases rapidly, usually to the detriment of the plant. The existence of an avoidance mechanism to prevent heavy metal uptake has not been observed (Ernst 1976, Antonovics et al. 1971).

The second pattern of uptake involves the rapid accumulation of ions to a uniformly high concentration as long as there is a minimal external concentration (Baker 1974). *Astragalus* species accumulate selenium but the high internal levels do not seem to affect plant growth (Shrift 1969). This pattern of ion uptake may also be responsible for plant growth on soils and spoils with a low nutrient status. The ability of plants to survive on infertile mine wastes implies a form of efficiency both in terms of selective uptake and utilization rates. Plants growing in acid soils are often adapted to lower levels of P (Rorison 1980) and have a lower Ca demand (Snaydon and Bradshaw 1969). These plants may not, however, grow as well as other populations of the same species in soils with normal amounts of the deficient nutrient (Jackson 1967).

In the third uptake pattern, the internal ion concentration(s) reflects the external concentration(s). Turner and Gregory (1967) found increasing Zn uptake by tolerant and non-tolerant Agrostis tenuis with increasing Zn concentrations in nutrient cultures. Roots accumulated more Zn than the shoots especially at the highest concentrations.

Regardless of the uptake pattern, it has been shown that metal tolerant plants contain more of the metal in their roots than non-tolerant plants (Bradshaw et al. 1965, Wainwright and Woolhouse 1975). If tolerant plants contain abnormally high levels of toxic ions there must be some internal mechanisms that render the ion non-toxic or buffer the plant against it.

A cell wall complexing system in the roots may bind large amounts of metals, but its capacity is probably finite and may not be capable of preventing all metal translocation and accumulation in aerial portions of the plant (Wainwright and Woolhouse 1975). Cytoplasmic resistance may also be important in metal tolerance. Other possible but less studied tolerance mechanisms may include changes in enzyme activity, the formation of altered enzymes with decreased affinity for inhibitors or increased relative affinity for the substrate compared to the competitive inhibitor and alternate metabolic pathways which bypass inhibited sites (Antonovics et al. 1971).

Plant tolerances are generally quite specific. If a plant is tolerant to more than one metal, there may be separate tolerance mechanisms for each metal. Tolerance mechanisms in a plant may be the cummulative effect of several physiological processes (Antonovics et al. 1971, Wainwright and Woolhouse 1975, Foy et al. 1978).

Laboratory Methods for Determining Plant Tolerances

Acid tolerance studies

Research on acid tolerance or plant sensitivity to soil acidity can be divided into two different types: solution culture and pot culture experiments.

The earliest solution culture experiments investigated the effect of acid or alkaline solutions on seedling root growth. Breazeale and Le Clerc (1912) used solutions of dilute H_2SO_4 and HCl as well as solutions of K_2SO_4, KCl and $NaNO_3$. Hoagland (1917) used nutrient solutions adjusted to various OH^- or H^+ concentrations with K_2PO_4 and K_2HPO_4 or KH_2PO_4 and H_3PO_4 respectively. Investigations by Arnon and Johnson (1942) studied the effect of H^+ on plant growth. Solution reactions ranged from pH 3 to pH 9. Aeration of the solution was achieved by rapid bubbling of air through porous carbon tubes. The large volumes of solution per plant and the weekly changes of solutions helped prevent large shifts in solution reaction and composition. A related study by Arnon et al. (1942) investigated the effects of H^+ on nutrient uptake.

Modern flowing culture techniques have been developed to overcome the problems of precise pH control and adequate nutrient levels at all pH levels (Asher et al. 1965, Edwards and Asher 1974). Plants are suspended in polyethylene pots through which the nutrient solution flows continuously. The solution entering all pots has the same common storage tank and is recirculated. The pH is adjusted, sometimes by automatic devices, mineral nutrients removed by the plants are replaced and the solution may be completely renewed at regular intervals. The flow rate depends upon the concentration of the solution and the rate of nutrient absorption by the plants (Islam et al. 1980) as well as the accuracy with which the concentration needs to be controlled (Edwards and Asher 1974). Generally the higher the concentration the slower the rate of flow.

The second type of laboratory technique involves greenhouse pot cultures. This technique is often used prior to field studies to estimate the amount and placement of liming materials needed for adequate growth of agricultural and pasture crops (Watenpaugh 1936, Schmehl et al. 1950, Brenes and Pearson 1973, Buchanan et al. 1975, Lundberg et al. 1977).

Metal tolerance studies

With the growing concern about environmental quality, researchers began looking into metal tolerances of vegetation found on mine wastes. Bradshaw (1952) observed that root growth of pasture populations of *Agrostis tenuis* were stunted when the plants were grown on Pb mine spoils but that plants originally found on the mine spoils produced relatively normal growth. Wilkins (1957) devised a method of assessing Pb tolerance of plants grown on or away from Pb contaminated spoils and soils. This method compared the rate of root elongation of plants in calcium nitrate solutions with varying Pb concentrations. High concentrations of Pb and other metals have a marked effect on root growth and Wilkins' method provided a convenient way to measure the effect of one metal on plant populations (Jowett 1964, Gregory and Bradshaw 1965). An index of tolerance developed by Jowett (1958 and 1964) compared root growth in toxic solutions with normal root growth in control solutions. This index provides an excellent means for quantifying plant tolerances.

Wilkins' experiment (1957) transferred tillers of one plant into a more toxic solution each day. The length of the longest root on each tiller was measured daily and the amount of root growth compared. In later experiments separate tillers were tested in each solution simultaneously. This parallel technique allowed for better comparisons between tolerant and non-tolerant populations (Wilkins 1978). The tolerance indices from these later experiments resulted in a plant tolerance ratio rather than a root tolerance.

Several researchers began utilizing this method of screening for plant tolerances to other metals such as Zn, Cu, Ni, Cd, and Al (Wu et al. 1975, Gregory and Bradshaw 1965, Chadwick and Salt 1969, Cox and Hutchinson 1980, Jarvis 1978). This method of immersing roots grown in tubes into solutions required changes to overcome inadequate aeration and concentration gradients.

Gadgil (1969) developed a drip culture apparatus that eliminated those problems. Tillers were placed in a glass T-piece and solution trickled down the tube. This allowed the roots to be constantly bathed with nutrient solution. The drip culture allowed adequate aeration and a uniform solute concentration. As large quantities of solution were used, nutrient concentrations remained relatively constant during the experiment.

Another frequently used technique for toxicity and deficiency studies is greenhouse pot culture. A simple method of screening populations for metal tolerances was used by several researchers (Walley et al. 1974, Gartside and McNeilly 1974) by sowing seed on potting soils made up of varying amounts of metalliferous mine waste and ordinary potting soil or on ordinary potting soil with known amounts of metal(s) added.

The Importance of Acid and Metal Tolerances in Land Reclamation

Toxic materials left by mining operations are visually unattractive and can be a source of pollution of surrounding areas (Jonas 1973, Draskovic 1973, Antonovics et al. 1971, Gemmell and Goodman 1978). There is a need to stabilize these sites. The establishment of vegetation has considerable advantage over other stabilization processes in terms of cost and permanence (Bradshaw et al. 1978). Agricultural techniques such as the addition of lime and fertilizers to ammeliorate the wastes' inimical properties are costly (Hutchinson and Kuja 1979). Covering toxic materials with a layer of non-toxic, neutral material may be impractical as well as very expensive (Bradshaw et al. 1978).

If vegetation can be established on these materials, the sites will be stabilized and visually improved. Metal and acid tolerant vegetation provide a feasible solution, as the plants are already capable of growing on mine wastes without amelioration. This vegetation may also be adapted to other environmental factors besides high metal and H^+ concentrations (Bradshaw et al. 1978, Antonovics et al. 1971).

Before revegetation begins careful analysis of site characteristics is required. A particular portion of a site may contain coarse-textured mine wastes that are extremely droughtly and low in nutrients (Bradshaw et al. 1978, Bradshaw and Chadwick 1980). Finer grained materials may or may not have adequate clay-sized particles for good water retention and such materials often contain high levels of heavy metals and acid producing minerals. Because metal tolerance is highly specific, the toxic metals must be identified so that the correct tolerant plants can be selected for revegetation (Antonovics et al. 1971, Cox 1979).

Site treatment may vary from minor regrading to more intensive treatments of adding and incorporating sewage sludge or mulches. Periodic fertilization may be necessary to replace nutrients lost by leaching, fixation by metals or organic chelates. Nevertheless, the use of tolerant vegetation can reduce post revegetation maintenance costs.

In western Montana and other mountainous states revegetation of abandoned mine wastes with tolerant vegetation may be the only solution. Reclamation programs usually have low budgets and the inaccessibility of derelict sites discourages frequent maintenance trips. Successful revegetation of acid collier wastes in the United Kingdom indicates that reclamation of acid hardrock mine wastes with tolerant vegetation has great potential.

METHODS AND MATERIALS

To evaluate the acid tolerance of plant species two decisions had to be made. First, what species would be evaluated and second, what evaluation techniques would be used. In this study the plants were selected from collected specimens found growing on hardrock mine sites. Two greenhouse trials were used; the first one was used to determine which of the collected species would be tested and the second one to evaluate acid tolerance of the selected species.

Native Plant Material Identification and Collection

During several trips to the mines at Champion and Porphyry Dike numerous plants were collected and/or identified. A species list for each mine site was developed. The major nomenclature source was Hitchcock and Cronquist's "Flora of the Pacific Northwest" (1978). Questionable plant identifications were verified or corrected by Montana State University herbarium staff. Appendix A contains the lists of plants and indicates where they were found.

Examples of the prominent plant populations were collected along with other less dominant plant species that were of interest. Living specimens were transported from the mines to the university in plastic bags with large root balls of tailings material. At the university greenhouse the plants were planted in plastic pots using a mixture of 50% Bozeman silt loam and 50% river washed sand.

The pH of a 2:1 paste of the tailings material around the roots of most of the collected plants (Appendix B) ranged from almost 2 to nearly 8. The plants growing in the most acid materials plus a few other major plants in less acid materials were chosen for the greenhouse trials and were vegetatively propagated to provide enough material for the experiments. Two plant populations from an earlier study of Zn and Cu tolerance by Surbrugg (1982) were also used. These plants had been collected from the Anaconda tailings in 1981 and maintained in the greenhouse in the potting mixture previously described. If plant tolerance to a certain element is a genetic trait, then vegetative propagation in normal, non-toxic soil will not alter or decrease the tolerance (Bradshaw 1977). This genetic trait associated with heavy metal tolerance should be applicable to acid tolerance as well.

Plant Species Selection

The selection of individual plants tolerant to acid tailings was achieved by means of tailings growth media trials. Researchers have used similar trials to identify plant populations tolerant of Pb, Cu or Zn tailings (Walley et al. 1974, Gartside and McNeilly 1974).

Collection and analysis of mine waste and tailings material

Five representative mine waste materials were collected. Three were collected from Champion Mine: one from the mine waste dump and two from the tailings pond. The remaining two materials came from the tailings dumps at Porphyry Dike.

The collected materials were prepared in the lab for chemical and physical analyses. The materials were air dried and passed through a 60 mm screen to remove large rocks and gravels. Samples of the screened materials were set aside; the remaining materials were disaggregated with a mortar and rubber policeman. They were then passed through a 2 mm sieve on a mechanical shaker.

The 60 mm screened samples were chemically analyzed for N, P, and K. Particle size analysis of the fine fraction ($\leq$ 2 mm) was carried out by the hydrometer method (Black 1965). Textures were determined using the U.S.D.A. soil texture triangle (Soil Survey Staff 1975). Table 2 lists the pH, a brief description and the collection location of each material. Appendix C, Table 20, lists the laboratory analyses performed on these materials and the procedures utilized.

Table 2. General description and location of tailings materials taken from Porphyry Dike (PD) and Champion Mine (CM).

PD01	A reddish orange, fine-textured tailings from the severely eroded tailings dump below the concentrator; pH 7.8
PD02	A white, gravelly, clayey tailings material containing a large amount of soft rock from the edge of the deep gully just below the mill building; pH 3.2
CM01	A mixture of layered yellow and white, clayey and loamy material from the tailings pond; pH 3.2.
CM02	Similar to CM01 but contains gravel-sized material and more of the white clayey material; pH 2.5.
CM03	Gray granitic and sandy material from the mine dump near the ore bins; pH 7.1.

Tailings preparation

The screened tailings materials were placed in greenhouse growth trays. Fertilizers (18-24-6 fertilizer, KH_2PO_4 and KI) were added and incorporated into the tailings. Additions were based upon previous N, P, and K analyses and calculated to raise the nutrient levels to those shown in Table 3. No micro-nutrients were added.

Table 3. Low to medium nutrient levels for plants (Dollhopf 1982).

NO_3-N	4 - 6 ppm
P	8 - 16 ppm
K	125 - 250 ppm

Screening criteria

The survival of plants in the acid tailings material would imply tolerances to any high metal levels present in the root zone material and to any micro-nutrient deficiencies and/or imbalances. The plants would also be tolerating high levels of hydrogen ions. One individual from each of the seventeen cloned populations (Appendix B, Table 19) were then planted in the trays. Waterings with tap water were scheduled to keep the soil moist and to prevent wilting. The tailings growth media trial was run for 4 weeks and monitored daily for plant health and survival.

Acid Tolerance Trials

The tailings growth media trial was only used to screen the plants for some degree of acid tolerance. However, there were several variables which could not be controlled such as chemical and physical differences between the tailings materials and the ability of some plants to modify the root zone in some fashion. The acid tolerance trials allowed for more control over the root zone pH and nutrient levels.

Plant material selection and preparation

A total of seven native populations representing five species showed variable degrees of tolerance to acid metalliferous mine and mill wastes in the screening test (Table 4). Matching commercial populations were established either from seed or cloned plants.

Table 4. Plant species and populations for the acid nutrient solution experiment.

Species	Populations
Agropyron trachycaulum (slender wheatgrass)	2 native (PD108, CM112), 1 commercial
Agrostis tenuis (colonial bentgrass)	2 native (CM105, AC100), 1 commercial
Deschampsia cespitosa (tufted hairgrass)	1 native (AC101), 1 commercial
Fragaria vesca (wild strawberry)	1 native (CM154), 1 commercial
Trifolium repens (white clover)	1 native (CM156), 1 commercial

Tillers from each native plant were assumed to be of the same genotype because they were reproduced vegetatively and not from seed. The commercial plants had some genetic variability since several plants from different seeds were used to acquire the needed number of tillers. Separate strawberry and clover plants (native and commercial populations) were used and genetic variability undoubtedly occurred.

Uniform sized tillers or individual plants were removed from the soil and the roots rinsed with tap water to remove all soil and foreign particles. The grass roots were trimmed to 20 cm, but the clover and strawberry roots were only trimmed to 40 cm as pre-trial tests had shown that these plants died or did poorly when trimmed to shorter lengths. The plants were then quickly immersed in standard Hoagland and Arnon's solution buffered to pH 6 with KOH. Adequate aeration was provided with air bubbled through the solution. The plants were left in the solution for 4 days to encourage the production of new roots.

The longest root and total plant weight of each healthy plant was measured and recorded. Tillers or plants as appropriate for each population were placed in the testing apparatus.

Experimental technique

A parallel technique similar to the method developed by Jowett (1958 and 1964) and later modified by other researchers (Gregory and Bradshaw 1965, McNeilly and Bradshaw 1968, Antonovics et al. 1971) for the study of metal tolerance was used for this study. This method allowed tillers of each genotype to root in similar solutions with known amounts of a heavy metal. Jowett's method has been modified for this acid tolerance trial to allow better control of pH and nutrient levels and to provide adequate aeration. This was achieved by changing from a standing solution to a flowing solution culture. This acid tolerance study, like those by Arnon et al. (1942) and Arnon and Johnson (1942) used a range of H^+ concentrations without a control.

Four individual plants or replications from each native and commercial population were tested for acid tolerance in three solutions with different levels of acidity. Due to the size limitation of the testing apparatus this acid tolerance trial was conducted in two runs; the first run consisted of Agropyron trachycaulum and Agrostis tenuis populations and the second run consisted of the remaining three species listed in Table 4. Each run was maintained for fourteen days.

Moderately low metal concentrations might suppress root growth but higher metal concentrations would totally inhibit root development (McNeilly and Bradshaw 1968). The production of plant biomass is also affected by toxic levels of heavy metals or nutrient imbalances. It has been assumed that this may be true for any toxic element including hydrogen ions. After each trial run the plant weight and root length were measured. Differences between plants grown in the most and least acid solutions would indicate some degree of acid tolerance.

Apparatus

The apparatus was adapted from one used by Surbrugg (1982) for testing metal tolerance. His apparatus was modified from a sophisticated drip culture apparatus used by Gadgil (1969). Gadgil's apparatus involved the placement of rooted tillers in a glass T-piece. Solution flowed into the growth chamber through the side arm of the T-piece. The growth chambers used in this study consisted of polyethylene tubes, 2.5 cm wide by 25.6 cm long, with a volume of 65.6 cm^3 (Surbrugg 1982). Acid washed sand provided the rooting medium for individual plants in each chamber.

The drip/sand culture apparatus supported 72 tubes separated into three treatments of 24 tubes each (Figure 4). One rack supported each treatment as shown in Figure 5. Separate reservoir buckets for each treatment were placed 80 cm above the racks so that the nutrient solution could flow by gravity syphon through polyethelene tubing to each individual tube. The drip rate was controlled by a flow meter calibrated to release the solution at a drip rate of 40 to 60 ml/hr.

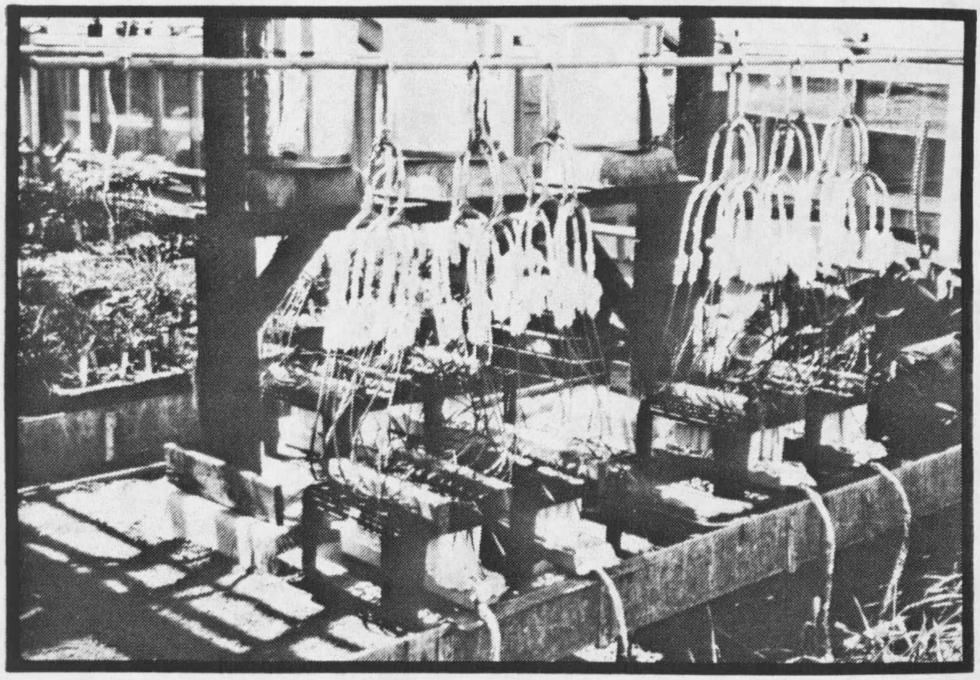

Figure 4. Drip/sand culture apparatus

Receiver troughs placed under the tubes collected the nutrient solution and funnelled it into a second reservoir. This container was emptied into the upper reservoir at six to eight hour intervals to recycle the solution and to maintain a constant head.

Nutrient solution pH levels

It was decided to use three test solutions ranging from pH 3 to 5 in increments of one pH unit. Experiments by earlier researchers (Hoagland 1917, Arnon and Johnson 1942) have indicated that pH 3 is at or below the lowest acid tolerance level of common agricultural crops. Additionally, the acid tailings from which the plants were collected had root zone pH levels near 3. Testing above pH 5 was deemed unnecessary since most plants grow well in near neutral soils.

Hoagland and Arnon's solution (1938) was used in this study. This balanced solution consists of four macro-nutrients and six

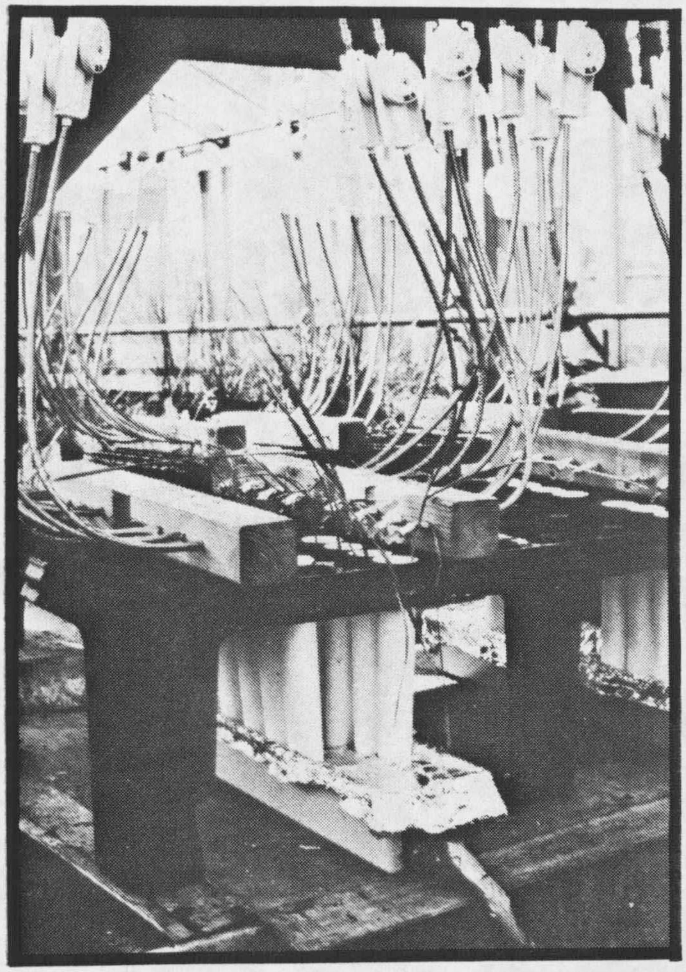

Figure 5. One treatment rack of the drip/sand culture apparatus.

micro-nutrients (Table 5). It was developed for standing sand cultures. In this type of plant growth experiment, a pH and nutrient gradient develops around the plant roots (Gadgil 1969). Flowing solution cultures do not allow the development of this gradient, therefore, the culture solution was diluted to one quarter strength. The dilute solution still contained adequate nutrients (Pagenkopf 1983) but would allow for a better test of acid tolerance.

Table 5. Hoagland and Arnon nutrient solution (1938).

Chemical	gm/l	mg/l
KNO_3	0.656	
$Ca(NO_3)_2 \cdot 4H_2O$	0.994	
NH_4HPO_4	0.115	
$MgSO_4 \cdot 7H_2O$	1.004	
H_3BO_3		2.86
$MnCl_2 \cdot 4H_2O$		1.81
$CuSO_4 \cdot 5H_2O$		0.08
$ZnSO_4 \cdot 7H_2O$		0.22
$H_2MoO_4 \cdot H_2O$		0.09
*$FeSO_4$ (0.5%)	] 0.6 ml/l 3 times weekly	
*Tartaric acid (0.4%)		

*replaced with FeEDTA (3.96 mg/l) to avoid iron precipitation.

Concentrated stocks were prepared from reagent grade chemicals and then mixed with large volumes of distilled water. The pH was adjusted by the addition of HCl or KOH. The nutrient solution was monitored daily for changes in pH and replaced every four days. The changes in pH varied less than 0.2 pH units for pH 3 and 4 solutions. The pH 5 solution varied less than 0.4 pH units. Solution replacements (four day intervals) maintained relatively constant levels of nutrients.

Sand preparation

River washed sand (99.7% SiO_2) was used as the rooting medium. It was first soaked in 1 N nitric acid for 48 hours. Then the sand was rinsed with distilled water until the effluent was similar in pH to distilled water. At this point the sand was divided into three equal portions. Each portion was soaked in the nutrient solutions at pH values of 3, 4, or 5 until the effluent remained at the pH level of the solution (Hewitt 1966).

Sand supported the plants inside the tubes and was coarse enough to allow free flow of the nutrient solution. A glass wool plug prevented the loss of sand from the bottom of the tubes yet permitted the nutrient solution to drain freely.

Statistical Analysis

The purpose of this study was to determine the acid tolerance of each species and each population. This experiment was set up as a factorial design. The set of treatments consisted of all possible combinations of two factors (Steel and Torrie 1980): pH and plant populations. Because of the variability in initial root lengths and plant weights and the lack of a control solution, the method of analysis utilized relative growth or percent change rather than tolerance indices as used by Jowett (1958 and 1964).

The main effect due to pH was of less interest than a significant Population or pH x Population interaction. A significant Population difference indicated that the amount of response differed between populations. A significant pH x Population response signified that the populations reacted differently to the addition of hydrogen ions and therefore differ in tolerance.

RESULTS

Tailings Analysis

Soil analyses (Table 6) indicated that all of the tailings materials were low in macronutrients except PD01 which was high in K and CM03 which contained adequate P. The consistent relatively low concentrations of metals and nutrients in PD02 was unusual. PD01 also had low levels of metals. PD01 and PD02 had the highest concentration of Na but the SAR was still low enough for plant growth. CM01 and CM02 contained higher levels of soluble Ca, Mg, Fe, Ni, Zn, Al, and Mn than the other tailings. CM02 had the lowest pH and as expected the highest concentrations of soluble metals.

Saturated paste extract analyses should indicate the amounts of water soluble metals and other nutrients available to the plants in the soil solution. Several studies have utilized water soluble or ammonium-acetate extracts for comparing nutrient levels to plant growth. The following water soluble levels were used in this study as "suspect" levels for classifying tailings as toxic: > 1 ppm Cu (solution culture, Jarvis 1978), > 2.5 ppm Ni (solution culture, Hogan and Ranser 1979), 7-10 ppm Zn (soil additions, Sakal et al. 1981), approximately 5 ppm Mn (Ouellette and Dessureaux 1958, Ward 1977) and > 1 ppm Al (solution culture, Lignon and Pierre 1932). Using these levels as guidelines Al was inhibitory in PD02 and CM03 but toxic to most plants in CM01 and CM02. Mn was toxic in CM01 and CM02, which also contained a toxic amount of Cu.

Table 6. Chemical characteristics of tailings materials.

	Soil pH							Saturated Paste Extracts (ug/g)										
	2:1 paste	H_2O extract	EC mmhos/cm	SAR	NO_3-N ug/g	P ug/g	K ug/g	Ca	Mg	Na	Fe	Cu	Pb	Al	Ni	Zn	Mn	B
PD01	7.72	7.82	0.75	0.25	3.5	0.6	250.0	58.2	42.2	10.20	0.06	0.06	<0.01	<0.50	<0.01	0.18	0.03	0.96
PD02	3.24	3.23	0.20	0.53	2.7	2.1	58.6	6.1	1.2	5.60	0.09	0.18	<0.01	1.80	<0.01	0.41	<0.03	0.30
CM01	3.19	3.20	1.40	0.04	2.8	3.1	43.0	169.0	66.4	2.65	0.59	0.73	<0.01	24.20	0.45	2.79	15.50	0.96
CM02	2.71	2.45	3.74	0.01	3.2	<0.1	23.4	432.0	136.4	1.34	9.80	5.08	0.02	80.00	1.08	5.92	11.00	0.90
CM03	7.60	7.07	0.40	0.10	2.8	7.0	54.7	68.4	5.9	3.07	0.02	0.05	0.01	1.40	0.01	0.19	0.06	1.50

These tailings materials were generally infertile and toxic. The silty and sandy textures (Appendix D, Table 22) indicated that the materials were susceptible to erosion making stabilization imperative to the reclamation of these two mines. Vegetation must be capable of tolerating the adverse chemical and physical characteristics and be able to establish rapidly to stablize the tailings materials.

Plant Species Selection

Plant responses were very different in the acid and non-acid tailings materials. The results of this trial are shown in Table 7. The variation in plant response may be due to differences in chemical composition and to the direct and indirect effects resulting from differences in pH.

One of the most startling and least anticipated developments was the death of all plants in the gray granitic material (CM03) from Champion Mine. It was not acid and an adequate amount of macronutrients had been provided. A few additional plants were planted in the tailings in separate pots and watered frequently to keep the material moist. These plants survived. It was assumed than that despite regular watering the CM03 material drained very rapidly and was droughty.

All of the plants survived in PD01. This material remained moist longer than the other tailings but settled and became hard when dry. Close inspection revealed numerous pores. New root growth was abundant in the tailings but was concentrated along the bottom and edges of the trays. This material apparently had no plant growth limiting chemical

Table 7. Number of plants surviving tailings growth trial.

		Tailings Material				
		Porphyry Dike		Champion Mine		
Key	Botanical Name	01	02**	01**	02**	03
*PD 108	Agropyron trachycaulum	3	2	0	1	0
PD 150	Anaphalis margaritacea	3	0	0	0	0
PD 111	Carex rossii	3	3	3	3	3
PD 151	Epilobium alpinum	3	0	0	0	0
PD 103	Juncus drummondii	3	3	3	3	1
PD 107	Trisetum spicatum	3	0	0	0	0
*CM 112	Agropyron trachycaulum	3	1	1	0	0
CM 118	Agrostis scabra	3	0	1	0	0
*CM 105	Agrostis tenuis	3	3	2	2	0
CM 152	Antennaria rosea	3	0	0	0	0
CM 103	Carex aquatilis	3	3	3	3	0
CM 104	Carex limnophila	2	0	0	0	0
CM 107	Deschampsia cespitosa	3	2	1	0	0
*CM 154	Fragaria vesca	3	1	2	2	0
CM 117	Poa compressa	3	0	2	0	0
*CM 156	Trifolium repens	3	2	2	0	0
*AC 100A	Agrostis tenuis	3	1	0	0	0
*AC 101	Deschampsia cespitosa	3	0	0	0	0
MC 101	Carex sp.	3	0	0	0	0

*Plants chosen for acid tolerance trials.
**Acid tailings.

chemical characteristics. The main growth limiting factor of this material would be its density and high erosion potential.

The three acid materials had noticeable effects on vegetation. Root tissue was frequently discolored and stunted. Nevertheless, the roots of surviving plants showed some new growth although it was not as abundant as that in PD01. Most of the grass species showed a purplish tinge which may be a symptom of P deficiency (Russell 1973).

More plants lived through the trials in PD02 than was anticipated because of the low nutrient levels. CM01 had the same number of survivors but a different combination of species. The scarcity of survivors in CM02 could be due to the extremely low pH which may have injured the roots as well as increased metal concentrations in this tailings material.

Fragaria vesca grew well in CM01 and CM02, showing a tendency to survive in acid soils if elevated levels of Ca were present. All of the strawberry plants experienced die-back after transplanting, but new foliage grew back quickly. Trifolium repens survived the acid environment of PD02 and CM01 but could not tolerate CM02. The elevated metal and H^+ levels apparently contributed to its demise; several of the grasses showed a similar response pattern.

The Carex and Juncus species survived in all three acid materials. Most of the remaining plants, especially the forbs, did not survive on any of the tailings materials, indicating no tolerance for acid and/or high metal levels. Antennaria rosea, which was found on a dry, acid site, probably did not survive because the tailings were too moist.

Acid Tolerance Trials

The acid tolerance trials were designed to evaluate the plant populations which showed some acid tolerance in the plant species selection trials. Unlike the tailings materials used in the selection process, however, the root zone pH was monitored to maintain a constant pH and nutrient level. The plants may have been able to modify the root zone pH in the tailings materials (Sutton and Hallsworth 1958) rather than relying on internal mechanisms to survive acid conditions.

Acid tolerance in Agrostis tenuis

The Anaconda tailings population of this species was found to be more acid tolerant than either the commercial population or the tailings population from Champion Mine. Tables 8 and 9 present the means of root growth and plant weight increases. Relationships between pH and root growth, and pH and plant weight are plotted in Figures 6 and 7 respectively. The vertical lines refer to standard deviations around each mean. Analysis of variance tables are found in Appendix E, Tables 23 and 24.

The most significant difference was found to be due to changes in pH. Root growth and plant weight increases for all populations were significantly less at pH 3. Although both responses were slightly greater at pH 4 than at pH 5, the differences were not significant.

Table 8. Percent root growth means for *Agrostis tenuis*.

	pH		
Population	3	4	5
Commercial	21.0 a A	136.3 ab B	98.3 a B
CM105	21.5 a A	102.0 a B	101.3 a B
AC100	47.0 a A	183.8 b B	169.0 b B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

Table 9. Percent increase in plant weight means for *Agrostis tenuis*.

	pH		
Population	3	4	5
Commercial	160.8 a A	305.2 a B	256.0 a B
CM105	165.8 a A	351.0 ab B	370.7 b B
AC100	226.3 a A	400.7 b B	362.2 b B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

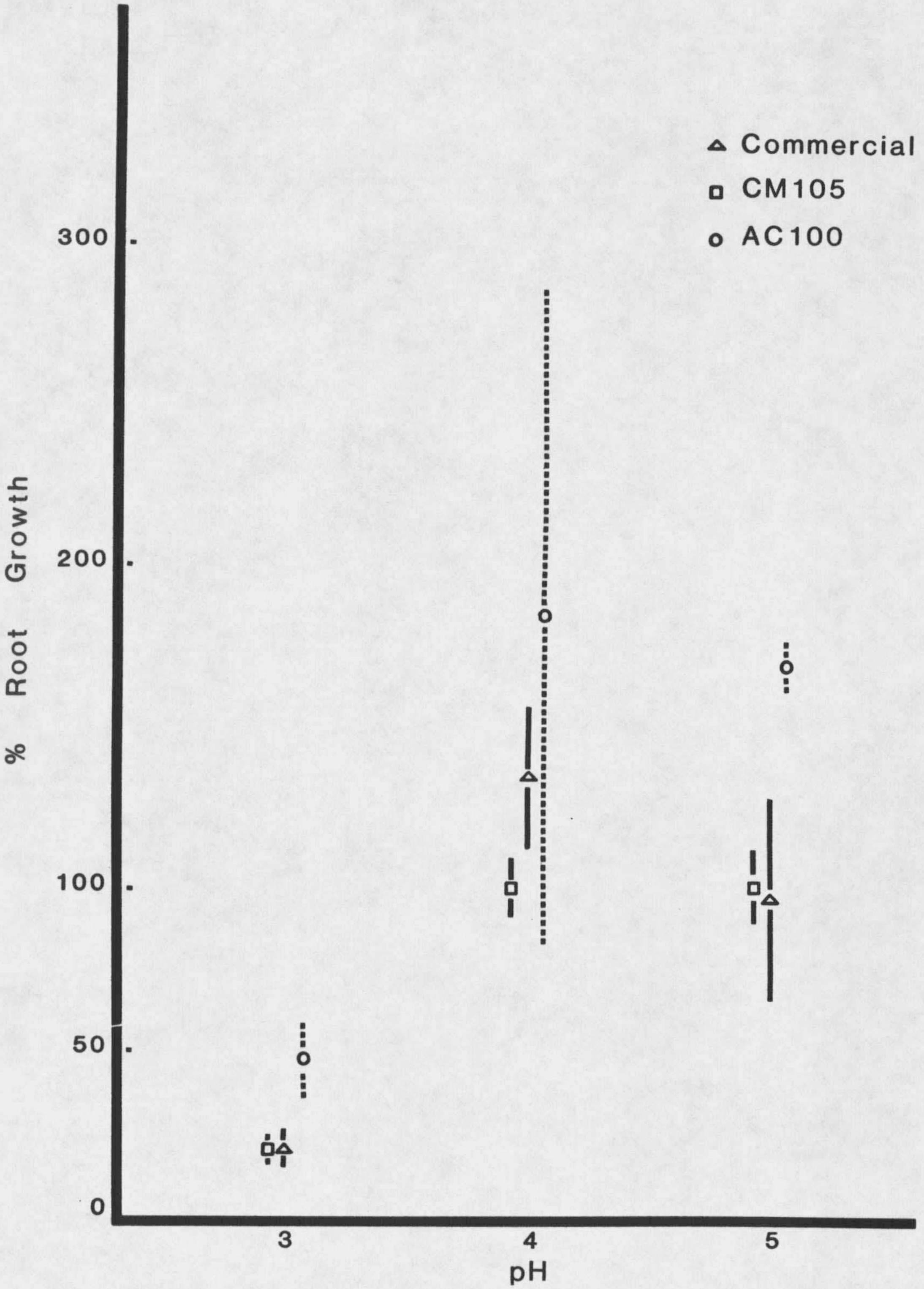

Figure 6. Means and standard deviations for percent root growth of Agrostis tenuis at three pH levels.

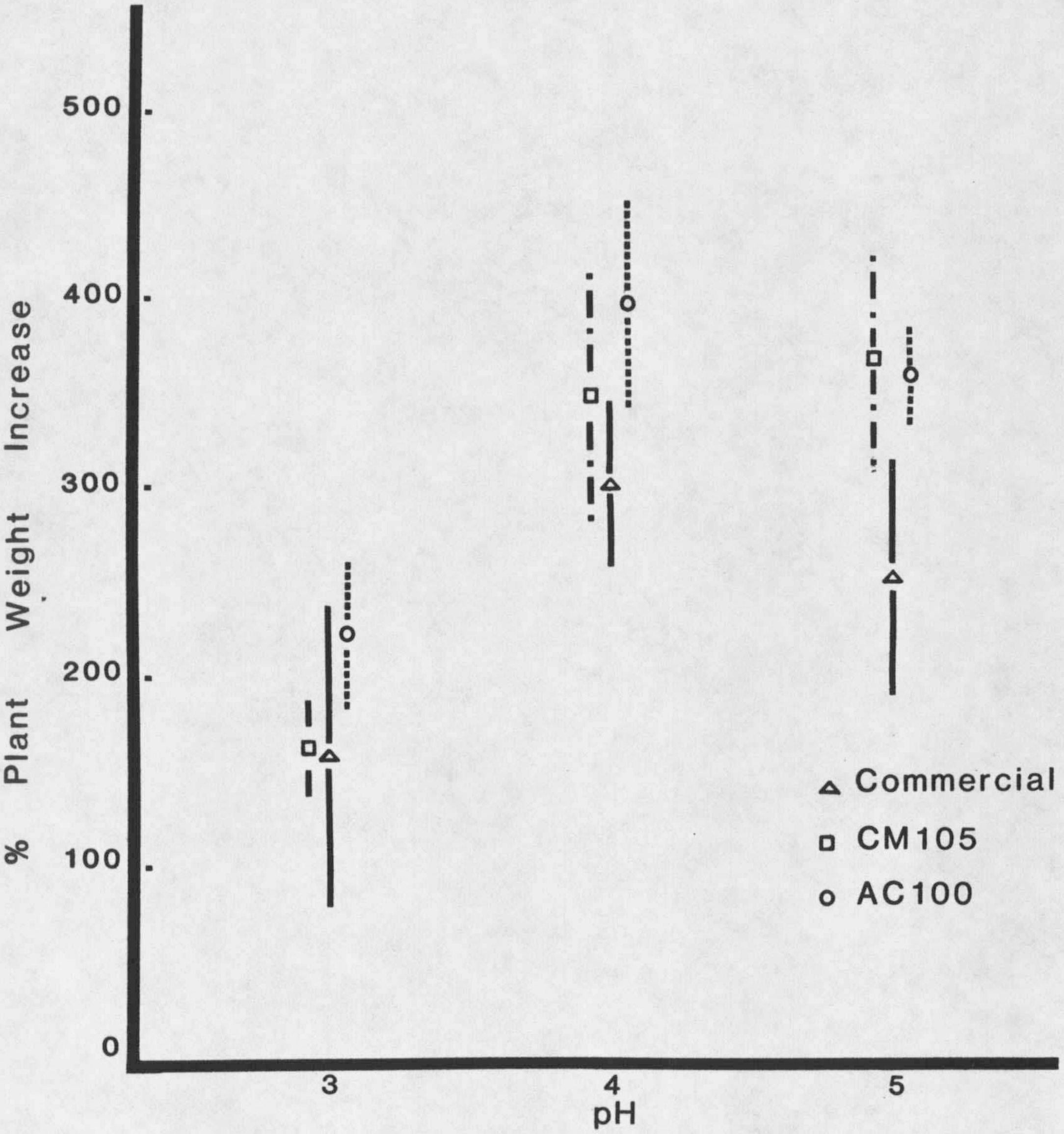

Figure 7. Means and standard deviations for percent increase in plant weight of <u>Agrostis tenuis</u> at three pH levels.

Significant differences were also found in the comparision of Populations. Partioning of the Population sums of squares revealed that the main source of variation for root growth was due to differences between the two tailings populations while the Tailings vs. Commercial partion accounted for most of the variation in plant weight increase. The plants noted as AC101 produced the most biomass and had the greatest root growth of all populations, although no significant differences were found at pH 3. The Agrostis noted as CM105 produced about the same amount of root growth and biomass as the commercial population but had significantly more biomass at pH 5. The lack of a significant pH x Population interaction indicates that the populations responded in a similar fashion to changes in pH.

When removed from the apparatus the roots for all Agrostis tenuis populations appeared healthy and had numerous root hairs on slender white roots in the pH 4 and 5 solutions. All populations had stunted, discolored roots at pH 3, but both tailings populations seemed to have more new growth than the commercial population. Most of the new roots were located at the base of the plant rather than branching from the original roots.

Acid tolerance in Agropyron trachycaulum

Surprisingly the two Agropyron trachycaulum tailings populations showed less acid tolerance than the commercial population. Means for this species can be found in Tables 10 and 11. Relationships between pH and root growth and pH and increases in plant weight are shown in Figures 8 and 9 respectively. Standard deviations around each mean are

Table 10. Percent root growth means for Agropyron trachycaulum.

Population	pH 3	pH 4	pH 5
Commercial	7.8 a A	151.3 b B	153.5 b B
PD108	11.0 a A	70.8 a A	73.3 a A
CM112	30.0 a A	95.5 ab B	90.5 ab B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

Table 11. Percent increase in plant weight means for Agropyron trachycaulum.

Population	pH 3	pH 4	pH 5
Commercial	86.5 a A	194.3 b B	212.5 b B
PD108	33.5 a A	63.8 a AB	140.8 a B
CM112	69.8 a A	78.3 a A	125.5 a A

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

indicated by vertical lines. Analysis of variance tables are found in Appendix E, Tables 25 and 26.

As with the Agrostis tenuis populations, the most significant source of variation for all Agropyron trachycaulum populations was pH. Root growth and increases in plant weight were substantially less at pH 3 than at other pH levels.

Significant Population differences were found for both root growth and plant weight. Partioning of the sums of squares indicated that a significant portion of the variation was between the tailings and commercial populations. Mean separations revealed that the commercial

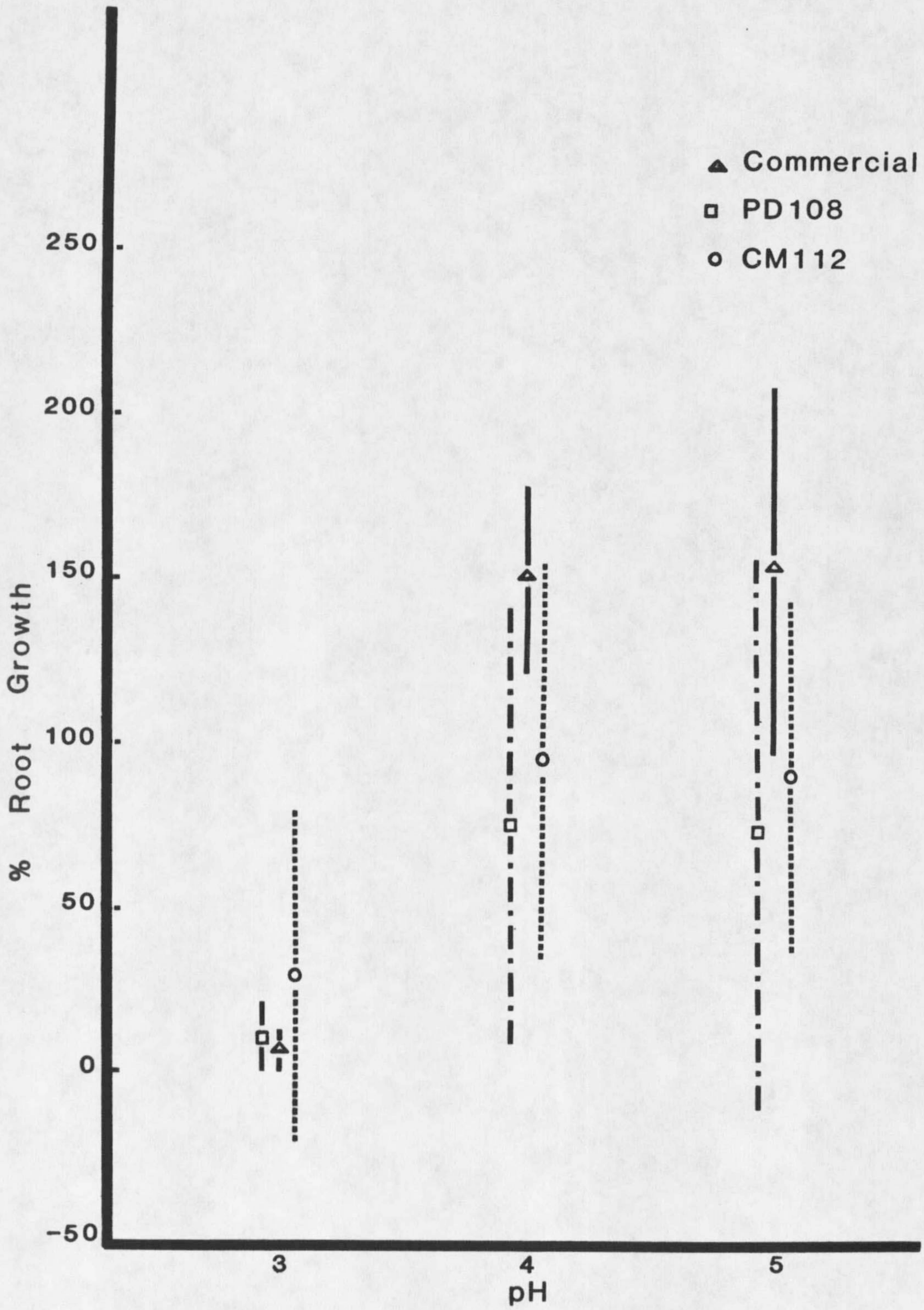

Figure 8. Means and standard deviations for percent root growth of Agropyron trachycaulum at three pH levels.

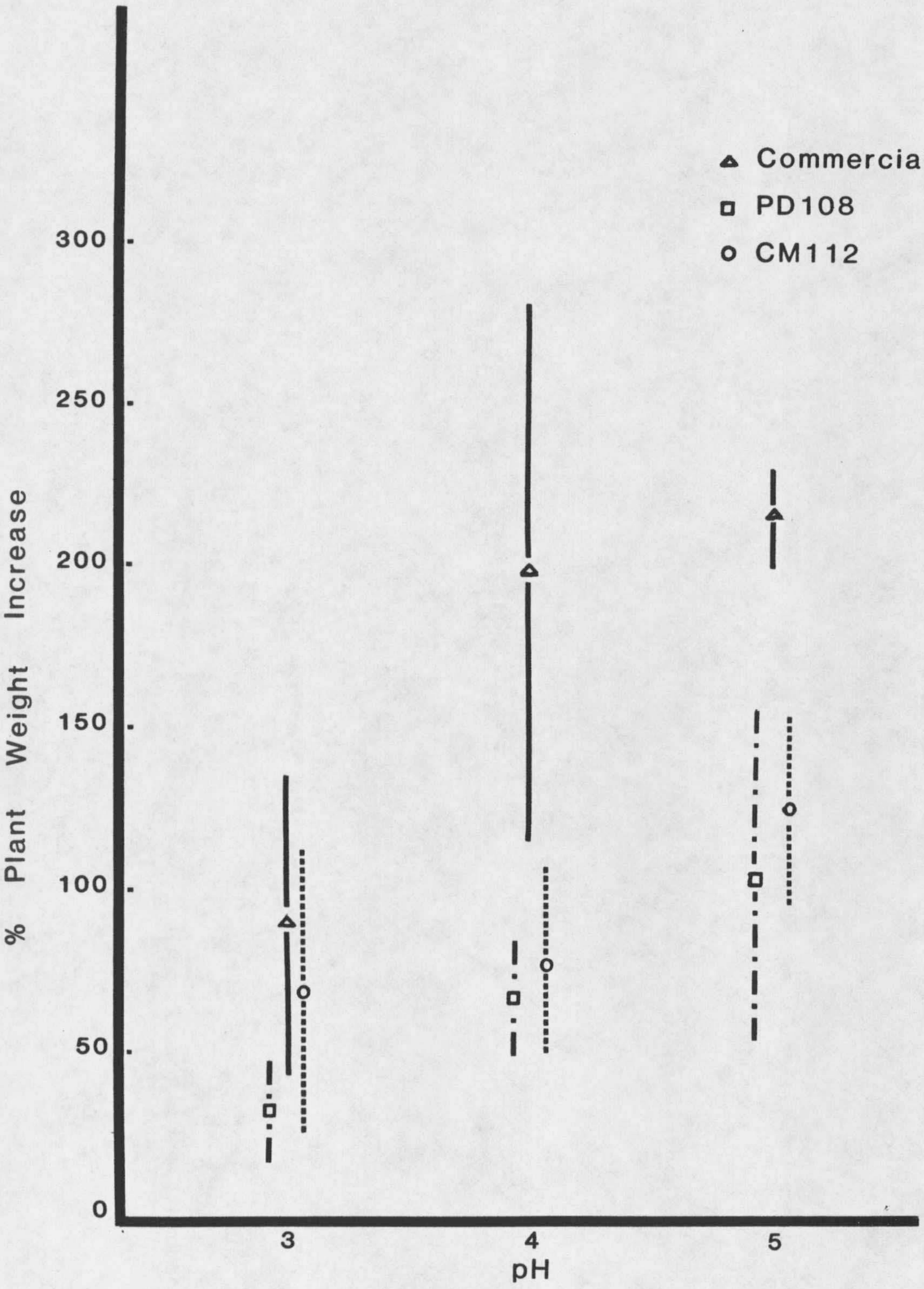

Figure 9. Means and standard deviations for percent increase in plant weight of Agropyron trachycaulum at three pH levels.

population produced significantly more root growth at pH 4 and 5 than the tailings population PD108 but not more than tailings population CM112. At pH 3 the commercial population produced the least amount of root growth, although it was not significantly different from the tailings populations. The commercial population also produced more biomass than the others at all pH levels but the difference was only significant at pH 4 and 5.

There was no significant pH x Population interaction. However, there was a significant pH x (Tailings vs. Commercial) variation indicating that the tailings and commercial populations reacted differently to changes in root zone pH.

The roots for all populations were extremely stunted and contorted at pH 3. Healthy new roots and root hairs were limited even in the less acid solutions. Tillers produced in the pH 3 solution were less rigid and had fewer leaves than pH 4 and 5 plants. Visually there was little difference between populations.

Acid tolerance in *Deschampsia cespitosa*

The mill tailings population of *Deschampsia cespitosa* from Anaconda (AC101) was found to be slightly more acid tolerant than the commercial population. Means for this species are found in Tables 12 and 13. Figures 10 and 11 show the relationship between pH and root growth, and pH and plant weight increase. Vertical lines indicate the standard deviation around each mean. Analysis of variance tables are located in Appendix F, Tables 27 and 28.

As with the previously discussed species, the main source of variation affecting root growth and plant weight was pH. Significantly

Table 12. Percent root growth means for <u>Deschampsia</u> <u>cespitosa</u>.

	pH		
Population	3	4	5
Commercial	-3.5 a A	46.8 a B	25.8 a AB
AC101	-2.0 a A	95.5 b B	83.0 b B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

Table 13. Percent increase in plant weight means for <u>Deschampsia</u> <u>cespitosa</u>.

	pH		
Population	3	4	5
Commercial	47.9 a A	321.0 b C	139.5 a B
AC101	16.5 a A	163.0 a B	146.3 a B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

more root growth and biomass were produced at pH 4 and 5 than at pH 3. Only the commercial population had significantly more biomass at pH 4 than at pH 5.

Another significant source of variation for both parameters was the Population interaction. The commercial population had significantly less root growth than the tailings population AC101 at pH 4 and 5. The commercial population, however, had significantly greater biomass than the tailings population at pH 4. The significant pH x Population interaction indicated that the populations reacted differently to changes in root zone pH.

The tailings population had the typical morphological features of metal tolerant plants: depauperite foliage but healthier roots than the

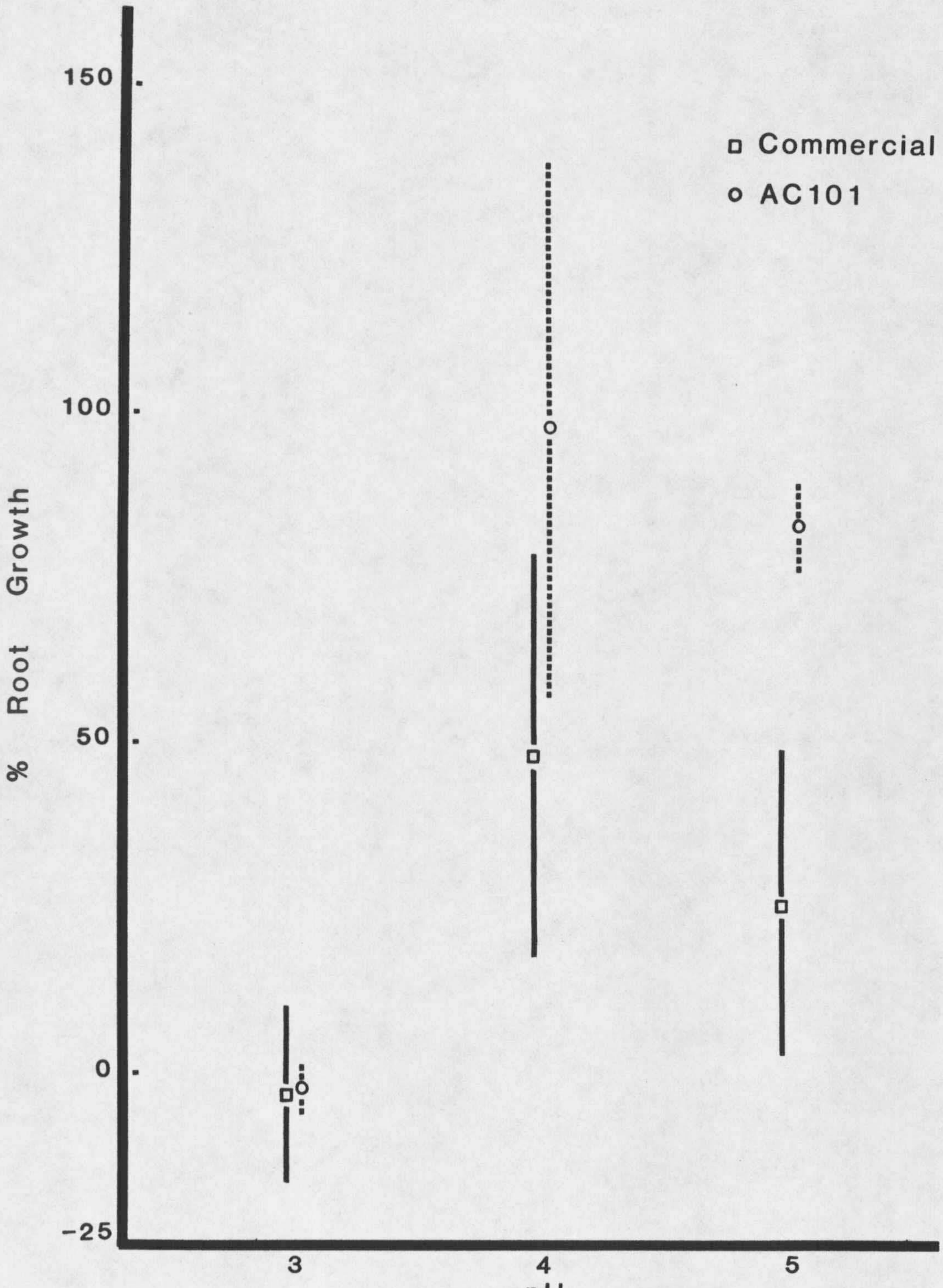

Figure 10. Means and standard deviations for percent root growth of <u>Deschampsia cespitosa</u> at three pH levels.

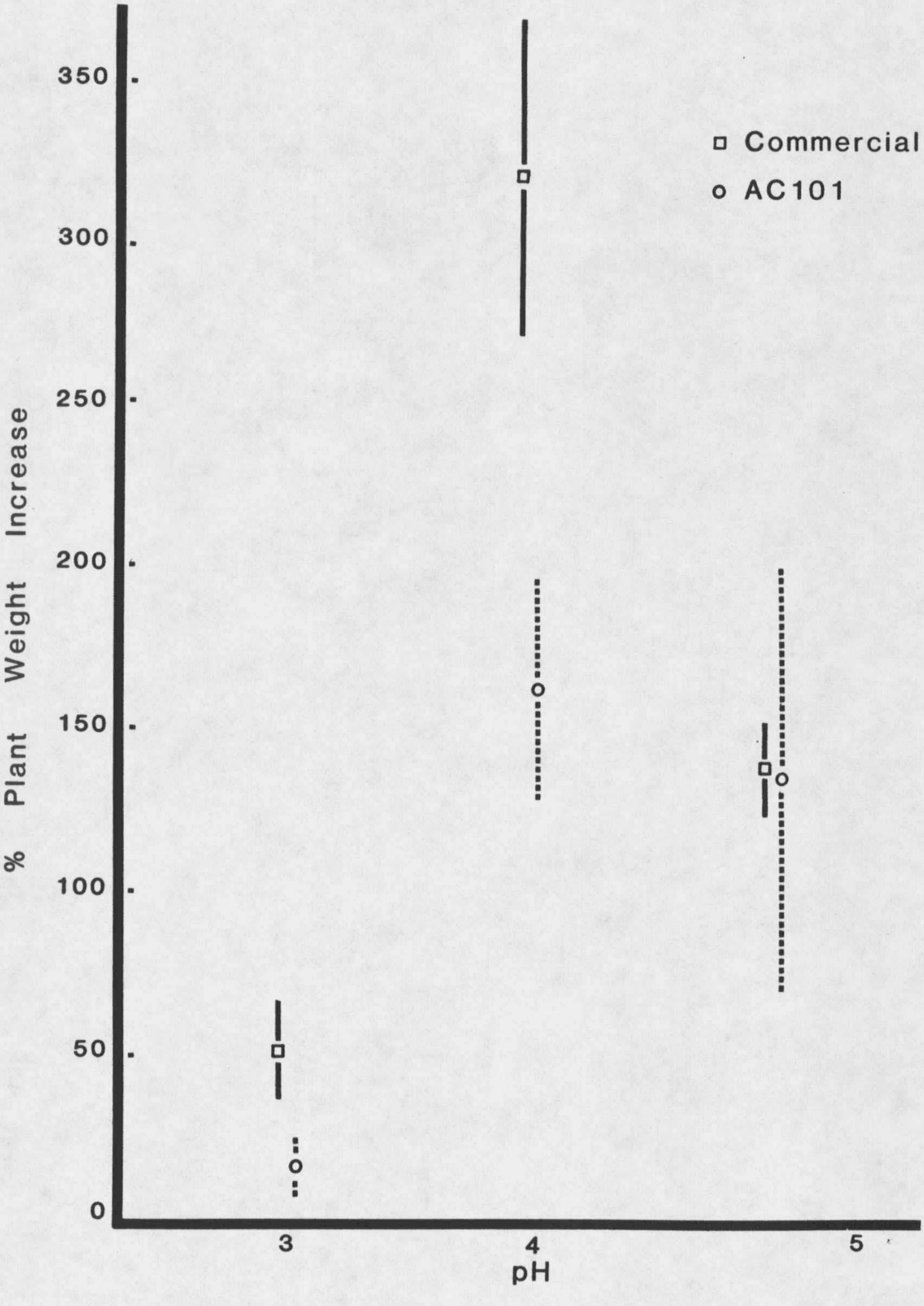

Figure 11. Means and standard deviations for percent increase in plant weight of Deschampsia cespitosa at three pH levels.

non-tolerant plants. Both populations produced numerous tillers especially in the two least acid solutions. Roots for both populations were stunted and slightly brown at pH 3, while root hairs and new roots were common at pH 5.

Acid tolerance in *Fragaria vesca*

The commercial population showed slightly more acid tolerance than the tailings population from Champion Mine, CM154. Tables 14 and 15 contain the means for *Fragaria vesca*. The relationships between pH and root growth, and pH and plant weight increase are shown in Figures 12 and 13. Standard deviations around each mean are indicated by vertical lines. Tables 29 and 30 in Appendix E contain the analysis of variance tables for *Fragaria vesca*.

As with the other species the most significant source of variation resulted from changes in pH. Root growth and plant weight increases were suppressed at pH 3 for both populations. The commercial population had significantly more root growth at pH 5 than at pH 4.

The Population interaction was another significant source of variation for root growth ($P \leq 0.05$) and for increases in plant weight ($P \leq 0.10$). Root growth for the commercial population was significantly higher at pH 4 and 5 than for the tailings population. Plant weight increase was also greater at pH 4 and 5 but the increase was only significant at pH 5. The significant pH x Population interaction ($P \leq 0.10$) indicated that the two populations reacted differently to changes in pH.

Plants from the tailings population were smaller and more compact than the commercial specimens. The foliage was also a darker green.

Table 14. Percent root growth means for Fragaria vesca.

	pH		
Population	3	4	5
Commercial	-5.5 a A	55.8 b B	89.8 b C
CM154	-3.8 a A	26.8 a B	51.0 a B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

Table 15. Percent increase in plant weight means for Fragaria vesca.

	pH		
Population	3	4	5
Commercial	-13.3 a A	139.3 a B	152.0 b B
CM154	6.5 a A	103.0 a B	97.3 a B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

The roots for both populations were beginning to rot in the most acid solution and there were very few new roots present and no visible root hairs. The roots were healthier at pH 4 and 5 but were still somewhat stunted and brown.

Acid tolerance in Trifolium repens

The tailings population of Trifolium repens (CM156) and the commercial population revealed comparable acid tolerance. Tables 16 and 17 illustrate the relationships between pH and root growth, and pH and plant weight respectively. The vertical lines refer to standard deviations around each mean. Analysis of variance tables are found in Appendix E, Tables 31 and 32.

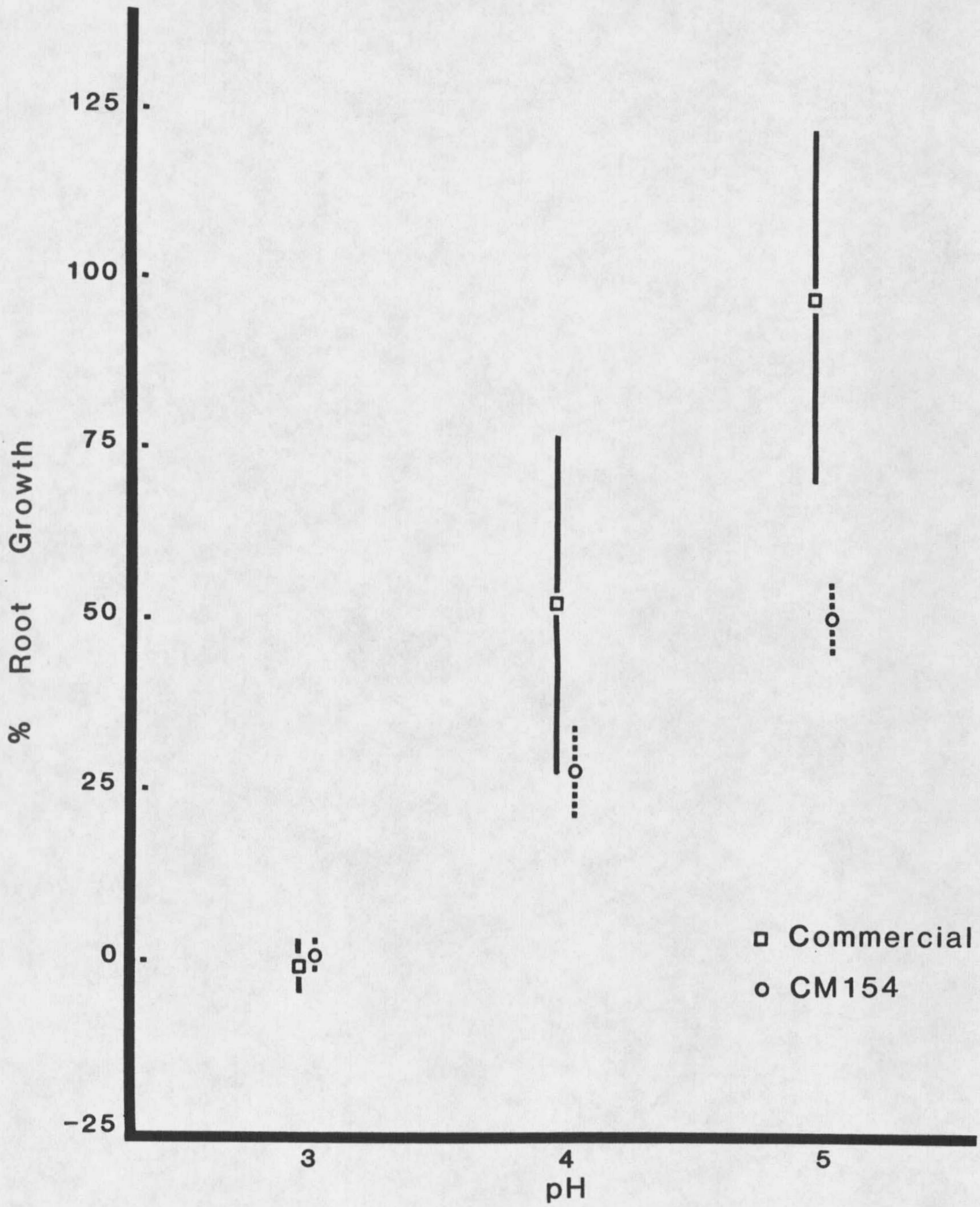

Figure 12. Means and standard deviations for percent root growth of Fragaria vesca at three pH levels.

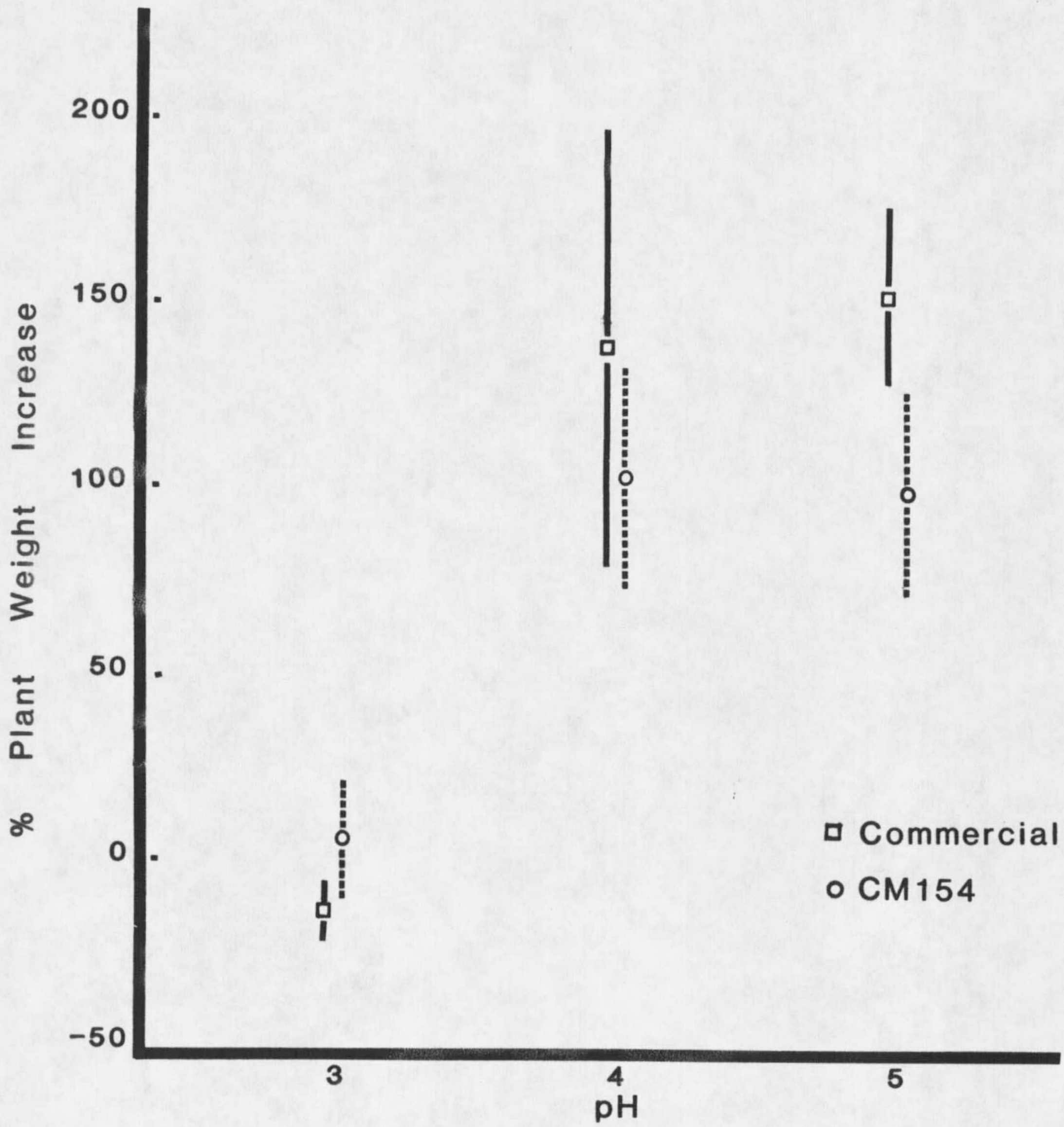

Figure 13. Means and standard deviations for percent increase in plant weight of Fragaria vesca at three pH levels.

Table 16. Percent root growth means for *Trifolium repens*.

	pH		
Population	3	4	5
Commercial	6.5 a A	95.0 a B	102.8 a B
CM156	-18.5 a A	188.8 b B	140.0 a B

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

Table 17. Percent increase in plant weight means for *Trifolium repens*.

	pH		
Population	3	4	5
Commercial	92.3 a A	559.5 a B	911.0 a C
CM156	1.3 a A	376.2 a B	780.2 a C

Means with different small letters in columns or capital letters in rows are statistically different at $P \leq 0.05$ level.

The major differences in this species were again due to changes in pH. There were substantial differences between pH 3 and the other two pH levels for root growth and between all pH levels for increases in plant weight for both populations.

Although there were no significant variations due to differences in Populations, CM156 produced significantly more root growth at pH 4 than the commercial population. At pH 3 the commercial population produced almost 100% more biomass than CM156 but it was not significant due to the small "n" and the large variation in samples. There was a significant source of variation in the pH x Population interaction for root growth ($P < 0.10$) which indicated that the two populations reacted differently to changes in root zone pH.

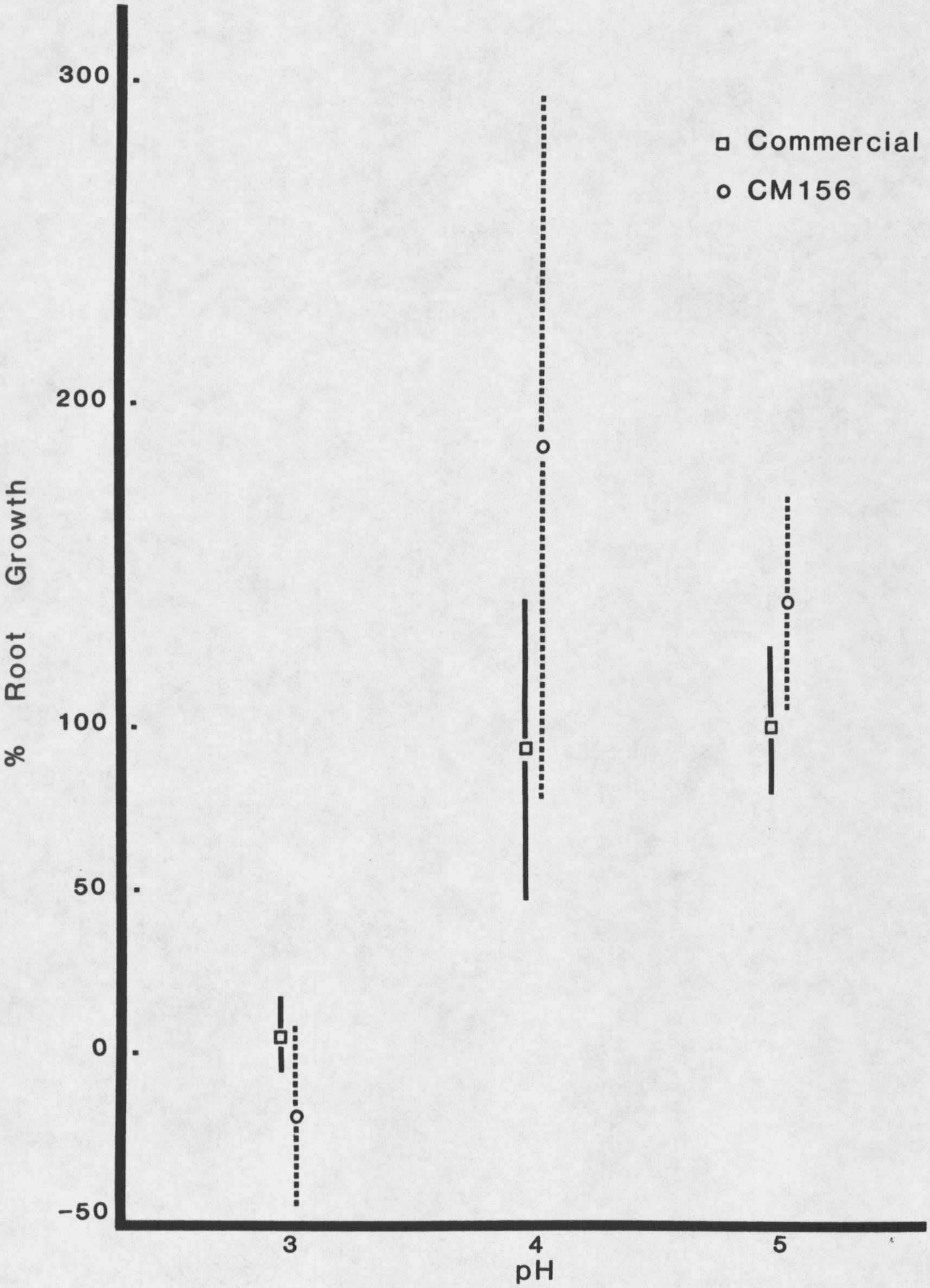

Figure 14. Means and standard deviations for percent root growth of <u>Trifolium repens</u> at three pH levels.

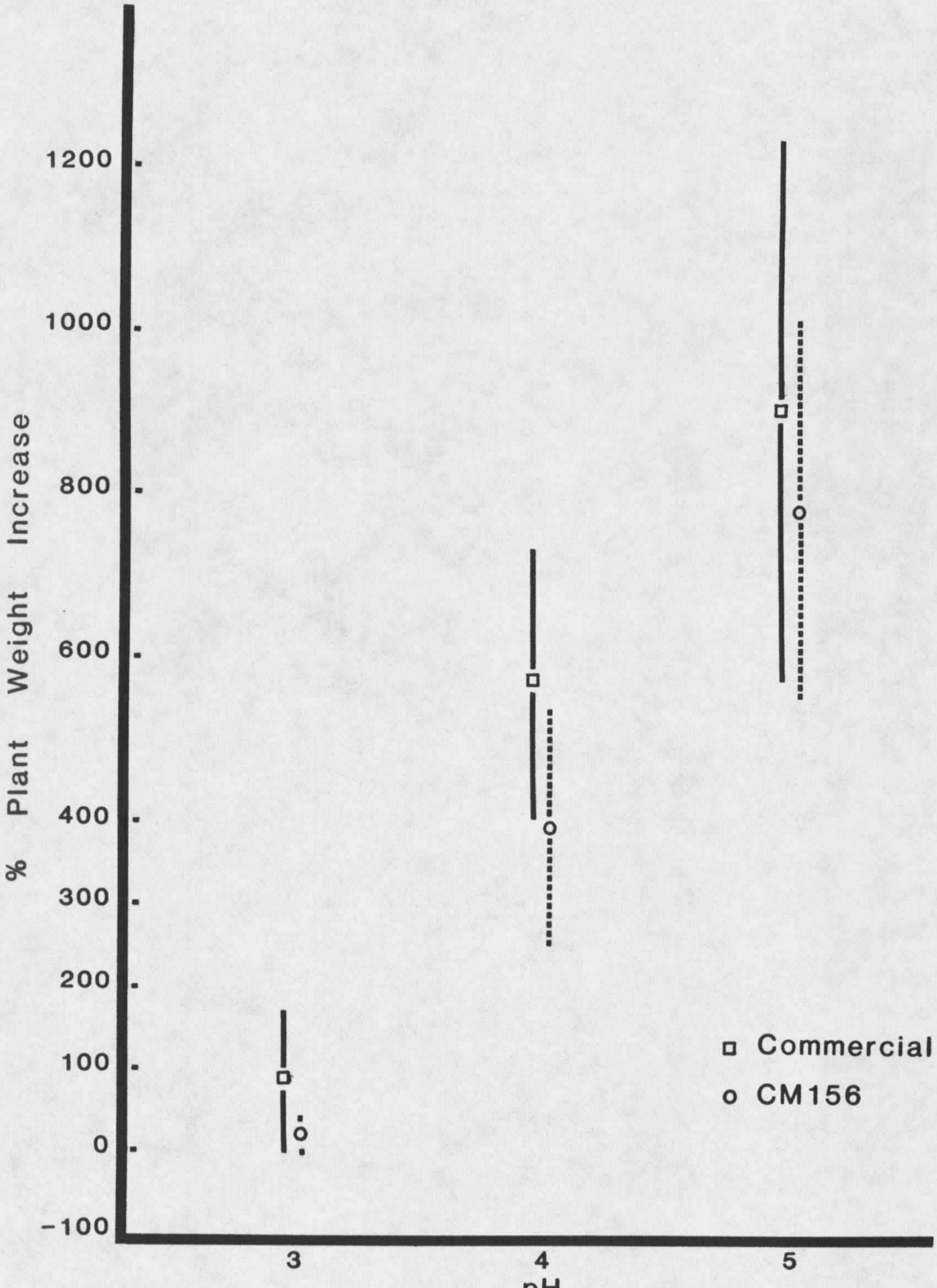

Figure 15. Means and standard deviations for percent increase in plant weight of Trifolium repens at three pH levels.

The commercial plants had extremely large leaves and long petioles compared to the tailings population. Root growth was stunted and discolored for both populations at pH 3 and nodules were very small and scarce. Nodules were larger and a healthy pink-white color in the two least acid solutions. At pH 4 most of the nodules were located only on the largest roots, but at pH 5 small nodules were developing on the smaller roots. Root hairs were present on the healthier roots at pH 4 and 5 but were very sparse at pH 3.

DISCUSSION

Many times it is difficult to determine if a plant is tolerating high levels of hydrogen ions, or one or more soluble metals. By excluding heavy metals from the solution culture test it was possible to omit that variable. However, as heavy metals are usually found in acid mine tailings other tests would be necessary to determine the range of tolerances a plant population had and thus its suitability for reclamation of specific sites.

Hydrogen ion injury

The most consistent response for all populations tested was a substantial decrease in root growth and biomass at pH 3. This response has been noted by other researchers (Arnon et al. 1942, Hoagland 1917, Islam et al. 1980) for a variety of species, mostly agricultural crops. Maximum root growth and increases in plant weight for most species in the acid tolerance test generally leveled off between pH 4 and 5. Both forbs, however, maintained a steady increase in root growth and probably would reach maximum growth at a higher pH level than was tested.

The amount of plant root injury appears to be related to the duration of exposure to low pH. Initial injuries have been shown to include impairment of ion transport (Jacobson et al. 1960) and damage of cell membranes allowing for the loss of absorbed nutrients (Fawzy et al. 1954, Jacobson et al. 1950). Other researchers (Arnon and Johnson 1942, Hoagland 1917, Islam et al. 1980, Sutton and Hallsworth 1958) have found that long term exposure to low pH levels results in the suppression of lateral root development and eventual death of root tips.

Visible effects of low pH on root growth were observed in all species at pH 3 and in some at pH 4. In general the damaged roots were "short, thickened, comparatively few in number, and discolored brown or dull grey" (Islam et al. 1980). Most new root growth was occurring at the base of the plant rather than branching off from existing lateral roots.

Legumes can be affected by an acid root environment due to direct effects on the plant roots (Joost and Hoveland 1986, Haynes and Ludecke 1981) as well as on the rhizobia needed for the fixation of nitrogen (Albrecht 1933, Jackson 1967). Rhizobia seldom survive below pH 4 and nodulation has been reported to be suppressed below pH 4.5 (Andrew 1976, Jackson 1967). Nodulation was suppressed for both Trifolium repens populations tested at pH 3 but was noticeable at pH 4 and 5 in this study.

Sutton and Hallsworth (1958) and other researchers (Albrecht 1933, Arnon et al. 1942, Moore 1974, Munns 1965) found that hydrogen ion toxicity could be reduced by higher levels of Ca and in some cases by higher levels of N. These moderating effects were severely limited when a solution culture was the growth medium rather than agar or sand. This could be the reason some of the tailings populations such as CM105, PD108, CM112, and AC101 showed minimal growth at pH 3 in the solution culture of the acid tolerance trial yet survived on acid tailings in the growth media trial as well as at the mine sites. The plants grow on these toxic root zone materials because of their ability to take up more anions than cations thus creating a more alkaline root environment to minimize hydrogen ion toxicity.

Acid tolerance

Of the three grass species tested, the commercial populations were as intolerant as their respective tailings populations to the most acid root environment with the exception of one Agrostis tenuis tailings population, AC100. This population revealed the greatest acid tolerance of all species and populations tested. Agrostis tenuis has been studied by several researchers (Jowett 1964, Gregory and Bradshaw 1965, McNeilly and Bradshaw 1968, Turner 1969) for tolerance to various metals found in acid mine spoils. This particular population is also known to tolerate high levels of Zn and Cu (Surbrugg 1982). It, therefore, shows a distinct ability to develop multiple tolerances.

Both forbs barely survived in the pH 3 solution. The tailings populations were no more tolerant to acid root zones than the commercial populations. The Trifolium repens, CM156, from Champion Mine had greater root growth than the commercial population at pH 4 and 5. Both clover populations had more root growth and greater increases in biomass than any of the other species listed.

Based on the limited range of pH levels tested, the acid tolerance of all species and populations tested was pH 4 except for the Agrostis tenuis AC100 which was slightly tolerant at pH 3. Testing at several pH levels between pH 3 and 4 would allow for more accurate delineation of acid tolerance where the root zone was not allowed to be modified by root activity. Additional testing in soils with known pH levels and no toxic metal levels would give a better indication of acid tolerances occurring in the field. Identification of metal tolerances for specific acid tolerant populations would allow for selective revegetation with plants tolerant to each site's specific characteristics.

SUMMARY AND CONCLUSIONS

There are hundreds of abandoned and inactive hardrock mines scattered throughout western Montana which are at best sparsely vegetated. These sites are potential sources of soil, air and water contamination. They may be extremely acid, contain high levels of heavy metals and are not physically suited for normal plant growth. Nevertheless, the most feasible way to stabilize and reclaim these sites is through the use of acid and metal tolerant vegetation which may be preadapted to climatic conditions and nutrient deficiencies.

The existence of metal tolerance in several plant species is well documented through field and greenhouse experiments. Much research also exists on the effects of acid soils and liming on crop growth. Unfortunately, most of this research is of little value to reclamation scientists since it addresses crop responses. The study of acid tolerance of non-agricultural crops is important to the reclamation scientist for the rehabilitation of acid mine wastes.

A tailings growth media trial was used to screen for metal tolerant and acid tolerant vegetation in plants collected from two derelict mine sites. Five species were selected: Agrostis tenuis, Agropyron trachycaulum, Deschampsia cespitosa, Fragaria vesca, and Trifolium repens. Commercial populations of each species were used as controls for assessing acid tolerance in a second greenhouse trial.

An "in parallel" technique was chosen for this second study. It used separate tillers of plants in three acid solutions. The apparatus was adapted from one developed by Surbrugg (1982). It was a sand/drip culture apparatus in which the sand provided support for the plants and when combined with a constant flow of nutrient solution provided excellent growth conditions.

The Agrostis tenuis tailings populations was more acid tolerant compared to the commercial variety of the same species. The commercial populations of Agropyron trachycaulum and Fragaria vesca, on the otherhand, was more tolerant of an acid root zone than the tailings populations. The Deschampsia cespitosa populations showed mixed responses to the different pH levels. The commercial population of Trifolium repens and the mine site population revealed comparable acid tolerances. This species produced by far the most biomass of all species tested.

Greenhouse trials are an excellent method of screening for acid and metal tolerances, but there is more control over the root environment than actually exists in the field. Field trials with the populations tested in this study would provide additional information on their adaptability to new site constraints and their applicability in acid mine waste reclamation.

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APPENDICES

APPENDIX A

Species Lists

Table 18. Species list for Champion Mine.

Botanical Name	Common Name	Location: On site	Location: Off site
Trees			
Picea spp.	spruce		x
Pinus contorta	lodgepole pine	x	x
Populus spp.	cottonwood	x	x
P. tremuloides	quaking aspen	x	x
Prunus virginiana	chokecherry		x
Pseudotsuga menzeisii	Douglas fir		x
Salix spp.	willow	x	x
Shrubs			
Alnus sinuata	Sitka alder	x	x
Arcostaphylos uva-ursa	kinnikinik		x
Ceanothus velutinus	snowbush		x
Juniperus spp.	juniper		x
Mahonia repens	Oregon grape		x
Rosa spp.	wild rose	x	x
Ribes spp.	gooseberry	x	x
Rubus idaeus	raspberry	x	x
Shepherdia canadensis	russett buffaloberry		x
Forbs			
Achillea millefolium	yarrow	x	x
Antennaria parviflora	Nutall's pussytoes	x	x
A. rosea	rosy pussytoes	x	x
Campanula rotundifolia	harebell		x
Cirsum vulgare	bull thistle	x	
Collinsia parviflora	maiden blue-eyed Mary		x
Epilobium angustifolia	fireweed	x	
Equisetum arvense	horsetail	x	x
Fragaria vesca	wild strawberry	x	x
Mertensia ciliata	mountain bluebell	x	x
Potentialla spp.	cinquefoil	x	x
Rumex acetosella	redsorrel	x	x
R. crispus	curley dock	x	x
Solidago spp.	goldenrod	x	
Taraxacum officinale	dandelion	x	x
Trifolium repens	white clover	x	x
Verbascum thapsus	mullein	x	

Table 18. continued

Botanical Name	Common Name	Location	
		On site	Off site
Grasses and Grasslike Plants			
Agropyron trachycaulum	slender wheatgrass	x	x
Agrostis scabra	ticklegrass	x	x
A. tenuis	bentgrass	x	x
Bromus marginata	mountain brome		x
Carex aquatilis	sedge	x	x
C. limnophila	sedge	x	x
C. pachystachya	sedge	x	x
C. rostrata	sedge	x	x
Deschampsia cespitosa	tufted hairgrass	x	x
Festuca spp.	fescue		x
Juncus balticus	rush	x	x
Oryzopsis exuiga	little ricegrass	x	
Poa compressa	Canadian bluegrass	x	x
P. palustris	bluegrass	x	
Sitanion hystrix	bottlebrush squirreltail	x	
Stipa viridula	green needle grass		x
Trisetum spicatum	spike trisetum	x	

Table 19. Species list for Porphyry Dike.

Botanical Name	Common Name	Location On site	Location Off site
Trees			
Abies lasiocarpa	subalpine fir		x
Picea spp.	spruce	x	x
Pinus albicaulis	limber pine	x	x
P. contorta	lodgepole pine	x	
Shrubs			
Alnus sinuata	Sitka alder		x
Ledum glandulosum	Labrador tea		x
Ribes spp.	gooseberry		x
Rubus idaeus	raspberry	x	x
Vaccinium scoparium	grouse whortleberry	x	x
Forbs			
Anaphalis margaritacea	pearly everlasting	x	
Antennaria rosea	rosy pussytoes	x	x
Epilobium alpinum	alpine willow weed	x	
E. angustifolium	fireweed	x	
Rumex crispus	curlydock	x	
Grasses and Grasslike Plants			
Agropyron subsecundum	bearded wheatgrass	x	
A. trachycaulum	slender wheatgrass	x	
Agrostis scabra	ticklegrass	x	x
A. tenuis	bentgrass	x	x
Calamagrostis canadensis	bluestem		x
Carex aperta	sedge	x	x
C. canescens	sedge	x	x
C. rossii	sedge	x	x
Deschampsia cespitosa	tufted hairgrass	x	x
Hordeum brachyantherum	meadow barley	x	
Juncus drummondi	rush		x
J. mertensianus	rush	x	x
J. parrvi	rush	x	x
Phleum alpinum	alpine timothy	x	x
Poa compressa	Canada bluegrass	x	x
Trisetum spicatum	spike trisetum	x	
Xerophylum tenax	beargrass		x

APPENDIX B

Collected Plant List

Table 20. Collected plants and root zone tailings pH.

Key	Botanical Name	Tailings pH
*PD 103	Juncus drummondi	4.3
PD 104/105	Carex rossii	4.1/4.5
PD 106	Agropyron subsecundum	6.5
*PD 107	Trisetum spicatum	6.9
*PD 108/109	Agropyrum trachycaulum	6.6/5.8
PD 110	Poa compressa	7.7
*PD 111	Carex rossii	3.6
*PD 150	Anaphalis margaritacea	5.7
*PD 151	Epilobium alpinum	5.2
PD 175	Vacinium scoparium	4.3
CM 101	Juncus balticus	4.1
*CM 103/108	Carex aquatilis	3.6/3.2
*CM 104/104A	C. limnophila	3.4/3.7
*CM 105	Agrostis tenuis	3.3
*CM 106/117	Poa compressa	3.1/4.4
CM 107/107A	Deschampsia cespitosa	3.8
CM 109/*118	Agrostis scabra	3.4/4.3
CM 110	Poa palustris	6.0
*CM 112/112A	Agropyron trachycaulum	6.9/3.2
CM 113	Oryzopsis exuiga	7.2
CM 114	Sitanion hystrix	7.2
CM 116	Poa compressa	7.4
*CM 152	Antennaria rosea	4.1
CM 153	A. parvifolia	4.1
*CM 154	Fragaria vesca	4.0
CM 155	Potentilla spp.	4.2
*CM 156	Trifolium repens	3.7
Plants collected from other mines:		
AC 100A	Agrostis stolonifera	-
*AC 100	A. tenuis	3.2
*AC 101	Deschampsia cespitosa	3.3
*MC 10	Carex spp.	-

*Used in tailings growth trial.

PD = Porphyry Dike; CM = Champion Mine; AC = Anaconda tailings pond; MC = Mclaren Mine tailings.

APPENDIX C

Analytical Methods

Table 21. Laboratory procedures for analysis of acid mine tailings

Parameter	Procedure	Reference
Nitrogen	Phenol 2,4 disulphonic acid method	Black et al. (1965) A.S.A. Monograph #9, part 2, p. 1216.
Phosphorous	$NaHCO_3$ extractable	Black et al. (1965) A.S.A. Monograph #9, part 2, p. 1044.
Potassium	NH_4OAc extractable	Black et al. (1965) A.S.A. Monograph #9, part 2, p. 903.
Ca, Mg, Na, Zn, Cu, Mn, Fe, Pb, Ni, and B	Water soluble- Saturated paste extract	Black et al. (1965) A.S.A. Monograph #9, part 2, p. 935.
pH	Water saturated past extract- electrode	Black et al. (1965) A.S.A. Monograph #9, part 2, p. 935.
SAR	Water soluble-calculated $Na/[(Ca + Mg)/2]^{1/2}$ meq/l	U.S.D.A. Handbook 60, p. 156.
E.C.	Water saturated paste extract	U.S.D.A. Handbook 60, p. 156.
Texture	Hydrometer method	U.S.D.A. Handbook 60, p. 123.

APPENDIX D

Tailings' Analysis

Table 22. Particle size analysis and soil texture

Sample	% Coarse Fragments	% Clay	% Silt	% Sand	Texture and qualifier
PD01	0.00	27.50	56.00	7.50	Silty clay loam
PD02	41.50	18.75	29.38	51.87	Sandy loam or loam, gravelly
CM01	3.20	23.75	40.00	36.25	Loam
CM02	59.10	17.50	20.00	62.50	Sandy loam, very gravelly
CM03	61.20	5.00	36.88	58.12	Sandy loam, very gravelly

Note: % Clay, % Silt and % Sand are for the fine soil materials (< 2 mm) only.

APPENDIX E

Statistical Tables

Table 23. Analysis of variance for percent root growth of *Agrostis tenuis* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	85,000	42,000	20.93*
Populations	2	23,280	11,640	5.73*
Tailings vs. Commercial	1	2,860	2,860	1.41
CM105 vs AC100	1	20,420	20,420	10.05*
pH x Populations	4	4,780	1,195	0.59
pH x (Tailings vs. Commercial)	2	1,350	675	0.33
pH x (CM105 vs. AC100)	2	3,430	1,715	0.84
Error	27	54,830	2,031	
Total	35	167,890		

$^*P \leq 0.05$

Table 24. Analysis of variance for percent increase in plant weight of *Agrostis tenuis* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	199,600	99,820	36.00*
Populations	2	48,520	24,260	8.75*
Tailings vs. Commercial	1	41,620	41,620	15.01*
CM105 vs AC100	1	6,900	6,900	2.49
pH x Populations	4	13,070	3,268	1.18
pH x (Tailings vs. Commercial)	2	7,555	3,778	1.36
pH x (CM105 vs. AC100)	2	5,515	2,758	0.99
Error	27	74,880	2,773	
Total	35	336,070		

$^*P \leq 0.05$

Table 25. Analysis of variance for percent root growth of Agropyron trachycaulum at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	64,140	32,070	11.43*
Populations	2	16,820	8,409	3.00**
Tailings vs. Commercial	1	14,340	14,340	5.11*
CM105 vs AC100	1	2,481	2,481	0.88
pH x Populations	4	12,210	3,054	1.08
pH x (Tailings vs. Commercial)	2	12,150	6,075	2.16
pH x (CM105 vs. AC100)	2	60	30	0.01
Error	27	75,780	2,807	
Total	35	168,950		

*P ≤ 0.05
**P ≤ 0.10

Table 26. Analysis of variance for percent increase in plant weight of Agropyron trachycaulum at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	43,030	21,510	12.06*
Populations	2	61,440	30,720	17.21*
Tailings vs. Commercial	1	58,030	58,030	32.51*
CM105 vs AC100	1	3,410	3,410	1.90
pH x Populations	4	11,510	2,878	1.63
pH x (Tailings vs. Commercial)	2	11,010	5,505	3.08**
pH x (CM105 vs. AC100)	2	500	250	0.14
Error	27	48,190	1,785	
Total	35	164,170		

*P ≤ 0.05
**P ≤ 0.10

Table 27. Analysis of variance for percent root growth of Deschampsia cespitosa at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	24,000	12,000	20.63*
Populations	1	7,704	7,704	13.24*
pH x Populations	2	3,609	1,804	3.10**
Error	18	10,470	582	
Total	23	45,783		

*P $\leq$ 0.05
**P $\leq$ 0.10

Table 28. Analysis of variance for percent increase in plant weight of Deschampsia cespitosa at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	176,600	88,310	60.30*
Populations	1	24,580	24,580	16.78*
pH x Populations	2	27,260	13,630	9.31*
Error	18	26,360	1,461	
Total	23	254,800		

*P $\leq$ 0.05

Table 29. Analysis of variance for percent root growth of *Fragaria vesca* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	22,870	11,400	41.24*
Populations	1	2,904	2,904	10.47*
pH x Populations	2	1,787	894	3.22**
Error	18	4,992	277	
Total	23	32,553		

*P ≤ 0.05
**P ≤ 0.10

Table 30. Analysis of variance for percent increase in plant weight of *Fragaria vesca* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	85,060	42,530	42.78*
Populations	1	3,384	3,384	3.40**
pH x Populations	2	6,019	3,009	3.03**
Error	18	17,900	994	
Total	23	112,363		

*P ≤ 0.05
**P ≤ 0.10

Table 31. Analysis of variance for percent root growth of *Trifolium repens* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	102,700	51,350	18.96*
Populations	1	7,491	7,491	2.77
pH x Populations	2	14,110	7,058	2.61**
Error	18	48,740	2,708	
Total	23	173,041		

*P ≤ 0.05
**P ≤ 0.10

Table 32. Analysis of variance for percent increase in plant weight of *Trifolium repens* at three pH levels.

Source of Variation	df	SS	MS	F
pH	2	2,555,000	1,278,000	34.50*
Populations	1	109,300	109,300	2.95
pH x Populations	2	8,564	4,282	0.12
Error	18	666,700	37,040	
Total	23	3,339,640		

*P ≤ 0.05

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