An Evaluation of *Deschampsia caespitosa* (L.) Beauv Populations for Metal Tolerances and Ecotypic Variation at Climax, Colorado

Paul Ellsworth Pratt
Utah State University

AN EVALUATION OF *DESCHAMPSIA CAESPITOSA* (L.) BEAUV POPULATIONS FOR METAL TOLERANCES AND ECOTYPIC VARIATION AT CLIMAX, COLORADO

by

Paul Ellsworth Pratt

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Paul E. Pratt

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by

Paul Ellsworth Pratt, Master of Science

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Major Professor: Dr. Cyrus M. McKell
Department: Range Science

Four populations of Deschampsia caespitosa (L.) Beauv (a Poaceae species), growing on disturbed and undisturbed sites at Climax, Colorado, were examined for metal tolerances (Zn, Cu, Pb, Al). Root growth assessments on parent plant and offspring material for each population were conducted using nutrient solutions containing elevated levels of Zn, Cu, Pb, or Al. Seed germination and seedling survival tests for each population were conducted on both alpine topsoil and mine spoil material.

Evidence is presented that supports the hypothesis that ecotypic variation exists within Deschampsia caespitosa and that this variation has allowed certain Deschampsia populations to become successfully established on alpine mine spoils. Acid mine spoil populations showed significantly less root growth retardation in the presence of zinc, copper or aluminum.

(78 pages)

INTRODUCTION

Revegetation of a disturbed alpine ecosystem is a difficult problem for the mining industry involved in the recovery of essential metals from this fragile environment. Successful reclamation can be assisted by the utilization of plant species tolerant to harsh conditions (Brown et al. 1978). Identifying plant species with ecotypes tolerant to the disturbed site is important in the revegetation effort.

Observation of alpine acid mine spoils at Climax, Colorado quickly leads to the conclusion that one Poaeae species, *Deschampsia caespitosa* (L.) Beauv (tufted hairgrass), is mainly responsible for the colonization of these drastically disturbed sites.

The primary objective of this study was to investigate if the *D. caespitosa* populations colonizing these spoils were specifically adapted to the adverse conditions.

The research hypothesis examined was: genotypic variation exists within *D. caespitosa* and this variation has predisposed certain *D. caespitosa* sub-populations to become successfully established on alpine mine spoils. The question whether the populations growing on mine spoil materials are ecotypes was considered.

Multielement soil/spoil analyses, hydroponic experiments with elevated concentrations of copper, lead, zinc, and aluminum salts, seed germination, and plant survival studies were conducted to evaluate the research hypothesis.

The acidification of mine spoils and the subsequent development of high concentrations of water soluble heavy metals within the spoil material is reviewed by Sorenson et al. (1980). In the process of metal recovery, overburden material containing pyritic minerals are exposed to air and water whereby various oxidation processes are initiated with sulfuric acid being one of the end products. When the pH of the spoil material is sufficiently lowered, certain heavy metals such as aluminum, zinc, and copper become soluble and may be present in quantities toxic enough to severely limit plant establishment and growth.

In the alpine-subalpine transition zone (3000 m elevation) of the Beartooth Plateau in southern Montana, mine spoil surface pH values were reported at 2.3 and lysimeter discharges through spoil material showed pH values from 1.8 to 2.9 (Johnson et al. 1975). These lysimeter samples were analyzed for heavy metal concentrations. The lowest values reported for zinc and copper were 11 ppm and 102 ppm, respectively. On overburden material at the Blackbird Mine in central Idaho, pH values range from 3.0 to 5.0 (Sorensen et al. 1980). These low pH ranges and high concentrations of heavy metals are characteristic of many alpine mine disturbances.

Microenvironmental conditions on the acid mine spoil site may greatly influence a plant's ability to become established on these sites. Howard (1978) studied the effect of Carex communities on the microenvironment at the McLaren Mine, near Cooke City, Montana. An important conclusion drawn from this study was that Carex communities

REVIEW OF LITERATURE

Four topics were considered in the literature review: (1) alpine acid mine spoil environments, (2) heavy metal tolerance and toxicity, (3) ecotypic differentiation within plant species, and (4) the plant, Deschampsia caespitosa.

Alpine Acid Mine Spoil Environments

Alpine environments represent a wide diversity of physical gradients (Billings 1974). These environments are typified by short growing seasons, high radiation loads, low average temperatures, long duration snow cover or wind swept areas with little available moisture. The extreme physical gradients which exist in the alpine ecosystem create an environment fragile to disturbance and hostile to non-adapted species (Billings 1974, Brown et al. 1976). These fragile alpine environments coupled with mine disturbances often produce new and harsh environments that are radically different from adjacent undisturbed communities.

Many alpine mine disturbances are characterized by extremely acidic spoil material that contains concentrations of heavy metals such as lead, copper, zinc, and aluminum, high enough to be toxic to many plant species. In addition to heavy metal toxicities and severe acidity, lack of nutrients and drought conditions in the acid mine spoil may also severely limit plant growth (Pinkerton and Simpson 1977).

significantly altered the microenvironment within the communities as compared to the adjacent bare areas, by reducing the harshness of the effects of wind speed, radiation, soil temperature, and by providing a richer source of plant nutrients. Favorable microenvironmental conditions may significantly increase a plants ability to survive and colonize a disturbed site.

Heavy Metal Tolerance and Toxicity

The physiology of metal tolerant plants is very complex and the mechanisms of tolerance are still under investigation. Turner (1968) reviewed heavy metal tolerance in plants and indicated that heavy metal tolerance does not generally involve differential ion uptake by the tolerant species. He suggested that tolerant plant cells exposed to high concentrations of heavy metals must either isolate the metal from interferring with metabolism or change the biochemical pathway to allow enzymes to function properly when exposed to toxic concentrations. In contrast, investigations by Mugwira and Patel (1977) and Mugwira and Elgawhary (1979) have suggested that root cation exchange properties may significantly result in differential ion uptake between aluminum sensitive and aluminum tolerant plant species. Mugwira and Elgawhary (1979) indicated that increased aluminum accumulation in aluminum sensitive wheat cultivars may be related to higher root cation exchange properties and lower pH root zone values as compared to aluminum tolerant wheat cultivars. That Mugwira and Elgawhary (1979) reported less consistent relationships between pH root zone values, and root cation exchange properties in aluminum sensitive cultivars of triticales may indicate that other physiological mechanisms such as those suggested by Turner (1968), could be responsible for aluminum tolerance in other plant species. Clearly, the biochemical pathways through which heavy metal tolerance in plants occurs is not yet fully understood.

Clarkson (1968) investigated the inhibitory effect of aluminum on root growth and the specific physiological mechanisms involved. While observing Agrostis tenuis seedlings grown in solutions with and without aluminum, he described the abnormal root morphology caused by aluminum toxicity. Excess aluminum caused the main axis root growth to be inhibited, followed by a series of laterals developing near the main axis root tips. The development of the laterals near the root tip is atypical and subsequently, the growth of these laterals is also inhibited. To further investigate the effect of aluminum on cell division, Clarkson conducted experiments with adventitious roots on onion (Allum cepa). From these experiments, Clarkson concluded that cell division may be the primary process that aluminum affects. Although no experiments were conducted utilizing aluminum tolerant ecotypes, he proposed that tolerance mechanisms either prevent aluminum from entering the cell or that some intercellular mechanism binds the aluminum or renders it to a non-toxic form.

The combination of certain metals on mine spoils has been found to create a synergistic toxic effect on plant growth (Hassett et al. 1976). However, zinc and copper tolerance mechanisms in Agrostis stolonifera appear to be specific and separate for each element and the cause of copper injury is apparently different in pure solutions than the cause of ion injury in balanced solutions (Wu and Antonovics

1975). Copper is extremely toxic to plants as indicated by severe retardation of root growth (Bennett 1971).

Pre-stress treatment of plant material has been recently found to be necessary in distinguishing, at the enzyme activity level, between some tolerant and nontolerant ecotypes. Cox and Hutchinson (1980) concluded that prior exposure to a toxic element was necessary in order to observe significant differences in root enzyme activity between tolerant and nontolerant *Anthoxanthum odoratum* and *D. caespitosa*. Wu et al. (1975) observed that significant differences in malate dehydrogenase activity between tolerant and nontolerant *Agrostis stolonifera* plants occurred after the plant material was pre-stressed to copper.

There have been relatively few studies conducted on the physiology of lead, copper, or zinc tolerance in plant species. Most research conducted on tolerances to these metals involve the detection or screening of tolerant populations or the evolutionary process whereby tolerant populations develop. Generally, some measurement of root growth (length or weight) is used to differentiate between tolerant and nontolerant populations.

Deschampsia caespitosa

Putnam (1971, p. 9) describes *D. caespitosa* as being "the only North American species with both a circumboreal and southern hemisphere distribution". The habitats for this species are varied. It is often a major constituent of wet alpine and arctic meadows. It is not restricted to wet meadow areas, however, as it is frequently found on dry roadsides or colonizing high altitude mine disturbances.

Putnam (1971) shows its range extending from New Mexico to Point Barrow, Alaska, and extending eastward into the New England states. D. caespitosa is often considered a high latitude or high altitude species as it is generally found in the higher elevations of the southern parts of its range.

Deschampsia caespitosa exhibits a high degree of morphological and cytological variability. This variability is evident in that taxonomists at different times have recognized at least thirty local races or variants (Kwano 1962), and that Lawrence (1945) separated the species into five climatic ecotypes. All plant specimens used in this study were classified as D. caespitosa according to the taxonomic description given by Cronquist et al. (1977).

Kwano (1966) notes that D. caespitosa reproduces sexually and Mitchell (personal communication, 1979) also considers it to be sexual and cross pollinated, but with a small possibility of self-pollination occurring. Cross pollination is an important genetic mechanism for maintaining intraspecific genotypic variation in a population.

Ecotypic variation in D. caespitosa has been attributed to disruptive selection due to mining operations as well as naturally occurring climatic gradients. Pearcy and Ward (1972) found that three different populations of D. caespitosa in the Rocky Mountains were significantly differentiated with respect to phenological development and growth, and that there were strong indications that these populations were ecotypic variants.

In a sand culture experiment where D. caespitosa seeds from a fluorspar mine waste and from a mixed woodland community were grown and subjected to fluoride solutions, the plant material from the mixed woodland community experienced significantly lower production (root and shoot dry weights) than the plant material from the fluorspar mine waste (Cooke and Johnson 1978). In an investigation on the long term effects of sulphur dioxide fumigations from smelter emissions, Freedman and Hutchinson (1980) reported that *Agrostis hyemalis* var. *tenuis*, *A. stolonifera* var. *major* and *D. caespitosa* colonized certain disturbed sites. Each of these species have been observed to exhibit a tolerance to heavy metals (Freedman and Hutchinson 1980, Jowett 1964, Wu et al. 1975).

The most recent investigation of multi-element tolerance in *D. caespitosa* was conducted at a nickel copper smelting complex near Sudbury, Ontario (Cox and Hutchinson 1980). They concluded that an increased tolerance of zinc, copper, lead, cadmium, and aluminum existed in the populations colonizing the smelter area. Furthermore, they presented evidence contrary to work by Gregory and Bradshaw (1965), which had suggested that tolerance of one metal does not confer tolerance to another.

Ecotypic Differentiation

Ecotypic differentiation in plant species has been studied since the early 1900's. The concept of an ecotype was defined by Turesson (1922a, 1922b, p. 345) as, "the product arising as a result of the genotypical response of an ecospecies to a particular habitat". Clausen, et al. (1940, p. 165) stressed that the, "basis for ecotypes is their environmental fitness", and included physiologic characteristics such as plant vigor, time of flowering, and the ability to survive in their investigation of ecotypes. Daubenmire (1947, p. 365) described a species with ecotypic variation as, "typically composed of a mosaic of populations, each of which differs in genetically based physiologic (and sometimes morphologic) features having survival value...". An important factor in the colonization of a new and unique habitat is, "the range of habitat variation a plant can endure", or its ecologic amplitude (Daubenmire, 1947, p. 366).

The probability of survival on the new habitat is higher for individuals with an environmental fitness (based on genetic combination) closely matching the specific site selection pressures (Daubenmire, 1947).

Clausen et al. (1940, p. 164) stated that, "the differentiation of an ecotype is a gradual process", and that, "its stage of development is expressed in the proportion of the major biotypes of which it is composed." Daubenmire, (1947, p. 368) in a little different language stated, "The differences among ecotypes may be no more than a matter of difference in frequency of certain alleles." Both authors essentially embrace the same concept.

Distinguishing between populations whose ecological amplitude overlap is a statistical consideration necessary to determine if the population means are significantly different (Ashby 1961). A more distinct separation of groups into ecotypes would be expected to develop when the population's environment substantially differs from the environment of the standard population.

The importance of ecotypic variation within alpine tundra species for adaptation to certain selection pressures was recognized by Turesson (1931) and Clausen (1963). Plant ecologists now recognize ecotypic variants of native species colonizing disturbed sites as an important element in the successful rehabilitation of disturbed sites (Brown et al. 1978).

Localized ecotypic variation is the result of interactions among natural variation, geneflow, and selection pressures (Jain and Bradshaw 1966). Walley et al. (1974) examined a commercial cultivar of Agrostis tenuis under increasing stressful soil conditions by increasing the ratio of copper contaminated mine soil in the potting compost. Through these experiments, Walley observed that certain individuals appeared to have a high tolerance to copper. Genetic variability may predispose certain populations to become successfully established on disturbed sites. Antonovics (1966) and McNeilly (1967) concluded that certain mine plant populations are the products of recent evolution by disruptive selection.

In a theoretical analysis (based upon field data) Jain and Bradshaw (1966) demonstrated that selection forces are able to maintain local differentiation with gene flow as high as 50 to 60 percent.

McNeilly (1967) concluded that selection forces on certain mine spoils were maintaining localized populations despite gene flow contamination. Selection pressures interacting with species variation may largely determine the extent of natural colonization success on the mine spoil material.

STUDY SITE

The study was conducted on plant and soil/spoil material collected from the Climax Molybdenum Mine at Climax, Summit County, Township 17S Range 79W, Colorado.

The mine properties cover approximately 57 square kilometers with elevations ranging from 3200 meters (m) to 4206 m above sea level. Historically, much of the area was disturbed by small precious metal mines in the early 1900's.

Four different areas containing populations of *D. caespitosa* plants were selected for study. These included three disturbed sites and one undisturbed, isolated alpine meadow which was used as the control. The disturbed sites were: 1) acid mine spoils, 2) a waste water intercept canal, and 3) an old access road.

The undisturbed alpine meadow site (control) is located approximately 1.6 km from the other sites. The site is geographically isolated from any major disturbances by a mountain ridge. The site is approximately 70 m^2, in size, 3718 m elevation, and has a northeasterly exposure. Plant species found on the site include *D. caespitosa*, *Geum rossii*, *Poa artica*, *Trisetum spicatum*, and *Carex* spp. This site is representative of the many alpine meadows in the area that receive surface moisture into the mid-summer from protected snow packs at higher elevations.

The acid mine spoil site is an overburden waste dump resulting from mining activity that probably occurred in the 1940's and 1950's.

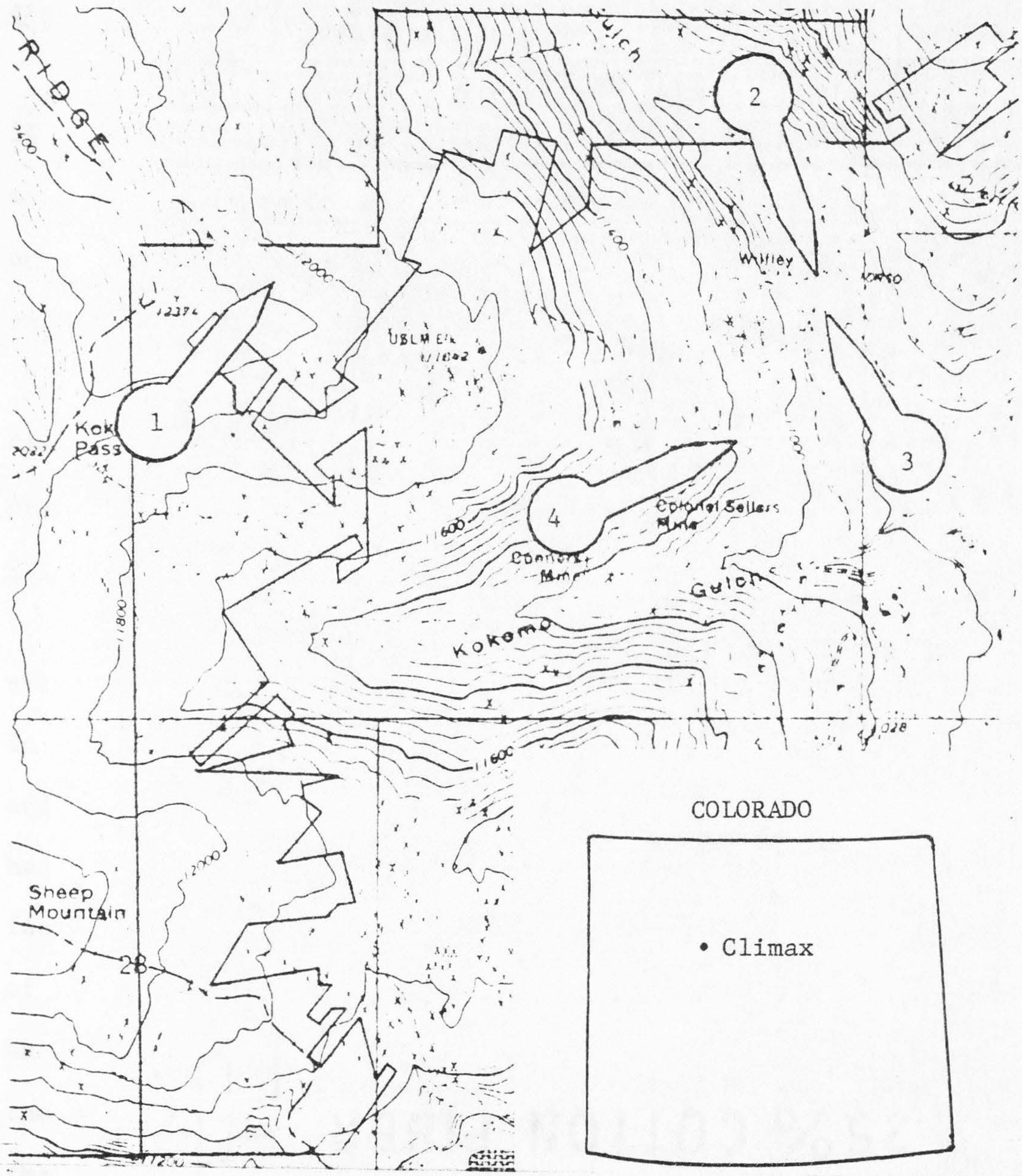

Figure 1. Locations of study areas at Climax, Colorado.
1. undisturbed alpine meadow (control)
2. acid mine spoil
3. waste water intercept canal
4. old access roads

The site is approximately 55 m^2 in size, at 3353 m elevation, and has a northeasterly exposure, and is solely colonized by D. caespitosa.

The waste water intercept canal site is a section (1 m wide and 50 m long) of a waste water collection canal that was built to isolate mine runoff water from downstream watersheds. The canal lies on a northeasterly exposure at 3350 m elevation. D. caespitosa is the only colonizing species.

The old access road site is a 20 m long section of a single lane dirt road that has been almost completely revegetated. The site is approximately 3533 m elevation and has an eastern exposure. D. caespitosa is the dominant colonizer on the site.

The concentrations of lead, aluminum, copper, and zinc in the soil/spoil and water extract samples taken from each site are shown in Table 1. Soil/spoil characteristics such as pH, particle size and surface moisture may greatly influence the concentration of heavy metal ions in soluble form. Because of these interrelated factors it is often difficult to interpret total ion concentrations of heavy metals in the soil/spoil samples. Therefore, the water extract analysis was used as an indication of the potential toxicity to which the root system was exposed. The concentration of each metal in the water-extract from the acid spoil site was substantially higher than the other three sites.

Extensive research has been conducted on plant adaptation to aluminum stress and the behavior of aluminum in relation to soil pH. It is well established that the solubility of aluminum increases

Table 1. Analysis of root zone material from each study site (each value is the average of six samples).

		Concentration (ppm)							
		Soil/Spoil (nitric perchoric digestion)				Water Extract			
Site	pH	Al	Zn	Cu	Pb	Al	Zn	Cu	Pb
Acid mine spoil	3.7	21993.00	664.05	113.26	4039.73	4.82	19.31	0.18	1.22
Waste water intercept canal	6.6	13752.62	1876.48	35.74	112.23	0.12	0.73	0.03	<0.18
Old access road	4.8	20036.26	122.53	22.45	73.22	0.72	0.27	0.03	<0.18
Alpine meadow	4.9	21771.00	643.39	50.58	1002.27	0.84	0.90	0.07	<0.18

as pH decreases (Lunt and Kofranek 1971). Thus, the low pH (3.7) of the mine spoil material indicates that aluminum may be present in toxic concentrations. Water extractable aluminum was 4.82 ppm in the acid mine spoil whereas in the other sites it was less than 1.0 ppm. Pratt (1966) suggested that for sensitive or nontolerant plant species, concentrations greater than 1.0 ppm in a soil saturation extract may be toxic. Aluminum tolerance in populations has been reported by Cox and Hutchinson (1980) for D. caespitosa populations occupying sites where the total extractable aluminum was 2.8 ppm. The concentration of water extractable aluminum (4.82 ppm) in the acid mine spoil could thus be considered sufficiently stressful to screen out sensitive or nontolerant individuals.

Water extractable zinc in the acid mine spoil material was 19.31 ppm whereas concentrations at the other sites was less than 1 ppm. Zinc, similar to aluminum and copper becomes more soluble at lower soil pH values. Zinc toxicity has been shown to occur in wheat seedlings in single salt concentrations at 10 ppm zinc (Clements and Putnam 1971). Zinc concentrations at 3.0 ppm and 5.0 ppm in nutrient solutions were used to test for tolerance in D. caespitosa populations (Cox and Hutchinson 1980). The same study reported zinc levels of 3.4 ppm in water extract samples from disturbed sites occupied by zinc tolerant D. caespitosa populations. This evidence suggests that 19.31 ppm of water extractable zinc in the acid spoil material creates a root zone environment stressful enough to limit colonization to zinc tolerant individuals.

Analysis of water extractable lead and copper indicate that relatively low levels of these elements were available for plant uptake at each site. The highest concentrations of water extractable lead (1.22 ppm) and copper (0.18 ppm) were found in the acid spoil material. Typically, lead found in the soil is insoluble and unavailable to plants (Cough et al. 1979) and concentrations seldom exceed 1 ppm of soluble lead (Brewer 1966). Cox and Hutchinson (1980) reported lead tolerance in _D. caespitosa_ populations where water extractable lead concentrations were 0.2 ppm. This level of lead wasn't considered stressful enough to cause the development of lead tolerant populations. The authors suggested that lead tolerance was conferred due to a common tolerance mechanism with another metal, possibly copper. It is questionable whether 1.22 ppm lead (acid spoil material) would create a root zone environment stressful enough for disruptive selection to occur. Copper tolerance in _D. caespitosa_ populations for which root zone material had water extractable copper concentrations of 8.4 ppm was reported by Cox and Hutchinson (1980). The acid spoil copper level (0.18 ppm) suggests that only a low, if any, copper tolerance should exist in any of the study populations.

METHODS

Multielement soil/spoil analyses, hydroponic experiments with elevated concentrations of copper, lead, zinc, and aluminum salts, seed germination, and plant survival studies were conducted to evaluate the research hypothesis.

Multielement soil/spoil analyses were conducted to determine if potentially toxic concentrations of heavy metals were present in the soil/spoil material as described previously. Hydroponic experiments were designed to examine if any of the four *D. caespitosa* populations (parent and offspring) exhibited tolerance to aluminum, zinc, copper or lead. Tillers and seedlings grown in nutrient solutions containing elevated levels of these metals were measured for root length. This measurement was used to assess metal tolerance. Germination and survival trials were conducted to examine if metal tolerance conveyed an advantage in germination and initial survival.

Plant Collection

All live plant materials (30 plants/site) were selected on a random basis. No apparent differences in morphology or vigor were noted among the study populations. Actual collection and transportation procedures followed are described by Whalley and Brown (1973). First, the entire plant was removed with minimum disturbance to the roots. The shoots were then clipped to about 10 percent of original height. The plant was then sealed in a zip-lock plastic bag containing a small amount of water and placed in a large cooler for transportation.

At the greenhouse, the soil around roots of each plant was separated and air dried for later use in soil/spoil analysis. The plant roots were gently washed and the plant transplanted in a standard 1:2, sand:peat potting mixture. Plants were grown under greenhouse conditions and maintained on a regular watering, fertilizer, and insect/disease control program.

Seed Collection and Preparation

Seed was collected from all sites during the summer of 1979 and 1980. All seed was dehulled prior to use for ease of handling and selection of nondamaged seed. Percent germination was determined for each population by sowing 250 seed per population in petri dishes containing No. 50 grade filter paper moistened with deionized water. A 14-day germination period was allowed before counts were made. The filter paper was kept saturated at all times during the germination period. All germination trials were conducted under greenhouse conditions with temperatures maintained at approximately 24^{o} C and under natural daylight. Only seedlings with a developed primary root and shoot were counted as germinated. Seed collected during 1979 had an unusually low percent germination (10 percent), therefore, only 1980 seed was used in experiments. Germination rates for the 1980 seed ranged from 72 percent (acid mine spoil population) to 84 percent (waste water intercept canal population).

Soil Analysis

Emission spectrometric analyses for 15 elements (P, K, Ca, Mg, Al, Fe, Na, Mn, Zn, Cu, B, Pb, Ni, Cr, Cd) was performed on the soil/spoil materials and deionized water extracts. All root zone material was air dried and sieved with a 2 mm screen prior to analysis. A slurry paste was used for all pH readings. Spoil/soil water extract was prepared by agitating 10 grams of material with 20 ml of deionized water for 15 minutes. After agitating, the solution was filtered (40 grade filter paper) and the resulting extract analyzed.

The multielement analyses were performed by the Research Analytical Laboratory, University of Minnesota on an ICP emission spectrometer. Nitric perchloric digestion was used for the soil/spoil analysis.

Topsoil and Mine Spoil Seed Germination Experiments

For each population, 520 seeds (260 seeds/replicate) were planted at equally spaced intervals on 6 cm-deep plastic trays containing either alpine topsoil or barren mine spoil material collected from the Climax Company mining area. The spoil material was amended with peat moss in a 1:3, spoil:peatmoss ratio. Each tray was placed and supported in a larger stainless steel tray to facilitate subirrigation. Deionized water was used for subirrigation. Soil moisture content was continually maintained near the saturation point. All trials were conducted during March and February under normal daylight in a controlled temperature greenhouse (approximately 24^{o}C).

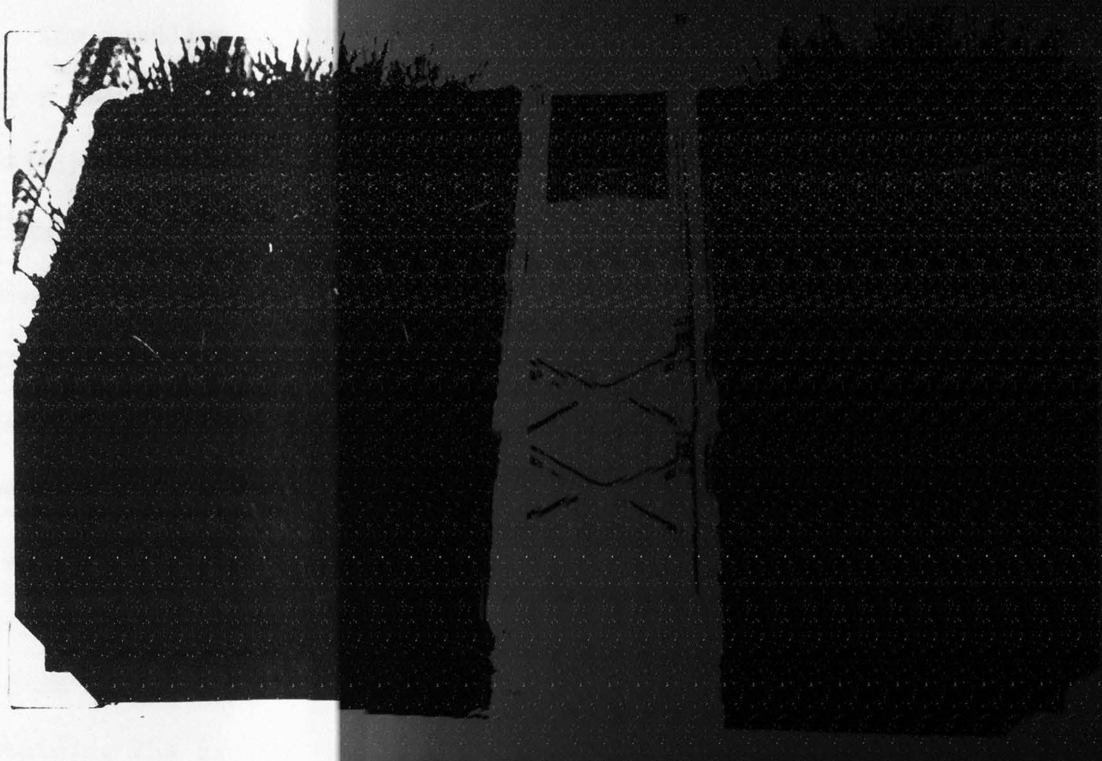

Figure 2. Germination of study populations on [illegible] material.

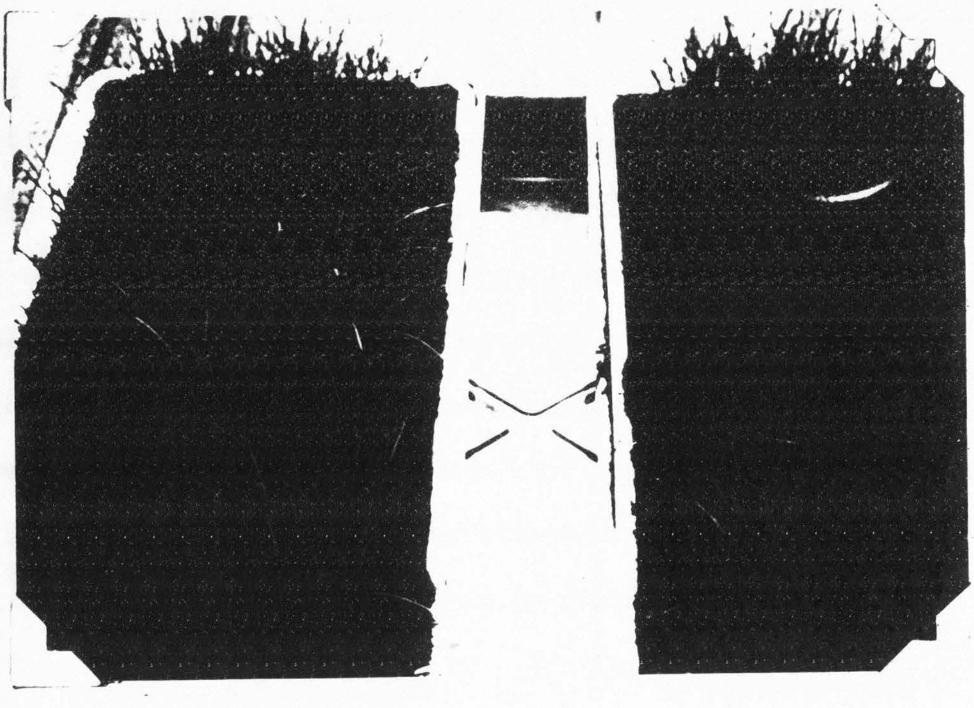

Figure 2. Germination of study populations on alpine topsoil material.

After initial planting, a 14-day germination period was allowed before counts were made. Only seedlings with a developed root and shoot were counted as germinated. Following the initial 14-day germination period, a 30-day growth period was allowed before counts of surviving seedlings were made.

Seedling Hydroponic Experiments

The seedling experiments were conducted as 4 x 4 factorials with four treatment levels for each metal and four D. caespitosa populations. Preliminary experiments were conducted to establish the level of each element necessary to cause significant retardation of root growth for the nondisturbed alpine meadow population, and to determine that root length rather than dry weight was the best measurement of metal tolerance. Preliminary experiments using 44 seedlings per treatment level were conducted to determine that the adequate sample size was 22 seedlings per population per treatment level.

Plexiglass platforms (7.5 cm in radius with 44-3 mm diameter holes on 6 mm centers) were floated in 3.78 liter, 16.5 cm radius containers containing the growth media. Flotation was provided by a 1/4" I.D. tygon tubing ring glued to the bottom of each platform. Each platform supported 44 seedlings or 11 seedlings per population.

Aluminum foil was wrapped on the outside of each container and the top surface of each platform was painted white to minimze light penetration and heat buildup in the solutions. Circular white cardboard rings with centers cut out were placed over each container to reduce light penetration from the interspace between the platform and container wall.

The basic growth media was a modified one-half strength Hoaglund's solution (Hoaglund and Arnon 1950). Preparation was followed as described by Langhans (1978). (For the solution recipe see Appendix A). Chloride salts of zinc, copper, and aluminum were used as amendments. However, lead nitrate was used due to the insolubility of lead chloride. Nutrient solutions were amended with three levels of aluminum (2, 5, and 10 ppm), lead (1, 2, and 4 ppm), zinc (3, 6, and 10 ppm), and copper (0.6, 1.2, and 4 ppm).

Seeds were germinated (using the same procedure as described under seed collection) and following a 14-day germination period, uniform seedlings were selected and planted on a random basis. Initial root length for each seedling was measured and recorded prior to placement in the nutrient solutions.

Following a 15-day growth period, root length was measured for each population of seedlings grown. Data for root length (mm) was then used for all analyses.

Solutions were changed and nutrient containers positionally rotated at two-day intervals. The aluminum solution was monitored daily for pH and adjusted as necessary with HCL and NH_4OH to maintain a pH level of 4.0 ± 0.05. All experiments were conducted separately and in a 80 x 120 x 150 cm reach-in Shearer growth chamber without any barrier between the light source and growth chamber. The irradiance at the top of the plants was 660 $uE \cdot m^{-2} \cdot S^{-1}$ (400-700 nm).

Photoperiod was 10 hours light and 14 hours dark with an abrupt change over period. Containers were placed in a random design in a chamber.

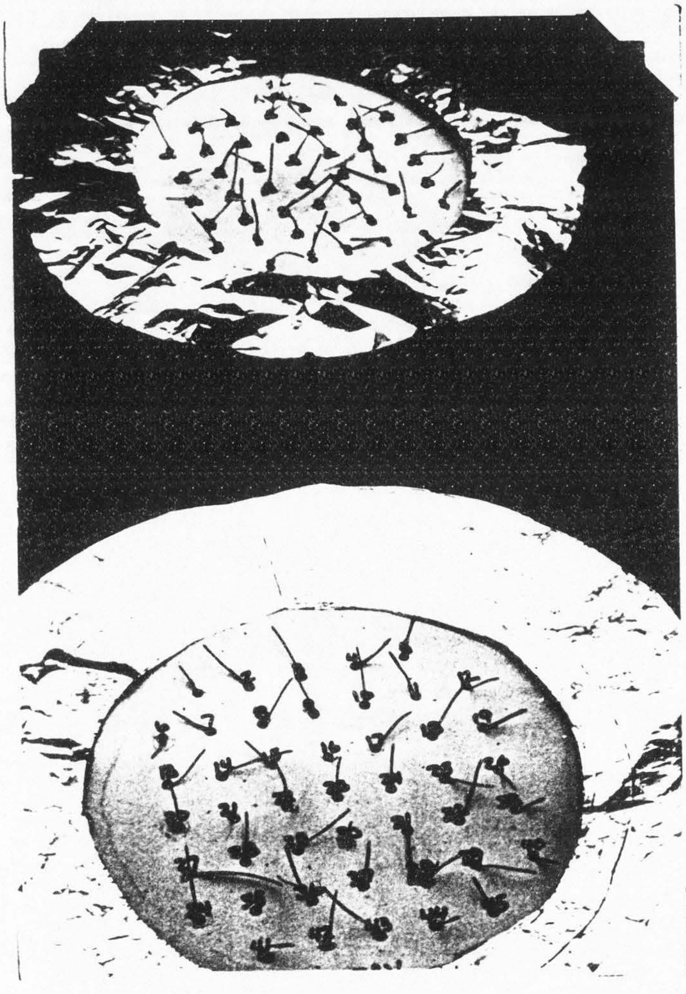

Figure 3. Plastic containers and apparatus utilized in the seedling hydroponic experiment.

Tiller Hydroponic Experiments

The nutrient solution and treatment levels in these experiments were identical to those in the seedling experiments except for zinc, for which the highest concentration was 20 ppm instead of 10 ppm. Two tillers per clone and 20 different clones per population were used for each treatment level.

Because of high plant mortality in the greenhouse, only ten plants were available from the old access road population. This population was not included in the final analysis because of low sample number. The data for the 0 ppm treatment level of aluminum and copper in the tiller experiments was lost due to tape recorder failure so the results do not include this treatment level.

White plexiglass platforms (19 cm x 39 cm with 180-4 mm diameter holes on 1.5 cm center) were supported on 20.7 liter aquaria wrapped in aluminum foil.

Individual tillers were prepared by separating them from the parent plant and then trimming the root to within 1/8" of the root base. Uniform tillers were selected for planting and randomly placed in the platforms. The tillers were grown for a 15-day period before actual root length (newly initiated roots) was measured.

Solutions were changed every four days and fresh media was added as necessary to compensate for any evaporative loss. Aluminum solutions were changed every two days. The pH was checked every other day and adjusted with HCL or NH_4OH as necessary to maintain solution pH at 4.0 ± 0.05.

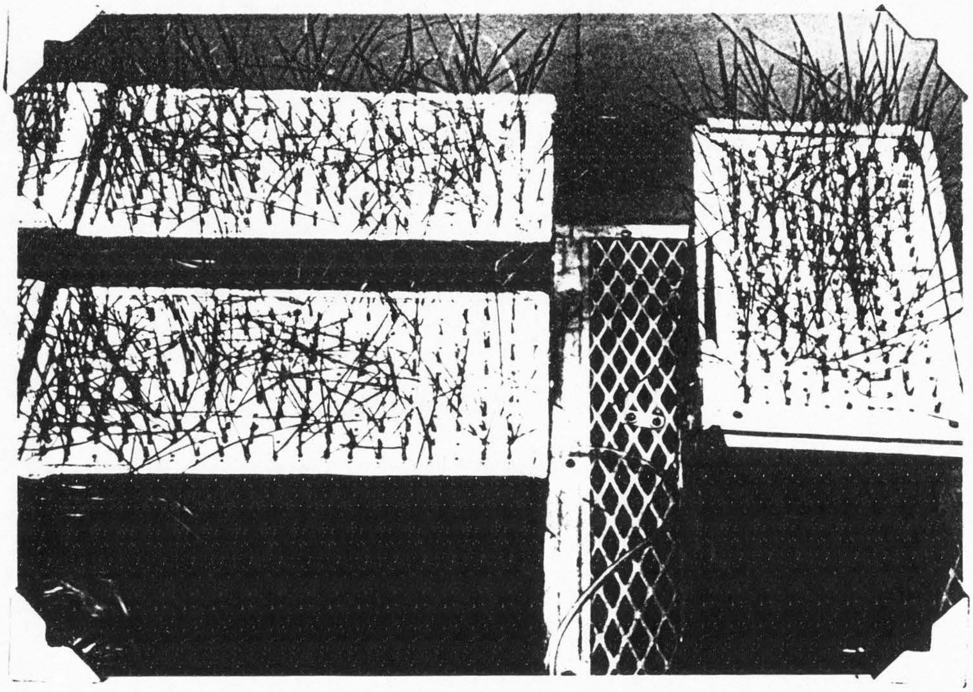

Figure 4. Glass aquaria and apparatus utilized in the tiller hydroponic experiments.

All experiments were conducted in the reach-in chambers, with conditions as reported in the previous section.

Table 2. Germination results on topsoil and mine spoil material of four *D. caespitosa* seed populations collected from the Climax, Colorado mining area. Two replicates of 260 seeds were sown in each material.

	Topsoil			Mine Spoil		
Population	Mean No. Germinated	s.e.	Mean % Germination	Mean No. Germinated	s.e.	Mean % Germination
Acid mine spoil	191	±10.03	72.0	111	± 3.01	43
Waste water intercept canal	210	± 4.01	81.0	159	±16.55	61
Old access road	201	± 0.50	77.0	164	± 6.52	63
Alpine meadow	210	±16.50	81.0	171	± 7.52	66

Table 3. Survival of four *D. caespitosa* populations collected from the Climax, Colorado mining area, when germinated and grown on topsoil and mine spoil material.

	Topsoil			Mine Spoil		
Population	Mean No. Alive	s.e.	% Survival	Mean No. Alive	s.e.	% Survival
Acid mine spoil	190	±11.03	99	84	± 3.50	76
Waste water intercept canal	206	± 5.01	98	137	± 9.53	86
Old access road	199	± 1.50	99	145	± 4.51	88
Alpine meadow	195	± 5.01	93	140	±10.00	82

produces seed. Unfavorable habitat conditions such as low moisture availability, low soil pH, low nutrient values, and high concentrations of certain heavy metals may affect the viability of a population. The acid mine spoil site is a more stressful environment than the other sites because of lower pH values and higher concentrations of potentially toxic heavy metals.

Seedling Hydroponic Experiments

Significant root length differences among populations were observed in the copper ($F_{.05,3/236}$ = 7.51, P <.0001), aluminum ($F_{.05,3/293}$ = 14.96, P <.0001), and zinc ($F_{.05,3/268}$ = 13.66, P <.0001) experiments. No significant differences in the lead experiment ($F_{.05,3/294}$ = 1.39, P = .28) were found (Figure 5). No significant interactions (α = .05) were indicated between populations and treatments.

Multiple mean comparisons were analyzed using Duncan's test (α = .01). The acid mine spoil population showed significantly greater root lengths at each aluminum concentration level than the other populations (Figure 5). Although generally the mean root lengths for the acid mine spoil populations were greater than the other populations at each zinc concentration level, the only significant differences (α = .01) occurred between the acid mine spoil and control (alpine meadow) populations at 0 ppm and 10 ppm zinc (Figure 6). The acid mine population also had greater root lengths in each copper concentration. However, significant differences were observed between the acid mine spoil population and the control population at 0 ppm, 0.6 ppm and 1.2 ppm. No significant

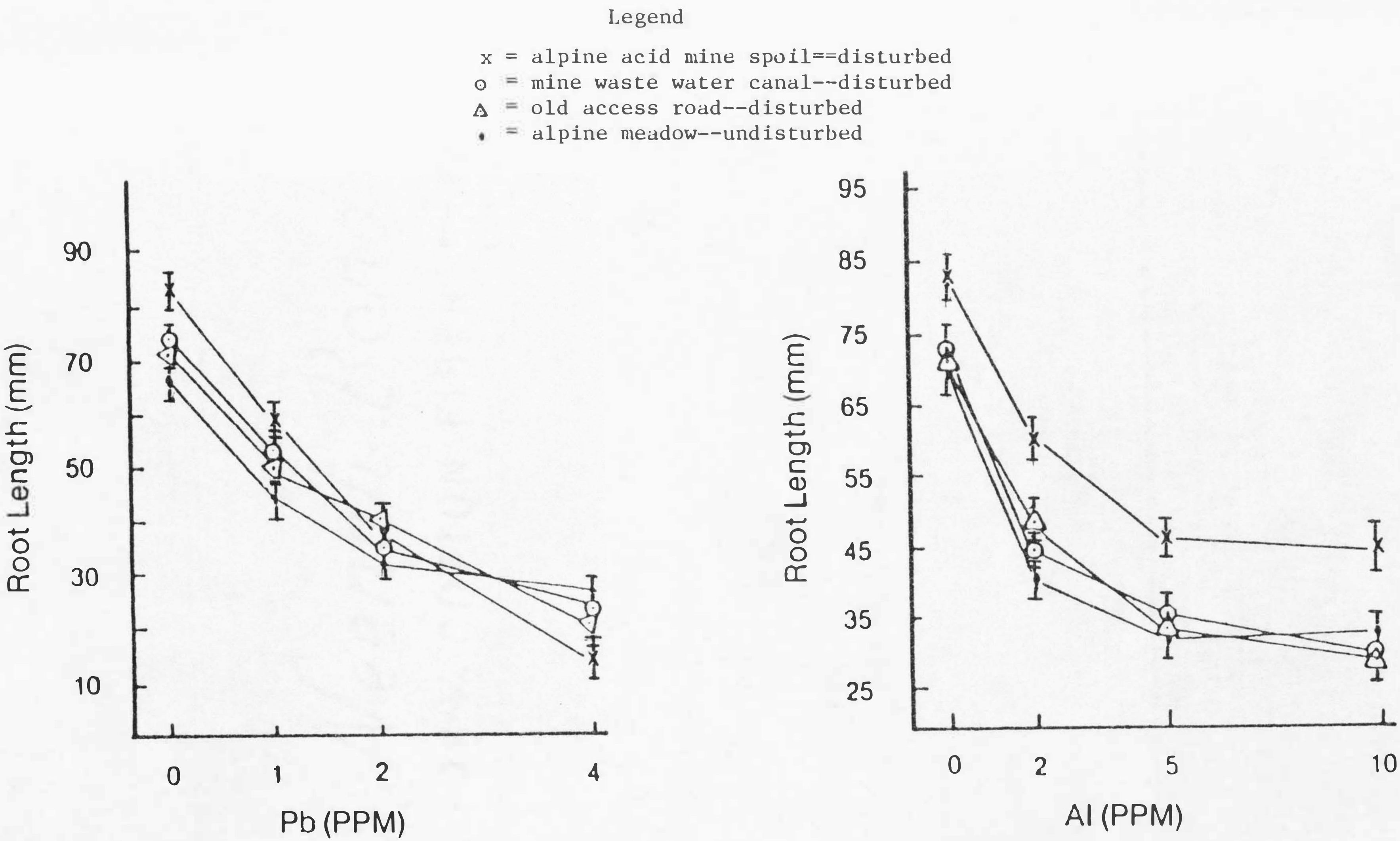

Figure 5. The effect of increasing concentrations of lead and aluminum in nutrient solutions on root length of four D. caespitosa seedling populations from Climax, Colorado. Vertical lines represent (±) one standard error.

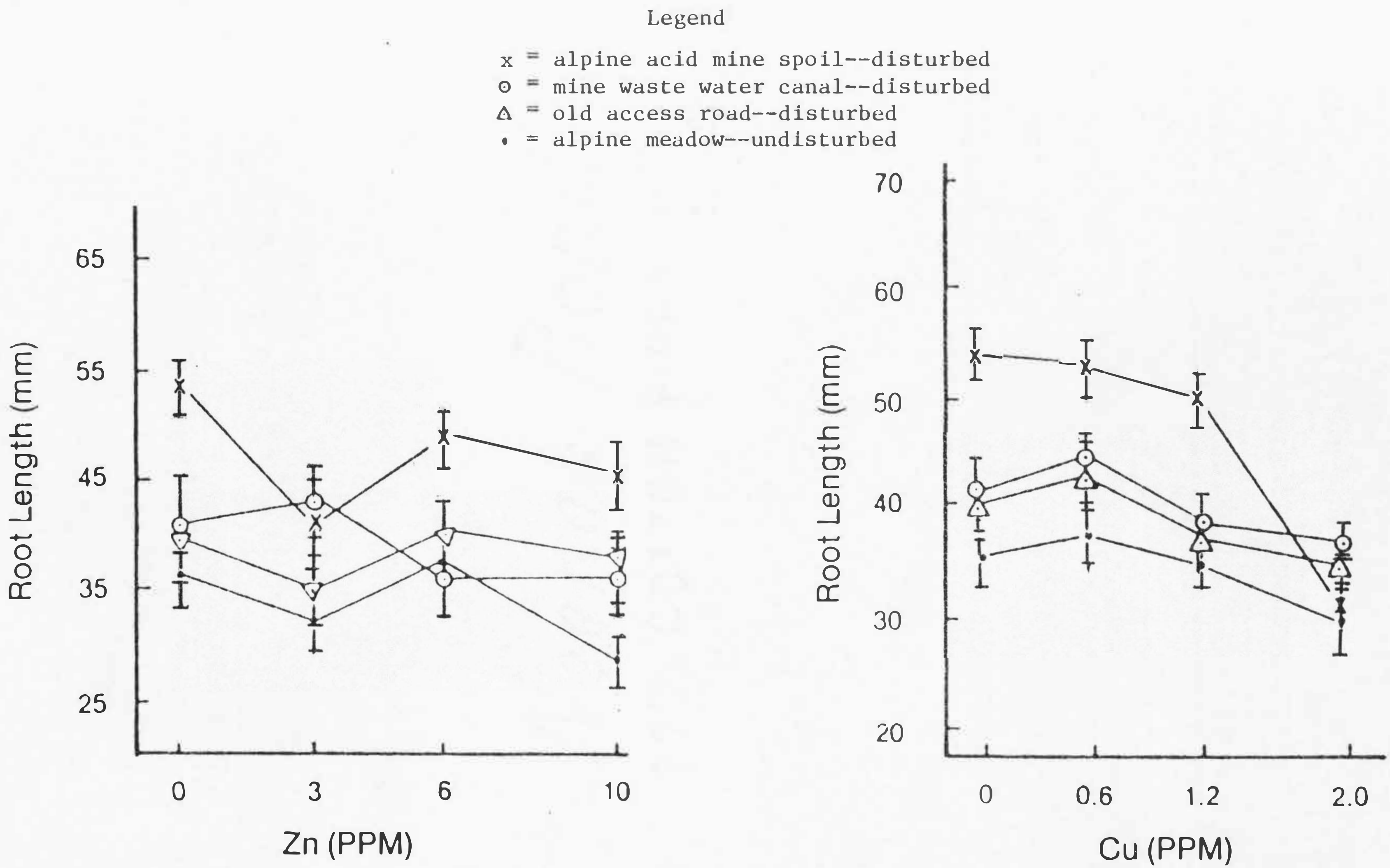

Figure 6. The effect of increasing concentrations of zinc and copper in nutrient solutions on root length of four *D. caespitosa* seedling populations from Climax, Colorado. Vertical lines represent (±) one standard error.

Legend

x = alpine acid mine spoil--disturbed
o = mine waste water canal--disturbed
• = alpine meadow--undisturbed

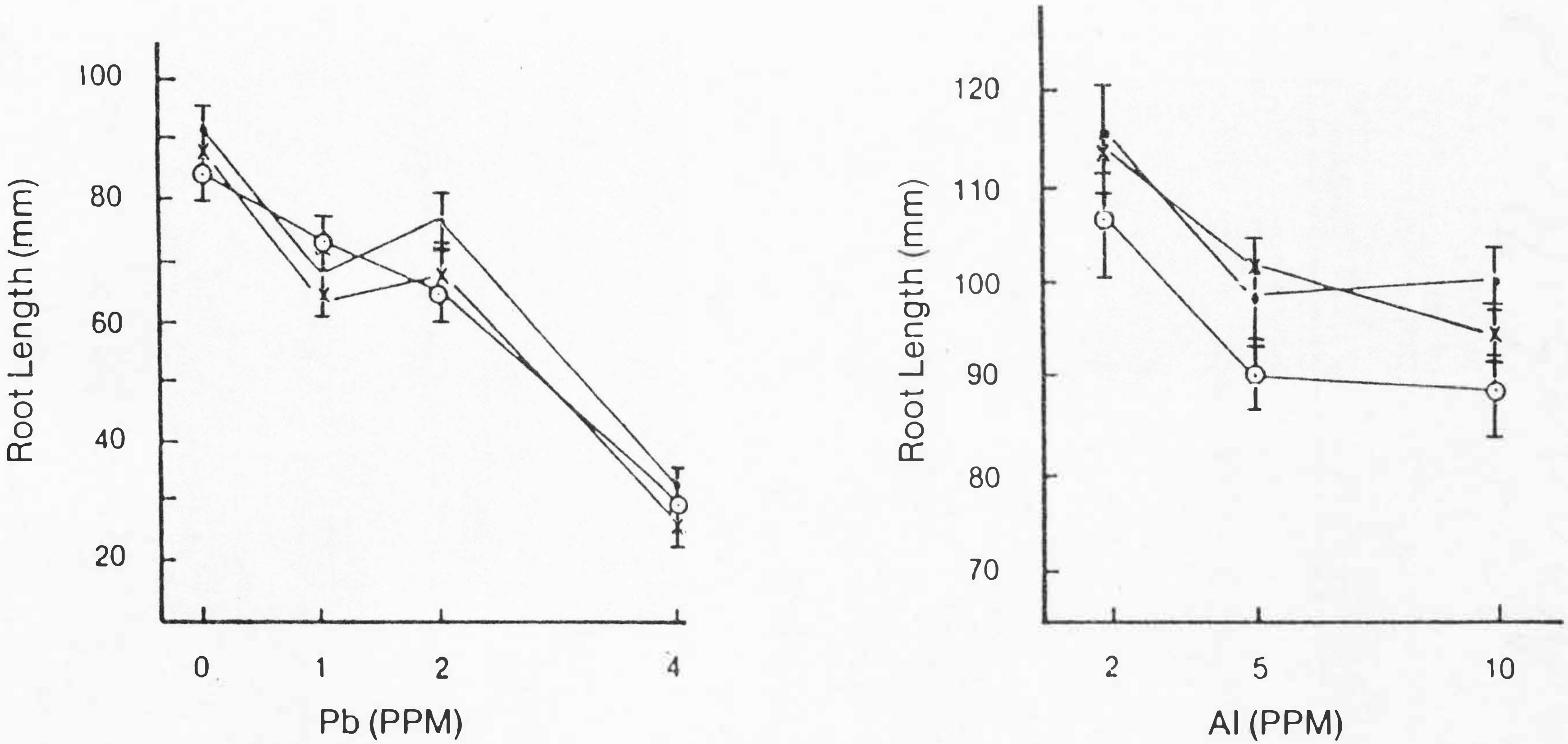

Figure 7. The effect of increasing concentrations of lead and aluminum in nutrient solutions on root length of three D. caespitosa tiller populations from Climax, Colorado. Vertical lines represent (±) one standard error.

Legend

x = alpine acid mind spoil--disturbed
o = mine waste water canal--disturbed
• = alpine meadow--undisturbed

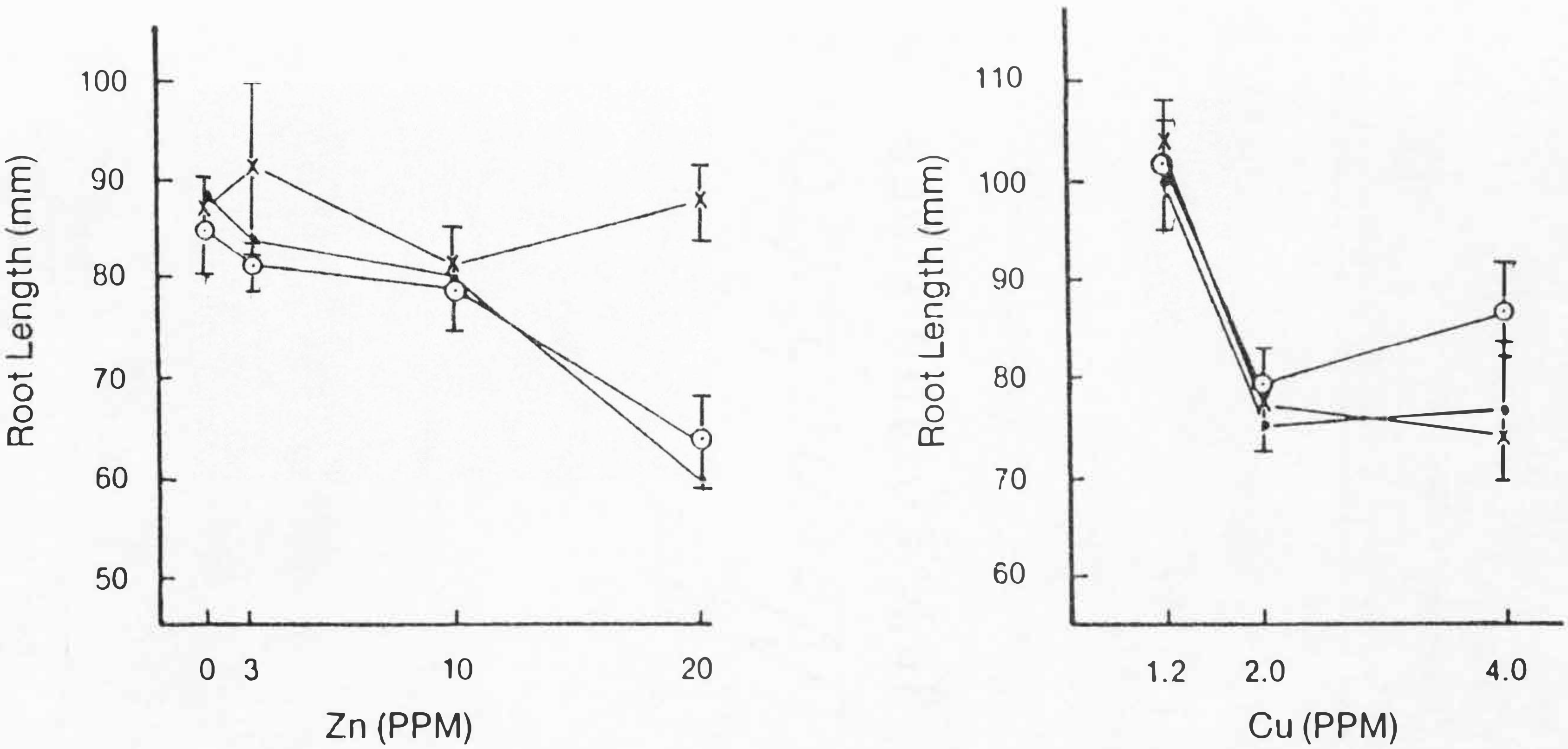

Figure 8. The effect of increasing concentrations of zinc and copper in nutrient solutions on root length of three D. caespitosa tiller populations from Climax, Colorado. Vertical bars represent (±) one standard error.

differences were found at the highest copper concentrations (2.0 ppm) (Figure 6).

Tiller Hydroponic Experiments

The only significant differences among populations occurred in the zinc experiments ($F_{.05,3/214} = 4.29$, $P = .0148$). Significant differences were not indicated in the copper ($F_{.05,2/152} = 1.10$, $P = .3334$), lead ($F_{.05,2/222} = 1.82$, $P = .1633$), or aluminum ($F_{.05,2/163} = 2.67$, $P = .0718$) experiments (Figures 7 and 8). No significant interactions ($\alpha = .05$) were indicated between populations and treatments.

Mean root lengths for the acid mine spoil population grown at the highest zinc concentration (20 ppm Zn) were significantly greater (Duncan's test, $\alpha = .01$) than either the waste water intercept canal population or the control population (Figure 8).

DISCUSSION

The *D. caespitosa* population colonizing the acid mine spoil site was observed to have significantly greater root lengths in the aluminum, zinc and copper experiments than the other populations. Plant populations colonizing mine disturbances have been considered the results of natural selection and of natural variation within the plant species (Jain and Bradshaw 1966). The principle involved in the development of ecotypic communities is that selection forces such as high concentrations of heavy metals or low quantities of available plant nutrients limit the colonization of the site to genotypes that are adapted to the particular high stress environment. These individuals then enhance the developing ecotypic community by providing a genetic composition tolerant to the specific site conditions.

The development of an ecotypic community through the interaction of disruptive selection and natural variation is supported by the observation that tolerant individuals are present in the non-tolerant alpine meadow (control) population. This suggests that a broad range of metal tolerances exists within *D. caespitosa* and that these tolerant individuals would be available for colonization of disturbed sites. The presence of natural selection is supported in that the acid mine spoil population is tolerant to the same heavy metals found in high concentrations in the spoil profile.

It was observed that the acid mine spoil population had greater root lengths at 0 ppm treatment level in the seedling experiments than the other populations. Furthermore, in the zinc seedling experiments where treatment levels were only significant at F prob. = .067 (Appendix D), the acid mine spoil population generally had greater root lengths than the other populations. This increased root length may be indicative that a complex of adaptations is occurring within the population. This complex of adaptations would be the result of numerous site selection pressures.

The rate of development of ecotypic communities colonizing disturbed mine sites appears to be a function of the frequency that metal tolerant individuals appear in the population and the severity of selection pressures at the site. High stress selection pressures coupled with low occurrences of tolerant individuals could preclude the site from being colonized except over extended time periods. However, the colonization of disturbed mine sites may occur in relatively short time periods given favorable site conditions for plant establishment and a sufficient number of fit individuals to colonize the site. The acid mine spoil disturbance at Climax has been colonized within the last 40 years by tolerant genotypes of D. caespitosa.

Successful seed germination and initial development of the root system and other vital life support systems may be dependent upon the individual's vigor and tolerance to high stress conditions. This short time period may be the most critical stage of development with respect to the plants exposure to high stress selection pressures. The nontolerant seedlings were observed to be more sensitive to lower concentrations of aluminum, copper, and zinc than the nontolerant tillers.

Stressful environments such as alpine acid mine spoils may seriously impair the parent plant's ability to produce high vigor offspring. Low nutrient conditions combined with harsh site conditions help create an environment in which seed production may be marginal. It is not unusual for alpine D. caespitosa communities to experience marginal seed production as is evident in 1979 when the germination rates for the D. caespitosa populations around Climax were less than 10 percent. The low percent germination of the acid mine spoil population compared to the other populations when planted in spoil material may be due to the adverse conditions under which the seed were produced.

It has generally been held that heavy metal tolerances are specific in response to the ions present in the soil or spoil material for which tolerance is observed and that the tolerance for one heavy metal does not confer tolerance for another heavy metal (Cox and Hutchinson 1980). Heavy metal tolerances then, are considered separate and selected in response to specific site conditions (Antonovics et al. 1971). Recently, Cox and Hutchinson (1980) have presented evidence and reviewed other researchers results such as Gregory and Bradshaw (1965) and Walley et al. (1974), that finds exception to this theory. Cox and Hutchinson (1980) observed elevated copper, nickel, lead, and zinc tolerances in the D. caespitosa populations (referred to as Coninston) growing around a smelting complex near Sudbury, Ontario although no elevated levels of zinc or lead were present in the soil. From this they suggest that "...some common physiological mechanism of tolerance for groups of metals, rather than the total independence of tolerance mechanisms for each metal (p. 644)", as a possible explanation for the observed multiple metal tolerances.

If, as Cox and Hutchinson (1980) suggested, a common physiological mechanism is responsible for group metal tolerances, then similar tolerances might be expected to occur in populations of D. caespitosa colonizing sites with similar disturbances. The Coninston population (Cox and Hutchinson 1980) is at a lower elevation and more northern latitude than the Climax acid mine spoil population. However, both populations have similar soil pH values (3.5-3.9) and water extractable lead (0.2-1.22 ppm). Water extractable copper in the Coninston root zone soils was 8.5 ppm which is substantially higher than the acid mine spoil root zone material at Climax (0.18 ppm).

Elevated tolerances for copper, zinc and aluminum were indicated in both the Coninston population (Cox and Hutchinson 1980) and Climax populations, however, lead tolerance was only observed in the Coninston population. In view that only very low levels of lead were available for plant uptake at the Coninston site, Cox and Hutchinson (1980) suggested that the lead tolerance was conferred by another metal tolerance, probably copper. They also point out that lead and zinc conferred tolerances have been associated with copper tolerances as shown by Allen and Shepard (1971) in a study investigating copper tolerant Mimulus guttalus.

The lack of a conferred lead tolerance in the Climax acid mine spoil population may be due to a low copper tolerance in the population. Copper tolerance in the acid mine spoil population was only observed in the seedling experiments and not in the tiller experiments.

The contrasting circumstances between the Coninston population, with a significant copper tolerance ($P<.001$) in tiller material, high levels of available copper (8.5 ppm) for plant uptake, and a conferred lead tolerance, and between the acid mine spoil population lends support to the idea that lead tolerance may be conferred through the development of copper tolerance. Until the physiological mechanisms of lead and copper tolerances are more adequately understood, it is only speculative to suggest what process is responsible for seemingly conferred metal tolerances.

In an excellent review on the genetic potentials of adapted ecotypes for reclamation of problem soils, Humphreys and Bradshaw (1976) show that the identification and use of existing, superior, tolerant ecotypes should be a high priority in mine reclamation. They stress that tolerant ecotypes colonizing mine disturbances represent an "integrated complex of adaptations" necessary for the colonization of the disturbed site. Multiple selection pressures such as low soil fertility, high soil surface temperatures, potential drought conditions, and heavy metal concentrations form the complex of selection forces present in the acid mine spoil environment.

These natural ecotypes adapted to a wide range of environmental stresses should be able to be utilized as commercial cultivars with only minimum, if any, artificial selection (Humphreys and Bradshaw 1976). Tolerant ecotypes colonizing mine spoils at Climax, Colorado could be utilized in a seed production program to provide adapted ecotypes to successfully revegetate other high altitude acid mine spoil sites.

As mining activity for the extraction of precious metals and energy related resources increase, new and unique environments such as large tailing dams are created which are often harsh for the establishment of plant life. Natural selection of tolerant ecotypes could be aided and the time requirement greatly reduced by the initiation of controlled selection programs on these new environments.

SUMMARY AND CONCLUSIONS

D. caesp*itosa* populations appear to be mainly responsible for the colonization of acid mine disturbances near Climax, Colorado. The possibility that this colonization was a result of genetic variation within D. caespitosa wherein individuals occupying the disturbed site were more fit to the particular harsh site led to the development of the research hypothesis: genetic variation exists within D. caespitosa and this variation has predisposed certain D. caespitosa populations to become successfully established on alpine mine spoils. Three disturbed populations and one undisturbed alpine meadow population (control) from Climax were examined for tolerances to aluminum, zinc, copper and lead. Hydroponic experiments were conducted wherein tillers and seedlings were grown in solutions containing elevated levels of these metals. Root length was used as the measurement of tolerance.

Spoil-soil material taken from the root zone of experimental plants was analyzed for multimetal composition and pH, in order to assess the root zone environment. In addition, germination and survival tests were conducted to examine if site specific tolerance conveyed an advantage in germination and initital survival on acid mine spoils.

Tolerances to aluminum, zinc and copper were observed in the D. caespitosa population colonizing the acid mine spoil site. Copper and aluminum tolerances were observed only in the seedling populations. Tolerance was observed for the same heavy metals found in high concentrations in the spoil profile. No conclusive results were obtained to indicate the role that site specific tolerance has on germination and initial seedling survival.

Genetic variation within D. caespitosa and the development of an ecotypic population on the acid mine spoil site is supported in that parents and offspring had a significantly higher number of individuals capable of initiating greater root growth under metal stress conditions than the other three populations. This increased fitness for metal tolerance is fixed and is maintained in propagated material and in seeds.

The fact that significant metal tolerance was observed in the parent and offspring of only the acid mine spoil population, and the fact that tolerant individuals were observed in the control population, supports the research hypothesis. The evidence suggests that the acid mine spoil population has a significant advantage over the control population for colonization of acid mine spoil disturbances. This tolerant population is worthy of further research to examine the potential of its use in reclamation of high altitude acid mine disturbances.

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APPENDIXES

Appendix A

Stock Concentrates Recipe of Nutrient Solution Used in Tiller and Seedling Hydroponic Experiments

Stock Concentrates Recipe
(From Langhans 1978)

Stock concentrate 1	Amount per liter
Potassium nitrate (KNO_3)	13.46 g
Potassium di-hydrogen phosphate (KH_2PO_4)	2.61 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	13.20 g
Sodium chloride (NaCl)	.005 g
Miconutrient concentrate	100 ml
Add water to make one liter and mix thoroughly to dissolve all salts.	

Micronutrient concentration	Grams per liter
Boric acid (H_3BO_3)	0.80
Manganese sulfate ($MnSO_4 \cdot H_2O$)	1.518
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.219
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	0.078
Molybdic acid (MoO_3)(85%)	0.020
Add water to make one liter and mix thoroughly to dissolve all salts.	

Stock concentrate 2	Grams per liter
Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$	59.17
Sequesterene 330 Fe	5.0

Approximate concentration of nutrients in final solution
(200:1 dilution of stock concentrates)

	NO_3^--N	$H_2PO_4^-$-P	K+	Na+	Ca++	Mg++	SO_4^--S
Atomic weight	14.01	30.97	39.10	22.99	40.08	24.31	32.06
ppm	49.44	3	30	.01	50.00	6.6	8.7

	Cl^-	Fe^{++}	B^{+++}	Mn^{++}	Zn^{++}	Cu^{++}	MoO_4-Mo
Atomic weight	35.45	55.85	10.81	54.94	65.37	63.54	95.94
ppm	.01	1	.07	.13	.025	0.01	.005

Amount per liter of ingredients were adjusted downward from original suggested level by Langhans 1978.

Appendix B

Soil/Spoil Water Extractable Metal Analysis

Soil/spoil water extractable analysis of root zone material from each site. Each value is the mean of six samples.

	A*		B*		C*		D*	
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$	s.e.
P	0.81	± 0.25	0.40	± 0.04	<0.28		0.80	± 0.16
K	14.66	± 2.83	9.7	± 1.08	7.20	± 1.07	10.52	± 1.19
Ca	19.55	± 2.54	95.64	± 14.29	44.03	± 6.34	23.56	± 3.25
Mg	3.11	± 0.47	20.35	± 2.61	9.43	± 1.78	4.63	± 0.73
Al	0.84	± 0.49	3.99	± 0.65	0.12	± 0.04	0.72	± 0.20
Fe	0.59	± 0.25	3.45	± 0.77	<0.01		0.65	± 0.12
Mn	0.81	± 0.38	12.20	± 2.23	1.51	± 0.54	0.49	± 0.09
Zn	0.90	± 0.27	19.31	± 3.13	0.73	± 0.23	0.27	± 0.08
Cu	0.07	± 0.01	0.18	± 0.01	0.03	± .000	0.03	± 0.01
B	0.17	± 0.02	0.12	± 0.01	0.12	± 0.01	0.16	± 0.03
Pb	<0.18		1.22	± 0.23	<0.18		<0.18	
Ni	<0.06		<0.06		<0.06		<0.06	
Cr	<0.01		0.02	± .000	0.01	± .000	<0.01	
Cd	0.03	± 0.01	0.25	± .06	0.03	± 0.01	0.01	± .01
Na	12.63	± 1.03	11.74	± 0.85	11.44	± 0.58	10.95	± 0.93

*Key: A = alpine meadow
B = acid mine spoil
C = waste water intercept canal
D = old access road

Appendix C

Soil/Spoil Nitric Perchloric Digestion Analysis

Total PPM

Soil/spoil nitric perchloric digestion analysis of root zone material from each site. Each value is the mean of six samples.

	A*		B*		C*		D*	
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$	s.e.
P	735	± 36	950	± 50	571	± 40	793	± 65
K	6174	± 109	4991	± 294	4168	± 655	7180	± 618
Ca	1739	± 159	1779	± 143	2458	± 205	1758	± 193
Mg	3280	± 72	3823	± 240	3276	± 460	6266	± 731
Al	21771	± 990	21993	± 1898	13752	± 2095	24520	± 2506
Fe	18145	± 662	25082	± 1154	19574	± 1513	19917	± 1756
Mn	755	± 82	462	± 55	609	± 69	325	± 29
Zn	644	± 28	664	± 41	1876	± 414	123	± 12
Cu	51	± 6.7	113	± 9	36	± 3.5	23	± 1.3
B	0.27		0.27		0.27		0.27	
Pb	1002	± 177	4040	± 1862	112	± 7	61	± 9.2
Ni	10.2	± 0.53	13.7	± 0.77	10.6	± 0.90	18	± 1.9
Cr	10.4	± 0.63	14.5	± 0.70	6.6	± 0.26	18	± 2.2
Cd	5.1	± 0.48	6.7	± 1.11	7.6	± 1.35	1.6	± 0.16
Na	220	± 14	245	± 14	216	± 17	218	± 9.0

*Key: A = alpine meadow
B = acid mine spoil
C = waste water intercept canal
D = old access road

Appendix D

Analysis of Variance Table D. caespitosa

Seedling Populations - Zinc

Analysis of variance table (two way unbalanced) of experimental results of four D. caespitosa seedling populations grown hydroponically in increasing concentrations of zinc.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	283	61,734.5775			
Treatment	3	1,334.9070	444.9690		
Population (Adj)		7,772.4908	2,590.8303	13.6607	<.0001
Population	3	7,732.8662	2,577.6221		
Treatment (Adj)	3	1,374.5316	458.1772	2.4158	.06683
Int	9	1,799.6244	199.9583	1.05	
Error	268	50,827.5554	189.6551		

Appendix E

Analysis of Variance Table D. caespitosa

Seedling Populations - Copper

Analysis of variance table (two way unbalanced) of experimental results of four *D. caespitosa* seedling populations grown hydroponically in increasing concentrations of copper.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	351	62,499.6627			
Treatment	3	4,815.7323	1605.2441		
Population (Adj)	3	4,835.7395	1611.9132	7.5121	.00008
Population	3	4,923.0646	1641.0215		
Treatment (Adj)	3	4,728.4071	1576.1357	7.3454	.00010
Int	9	2,208.4810	245.3868	1.14	
Error	236	50,639.7099	214.5750		

Appendix F

Analysis of Variance Table D. caespitosa

Seedling Populations - Aluminum

Analysis of variance table (two way unbalanced) of experimental results of four D. *caespitosa* seedling populations grown hydroponically in increasing concentrations of aluminum.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	308	163,376.62			
Treatment	3	81,685.01	27,228.34		
Population (Adj)	3	10,717.45	3,572.48	14.96	<.0001
Population	3	10,003.13	3,334.38		
Treatment (Adj)	3	82,399.33	27,466.44	115.05	<.0001
Int	9	1,023.18	113.69	0.48	
Error	293	69,950.98	238.74		

Appendix G

Analysis of Variance Table D. caespitosa

Seedling Populations - Lead

Analysis of variance table (two way unbalanced) of experimental results of four D. caespitosa seedling populations grown hydroponically in increasing concentrations of lead.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	309	220,889.2548			
Treatment	3	115,300.1433	38,433.3811	114.3044	<.0001
Population	3	1,306.8876	435.6292	1.2956	.27609
Int	9	5,566.7955	618.5328	1.83	
Error	294	98,853.6988	336.2371		

Appendix H

Analysis of Variance Table D. caespitosa

Tiller Populations - Zinc

Analysis of variance table (two way unbalanced) of experimental results of three *D. caespitosa* tiller populations grown hydronically in increasing concentrations of zinc.

Analysis of Variance (Method of Weighted Squares of Mean)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	225	125,202.3761			
Treatment	3	9,133.5313	3,044.5104	6.1035	.00053
Population	2	4,282.6688	2,141.3344	4.2928	.01486
Int	6	5,322.0263	887.0044	1.78	
Error	214	106,746.4726	498.8153		

Appendix I

Analysis of Variance Table D. caespitosa

Tiller Populations - Copper

Analysis of variance table (two way unbalanced) of experimental results of three D. caespitosa tiller populations grown hydroponically in increasing concentrations of copper.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	160	88,814.3199			
Treatment	2	21,552.5873	10,766.2936		
Population (Adj)	2	951.2381	475.6190	1.1032	.33444
Population	2	1,029.5241	514.7621		
Treatment (Adj)	2	21,474.3012	10,737.1506	24.9054	<.0001
Int	4	780.7437	195.1859	0.45	
Error	152	65,529.7508	431.1168		

Appendix J

Analysis of Variance Table D. caespitosa

Tiller Populations - Aluminum

Analysis of variance table (two way unblanced) of experimental results of three D. *caespitosa* tiller populations grown hydroponically in increasing concentrations of aluminum.

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	171	112,303.5116			
Treatment	2	11,409.3367	5,704.6683		
Population (Adj)	2	3,190.3926	1,595.1963	2.6761	.07185
Population	2	3,107.7045	1,553.0523		
Treatment (Adj)	2	11,492.0247	5,746.0124	9.6395	.00011
Int	4	541.4499	135.3625	0.22	
Error	163	97,162.3325	596.0879		

Appendix K

Analysis of Variance Table D. caespitosa

Tiller Populations - Lead

Analysis of variance table (two way unbalanced) of experimental results of three *D. caespitosa* tiller populations grown hydroponically in increasing concentrations of lead.

Analysis of Variance (Method of Fitting Constants)

Source	Df	SS	MS	F-Ratio	F-Prob.
Total	233	179,502.8846			
Treatment	3	105,513.4048	35,171.1349		
Population (Adj)	2	1,172.6805	586.3402	1.8270	.16330
Population	2	1,066.9669	533.4834		
Treatment (Adj)	3	105,619.1184	35,206.3728	109.7031	<.0001
Int	6	1,571.6349	261.9391	0.82	
Error	222	71,245.1645	320.9242		

VITA

PAUL ELLSWORTH PRATT

Candidate for the Degree of

Master of Science

Thesis: An Evaluation of *Deschampsia caespitosa* (L.) Beau Populations for Metal Tolerances and Ecotypic Variation at Climax, Colorado

Major Field: Range Science

Biographic Information:

Personal Data: Born at Elizabethtown, New York, August 19, 1951, son of Charles and Delia Pratt.

Education: Graduated at Southeast High School, Greensboro, N. C. in 1969. Attended North Carolina State University, Raleigh, N.C. (1969-1971). Received Bachelor of Science in Environmental Studies from Utah State University in 1979; completed requirements for the Master of Science Degree in Range Science from Utah State University in 1982.

Professional Experience: Range research technician with USFS, Intermountain Forest and Range Experiment Station, Logan, Utah, 1975-1981. Graduate Research Assistant in Range Science Department, Utah State University, Logan, Utah, 1980-1982. Land specialist with the State of Utah, Division of State Lands and Forestry, Moab, Utah, 1981-present.