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THE ECOLOGICAL AND PHYSIOLOGICAL IMPACT OF SO_2 ON PLANTS, PARTICULARLY MOSSES

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "THE ECOLOGICAL AND PHYSIOLOGICAL IMPACT OF SO_2 ON PLANTS, PARTICULARLY MOSSES" submitted by WILLIAM E. WINNER in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The ecological impact of SO_2 emitted from a natural gas refinery was measured. The vegetation in ecologically similar forest stands dominated by white spruce was assessed by using standard synecological techniques which showed both an angle-dependent and a distance-dependent gradient of air pollution stress. In heavily stressed stands, terrestrial mosses appeared to be more sensitive to SO_2 than were vascular plants found in the understory. As the stress increased the moss carpet deteriorated first in depth and then in coverage. The invasion of weeds, as well as the trend for a few subordinate moss species to become more prominent, was indicated by local increases in community diversity; however, this change was not reflected by the Index of Atmospheric Purity.

Stable sulfur isotope analysis showed that mosses contained a greater relative abundance of sulfur originating from the refinery than did conifer needles. Also, the sulfur isotope ratios of sulfur extracted from mosses reflected the gradients of ecological perturbation. Experiments which involved the transfer of potted plants verified the potential for the isotopic composition of plants to change when exposed to SO_2. Isotopic analysis

further showed that Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis, the dominant species of the white spruce stands, accumulated SO_2 at similar rates.

Although the feather mosses had similar rates of SO_2 uptake, the field studies indicated that Pleurozium schreberi was the species most sensitive to increasing SO_2 stress. The feather mosses were fumigated in controlled laboratory conditions while their photosynthetic and respiratory rates were monitored. Following exposure to several experimental conditions involving SO_2 application, Pleurozium schreberi was the species which showed the greatest immediate impairment to photosynthesis. SO_2 did not appear to influence respiration, however. These species also showed the same trends when water stress was increased. The feather mosses can survive cycles of desiccation and rehydration, and I have considered relationships between increasing SO_2 stress and water stress in the ecological and physiological contexts.

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During the course of these studies, I have become deeply indebted to my wife, Janet, who has assisted with field studies, laboratory experiments, and the preparation of manuscripts.

DEDICATION

I dedicate this thesis to the members of my family. They have continually encouraged scholorship, volunteered their resources whenever necessary, and have always maintained a broad, firm base of support which stemmed from genuine interest and concern.

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PREFACE

The Magnitude and Expense of SO_2 Pollution

SO_2, NO_x, CO, and hydrocarbons are all air pollutants, but SO_2 seems to be the only pollutant for which man is almost totally responsible (Whelpdale and Munn 1976). Volcanoes provide a natural source of SO_2, but in quantities which are negligible (Whelpdale and Munn 1976).

Historically, there has never been so much SO_2 emission as there is today. On a global basis, there has been an exponential increase in the rate of SO_2 emissions from 5 million tons in 1860 to more than 150 million tons in 1970 (Bibbero and Young 1974). Of this latter value, 96% is produced in the Northern hemisphere and 24% is produced in the United States alone (Bibbero and Young 1974). SO_2 emission rates are projected to be more than 300 million tons annually by the end of this century.

The dispersion of SO_2 is influenced by meteorological and geographical factors which determine the concentrations of SO_2 which eventually reach ground level. Although ambient concentrations of this gas are from 0-0.2 ppb (Whelpdale and Munn 1976), concentrations of over 1 ppm often are recorded in

cities (Seinfeld 1975). From December 1-5, 1930, the Meuse Valley in Belgium had SO_2 concentrations which ranged from 9.6 ppm to 38.4 ppm (Seinfeld 1975). This is the most severe SO_2 pollution episode which I have found to be documented.

SO_2 damages cost the United States an estimated $14 billion in 1977. All other air pollutants combined were estimated to have caused about $10 billion in damages during that year (Bibbero and Young 1974). In 1968, when the damage from SO_2 was estimated at $8 billion, the impact upon vegetation alone was estimated at $13 million. The remaining damages from SO_2 are attributed to human health, property, and materials (Bibbero and Young 1974). Since the costs of SO_2 damages increased between 1968 and 1977, it seems reasonable to predict that as emissions increase in the future, so will the expense.

The Ecological Impact

The observation that air pollutants influence the distribution and appearance of plants was made in the late 1800's. In 1892 Arnold observed that certain moss species seemed to be disappearing from the city of Munich. Until 1958 (Barkman) the paucity of cryptogams around industrial areas received only casual mention in vegetational studies. Since the middle 1960's certain epiphytes, including moss

species, have been shown to be absent from the urban centers of Newcastle (Gilbert 1965, 1968), New York (Brodo 1967), Stockholm (Skye 1968), and Montreal (LeBlanc and DeSloover 1970). Additionally, field studies around industrial sites far from cities reveal depauperate cryptogam populations (Rao and LeBlanc 1967; Hoffman 1974). The absence of mosses and lichens from these areas has strongly implicated air pollutants as the agents deleterious to vegetation. Rydzak (1958) and Geiger (1965) have postulated that changes in relative humidity and higher temperatures are the reasons for industrial-caused "lichen deserts," but LeBlanc and Rao (1973) later refuted this argument.

Field studies show that the distribution of air pollution bioindicators can be correlated with air pollution concentrations, and that they further reflect a concentration gradient of emissions downwind from a pollution source (Hawksworth 1973). The decline in diversity of cryptogam communities around industrial sites that recently have become operative shows that there is a relationship between the absence of sensitive plants and industrial activity (Coker 1967; Barkman 1969; Gilbert 1971a; LeBlanc and Rao 1973). Synecological analyses have shown some moss species to be as sensitive to air pollution as lichens are (Inglis and Hill 1974) and that they are equally useful as bioindicators for mapping the extent of air pollution

damage to vegetation (Barkman 1969; Gilbert 1971b). Although more extensive field work has been done with epiphytic cryptogam species, some terricolous and saxicolous moss species have been described as clearly being air pollution-sensitive (Gilbert 1970a, 1970b; Nash and Nash 1974).

Perhaps the most glaring and persistent flaw in associating the distribution of cryptogam bioindicators with pollution sources is the failure to describe the higher plant vegetation types occurring within the study area. Changes in the vegetation type of higher plants around a pollution source must surely result in an alteration in the composition of cryptogam communities, yet no one has ever used synecological analysis to document vegetational homogeneity.

Physiological Injury

In some circumstances, air pollution can cause the death of all exposed vegetation (Gordon and Gorham 1963; Hawksworth 1973). However, acute damage may not be the most significant since this is usually restricted to a relatively small area of vegetation adjacent to the pollution source. Vegetation farther from the source may appear normal but may actually be suffering from covert forms of physiological damage. Such injury may lower the metabolic capacity of plant communities as well as alter the resistance to cold

and water stress of particular species (Linzon 1971; Bleasdale 1973). Consequently, definition of the physiological effects of air pollution is necessary in order to determine the cause of death to plants and to monitor its actual impact upon the phytocoenosis.

In the laboratory, where experimental conditions are carefully controlled, research shows oxidized forms of sulfur to be very toxic to plants (Coker 1967; Dässler and Ranft 1969; Bortitz and Ranft 1972; Nash and Nash 1974). Biological toxicity of SO_2, which is the oxidized form of sulfur most dangerous to living systems (Williamson 1973), has been shown to increase with relative humidity (Couey and Uota 1961), temperature (Couey 1965), duration of exposure (Bleasdale 1973), and decrease in pH (Inglis and Hill 1974). Although few attempts have been made to correlate the extent of physiological damage with the expression of visible symptoms, a growing body of experimental data suggests that physiological systems of mosses are affected by SO_2 gas.

Bressan, et al. (1978) have shown that vascular plants can absorb SO_2 gas through their leaves. In addition, cultivars of the Cucurbitaceae family which have apparent variances in SO_2 resistance actually differed in SO_2-uptake rates. SO_2 resistance was similar among the cultivars when uptake was similar. These studies also showed that young and old cucumber

leaves have similarities in uptake rates but differences in SO_2 resistance.

The uptake of atmospheric pollutants by vascular plant foliage has been demonstrated by Garsed and Read (1974). They exposed young bean plant leaves to $^{35}SO_2$. Uptake through stomates was presumed to occur because more readioactivity was found in plants fumigated in the light than was found for those fumigated in the dark (Garsed and Read 1977a). The ^{35}S label was transported throughout the plant. None of the $^{35}SO_2$ could be recovered from irradiated plants 24 hours following the exposure, but this was not the case for plants in the dark (Garsed and Read 1977b). The rapid conversion of $^{35}SO_2$ to other products also was observed by Gilbert and McWeeny (1976).

The observation that the algal components of SO_2-fumigated lichens are killed (Rao and LeBlanc 1965), coupled with the demonstration that chlorophyll is irreversibly oxidized after SO_2 exposure (Syratt and Wanstall 1969), has resulted in the focusing of physiological studies upon the photosynthetic system.

Cryptogams clearly show reduced photosynthetic capacity following SO_2 exposure (Puckett, et al. 1973, 1974; Hill 1974; Inglis and Hill 1974) although most experimenters immerse the plant material in SO_2 solutions. Although attempts have been made to correlate the immersion of plants in solutions of known

SO_2 concentrations with gaseous fumigation concentrations (Saunders 1970; Nieboer, et al. 1977), the comparison of these techniques seems dubious (Showman 1972; Türk, et al. 1974; Nash and Nash 1974). In the immersion technique, the depression of ^{14}C fixation rates following the addition of ^{14}C-emitting carbonate-bicarbonate mixtures is measured. Epiphytic lichens and mosses may not photosynthesize normally during immersion; further, the relationship between nonphotosynthetic carbon fixation and light-induced fixation has yet to be determined.

The mechanism of photosynthetic disruption in mosses and lichens may be due to the oxidation of chlorophyll to phaeophytin (Rao and LeBlanc 1965; Pearson and Skye 1965; Puckett, et al. 1973; Nash and Nash 1974). However, low concentrations of SO_2 that do not alter chlorophyll levels are also known to lower photosynthetic activity (Showman 1972). No research to date has shown that reductions of total chlorophyll and photosynthetic capacity are functionally related. If the quantity of chlorophyll within a cell is not the limiting factor in photosynthetic metabolism, then no relationship need exist. Isolated evidence shows that exposure to SO_2 affects other aspects of photosynthesis. SO_2 within a plant cell seems to interfere with the redox reactions of photosynthesis, particularly within (1) the electron transport

system (Schmidt and Trebst 1969) and (2) the chloroplast coupling factor involved with photophosphorylation (Kyrie and Jagendorf 1971). Disruption of the Calvin Cycle is also apparent because intercellular SO_3^{-2} exhibits competitive inhibition of ribulose-1,5 diphosphate carboxylase with HCO_3^{-2} (Ziegler 1972).

The effect of SO_2 upon respiratory metabolism is unclear. Isolated phycobionts and mycobionts of Cladonia cristatella show reduced oxygen consumption after gaseous SO_2 exposure, but respiration of the intact lichen is accelerated after fumigation (Showman 1972). Other reports show that SO_2 inhibits the respiration of intact lichens immersed in SO_2 solutions (Baddeley, et al. 1971, 1972).

Under anaerobic conditions SO_2 is known to inhibit phenolase, ascorbate oxidase, and pyruvate decarboxylase, which thereby increases the storage life of plant foods (Haisman 1974). Degradation of these enzymes, and perhaps of other oxidizing enzymes, offers evidence that SO_2 may cause depression of respiration. The fact that isolated mitochondria from both an SO_2-sensitive plant (bean hypocotyls) and an SO_2-tolerant plant (maize coleoptiles) suffer similar reduction in ATP production following SO_2 exposure (Ballantyne 1973) suggests that in some plants cytoplasmic factors may detoxify oxidized sulfur.

It further suggests that observed enzyme "degradation" may be due in part to normal enzyme turnover combined with reduced enzyme synthesis found in cells with low ATP pools.

Further evidence that cells exposed to oxidized sulfate synthesize compounds essential for metabolism at reduced rates is offered through examination of amino acid pools and the protein synthesizing machinery after SO_2 exposure. Primary leaves of bean show a pronounced increase in the concentration of free amino acids (Godzik and Linskens 1974) which suggests reduced anabolism. Additionally, SO_2 exposure results in reduced mitotic activity (Bleasdale 1973) and in a slower rate of mRNA translation caused by mutation of tRNA (Furuichi, et al. 1970). Experiments show that SO_2 in solution alters the structure of the uracil and isopentenyladenosine residues adjacent to the 5' and 3' ends of the yeast tRNA (tyrosine) anticodon. The altered tRNA is not rendered inactive but has a lower translation rate. Also, SO_2 is known to convert cytosine to uracil (Shapiro, et al. 1970) and to have modifying effects upon methionine (Inoue and Hayatsu 1971), the cytosine-guanine bridge of bacterial DNA (Mukai, et al. 1970), and the minor nucleoside, 4-thiouridine (Hayatsu 1969).

Research Approach

It seems clear that SO_2 is becoming more prominent in the air. The considerable damage attributed to this pollutant warrants its study. Biological organisms, which can suffer physiological damage or death following SO_2 exposure, can provide insights into the mode of SO_2 toxicity and thereby can contribute to our understanding of its impact. Only in this way can the implications of large scale emissions be elucidated.

Although damage to vegetation is apparent in the field and can be demonstrated in the laboratory, few attempts have been made to correlate these observations. Thus, when bioindicators of SO_2 are identified in the field, these plants are rarely studied in the laboratory. Similarly, when a biochemist observes the effect of SO_2 on a biochemical event, rarely does he ask whether the phenomenon occurs in the field.

Therefore, I will attempt to use ecological techniques to assess the impact of SO_2 stress on the structure of a plant community. Attempts to associate SO_2 with ecological perturbation will be made. Plants in the study area will be surveyed with regard to their apparent responses to SO_2, and these findings will be exploited in the laboratory to determine if SO_2 could account for ecological distribution of

plants at the study site. Physiological comparisons between species which appear to vary in SO_2 tolerance will be made during controlled fumigations in order to help clarify the processes of SO_2 toxicity.

Considerable attention will be paid to terrestrial mosses. These plants, which have been classified as SO_2-sensitive, play a prominent role in northern ecosystems. The moss carpet is extensive in the Boreal Forest and is thought to (1) aid in the process of soil formation, (2) prevent soil erosion, and (3) play a prominent role in the cycling of water and nutrients in ecosystems. Therefore, the ecological consequences of removing this understory component may be wide-ranging.

As an experimental organism mosses have many advantages, particularly because they are readily collected and survive storage. Studying the effects of air pollutants also is facilitated by the fact that gases enter the moss over the whole leaf (phyllidia) surface: there are no specialized coverings or openings on the phyllidia which are similar to the cuticle and stomates of vascular plants. Lichens are useful to indicate the presence or absence of pollutants. However, the value of lichens as experimental organisms is somewhat limited because they are a composite of algae and fungi growing in association, and the

interactions between the components make lichens difficult organisms to study from the physiological standpoint. Further, lichen metabolism is unique and involves pathways and compounds found in no other plant.

Bryophytes, on the other hand, are potentially useful air pollution bioindicators. [9] Moss metabolism, which can be easily studied, is similar to that of most higher plants. I intend, therefore, to develop bryophytes as an organism for the study of the physiological effects on plants of air pollution.

Chapter 1

INTRODUCTION TO THE ECOLOGICAL ANALYSIS

Field studies on the distribution of air pollution bioindicators (particularly epiphytic cryptogams) have shown that pollutants often reduce the ecological importance of species to which they are toxic. Furthermore, community diversity (taken as a measure of ecosystem stability) is lowered when a community becomes simplified due to the removal of pollution-sensitive species. Several studies have been conducted on the effects of removal of select species from plant communities. These studies have been concerned with species removal from old fields where successions are still progressing (Pinder 1975; Allen and Forman 1976), removal of dominant overstory species by the use of herbicides (Niering and Goodwin 1974), insect attack (Collins 1961), or disease (Keever 1950; Good 1968). However, except for the study of Wood and Nash (1976), air pollution impact upon plant community structure has received little attention.

Synecological studies have been used to determine the phytosociological impact of heavy metals, NO_x, HF, O_3, and SO_2 on epiphytic cryptogams within cities (Gilbert 1965, 1968; Brodo 1967; Skye 1968; LeBlanc and DeSloover 1970) and around industrial sites far from urbanization (Rao and LeBlanc 1967; Hoffman 1974). These field studies

demonstrate that such species (particularly epiphytic lichens) act as air pollution bioindicators and that their distribution can be correlated with air pollution concentrations (Coker 1967; Barkman 1969; Gilbert 1970a; LeBlanc and Rao 1973). Some terricolous and saxicolous moss species have also been described as being sensitive to air pollution (Gilbert 1970a, 1970b; Nash 1972; Nash and Nash 1974).

In one phase of this study I analyzed the synecological changes which occur as understory species are removed from a single "climax" plant association subjected to defined gradients of increasing air pollution stress. More specifically, I have followed the changing ecological relationship between terrestrial mosses and vascular plants in a white spruce association understory that has been fumigated with emissions containing predominantly SO_2. In addition, I analyzed in detail the ecological status of the terrestrial mosses in order (1) to assess the merits of terrestrial mosses as bioindicators of air pollution stress; (2) to determine if SO_2-caused changes in the coverage of mosses compare with changes in other ecological parameters (e.g. frequency, biomass, and turf depth); (3) to evaluate the vulnerability of mosses to SO_2 throughout their lifecycles; (4) to define the limitations of the Index of Atmospheric Purity (IAP) in indicating the impact of air pollution upon a plant community; and (5) to propose the criteria for an Index of Community

Vigor (ICV).

THE STUDY AREA

Location and Plant Physiognomy

The study area is located at 54°20' latitude and between 117°00' and 116°35' west longitude in west-central Alberta. The town site of Fox Creek, Alberta, emits no pollutants and is located 10 km northeast of the Kaybob I and II refineries. The Kaybob refineries are twin, adjacent natural gas processing plants which share a single SO_2 dispersion stack and which are located in the mixed wood section of the Boreal Forest (Rowe 1972). The study area is therefore composed of both deciduous and coniferous plant associations, and Picea glauca dominates the overstory wherever succession progresses on gentle, well-drained slopes.

Meteorology

The study area receives nearly 50 cm of precipitation per year, most of which falls during the warm summer months. Averaged data from 1964 to 1973 show that July is the wettest (11.4 cm precipitation) and warmest (mean daily maximum 22°C) month of the year. The months from October through March can be very dry; precipitation ranges from 0 to 3.2 cm throughout this period, and the mean daily temperatures are below freezing.

Wind direction, frequency, and velocity were

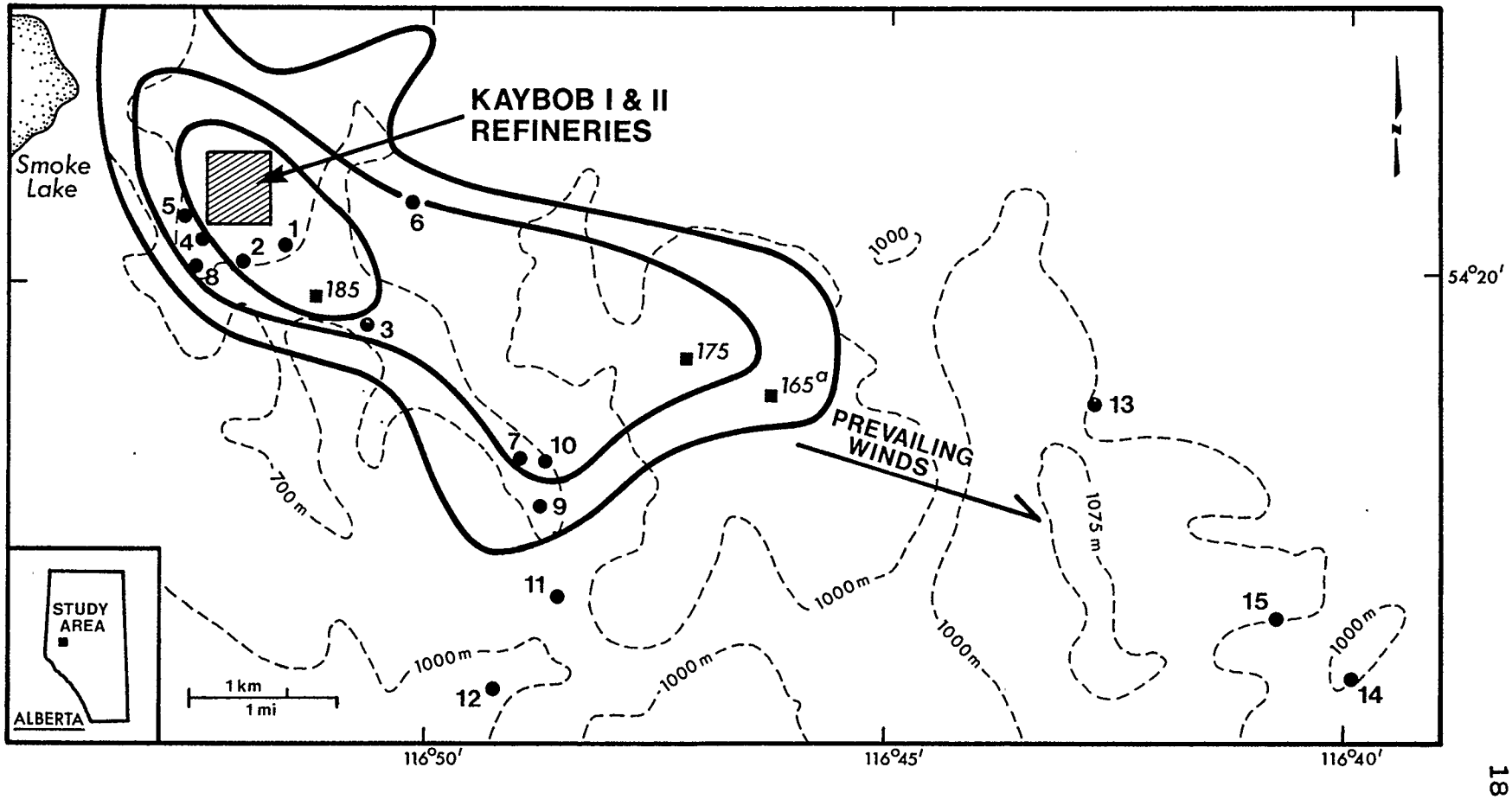
measured at Whitecourt, Alberta, which is located 80 km southeast of the study area, during 1973. Detectable air currents existed 80% of the time and averaged 7.8 km per hour. Data compiled from 24 windroses representing wind directions at four-hour intervals throughout the day during the months of January, April, July, and October are shown in Figure 1. Wind prevailed from the northwest quadrant of the compass more than 72% of the time that air movement was measurable. All of the meteorological data was gathered by Intera Corporation, Calgary.

SO_2 released from the Kaybob refineries is usually carried in a southeasterly direction and two stress gradients are produced by this emission dispersion. One gradient is dependent upon the distance any site is positioned from the emission source, and those close to the SO_2 source receive higher concentrations of emissions than do those located farther away (Fig. 1). The second SO_2-stress gradient depends upon the angle at which a site is positioned from the vector of the prevailing winds which pass through the refinery.

Topographic and Edaphic Factors

The study area gently increases from 600 m to 1075 m in elevation (Fig. 1). Breaks in topography are the result of Pleistocene glaciation and could influence the degree of SO_2 stress at any site within the study area. Soils of the study area are alfisols, and edaphic factors

Figure 1. Map of the study area showing the position and number (in order of increasing percentage of moss coverage - Table 2) of each macroplot (o) in relation to the Kaybob refineries. The isobar values (0.185, 0.175, and 0.165) represent the average quantity (mg $SO_3 \cdot 100\ cm^{-2} \cdot day^{-1}$) of atmospheric sulfur, excluding dustfall, that was captured by sulfation cylinders (X) during the year 1972. The broken lines indicate elevation and topography and the dark lines are sulfation isobars.


KAYBOB I & II
REFINERIES
Smoke
Lake
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
185
175
165a
1000
700 m
1000 m
1075 m
PREVAILING
WINDS
54°20'
116°50'
116°45'
116°40'
STUDY
AREA
ALBERTA
1 km
1 mi

are not related to the altered plant community structure which was observed.

The Air Pollution

Since natural gas refineries do not emit large amounts of NO_x (Table 1), heavy metals, or O_3 (Klemm 1972), they offer a unique opportunity to study in relative isolation the effects of SO_2 as a pollutant. All natural gas refineries located along the foothills of the Rocky Mountain Range, including Kaybob I and II, emit effluent gases similar in composition to the data shown in Table 1. The Kaybob refineries have been operational since 1968, and from 1973 to 1975 have emitted 71,000 kg of SO_2 per day (personal communication from Mr. Robert Brock of Hudson's Bay Oil and Gas Company, Ltd., Calgary).

Located around the Kaybob refineries are 38 sulfation cylinders. Data from 17 of these, for which the locations are given in Figure 1, were used to verify the SO_2 distribution patterns found downwind from Kaybob I and II. The isobars in Figure 1 reveal the patterns of SO_2 dispersion in the study area. The isobar values given on Figure 1 represent the average sulfation in mg $SO_3 \cdot 100\ cm^{-2} \cdot day^{-1}$ during 1973. These data were taken from a figure submitted by Hudson's Bay Oil and Gas Company, Ltd., Calgary, to the Air Quality Control Branch of Alberta Environment, Edmonton, Alberta.

Table 1. The quantitative analysis of gasses emitted[a] from the stack of a natural gas refinery[b] located near Calgary, Alberta. These data are converted to ppm (v/v) on a dry weight basis[c].

Gas	Quantity
N_2	845,900
CO_2	91,700
O_2	43,400
Ar	10,000
SO_2	8,000
CO	800
NO_X	163

a on a wet basis the concentration of H_2O is 211,900 ppm (v/v) and H_2, C_1, C_2, H_2S, COS, and CS_2 are not detectable

b when the average stack gas temperature is 668°C

c unpublished data from Rowe, R.,Western Research and Development, Ltd., Calgary, Alberta.

METHODS

All the mature white spruce stands in the study area, as delimited by an Alberta Forest Service cover type map (1972), were investigated, and in the summer of 1975 15 macroplots (10 m x 25 m) were established at various distances from the Kaybob refineries (Fig. 1). The necessary number of macroplots was determined by sampling until the addition of macroplots resulted in a small increase in the total number of species encountered (Fig. 2). The criteria for macroplot selection were the following: (1) macroplots could slope no more than 5%; (2) there could be no evidence of hiking, camping, logging, or disturbances other than air pollution; (3) stands could have no history of recent fires; (4) the overstory of each macroplot had to be dominated by Picea glauca; (5) macroplots had to be positioned at least 50 m from the nearest road.

Macroplots numbered 1, 3, 7, 9, 11, 12, 13, and 15 on Figure 1 represent the distance-dependent gradient of decreasing SO_2 stress. Macroplots 1, 2, 4, and 5 reflect the angle-dependent gradient of decreasing SO_2 stress. Macroplot 8, which was positioned at the base of a slope, and macroplots 10 and 14, which were associated with ridges, were omitted from either gradient. These macroplots, although situated on flat ground, are influenced by topographic features; data from them are presented only

where appropriate.

With the exception of lichens, fungi, and hepatics, all the macroscopic vegetation within each macroplot was sampled. The stems of all trees and erect shrubs were counted and classified within the following diameter ranges at breast height (dbh): 0-0.4 dm, 0.5-0.9 dm, 1.0-1.9 dm, 2.0-2.9 dm, 3.0-3.9 dm, 4.0-4.9 dm, 5.0-5.9 dm (Daubenmire 1968). The number of tree seedlings (plants less than 0.5 dm dbh) was recorded also. The percent basal area coverage for each species encountered within any macroplot was calculated by multiplying the number of individuals in each size class by the area assumed by the size class median. These values were summed, divided by the macroplot area, and multiplied by 100.

Understory coverage in each macroplot is considered to be a measure of ecological importance and was sampled according to the technique of Daubenmire (1968). Forty 2 dm x 5 dm plotframe settings were located along the sides of two 25 m transects at 1 m intervals. The cover for each plant was classified using the following categories: 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%. From these data the percentage of cover was determined for each species. Coverage was calculated for all plant species in individual macroplots by multiplying the midpoint of each observed cover-class range by the area of the plot-frame. These products were summed after 40 plotframe placements, divided by the sampled area (4.0 m^2 in this

case), and multiplied by 100 to give the percentage. Frequency was derived by calculating the percentage of placements in which any species was encountered (Daubenmire 1959).

The coverage measuring system was also employed to quantify the phytosociology of the terrestrial bryophytes within each macroplot according to the methods of Stringer and Stringer (1974). Sixteen 3 dm x 10 dm plotframes were located systematically along two 25 m transects, and following each plotframe placement the coverage of each bryophyte species was identified as being the following: 0-0.9%, 1-5%, 6-15%, 16-25%, 26-50%, 51-75%, 76-100%. Bryophyte species on tree roots were recorded if they were not above 5 cm from the ground. Frequency and coverage calculations were made such as those described for the vascular plant component of the understory.

All the bryophyte species encountered within each stand and all the specimens of plants requiring microscopic examination for taxonomic verification were collected. The vascular plant nomenclature follows Hitchcock and Cronquist (1973) or Moss (1959), and the bryophyte nomenclature follows Crum, Steere, and Anderson (1973) except for the Mniaceae (Koponen 1974) and the Dicranaceae (Peterson 1976, personal communication). For some specimens of the genera Salix, Alnus, Rosa, Viola, Ribes, and Carex, as well as for the families Compositae and Gramineae, identification of the species name was not

feasible. These plants were identified to the most specific taxonomic group possible and were counted as species in data processing.

Diversity was measured using the Shannon-Weaver index (Shannon and Weaver 1963) as follows to obtain an indication of community organization:

$$\bar{H} = \sum_{i=i}^{-S} P_i \log_2 P_i$$

where

$\bar{H}$ = Diversity

P_i = Importance value of ith species (the importance value is calculated as ni/N where n_i = coverage of the ith species and N = total coverage

S = Total number of species

During the summer of 1976 I revisited 15 macroplots which in 1975 had been positioned in the Kaybob I and II study area located near Fox Creek, Alberta. Transects were constructed in each macroplot, and all macroplots were 10 m x 25 m in area. A 3 dm x 10 dm plotframe again was used 16 times along the transects which were used to measure terrestrial moss coverage. After each plotframe placement the depths (to the nearest 2.5 mm) of the chlorophyllose (green) portion of the moss turf (which is presumed to be active) were recorded. The mean turf depth was calculated by dividing the sum of the measurements in a macroplot by

the number of plotframe placements in which a moss carpet grew. The number of moss capsules also was recorded for each species and this number was interpolated mathematically to represent the capsule count per square meter.

To estimate moss biomass the entire moss carpet was collected from beneath the second, eighth, and sixteenth plotframe placements in each macroplot. This was air-dried until samples of the carpet placed into an oven at 110°C for six hours lost no more weight. The collection from each macroplot was cleaned of debris, combined, and weighed. The percentage of green moss was calculated for each macroplot by (1) selecting a portion weighing at least 100 grams dry weight (which was done by combining four samples randomly drawn from an opaque bag); (2) isolating and weighing the green part of the turf; (3) dividing this weight by the dry weight of the portion; and (4) multiplying by 100 to derive a percentage.

Calculations of the Index of Atmospheric Purity for the understory components in each macroplot of the study area were made by using the following modification of LeBlanc and DeSloover's formula (1970). The following expression was used:

$$IAP = \sum_{n}^{i=1} Q_i (cf) 100^{-1}$$

where

IAP = Index of Atmospheric Purity

Q_i = the ecological index (resistance factor) of the ith species calculated as the average number of species which occur with the ith species in any macroplot in which it is found (LeBlanc and DeSloover 1970)

c = the actual coverage percentage of a species

f = the actual percent frequency of a species

n = the total number of species

The mean Q_i value for all the plant species in each macroplot also was calculated to test for changes in the ecological resistance of vegetation as SO_2 stress increased.

Soil samples were collected to determine if edaphic factors could account for the distribution of plants in the study area. Five soil cores, about 4 cm in diameter and 10 cm deep, were collected from each macroplot, combined, oven-dried, passed through a 2.0 mm mesh, and divided for analysis. A sedimentation method (Pettijohn 1938) was used to analyze soil textures. To ascertain soil pH, a 16 g portion of soil was added to 40 ml of distilled H_2O, stirred at 5 minute intervals for 30 minutes and measured with a pH meter. The method of Walkley and Black (Chapman 1961) was used to analyze the percentage of organic matter.

RESULTS

Species Numbers

A total of 6 tree species, 44 vascular understory species, and 39 moss species were encountered during the

sampling (see Appendix 1). The 39 moss species compare favorably with the 52 moss species identified by LaRoi and Stringer (1976) during the sampling of 34 white spruce-fir stands located across Canada.

Macroplots 1 and 2, potentially the most stressed macroplots (Fig. 1), contain 6 and 16 species respectively (Table 2). Hence, the curve in Figure 2 results from (1) the typical species area curve which indicates sampling adequacy, and (2) the depletion of species related to pollution stress.

The Overstory

Since the macroplots were selected to eliminate variation in the overstory, all are uniformly dominated by Picea glauca. The number of trees in any macroplot did not differ significantly (Chi square values show $p \leq 0.05$) from the mean expected number of trees in each size class larger than 0.5 dm dbh (0.5-0.9 dm = 3.2 ± 0.72 SE, 1.0-1.9 dm = 11 ± 0.96 SE, 2.0-2.9 dm = 8.0 ± 1.0 SE, 3.0-3.9 dm = 3.7 ± 0.45 SE, 4.0-4.9 dm = 0.80 ± 0.26 SE). Table 3 shows that the percent coverage of the basal area of Picea glauca ranges between 0.65% and 0.40% (mean = $0.51\% \pm 0.01$ SE) and further documents the overstory homogeneity.

The number of Picea glauca seedlings, however, shows high variability between macroplots. For example, macroplots 1 and 14 have nearly identical numbers of trees

Figure 2. The species response curve showing the increase in species numbers during the sampling of the macroplots.

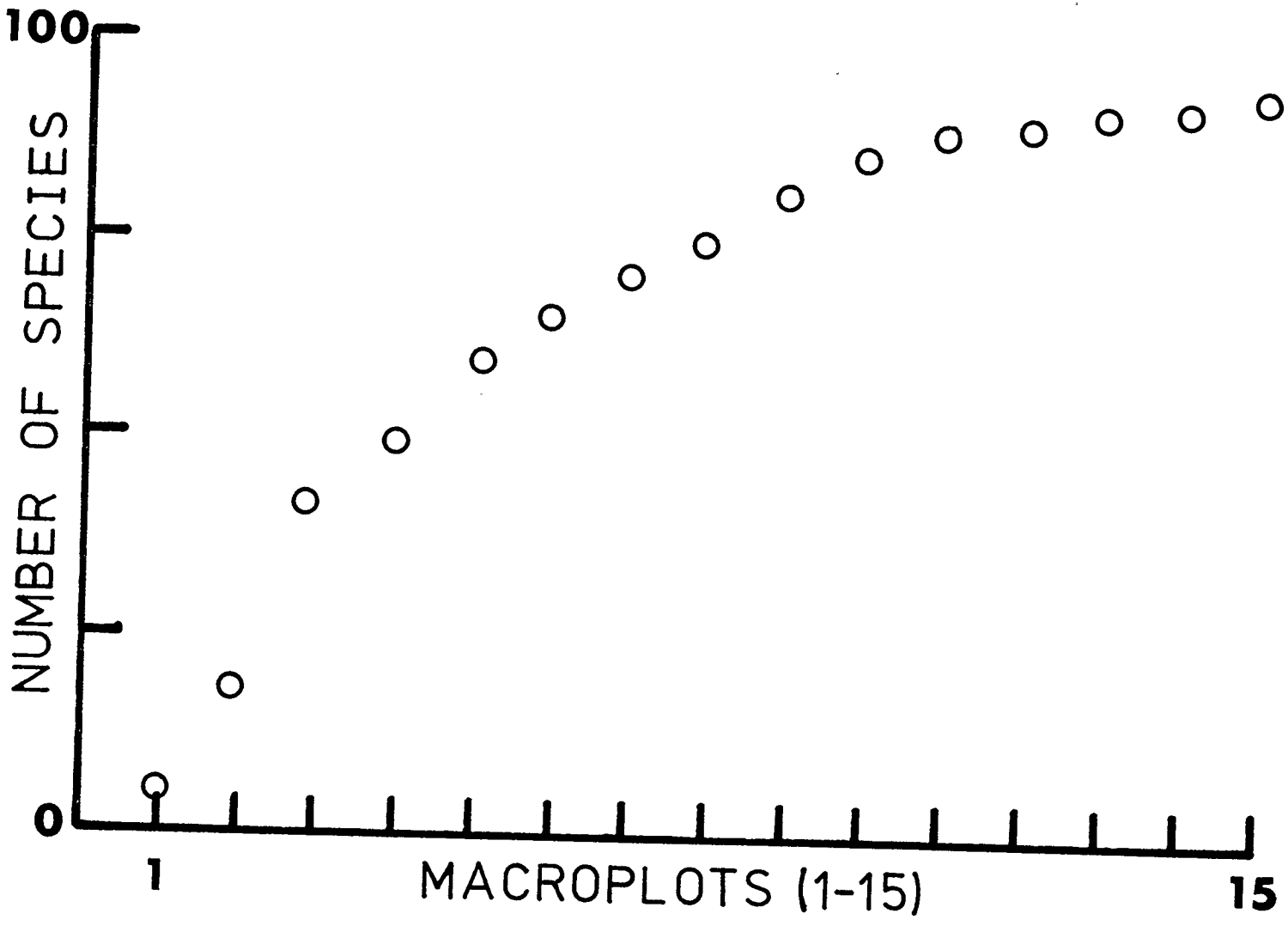

100
0
NUMBER OF SPECIES
1
MACROPLOTS (1-15)
15

Table 2. The number and the percent coverage of the overstory and understory species encountered within each of the macroplots. The percent coverage is based upon the basal area and the canopy coverage data for the overstory and understory species, respectively.

Macroplots	Number of Species				Percent Coverage			
	Trees	Vascular Understory	Mosses	Total	Trees	Vascular Understory	Mosses	Vascular Understory and Mosses
1	1	5	0	6	.56	7.20	0.00	7.20
2	4	7	5	16	.53	31.5	9.59	40.0
3	2	21	17	40	.66	76.2	23.9	100
4	2	15	9	26	.55	81.6	30.5	112
5	4	23	15	42	.49	74.6	33.6	108
6	2	25	17	44	.47	59.4	43.3	103
7	2	18	14	34	.58	88.9	48.4	136
8	4	21	7	32	.52	63.4	50.6	114
9	3	25	13	41	.67	78.0	53.3	131
10	4	26	14	44	.57	103	57.0	161
11	2	30	19	51	.50	102	57.3	159
12	3	19	16	38	.55	80.6	61.7	142
13	2	27	15	44	.59	110	61.8	171
14	3	21	18	42	.78	82.6	64.0	147
15	3	26	23	52	.62	136	64.6	200

Table 3. The actual number (N^a), and the percent basal area coverage (C^b), for the two most important tree species in the study area.

Species	Macroplots 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C	N / C
Picea glauca	27 / .56	28 / 142	71 / .65	19 / 147	24 / .46	169 / .40	510 / .54	37 / .50	64 / .62	136 / .49	33 / .49	186 / .53	110 / .53	250 / .55	119 / .47
Abies balsamea		241 / .10	37 / .01	252 / .08	77 / .02	9 / .07	176 / .04	26 / .01	176 / .03	358 / .08	47 / .01	87 / .02	185 / .06	491 / .22	474 / .13

a Number of trees in each macroplot

b Coverage, in percent, within each macroplot

in size classes larger than 0.5 dm dbh but contain 0 and 220 seedlings respectively. Macroplots 1, 2, 4, 5, 8, and 11 have fewer than 11 seedlings. The variability in seedling numbers between macroplots is masked in Table 3 because seedlings, even in large numbers, contribute little basal area coverage to the stand.

Abies balsamea occurs with a low basal area coverage in all sampled stands with the exception of macroplot 1 (Table 3). The large numbers of trees associated with these low basal area values indicate that Abies balsamea occurs primarily as seedlings. Betula papyrifera, Pinus contorta, Sorbus scopulina, Populus tremuloides, Salix spp., and Alnus spp. are scattered in 8 of the 15 macroplots sampled, and because these species never attain more than 0.02% basal area coverage, their ecological importance is considered to be small.

Understory Coverage

The understory of the white spruce association is well developed, and the coverage of vascular plants and mosses is sometimes greater than 100% due to overlapping canopies. The prostrate vascular plants Cornus canadensis, Linnaea borealis, and Mitella nuda are in direct contact with the terrestrial mosses and have the highest coverage and frequency values (Table 4). These three species jointly have more than 25% coverage in all the macroplots

Table 4. The percent coverage of the dominant vascular plant species encountered within the understory (less than 1m tall) of the macroplots within the study area.

	Macroplots														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cornus canadensis	0.40	13	19	27	17	13	18	14	15	25	11	11	20	30	26
Linnaea borealis		14	11	30	14	1.4	21	22	9.3	26	20	32	18	12	30
Mitella nuda		1.3	3.9	3.5	3.0	5.4	17	5.7	10	3.0	8.3	9.5	5.4	2.9	5.1

with the exception of numbers 1 and 6, and also have the highest coverage value of 61% in macroplot 15.

Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis are the terrestrial moss species that have the greatest phytosociological importance (Table 5). They account for a mean of 90% (± 1.57 SE) of the total coverage of terrestrial mosses in macroplots 2 through 15. The coverage of Hylocomium splendens and Ptilium crista-castrensis tends to increase in macroplots 15 through 7 (Table 5) and thus parallels increased stress along the distance-dependent gradient of macroplots. This increase in coverage of subordinate moss species may be related to the decreasing ecological importance of Pleurozium schreberi in macroplots 15 through 5 (Table 5). In macroplots 4, 3, and 2 the actual coverage of all three species taken together decreases.

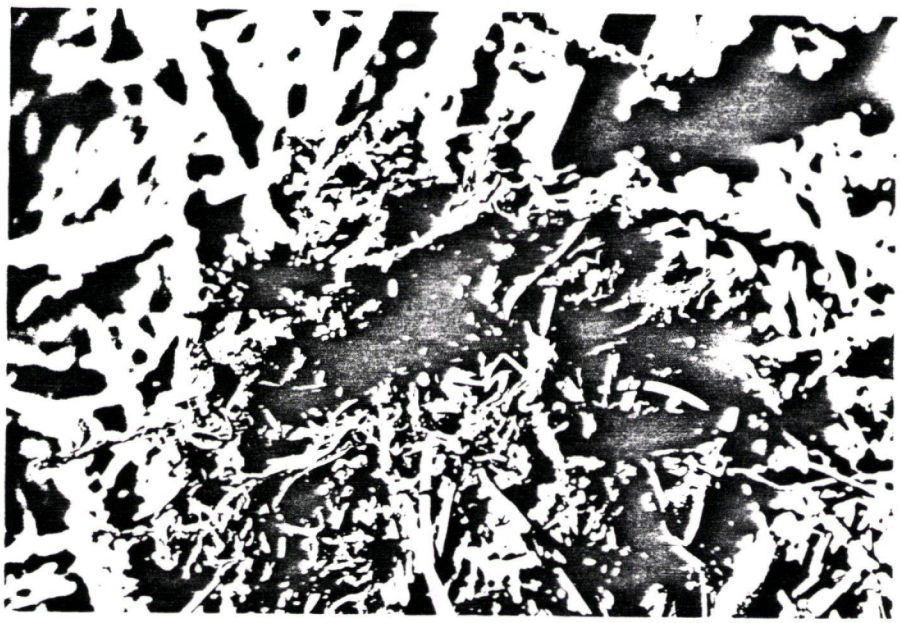
No terrestrial mosses occur in macroplot 1. Proof that macroplot 1 is indeed an altered forest stand which is part of the white spruce association is confirmed by the normal distribution and dominance of Picea glauca (Table 3); by the presence of Cornus canadensis, which is normally the dominant understory species (Table 4); and by the dead remnants of once prevalent Hylocomium splendens and Pleurozium schreberi (Plate 1).

The relative coverage (coverage values in macroplot 15 are considered to be 100%) of the understory vegetation increases in the macroplots positioned farther from the

Table 5. The percent coverage of the dominant terricolous moss species encountered within the macroplots of the study area.

Species	Macroplots														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pleurozium schreberi		5.6	9.5	9.8	9.7	4.8	16	11	23	20	9.1	22	46	25	40
Hylocomium splendens		1.6	4.1	16	8.5	17	20	20	21	9.8	43	29	9.1	12	13
Ptilium crista-castrensis		2.3	7.5	4.4	11	13	7.8	11	5.1	18	2.9	5.6	2.7	19	6.4

Plate 1. Specimens of dead Hylocomium splendens and Pleurozium schreberi at macroplot 1. Photograph by W. E. Winner.



refineries (Fig. 3A). Figure 3A shows that the relative coverage of the mosses remains constant until that of the vascular plant component of the understory drops to 65% (an absolute value of about 100%; see Table 2). The increase in relative coverage of the understory is the most extreme at distances between 0.5 and 1.3 km from the refineries. Relative coverage values for mosses decline over a greater distance along the distance-dependent stress gradient (0.0-3.8 km) than do those for the vascular plants (0.0-1.2 km). This decline indicates that mosses are the more pollution-sensitive component of the understory (Fig. 3A).

Along the angle-dependent gradient there seems to be little change in the total percent coverage between macroplots located at 90° and 120° (macroplots 4 and 5 respectively) to the prevailing winds (Fig. 3B). However, macroplots positioned at angles smaller than 90° show a dramatic decline in understory coverage with increased SO_2 stress. At this point, decline in moss cover parallels that of vascular plants (Fig. 3B).

Understory Diversity

Separate diversity indices calculated for the vascular plant and moss components of the understory in each macroplot show the effect of SO_2 stress upon diversity (Table 6). Except in macroplot 2, diversity values for the vascular plant component of the understory are higher

Figure 3. The relative coverage of the understory (o), moss (□), and vascular plant (X) components along the distance-(A) and angle-(B) dependent gradients.

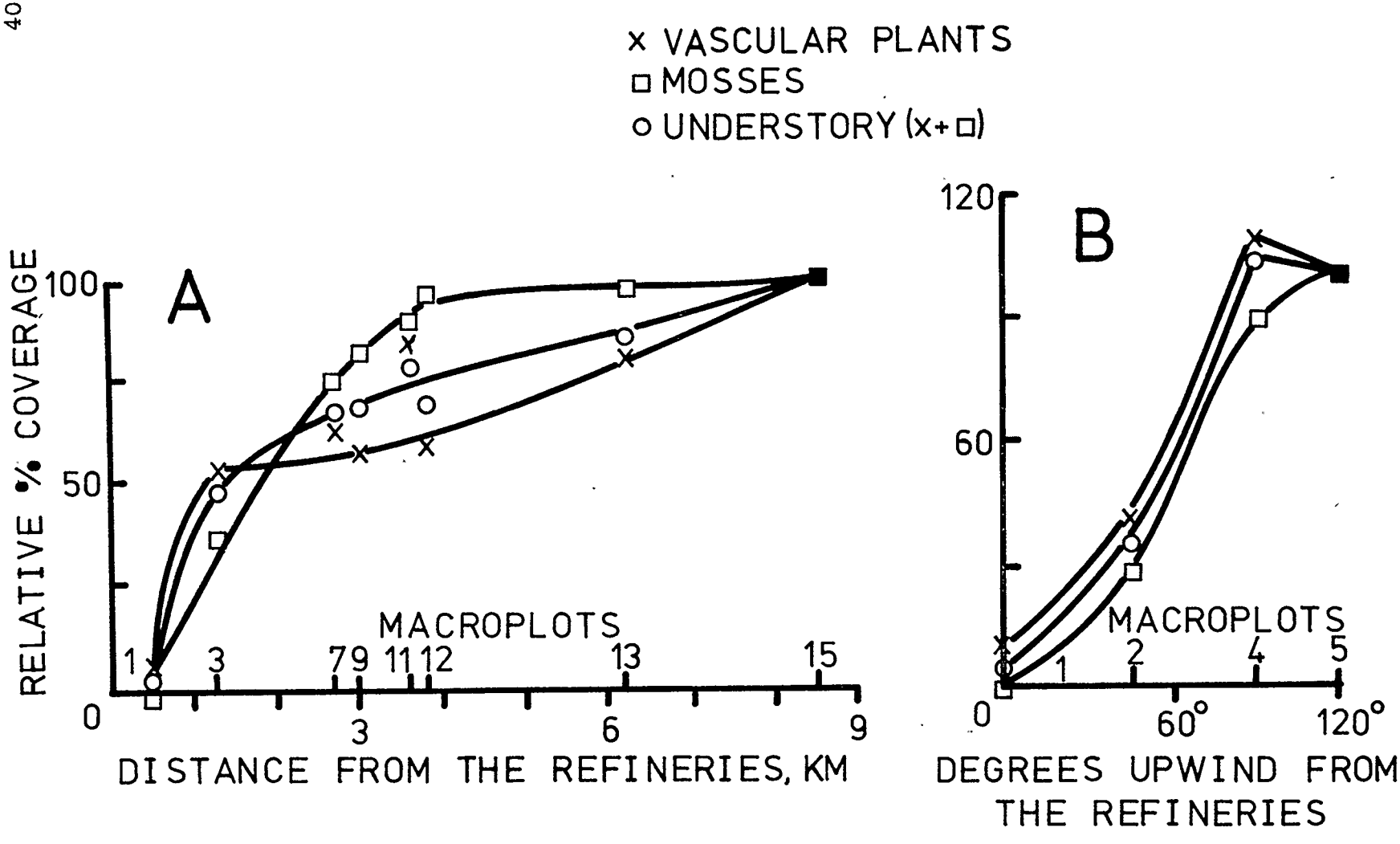

× VASCULAR PLANTS
□ MOSSES
○ UNDERSTORY (×+□)
A
RELATIVE % COVERAGE
100
50
0
MACROPLOTS
1
3
7
9
11
12
13
15
3
6
9
DISTANCE FROM THE REFINERIES, KM
B
120
60
0
MACROPLOTS
1
2
4
5
60°
120°
DEGREES UPWIND FROM THE REFINERIES

Table 6. Shannon-Weaver's index of diversity for the understory, vascular plant, and moss components within each macroplot.

Macroplots	Shannon-Weaver Index		
	Vascular Understory	Mosses	Vascular Understory and Mosses
1	1.39	0.00	1.39
2	1.45	1.45	2.38
3	3.70	2.19	4.08
4	2.86	1.51	2.95
5	3.73	2.30	4.15
6	3.69	2.30	4.13
7	3.05	2.08	3.63
8	3.03	2.08	3.57
9	3.19	1.90	3.62
10	3.21	2.22	3.83
11	3.93	1.22	4.02
12	3.18	1.90	3.53
13	4.04	1.35	4.05
14	3.10	2.14	3.66
15	3.39	1.93	4.74

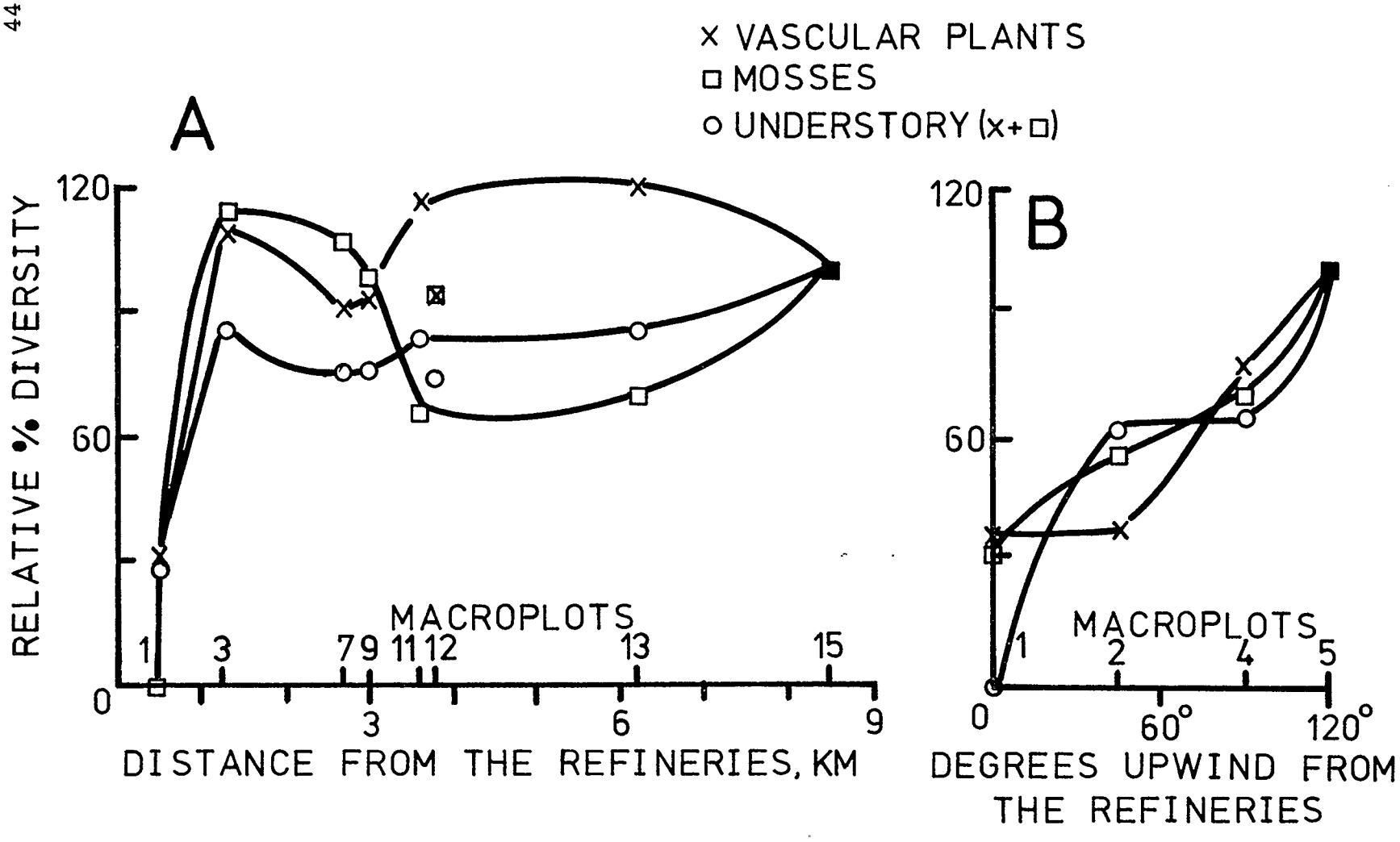
than are those values for the terrestrial mosses. Neither understory component is as diverse as the composite understory (vascular plants and mosses together). The highest diversity value for the terrestrial mosses is 2.30 in macroplots 5 and 6; however, 13 macroplots have higher diversity values for vascular plants (Table 6).

The relative diversity of the understory along the distance-dependent stress gradient is nearly constant (Fig. 4A) despite the trend for coverage to change along it (Table 2 and Figure 3A). Also, the divergence of the relative diversity values of the two understory components--mosses and vascular plants--is masked by the consistency of the relative diversity found for the whole understory (Fig. 4A).

The increase in relative diversity values along the distance-dependent gradient for the vascular plant understory component in macroplots located closer than 2.6 km to Kaybob I and II is due to numerous "weed" species which invade openings in the white spruce association when SO_2-sensitive species are eliminated. Ultimately, if SO_2 stress increases, most of the vascular plant species which move into such openings also are eliminated from white spruce stands. Thus, in the most heavily stressed stand (macroplot 1), the five vascular plant species which persist (Table 2) also are encountered in the least stressed stands along both SO_2 gradients.

The initial decline in relative diversity of other

Figure 4. The relative diversity (the values in macroplot 15 are considered to be 100%) of the understory (o), moss (□), and vascular plant (x) components along the distance- (A) and angle- (B) dependent gradients.


× VASCULAR PLANTS
□ MOSSES
○ UNDERSTORY (×+□)
A
RELATIVE % DIVERSITY
120
60
0
MACROPLOTS
1 3 7 9 11 12 13 15
3 6 9
DISTANCE FROM THE REFINERIES, KM
B
120
60
0
MACROPLOTS
1 2 4 5
0 60° 120°
DEGREES UPWIND FROM THE REFINERIES

terrestrial mosses which occurs with increasing SO_2 stress along the distance-dependent gradient (Fig. 4A) reflects the decline in species number from a high count of 23 in macroplot 15 to a mean of 16 species in macroplots 3, 5, 7, 9, 11, 12, and 13 (Table 2). Relative diversity increases in macroplots 11 to 3 because the coverage (ecological importance) of the dominant moss, Pleurozium schreberi, decreases and because the coverage of the subordinate mosses, Hylocomium splendens and Ptilium crista-castrensis, increases (Table 5). Therefore, the relative diversity for the mosses is highest in heavily stressed macroplots due to the more even distribution of coverage amongst the species which are present.

Along the angle-dependent gradient at 0.5 km from the SO_2 emission source, the relative diversity decreases in forest stands positioned more directly downwind (Fig. 4B). Although the relative coverage of the understory components is constant between 120° and 90° (plots 5 and 4) from the vector of the prevailing winds (Fig. 3B), the relative diversity value drops markedly (Fig. 4B). The relative coverage of the mosses changes from 90% to 28% between macroplots 2 and 4 (Fig. 3A) but the relative diversity value of the mosses remains constant (Fig. 4B). Between macroplots 2 and 1 the relative diversity values for the mosses decline much more than do those for the vascular plant component of the understory.

The Moss Turf

Relative coverage of mosses diminishes as SO_2 stress increases along both stress gradients (Figs. 5A and 5B), and the species Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis account for 90% of the canopy coverage of the terrestrial moss in all 15 macroplots. Although these feather moss species have lowest frequency percentages in macroplots 5 and 13 (Table 7), coverage analysis shows these macroplots to be lightly stressed (Figs. 5A and 5B).

The frequency percentages of the feather mosses generally remain high in macroplots where green mosses are detected (Table 7). For example, relative moss coverage (relative values are related to macroplot 15) in macroplot 3 is about 33%, but relative frequency (based upon averaging feather moss values from Table 7) is about 79%. Therefore, the moss carpet which persists in heavily stressed macroplots has low relative coverage and the gametophytes of the dominant species, which have high frequency percentages, are numerous, small, and widely scattered.

The depth of the green moss turf changes along both SO_2-stress gradients. In macroplot 15, the least stressed macroplot in the study area, the depth of active moss averages 2.8 cm. However, the moss carpet is deepest in macroplot 13 where a value of 8.0 cm is recorded and where the average depth is 3.9 cm (Fig. 5A). The decline

Figure 5. The relative percent coverage (●) and mean active turf depth (■) for mosses in macroplots along the distance- (A) and angle- (B) dependent SO_2-stress gradients. (SE is less than the size of the symbols.)

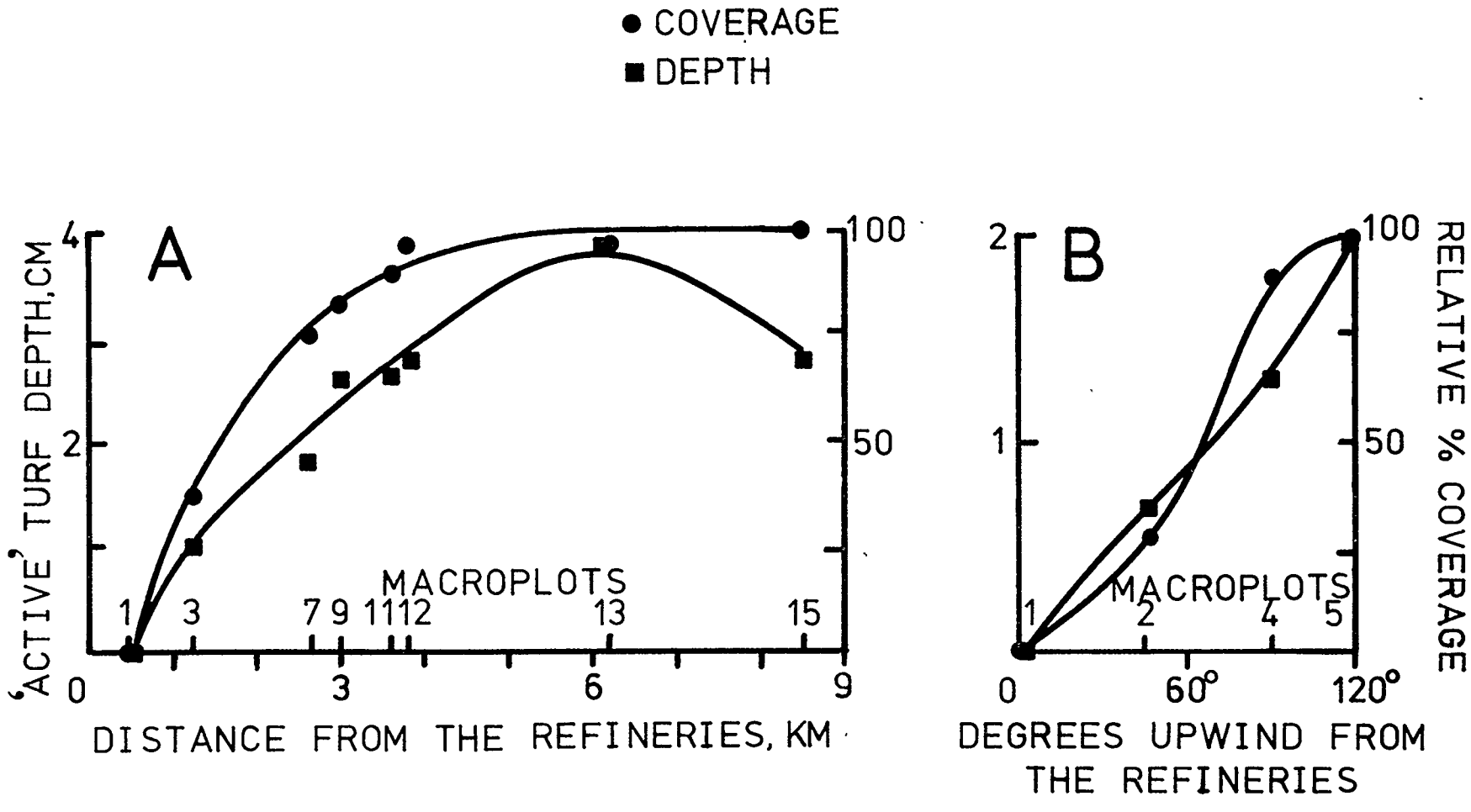

● COVERAGE
■ DEPTH
A
'ACTIVE' TURF DEPTH, CM
4
2
0
100
50
MACROPLOTS
1 3 7 9 11 12 13 15
3
6
9
DISTANCE FROM THE REFINERIES, KM
B
2
1
0
100
50
RELATIVE % COVERAGE
MACROPLOTS
1 2 4 5
60°
120°
DEGREES UPWIND FROM THE REFINERIES

Table 7. Frequency percentages calculated for the feather moss species in macroplots along the distance- and angle-dependent SO_2 stress gradients.

	Frequency Percentages											
	Distance-Dependent Gradient								Angle-Dependent Gradient			
Macroplot	1	3	7	9	11	12	13	15	1	2	4	5
Pleurozium schreberi	*	75	94	100	100	100	100	100	*	88	100	69
Hylocomium splendens	*	69	94	100	100	100	75	94	*	44	100	38
Ptilium crista-castrensis	*	75	100	75	88	100	38	88	*	69	100	81

* only dead specimens observed.

in relative coverage and active turf depth is abrupt along the angle-dependent gradient of increasing SO_2 stress (Fig. 6B).

The dry weights of the green parts of the collected moss turfs also tend to decline as SO_2 stress increases along both gradients (Figs. 6A and 6B). The highest dry weight is not found in the least-stressed macroplot of the gradients but rather in macroplots 13 and 4 of the distance- and angle-dependent gradients respectively. Values from 22 kg to 26 kg dry weight of green moss per 250 m^2 are found in macroplots 7, 11, 12, and 15. These values give an indication of the large amounts of terrestrial moss which can develop in this white spruce association.

The percentage of green moss found in the turf of each macroplot, based on dry weights, further describes the impact of SO_2 upon the terrestrial mosses. Figure 7A shows that along the distance-dependent gradient over 33% of the moss turf is active in macroplots 15, 13, and 11. This value declines to less than 20% in macroplot 3 and to zero in macroplot 1 where only non-chlorophyllose moss is found. Although the highest percentage of green moss along the angle-dependent gradient is only 23%, this declines in a pattern which is similar to that of the distance-dependent gradient (Fig. 7B).

The correlation between percentage cover and biomass of green moss is very high (Fig. 8). These

Figure 6. The biomass (kg dry weight) of active (chlorophyllose) terrestrial moss found in macroplots along the distance- (A) and angle- (B) dependent stress gradients.

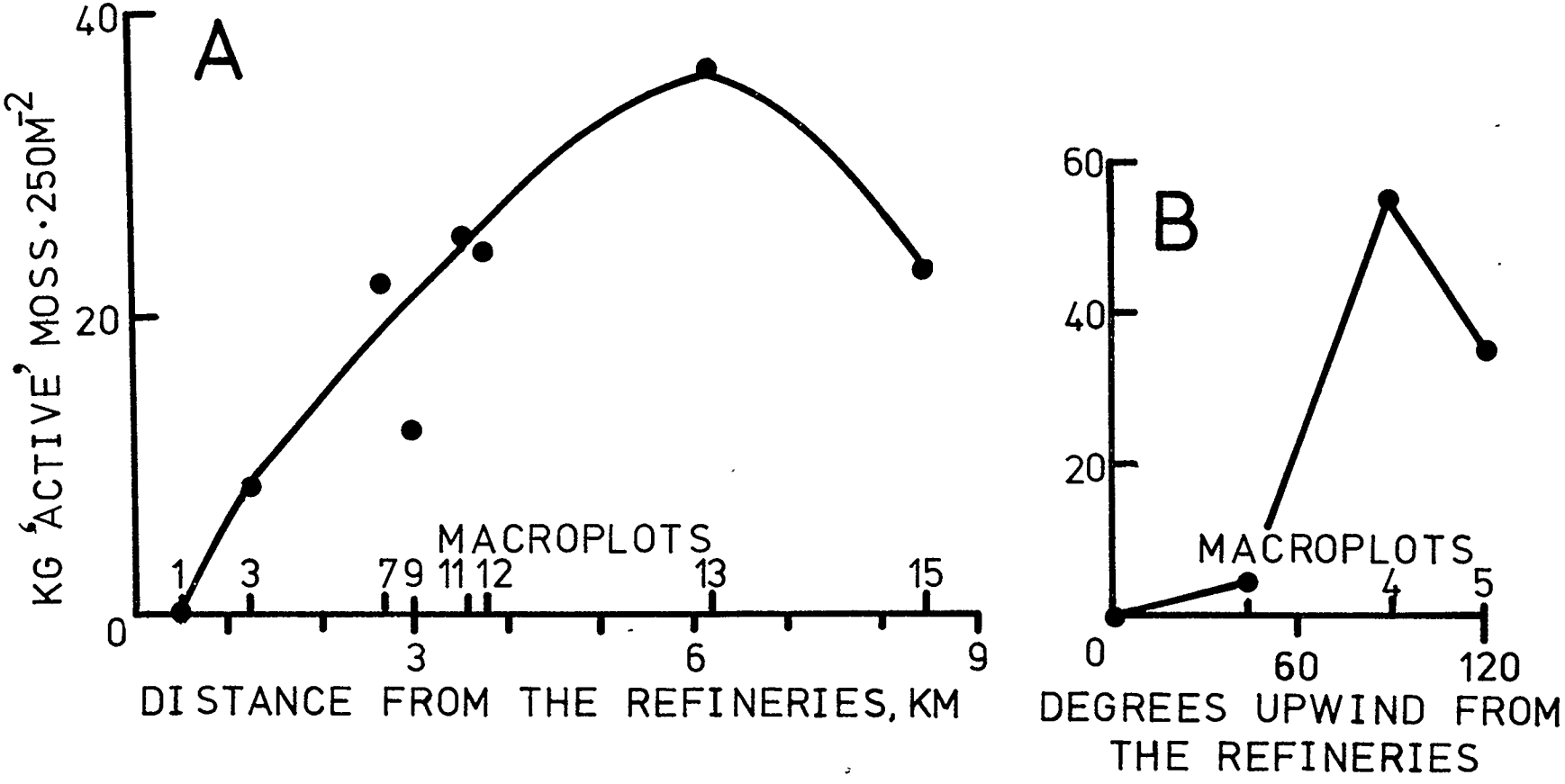

A
40
20
0
KG 'ACTIVE' MOSS·250M⁻²
MACROPLOTS
1
3
7
9
11
12
13
15
3
6
9
DISTANCE FROM THE REFINERIES, KM
B
60
40
20
0
MACROPLOTS
4
5
60
120
DEGREES UPWIND FROM THE REFINERIES

Figure 7. The percentage of active (chlorophyllose) moss (taken as a proportion of the entire moss turf), on a dry weight basis, calculated for each macroplot along the distance- (A) and angle- (B) dependent gradients of SO_2 stress.

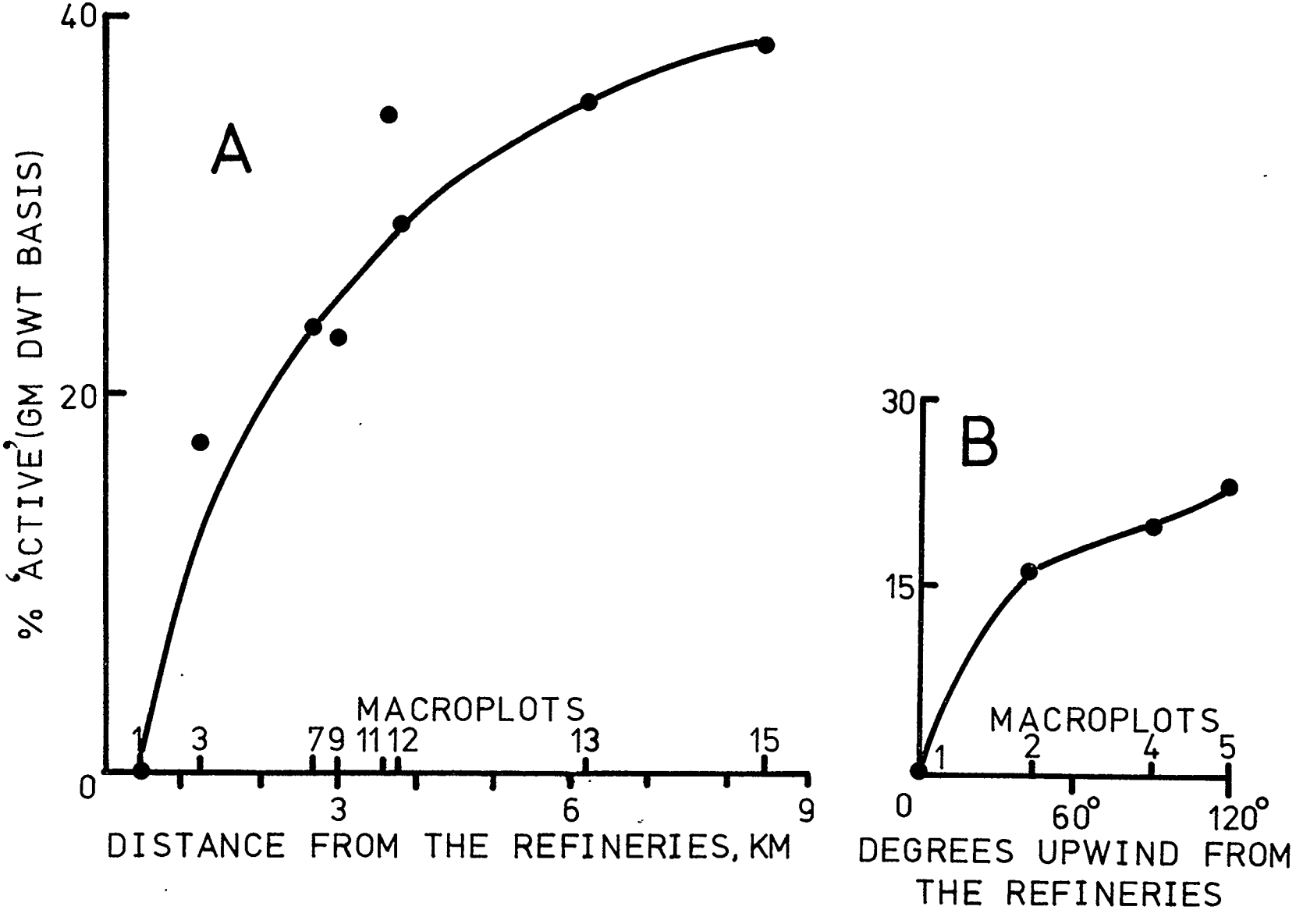
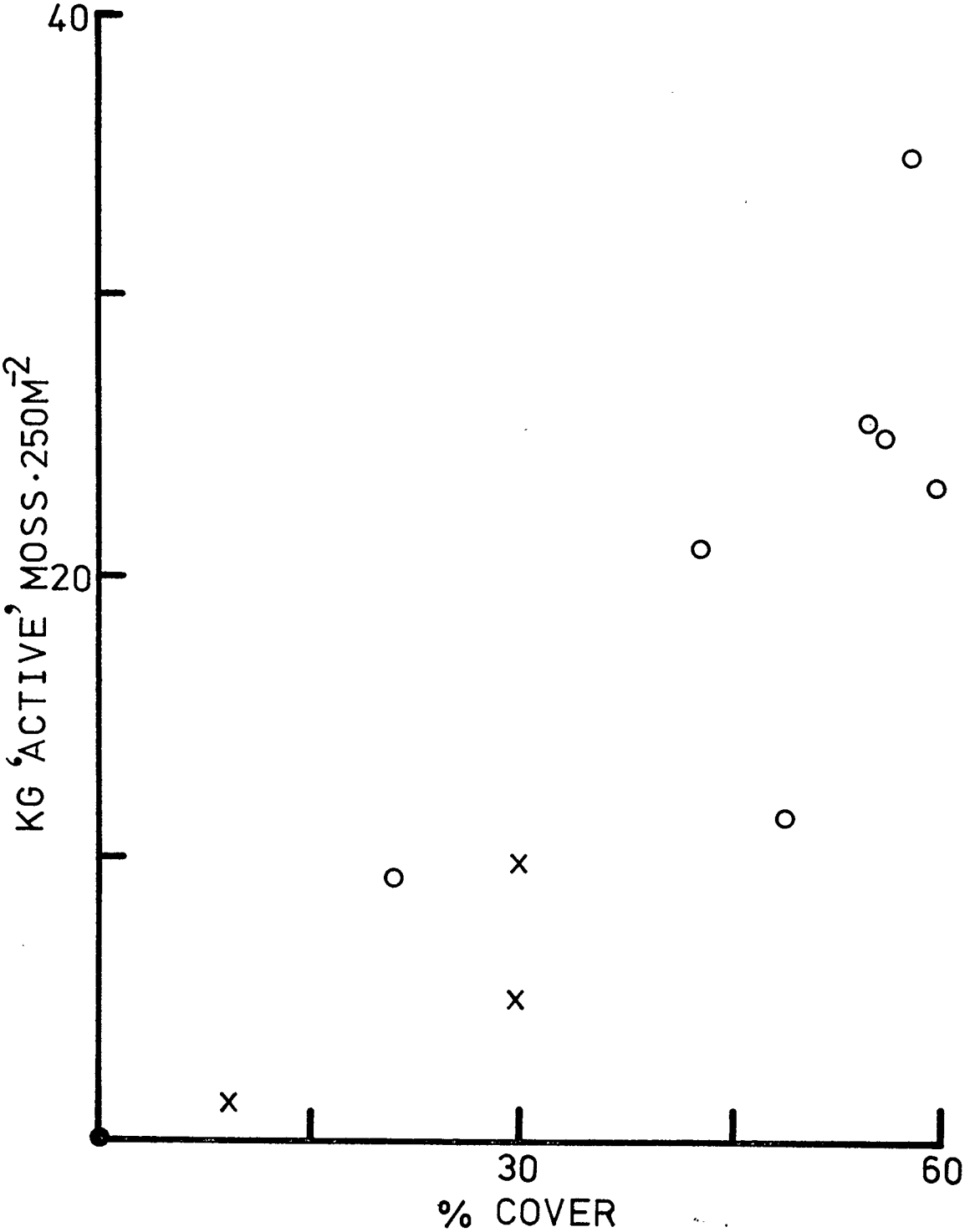

A
% 'ACTIVE' (GM DWT BASIS)
40
20
0
MACROPLOTS
1
3
7 9 11 12
13
15
3
6
9
DISTANCE FROM THE REFINERIES, KM
B
30
15
0
MACROPLOTS
1
2
4
5
60°
120°
DEGREES UPWIND FROM THE REFINERIES

Figure 8. The correlation between the biomass of active (chlorophyllose) moss (kg dry weight) and percentage coverage for mosses in macroplots along the distance- (o) and angle- (x) dependent stress gradients. F for data plotted on either linear ordinates or X versus log Y is 12.4 and 25.2 respectively. Spearman's r = 0.915.


40
20
KG 'ACTIVE' MOSS·250M⁻²
30
60
% COVER

ecological parameters were plotted to determine the effects of SO_2 stress upon their relationships. The F values for these data are higher when plotted on semilog ordinates than when plotted on linear ordinates. Therefore, they show that as SO_2 stress initially increases, biomass declines more abruptly than does coverage. In the zone of intense SO_2 stress, coverage declines more abruptly than does biomass, which is already low (Fig. 8). When all the macroplots are considered together these trends appear to be true along both stress gradients.

Moss Capsule Frequency

An analysis of the frequency of moss capsules in the study area was undertaken to help explain the manner in which increasing SO_2 stress brings about the declining ecological status of terrestrial mosses. Capsules were encountered for only 8 of the 39 bryophyte species which are known to occur in the study area. Along the distance-dependent gradient, Dicranum fuscescens and Pohlia nutans produced the largest number of capsules (Table 8). Among the feather mosses, Pleurozium schreberi produced half the number of capsules that Hylocomium splendens and Ptilium crista-castrensis did. Longton and Green (1969) observed Pleurozium schreberi to be sterile in most areas because male plants are rare. The production of low numbers of capsules for this species, therefore, may be unrelated to the presence of SO_2.

Table 8. The number of moss capsules per square meter counted in macroplots positioned along the distance-dependent gradient of SO_2-stress.

Macroplot	1	3	7	9	11	12	13	15	Total
Pleurozium schreberi				6.0			.80		6.8
Hylocomium splendens			1.6	.60	2.0	3.6	5.6		13
Ptilium crista-castrensis				1.8			13	.20	15
Dicranum fuscescens		.20	3.6	1.0	1.8	6.6	11	2.4	27
Pohlia mutans		3.0				13	8.2		24
Mnium spinulosum					.80	2.2		1.2	4.2
Drepanocladus uncinatus					9.0				9.0
Plagiomnium ellipticum							1.0		1.0
Total		3.2	5.2	9.4	14	25	40	3.8	

The decline in capsule number along the distance-dependent stress gradient (Table 8) roughly parallels the decline in moss coverage (Fig. 5A). Macroplot 15 is an exception because only a few more capsules were produced here than in macroplot 3. Hylocomium splendens and Ptilium crista-castrensis produced fewer capsules as SO_2 stress increased in macroplots 15 through 7, despite their tendency to increase in coverage. The high number of capsules for Dicranum fuscescens and Pohlia nutans in macroplots 13 and 12 respectively (Table 7) did not correspond with their very low coverage of 0.22% and 0.06% respectively.

Capsules were detected only in macroplot 4 along the angle-dependent SO_2-stress gradient, where in each square meter Hylocomium splendens had 1100 capsules, Ptilium crista-castrensis had 250 capsules, and Mnium spinulosum had 800 capsules.

Index of Atmospheric Purity

The Index of Atmospheric Purity (IAP), first proposed by DeSloover (1964), has long been accepted as an index by which zones of air pollution intensity can be delimited after determining the status of bioindicator populations throughout a study area. The use of vegetational zonation as a tool for documenting the impact of air pollution has been applied principally to epiphytic cryptogams (Hawksworth 1973), and except for Gilbert (1968),

Nash (1972), and Taoda (1972, 1976), bryophytes have not been studied carefully. I felt that the calculation of IAP values would define more clearly the status of the moss component in the understory and would reflect with a single index value the degree of SO_2 stress at any macroplot.

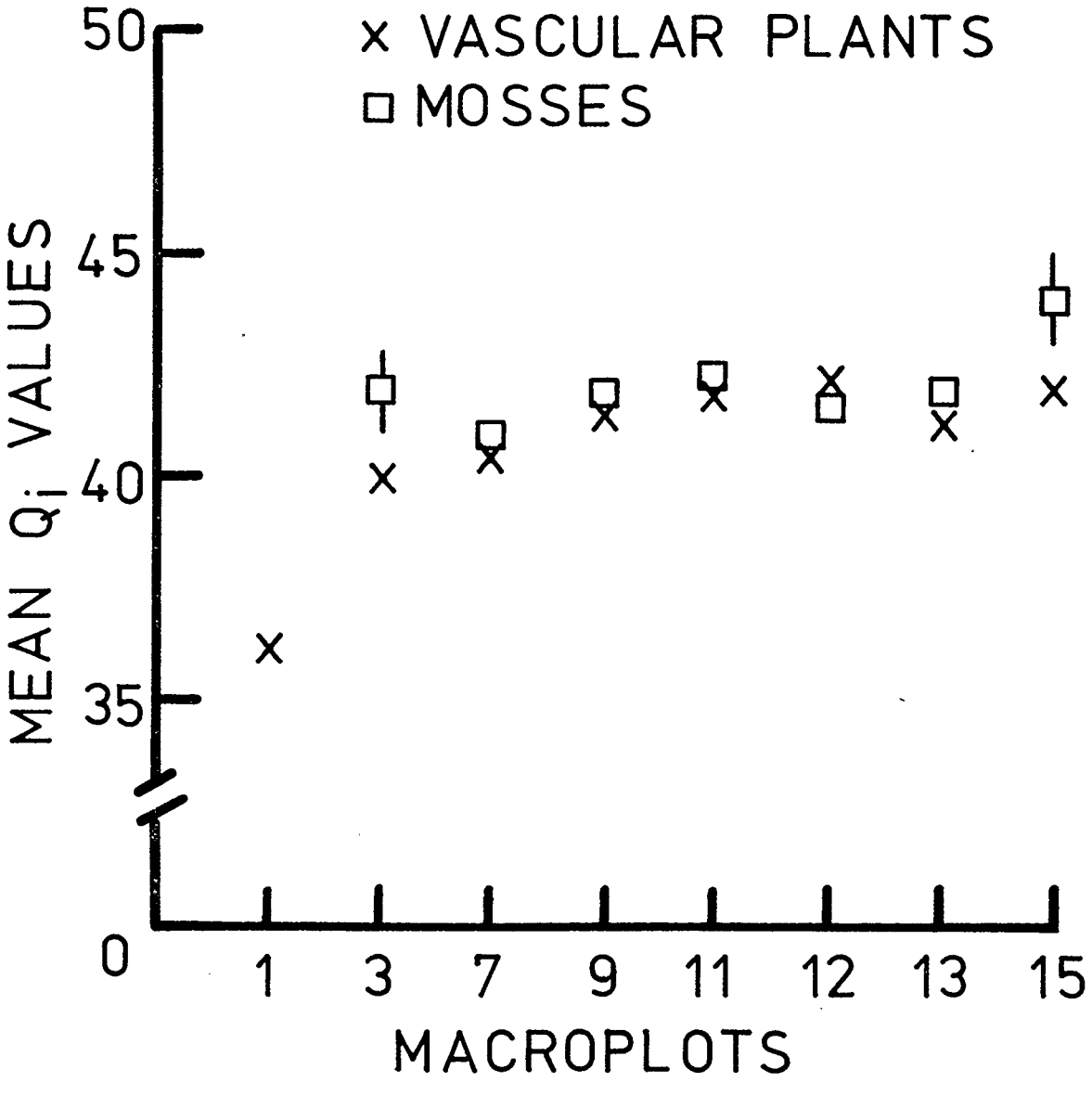
The IAP values for each macroplot (Table 9) are only partially effective in describing SO_2 intensity and impact upon the vegetation. However, values decline for both of the understory components as SO_2 stress increases along the angle-dependent gradient and in the most heavily stressed portion of the distance-dependent gradient. Yet the changes in moss coverage, active turf depth, biomass, and percentage of active moss which are seen in macroplots located farther than 1.3 km from the refineries are not reflected by the IAP. The same trends also occur for the vascular plant component of the understory and for the understory as a whole (Table 9) in spite of the fact that these vegetational units also decline in percent coverage as SO_2 stress increases along both gradients.

The Q_i factor in the IAP formula is taken to be the inverse of pollution resistance afforded by a species; those species with high Q_i values are considered to be pollution-sensitive and those with low Q_i values are considered to be pollution-tolerant. The mean Q_i values for all understory species were calculated along the distance-dependent gradient and were plotted (Fig. 9)

Table 9. Index of Atmospheric Purity values calculated for the vascular plants, terrestrial mosses, and entire understory for macroplots along the distance- and angle-dependent gradients of SO_2 stress.

	IAP Values											
	Distance-Dependent Gradient								Angle-Dependent Gradient			
Macroplot	1	3	7	9	11	12	13	15	1	2	4	5
Terrestrial Mosses	0.00	171	262	207	239	262	189	285	0.00	89.7	147	183
Vascular Understory Plants	37.9	298	222	245	336	231	369	274	37.9	56.8	180	271
Vascular Understory Plants and Mosses	37.9	469	484	452	576	494	557	558	37.9	146	327	454

Figure 9. The mean Q_i value ($\pm$ SE, where difference is significant) for the vascular plant (X) and moss (▫) components of the understory in macroplots along the distance-dependent stress gradient.


x VASCULAR PLANTS
□ MOSSES
MEAN Qi VALUES
50
45
40
35
0
1
3
7
9
11
12
13
15
MACROPLOTS

to compare values of understory components in stressed and unstressed macroplots. Mean Q_i values tend to be higher for mosses than for vascular plants, which suggests that mosses are more sensitive to SO_2, although they are significantly so only in macroplots 1 (moss mean $Q_i = 0$), 3, and 15. Except for the macroplots of the gradient extremes, there is little change in the mean Q_i values for macroplots positioned along the distance-dependent gradient. Thus, except for macroplots 1 and 15, the inclusion of Q_i into the IAP formula has the approximate effect of a constant multiplication factor.

Soils

In an effort to determine whether edaphic changes could account for the distribution of vegetation in the macroplots, soil samples were collected and their texture, pH, and percentage of organic matter were analyzed (Table 10). Textural analysis showed the soils of the study area to be alfisols, and in spite of the variation in sand, silt and clay percentages, neither texture nor pH of these samples appeared to be related to the patterns of plant community structure shown by canopy coverage data.

The high percentages of organic matter and the high concentration of sulfate sulfur found in macroplot 1 appear to be the only edaphic measurements related to the state of the vegetation in the study area. It is

Table 10. Analysis of soil cores collected from each macroplot.

Macroplot	Texture Analysis* Sand	Silt	Clay	pH	% Organic matter
		Percentage			
1	32.86	49.80	17.34	6.1	12.9
2	42.56	49.92	7.67	5.5	0.1
3	52.64	28.76	18.61	5.5	2.1
4	40.22	33.11	26.67	5.5	1.2
5	Soils not analysed				
6	34.48	53.44	12.09	5.6	0.7
7	19.59	56.81	24.10	6.0	2.9
8	37.20	51.97	10.83	5.5	0.5
9	38.39	54.34	7.27	5.6	0.9
10	43.02	44.08	12.90	5.5	0.3
11	45.15	43.92	10.93	5.5	0.8
12	43.95	53.32	13.73	6.0	1.9
13	39.72	43.24	17.00	6.3	1.1
14	28.84	60.38	10.78	5.1	1.1
15	34.58	52.38	13.04	5.1	0.1

*Mean of triplicate samples with less than 1% difference between samples.

N/D = None Detected Gravimetrically.

possible that the high percentage of organic matter found in macroplot 1 is constituted from dead and decaying vegetation found there.

DISCUSSION

The low number of Picea glauca seedlings associated with heavily stressed macroplots suggests that this species may not be reproducing effectively in stands near the refineries. In similar macroplots located where air pollution stress is low, sexual reproduction is successful. If reproductive failure results from SO_2 stress, then several interesting factors may be involved. Sublethal concentrations of air pollution are known to reduce conifer growth rates (Dochinger and Seliskar 1970; Westman 1974), and Houston and Dochinger (1977) show that one consequence of this in Pinus spp. is the production of fewer seeds per cone and a reduction in seed weight. The germination percentage of seeds produced by Pinus resinosa Ait. which are subjected to air pollution stress also is lower than is that for control plants. The failure of moist pollen to germinate following air pollution stress (Karnosky and Stairs 1974; Masura, et al. 1976) may reduce fecundity. McGee (1975) suggests that when canopy cover is removed the accompanying phenological changes make the seedlings more susceptible to frost. Perhaps in this study area changes in the understory canopy

render Picea glauca seedlings more susceptible to SO_2 stress. Such forms of indirect air pollution damage also may account for many of the synecological alterations seen in the understory of this study area.

The reproductive strategies of the understory components account for some of the patterns of community perturbation which are documented. The invasion of the "weedy" angiosperms occurs at a distance of 6 km from the refinery, a location where SO_2 stress is low. Openings which occur within the vascular plant component of the understory are apparently filled by seeds dispersed from angiosperms. Subordinate species of terrestrial mosses which are more tolerant of SO_2, which exhibit limited sexuality, and which lack methods of spore dispersal that are competitive with mechanisms of seed dissemination seem to fill openings in the moss component of the understory by vegetative growth. For the sake of convenience I have considered the understory components separately. However, it is reasonable to expect that when a pollution-sensitive vascular plant is eliminated from a forest stand the opening which results will not be occupied exclusively by another vascular plant.

Many ecological and physiological studies have shown epiphytic cryptogams, including mosses (Gilbert 1970a, 1970b; Nash 1973; Nash and Nash 1974; Taoda 1976), to have low tolerances of air pollutants. In this study I found that moss coverage was not reduced until SO_2 stress

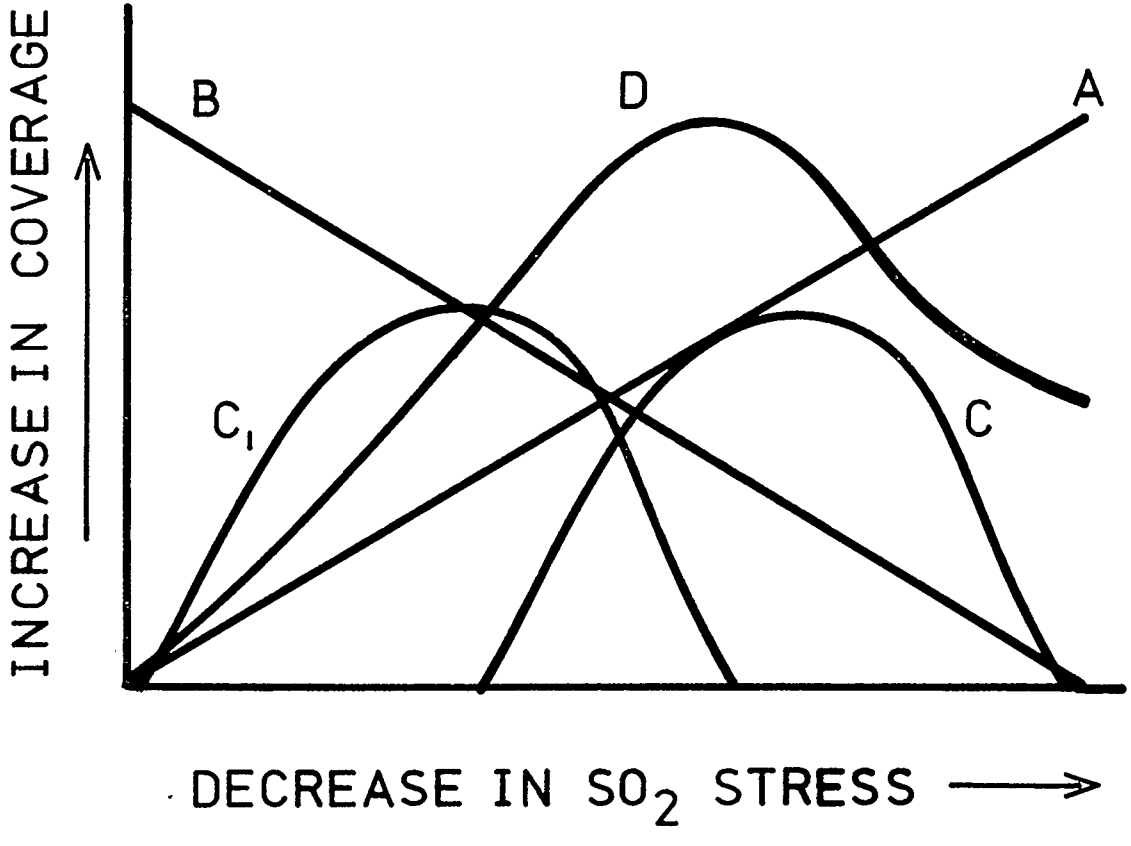
caused the relative coverage of vascular plants to drop to 65%. Mosses located 0.0-3.0 km from the refinery then exhibit greater sensitivity to increases in air pollution. They may be exposed to SO_2 in a number of ways, e.g. uptake from the air or rainwater through phyllidia which lack a waxy cuticle or from snow as SO_2 is trapped and then released during spring thaws prior to the growth of vascular plant species in the forest understory (Elgmork, et al. 1973; Hagen and Langeland 1973).

The response of any species to an SO_2 gradient may assume one of the four patterns represented in Figure 10. The data in Tables 4 and 5 allow the assignment of SO_2-response patterns (Fig. 4) to the dominant species encountered in the understory. The changes in coverage along the SO_2-stress gradient for Cornus canadensis, Linnaea borealis, and Mitella nuda (Table 4) and Pyrola asarifolia (data not presented) roughly resemble the A pattern and tend to decline at a constant rate. The Gramineae and Equisetum arvense exhibit the B response pattern, and these species appear to increase in coverage with an increase in air pollution stress. Species such as Adenocaulon bicolor, Osmorhiza occidentalis, Vaccinium vitis-ideae, and Lonicera villosus have coverage trends which resemble the C and D patterns and have high coverage at localized regions of a stress gradient.

With the exceptions of Hylocomium splendens and

Figure 10. A model illustrating the patterns of changing coverage which individual plant species might exhibit along a gradient of SO_2 stress. The A pattern represents the decline of coverage of SO_2-sensitive species. The B pattern indicates species that invade into disturbed stands and/or have a requirement for high levels of atmospheric sulfur. Species which show localized increases and subsequent declines in coverage with increasing SO_2 stress may be either weeds (C patterns) or endogenous community members (D pattern).

COVERAGE RESPONSE MODEL



Ptilium crista-castrensis, the terrestrial moss species decrease in coverage with increasing SO_2 stress and thus resemble the A pattern. Both of the excepted species mentioned above reflect the D pattern and initially increase in coverage when the more pollution-sensitive and dominant Pleurozium schreberi diminishes in ecological importance along the distance-dependent stress gradient (Table 5).

The patterns of synecological change along the angle- and distance-dependent gradients differ. For example, the relative diversity continually declines as SO_2 stress increases along the angle-dependent gradient. However, this is not true for the distance-dependent gradient. One possibility in explaining these differences is that the array of macroplots uniformly positioned at 0.5 km from the refinery reflects the same stress gradient found between macroplots 1 and 3 of the distance-dependent gradient. Hence, the patterns of descending relative diversity and relative coverage associated with increasing SO_2 stress are similar if inspection of the distance-dependent gradient is restricted to distances less than 1.2 km from the refinery. Alternatively, while changes along the distance-dependent gradient may result from progressive dilution of SO_2, those changes along the angle-dependent gradient may result from the frequency of fumigation episodes. It is possible that along the latter gradient all the macroplots are fumigated with relatively

high SO_2 concentrations, but that macroplot 5 is fumigated less frequently than is macroplot 1 (Fig. 1). Intermittent fumigations at high concentrations may be sufficient to prevent successful "weed" invasion or the increase of the ecological importance of the subordinate moss species.

Synecological changes caused by species removal due to SO_2 stress are complex. The tendency for diversity to increase with SO_2 stress for both understory components along sections of the distance-dependent gradient is surprising, particularly since many studies of epiphytic cryptogams show simple patterns of declining species number, plant vigor and vitality, and canopy coverage, all of which are related to increases in air pollution stress.

The selective removal of plant species from assorted plant communities has not always resulted in substantial loss of vegetative production, stability, or the ability of the plant association to recover from stress. Allen and Forman (1976) have discussed the results of studies in which selective species removal by clipping, logging, herbicide application, insect attack, and experimental applications of SO_2 have led to increased productivity of other plant species in the community. The ability of communities to maintain stability or to recover from these perturbations depends upon many factors pertinent to the species which are removed. These factors include abundance, canopy shape, canopy position above the

ground, and reproductive strategy. Vertical organization of the association, size of the area under stress, duration of the stress, and meteorological factors also may influence the responses of plant associations under stress.

In this study of the white spruce association, the ecological importance (coverage) of many understory species changes in relation to SO_2 stress, but even in the most heavily fumigated macroplot the persistence of the species forming this plant association is apparent. Hence, the white spruce association shows resilience such as that defined by Holling (1973) for spruce forests attacked by spruce bud worm. The capacity for this study area to recover following cessation of SO_2 fumigation must remain unknown at this point.

Braun-Blanquet's (1932) method for assessing the "ecological importance" of plants by measuring canopy coverage was quantified by Daubenmire (1959) and has been recently extended to terrestrial mosses (Stringer and Stringer 1974). Seasonal changes in canopy size and the unknown role that underground plant parts play in determining the ecological relations between individuals and species are characters which may obscure the measurement of the ecological importance of vascular plants (Daubenmire 1968). These shortcomings are minimized when canopy coverage analysis is applied to mosses because these plants have little subsoil structure and are

persistent perennials. Consequently, the use of canopy coverage measurements to provide synecological analysis of terrestrial mosses seems appropriate.

The decline in moss canopy coverage as SO_2 stress increases along both gradients (Figs. 5A and 5B) roughly parallels the decline in both the green turf depth (Figs. 6A and 6B) and the proportion of the green moss biomass (Figs. 7A and 7B). Hence, I consider the status of terrestrial mosses in this study area to be a reflection of the severity of SO_2 stress at any site. Coverage is a useful parameter for assessing the impact of SO_2 upon vegetation because it is convenient to measure and does not involve damage to the moss turf. I conclude, therefore, that terrestrial mosses can be useful bioindicators of air pollution.

The deterioration of the moss turf occurs simultaneously in two dimensions. A relatively low degree of SO_2 stress affects the vertical structure of the turf, as is shown by the green turf depth (Figs. 5A and 5B), green biomass (Figs. 6A and 6B), and percentage of green moss (Figs. 7A and 7B), more dramatically than it does the horizontal structure, as is shown by coverage (Fig. 8). In macroplots where SO_2 stress is relatively high, the reverse is true.

The chlorophyllose moss turf in macroplot 13 is thicker (Fig. 5A) and has more biomass (Fig. 6A) than does that in macroplot 15. Coverage of the moss in both

macroplots is similar (Fig. 5A). The increase in the prominence of mosses in macroplot 13 may be due to a decline in relative coverage of the vascular plants from 100% to 81% between macroplots 15 and 13. Hence, mosses in macroplot 13 compete against fewer vascular plants but are still "protected" by a substantial vascular plant canopy. Wood and Nash (1976) have also noticed "positive" vegetational responses to increases in pollution stress produced by a copper smelter in the Sonoran Desert, but they did not offer an ecological explanation.

The decline in capsule numbers in macroplots with increasing SO_2 stress along the distance-dependent gradient is consistent with the observation of the moss species Leskea polycarpa made by LeBlanc and DeSloover (1970). These observations suggest that during their life history mosses may be vulnerable both when capsules form and when protonema develop (Gilbert 1968; Nash and Nash 1974). Most bryophytes are long-day plants and fruit more frequently at high light intensities and warm temperatures (Benson-Evans 1964). Hence, thinning of the herbaceous component of the understory in this study area due to increasing SO_2 stress should generally stimulate moss capsule production. The more xeric conditions which result from understory thinning may reduce capsule formation by restricting sperm mobility. Laboratory experiments will be necessary to separate the direct and indirect effects of SO_2 upon moss capsule production.

When the decline in moss coverage occurs most rapidly, the number of capsules present is already low in relation to the number of capsules encountered in lightly stressed macroplots. Consequently, the degree to which SO_2 stress reduces moss coverage by influencing capsule formation is probably small. As SO_2 stress increases in macroplots 9 through 15, the coverage of Hylocomium splendens and Ptilium crista-castrensis increases. Coverage increases, therefore, must be the result of vegetative growth processes which are somewhat resistant to air pollution stress.

The IAP formula has changed frequently since its inception (DeSloover 1964). Some formula modifications are the result of a wide range of synecological methods which generate so many forms of data that a single IAP formula is not applicable. Other mathematical modifications have been made in order to refine the IAP. Hoffman (1974) increased the amount of ecological information contained within the IAP value by adding a qualitative sociability estimation with a vigor-vitality value, and then used this sum as the term "f" in LeBlanc and DeSloover's IAP formula (1970). Hoffman (1974) also discussed the relative merits of the ecological factor Q_i and used this factor in his IAP expression. Modifications to the IAP formula unfortunately, however, prohibit comparison of air pollution impact studies.

The IAP appears to have limitations even within a

single study. Although the IAP values in Table 3 clearly show the result of SO_2 stress along the angle-dependent gradient, these indices hide the more subtle SO_2-induced canopy coverage alterations along the distance-dependent gradient. Coverage data are included in the IAP but apparently are obscured by mathematics. Since ω_i values are generally constant throughout the study area they have little effect on the IAP in this study, and since the macroplots are numbered in order of increasing coverage of the terrestrial mosses, the discrepancy between IAP trends and observed responses along the distance-dependent gradient must be the result of the manipulation of the coverage and frequency data. A high c x f product, therefore, can mean one of two things: (1) the site has not been heavily stressed, or (2) a moderate stress has allowed either weeds or relatively pollution-tolerant but subordinate species to increase in phytosociological importance. Both of these latter phenomena occur along the distance-dependent SO_2-stress gradient. The IAP, then, seems applicable when lichens are the only organisms under investigation or when, in similar situations, weedy or subordinate species fail to increase in coverage with increases in air pollution stress.

The IAP is an estimation of vegetational perturbation but it falls short of being an accurate "Index of Community Vigor" (ICV). This suggested change

in terminology would accent the affected plant communities, whereas the IAP indirectly describes air quality.

In assessing air pollution impact upon vegetational communities ecologists should utilize as much quantitative information as possible. Such data may include species number, coverage, and frequency. Community diversity should also be considered as an expression capable of showing the effects of air pollution upon an association of species. As these data components in themselves can be inadequate expressions of the status of vegetation at any site, an unbiased mechanism to weigh coverage and diversity measurements is appropriate. The "ecological factor" Q_i is already used, perhaps incorrectly, in this and in other studies as such a device. The ICV should be deduced theoretically and made statistically justifiable so that the equation exists as a firm expression resistant to convenient alterations made on an arbitrary basis.

Chapter 2

INTRODUCTION TO STABLE ISOTOPE STUDIES

Establishing cause and effect relationships between an air pollutant and apparent ecological damage is difficult (for examples see Nash 1975, Wood and Nash 1976). This study, however, will show how I have used stable sulfur isotopes to trace SO_2 from an emission source in the surrounding vegetation. By using stable sulfur isotope ratios I have (1) determined some of the fates of the pollutant when it is released into the ecosystem, and (2) identified some of the ecological factors which influence SO_2 accumulation by ecosystem components.

Atmospheric SO_2 in Alberta has $\delta^{34}S$ values* between +5 and +30. This range corresponds to that of H_2S in natural gas wells and is higher than that for sulfur from non-industrial sources in the study areas.

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*Terrestrial sulfur isotope abundances are defined on a $\delta^{34}S$ scale which for a given sample expresses the deviation in parts per thousand (o/oo) of its $^{34}S/^{32}S$ abundance ratio from that of meteorite troilite, i.e.:

$$\delta^{34}S = \left[\frac{[^{34}S/^{32}S]\ \text{Sample}}{[^{34}S/^{32}S]\ \text{Meteorite}} - 1 \right] \times 10^3$$

The $\delta^{34}S$ value of the H_2S varies with the depth, stratum, etc. (Krouse 1977a). In gas refining the H_2S is converted to elemental sulfur by the Claus reaction, and during this conversion a small percentage of the reaction products is emitted as SO_2.

In contrast to the high $\delta^{34}S$ values of SO_2, those of sulfur in unpolluted soils, air, and vegetation in many regions of Alberta tend to be nearer to 0 and even as low as -30 (Lowe et al. 1971; Hitchon and Krouse 1972). Therefore, isotopic analysis of sulfur extracted from ecosystem components can be used to determine that proportion of sulfur which originated as air pollution emitted from natural gas refineries.

STUDY AREAS AND METHODS

SO_2-Stress Gradients

Field work was carried out during the summers of 1975 and 1976 in the Boreal Forest near Fox Creek in central Alberta. The Kaybob I and II natural gas refineries are located in the study area and emit about 71 metric tons of SO_2 per day. I located all the forest stands dominated by a white spruce overstory within 10 km of the refineries. These stands were ecologically similar and undisturbed except for the effects of SO_2. At least one macroplot (10 m x 25 m) was established in each stand and altogether 15 macroplots were delimited in the study area.

The canopy coverage of the terrestrial moss carpet in each macroplot was measured with a plotframe, as is described in Chapter 1. In this way I examined the two SO_2-stress gradients associated with the Kaybob I and II emission source (see Chapter 1).

Samples of terrestrial mosses, conifer needles, soil, and air were collected from the study area for isotopic analysis. Five samples of moss turf, each about 4 dm^2, were collected from near the corners and center of each macroplot. These samples were air-dried and the collections from each macroplot were combined in opaque bags. Five 0.2 g samples were selected randomly from each of the 15 bags and were prepared for sulfur isotope analyses.

Needles of Picea glauca and Abies balsamea were collected in November, 1976, from heavily, moderately, or lightly stressed macroplots along the distance-dependent gradient. They were collected by harvesting branches at breast height on the outer edge of the canopy which faced the refineries and then were sorted into age classes.

To investigate the variance of $\delta^{34}S$ values in conifer needles in relation to their height in a Picea glauca canopy, I collected needles at various heights from a 31 m tall tree. The tree grew about 100 m from macroplot 2 and had been uprooted during road construction started in November, 1976.

Five soil cores, about 4 cm in diameter and 10 cm deep, were collected from near the corners and center of macroplots 9, 7, 2, and 1. The cores were combined to make up a sample representative of each macroplot and then were air-dried and passed through a 2.0 mm mesh; a 0.2 g portion, selected by splitting, was obtained for isotopic analysis. Total sulfur was determined by the method of Siegfriedt et al. (1951).

High volume air samplers, equipped with a fiber-glass filter (for trapping particulates in the air), and a KOH-triethanolamine-impregnated cellulose filter (for absorbing atmospheric SO_2) were operated in the study area from November 15 to 18, 1976. The air samplers were positioned at three sites which varied in SO_2 stress and were either near the refineries or near a station equipped with an SO_2 analyzer which verified the presence of industrial emissions.

Potting Experiments and Laboratory Treatments

I altered the $\delta^{34}S$ values of plants in both the field and laboratory by exposing them to SO_2. Three- to four-year-old Pinus contorta specimens were collected from an SO_2-stressed or -unstressed site near the Ram River natural gas refinery (200 km northwest of Calgary). They were potted in their native soils when collected in early May before needle flush and were maintained in a greenhouse until bud break in early

June. The $\delta^{34}S$ values of conifer needles were determined just prior to transferring the trees into the field and represent native sulfur compounds and those compounds taken up during greenhouse care. Some pots were covered with a 1 cm layer of either peat or clipped, living specimens of the terrestrial moss Tortula ruralis (collected 20 km west of Calgary). Nylon mesh was laid between the potting medium and covering material and the layer of Tortula ruralis, which could have blown out of the pots, was contained with cheesecloth. Half-strength Hoagland's solution (without SO_4^{-2}) was used to supply water and nutrients and was delivered through irrigation tubes extending above and below the pot covers. The tubes were stoppered between bi-weekly watering treatments.

The initial isotopic composition of the needles, potting medium, and pot covers was determined prior to setting out the plants in early June, 1976. The pots were placed upon exclosures (elevated 0.5 m above the ground) to eliminate herbivory at the following two study sites: (1) an area of high SO_2 stress located 2 km southwest of the Balzac natural gas refinery (35 km northeast of Calgary), and (2) an area of low stress located at the University of Calgary Environmental Research Center (80 km southwest of Calgary).

Six 0.25 g air-dried portions of each of the feather moss species--Pleurozium schreberi,

Hylocomium splendens, and Ptilium crista-castrensis--were obtained from macroplot 3, hydrated for 24 hours, and taken to a fresh weight of 1.25 g by blotting. The sulfur content of three portions of each moss species was determined by the method of Siegfriedt et al. (1951). The moss samples were oxidized by O_2 under pressure in a Parr bomb so that all the sulfur was converted to sulfate. When all the SO_4^{-2} had been precipitated by $BaCl_2$ titration, any additional Ba^{+2} which was added formed a complex with tetrahydoxiquinone to indicate the titration end point. The $BaSO_4$ obtained was combined and isotopically analyzed.

Three portions of each moss species were fumigated with clean, dried air and mixed with SO_2 supplied from a cylinder. The gas mix was delivered at a flow rate of 1. $l \cdot minute^{-1}$ through glass or FEP teflon tubing for a period of 15 minutes. The SO_2 concentration entering the glass fumigation chamber was assayed by the method of Huitt and Lodge (1964) (See Chapter 3 for the specific procedures.) The total sulfur contents and $\delta^{34}S$ value were again obtained for the fumigated plants. Photosynthetically active radiation was less than 9 $\mu E \cdot m^{-2} \cdot second^{-1}$ during the fumigations. The $\delta^{34}S$ value of the SO_2 in the cylinder was determined by bubbling the gas in water, by oxidizing the dissolved sulfite to sulfate with 10% H_2O_2, and by obtaining $BaSO_4$ for analysis by adding a 10% solution of $BaCl_2$.

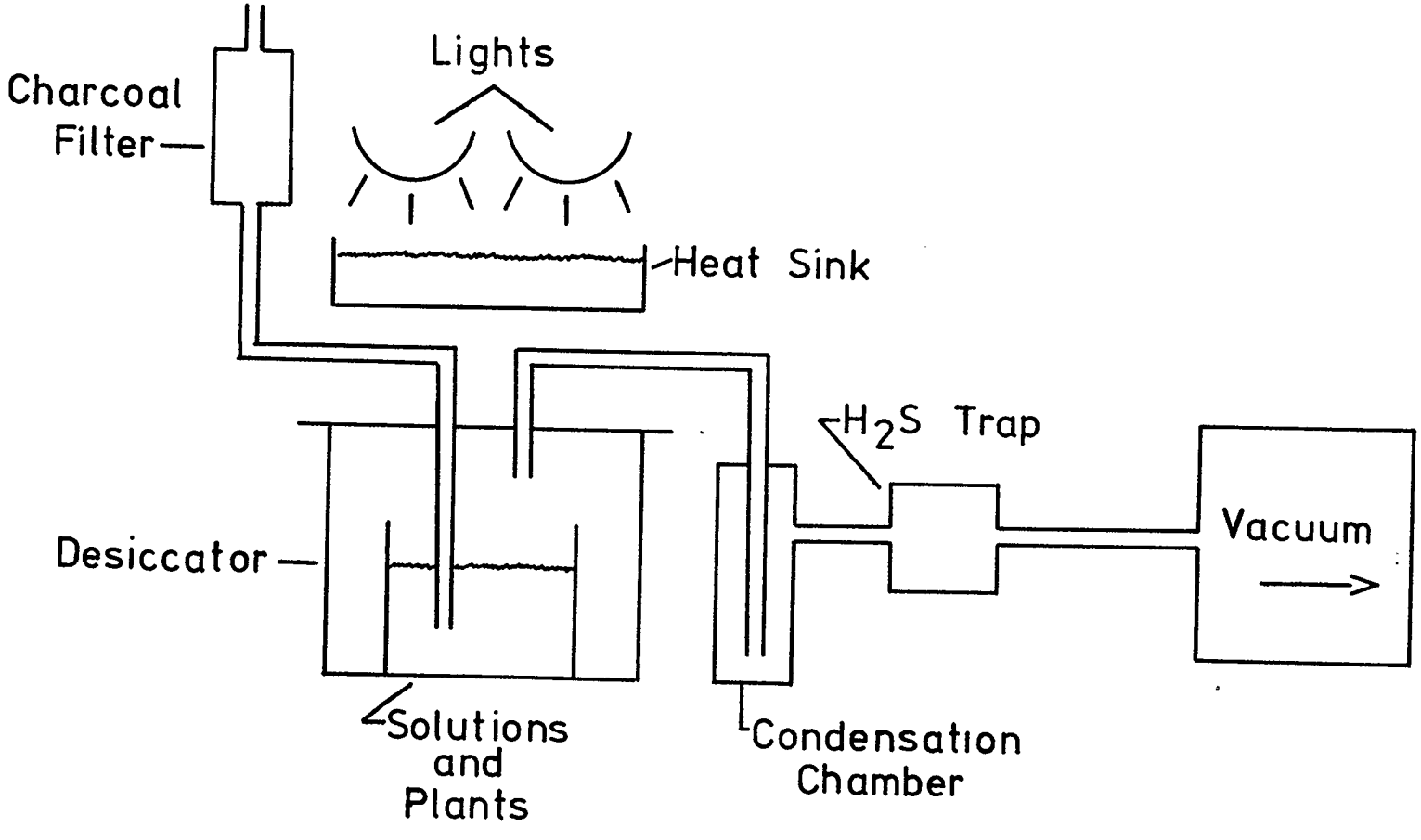
H_2S Emissions

It has recently become known that H_2S gas is emitted from a wide variety of plants when their leaves are irradiated and their roots are placed in solutions of sulfate or bisulfite ions (Wilson, et al. 1978). Since these conditions exist for plants at Fox Creek, it seemed conceivable that they could alter their $\delta^{34}S$ values by preferentially releasing one of the stable sulfur isotopes during the H_2S emission process.

To test for isotopic fractionation during the process of H_2S emission, a system was assembled to capture, for isotopic analysis, any H_2S gas emitted by test plants (Fig. 11). Air from the laboratory was pulled through a series of chambers by vacuum. The flow rate was monitored by a Matheson 7264 rotameter at 2-4 $l \cdot minute^{-1}$. The air was filtered through charcoal before it entered the experimental chamber, bubbled through the test solution, and passed over the plants. Radiation was supplied by two 300-watt incandescent bulbs and a bank of Sylvania cool-white fluorescent tubes. Irradiation from the lighting was filtered through 3-4 cm of water which served as a heat sink. Photosynthetically active radiation was measured at 150 $\mu E \cdot m^{-2} \cdot second^{-1}$ with a Lambda LI 192S quantum sensor connected to a voltmeter. The air, including any H_2S which had been emitted, passed through

Figure 11. The system by which H_2S gas emitted by plants was captured as Ag_2S for isotopic analysis.

H_2S Emission and Capturing System



the chamber containing plants, through a condensation chamber, and into contact with $AgNO_3$ adhering to quartz wool. The $AgNO_3$ reacted with H_2S to form Ag_2S which I isotopically analyzed.

Test solutions of oxidized sulfur were prepared by adding distilled water to either $Na_2S_2O_5$ or Na_2SO_4 obtained from the Sigma Chemical Company or Na_2SO_4 prepared by passing air over burning sulfur in a Thunberg tube. The SO_2 was dissolved in distilled water and was then oxidized to SO_4^{-2} by using a 10% solution of H_2O_2. The Na_2SO_4 was obtained by adding NaOH and evaporating the mixture to dryness. A 500 ml beaker was used to contain the roots of experimental plants and was filled to the 400 ml mark with a test solution.

H_2S was generated in the experimental chamber to determine if there was any isotopic preference by the H_2S trap. This was done by placing FeS in the chamber, adding concentrated HCl, and then by flushing the chamber with filtered air.

Seven to nine cucumber plants (cv. Straight Eight) which were from five to seven weeks old were used in an eight-hour experimental run. These plants were raised from seeds in soil of equal parts peat, vermiculite, and Terragreen (ceramic chips) and were watered from a tap. The plants were raised from seeds which were planted about 5 cm apart in flats which were

kept in a greenhouse. The flats were placed about 0.5 m below a bank of Sylvania Gro-Lux fluorescent tubes which were set at a 14-hour photoperiod. The plant roots were washed clean prior to experimentation. Three to five runs were necessary to obtain enough Ag_2S for isotopic analysis.

Isotopic Analysis

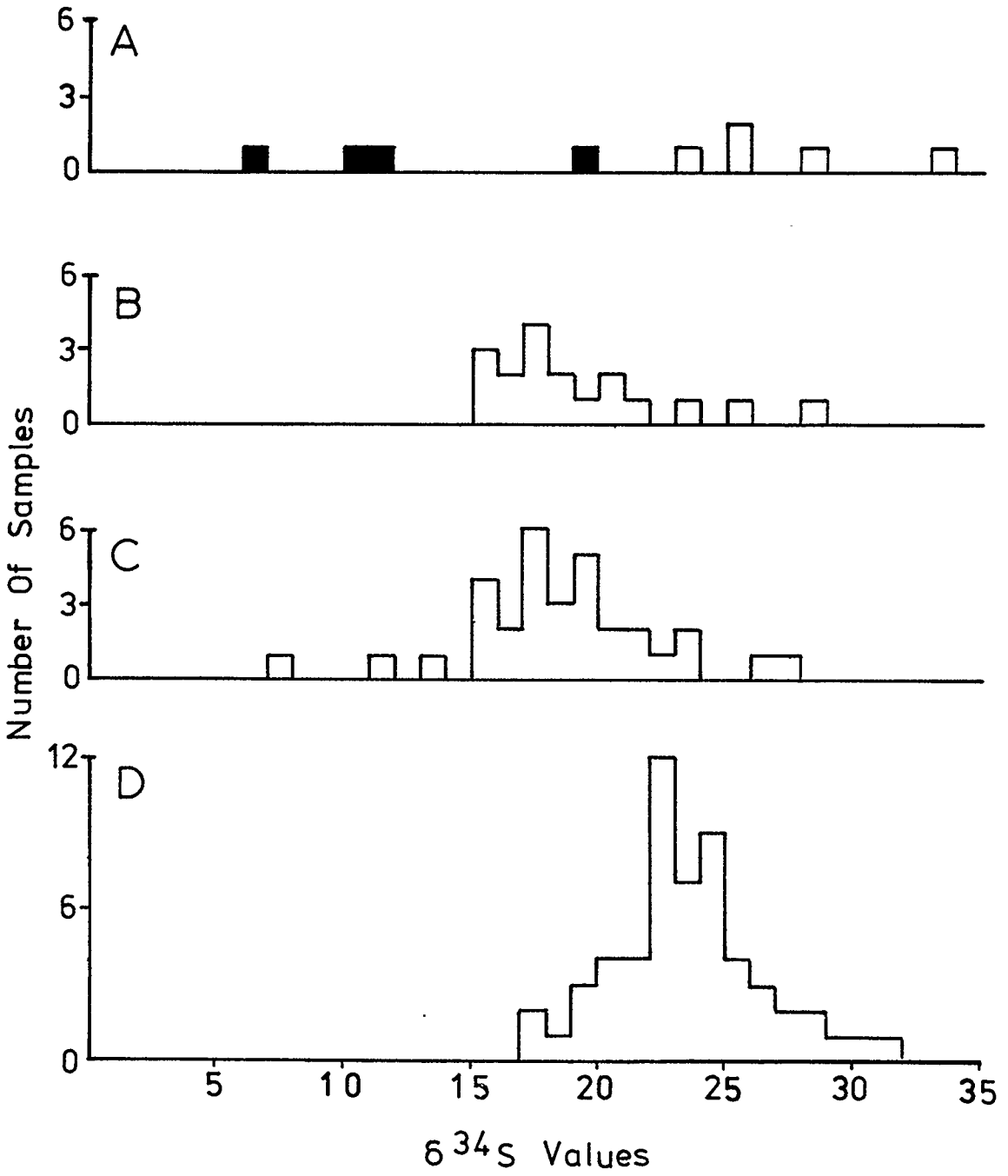
Samples for isotopic analysis were oxidized in 25 atmospheres of O_2 in a Parr bomb which converted all forms of sulfur to sulfate. The bomb, crucible, and electrodes were rinsed several times with distilled water which was collected. Ba^{+2} was added to precipitate $BaSO_4$, which was converted to Ag_2S (Thode et al. 1961). Dried Ag_2S was converted to SO_2 for isotopic analysis by reacting it with Cu_2O at 900°C. Ion currents of mass 66 and 64 + 65 were measured with an isotope ratio mass spectrometer built around a Micro-mass 602 analyzer.

RESULTS

The Fox Creek Study Area

The mean $\delta^{34}S$ value of sulfur collected by SO_2 absorbent filters in the high volume air samplers positioned around the Kaybob refineries is about +25 (Fig. 12A). This is within the range of values for SO_2 associated with other natural gas refineries which

Figure 12. The $\delta^{34}S$ values of (A) soils ■ and air □ , (B) Abies balsamea needles, (C) Picea glauca needles, and (D) terrestrial mosses, collected at the Fox Creek study area.


A
B
C
D
6
3
0
12
Number Of Samples
5
10
15
20
25
30
35
δ 34S Values

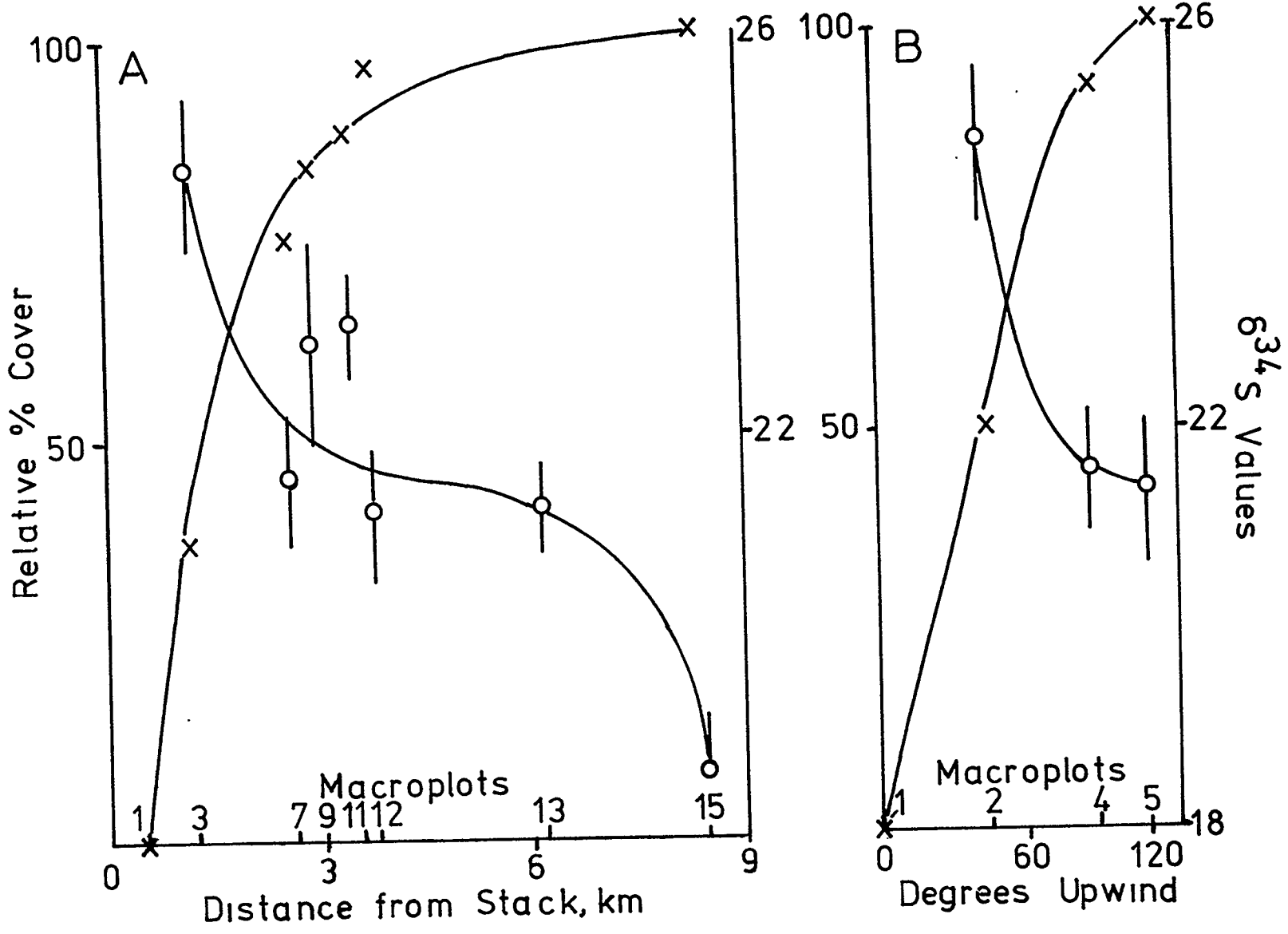
process H_2S gas (Krouse 1977a, b).

The sulfur concentration of the collected soils was greater than 0.7 ppm (the minimal concentration required for isotopic analysis) in only four macroplots. Except for the macroplot most heavily stressed by SO_2, where the soil sulfur concentration was 16.5 ppm and the $\delta^{34}S$ value was +20, the $\delta^{34}S$ values of soils collected from moderately stressed macroplots range from +7 to +12 (Fig. 12A).

In this study area, $\delta^{34}S$ values for terrestrial mosses range between +16 and +32 with an approximate mean value of +24 (Fig. 12D). The $\delta^{34}S$ values for conifer needles also vary, but the mean is about +16 (Figs. 12B and 12C). This suggests that mosses absorb airborne sulfur while conifer needles derive their sulfur from both the air and soil. These data are consistent with those cited by Krouse (1977b) for the Ram River area of Alberta, although the $\delta^{34}S$ values of conifer needles and SO_2 are naturally lower than are those in the Fox Creek study area.

The feather moss species, Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis, which were collected from each macroplot along the distance- and angle-dependent stress gradients, were analyzed for their isotopic composition (Figs. 13A and 13B). Their $\delta^{34}S$ values range from +18 to +24. These data confirm the uptake of industrially processed sulfur

Figure 13. The change in relative percent cover (X) and $\delta^{34}S$ values (0) of moss in macroplots along the distance- (A) and angle- (B) dependent SO_2 stress gradients in the Fox Creek study area. Isotopic values are means, $\pm$ SE where n = 5.


A
100
50
Relative % Cover
26
22
Macroplots
1
3
7
9
11
12
13
15
0
3
6
9
Distance from Stack, km
B
100
50
26
22
18
Macroplots
1
2
4
5
0
60
120
Degrees Upwind
δ34S Values

by mosses and are similar to the values obtained from samples represented in Figure 12. However, the mean $\delta^{34}S$ values of the most lightly stressed macroplot along the distance-dependent gradient declines to +18 (Fig. 13A) and only to +22 along the angle-dependent gradient (Fig. 13B).

Changes in the synecological relations of the vegetation at Fox Creek have been previously attributed to SO_2. I associated trends from the isotopic analyses of sulfur extracted from feather mosses with the relative percent coverage of terrestrial mosses along the SO_2-stress gradients. Figures 13A and 13B show that the ecological status of the terrestrial mosses is inversely related to the $\delta^{34}S$ value along both stress gradients. When the coverage of the moss carpet is extensive, the $\delta^{34}S$ value is low and vice versa.

Canopy coverage data show that Pleurozium schreberi is the feather moss most sensitive to increasing SO_2 stress (Table 5). Isotopic analyses of sulfur extracted from the three feather moss species were made for each macroplot in the study area to determine whether variance in the ecological responses of moss species corresponded to a similar variance in the accumulation of industrially processed sulfur. No significant differences in $\delta^{34}S$ values were found between the three feather moss species, however. The mean $\delta^{34}S$ values ($\pm$ SE) for Pleurozium schreberi,

Hylocomium splendens, and Ptilium crista-castrensis were +22.5 ± 0.7, +22 ± 1.0, and +21 ± 1.0 respectively.

The variance in $\delta^{34}S$ values is greater for conifer needles than for mosses (Fig. 12), and analyses were made to determine why this is so. Needles were collected from Picea glauca and Abies balsamea in each macroplot along the distance-dependent SO_2-stress gradient. The mean $\delta^{34}S$ value for both species was found to be +18, which is similar to that obtained from specimens represented in Figure 12. No significant difference was observed either between the two species or between macroplots along the stress gradients. However, Picea glauca needles collected beyond 8 km southeast of the study area (i. e. 18 km from the emission source) have $\delta^{34}S$ values of about +10, a value significantly lower than for those obtained within our study area (J. Case, personal communication).

The $\delta^{34}S$ values of Picea glauca needles tend to decline with needle age (Table 11). These data support the observation of Guderian (1977) that in SO_2-stressed sites, conifer needles decrease in sulfur content as needle age increases. The isotopic composition of conifer needles collected from the same tree but at different heights above the ground is not uniform. One-year-old needles at the top of the tree have a $\delta^{34}S$ value of +26 (Table 12) which is similar to that of emitted SO_2 (Fig. 12). The mean of the $\delta^{34}S$ values

Table 11. The $\delta^{34}S$ values* of sulfur extracted from Picea glauca needles of different ages.

Needle Age, Years	Mean $\delta^{34}S$ Value
1	+22 ± 1.5
2	+18 ± 1.8
3	+17 ± 2.0

*representing mean of five samples ± SE.

Table 12. The $\delta^{34}S$ values* of sulfur extracted from Picea glauca needles collected at various heights.

	Needle Height In Tree, Meters				
	4	11	17	21	31
Needle Age, Years					
1		+16	+17	+18	+26
2	+15	-	+13	+18	+15
3	+15	+19	+18	+17	+17

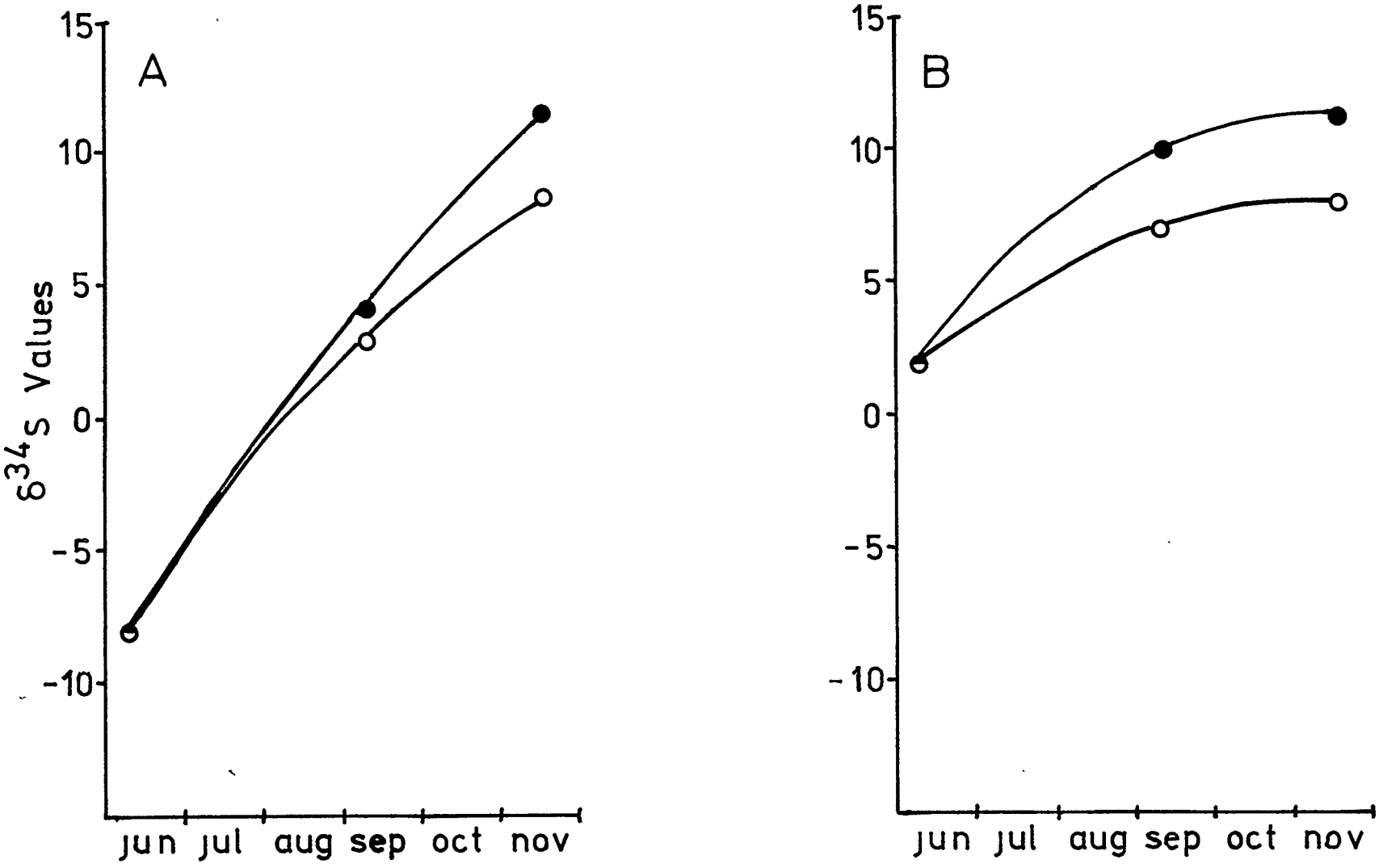
*representing analysis of one sample.

obtained for the other needles of this tree is +17 (± 0.5 SE) and is similar to that of Picea glauca needles shown in Figure 12.

Controlled Exposure Experiments

To tie more closely the variance in $\delta^{34}S$ values with SO_2 pollution, I devised manipulative field experiments involving the transfer of potted plants to an area of either high or low SO_2 stress. Hence, $\delta^{34}S$ values were determined for plants prior to and following their transfer. The $\delta^{34}S$ value of needles from Pinus contorta collected from the relatively unstressed Ram River site was -8 in June, 1976. These tree needles, after their transfer to a position downwind from the Balzac SO_2 emission source, obtained a $\delta^{34}S$ value of +12 by the middle of November, 1976 (Fig. 14A). Needles of trees from the same Ram River site but which were transferred to Kananaskis, an area of less SO_2 stress than the Balzac site, had a final $\delta^{34}S$ value of only +8 at the end of the growing season. The needles of a group of trees collected from another Ram River site with higher SO_2 stress started the experimental period with a $\delta^{34}S$ value of +2 (Fig. 14B). After transfer to either Balzac or Kananaskis the values rose to +12 and +8 respectively (Fig. 14B). Thus, the $\delta^{34}S$ value for conifer needles changed in relation to the availability of isotopically heavier SO_2.

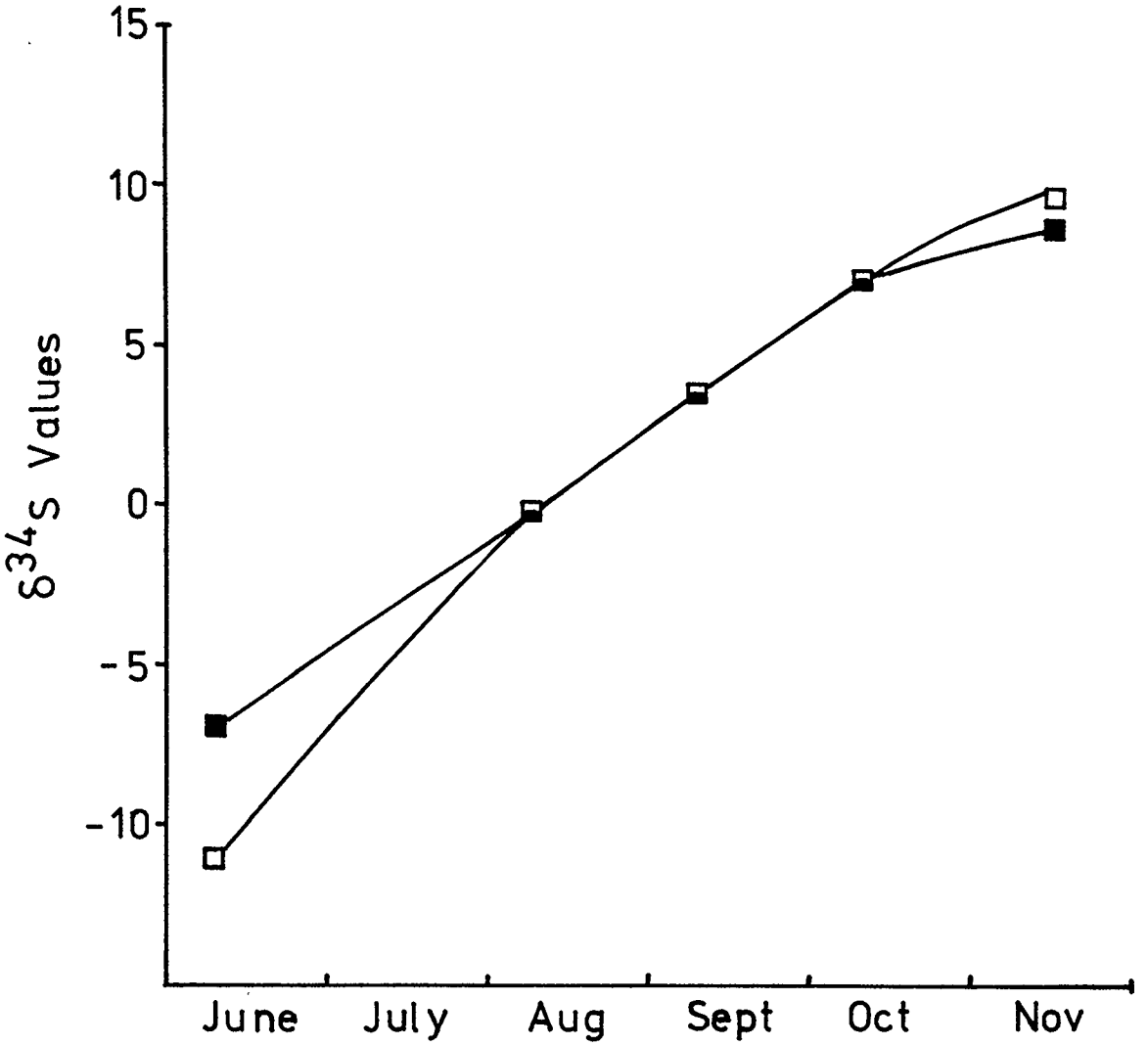
Figure 14. The isotopic changes during the 1976 growing season in Pinus contorta needles originating from the Ram River sites of either low (A) or high (B) SO_2 stress and which were transferred to either Kananaskis (0) or Balzac (●). Values represent a single analysis.


A
B
15
10
5
0
-5
-10
$\delta^{34}S$ Values
jun
jul
aug
sep
oct
nov

Potting experiments involving isotopic analysis also were devised to determine the potential contribution of atmospheric sources of sulfur to the extractable sulfur of soils. A group of potted trees from the lightly stressed Ram River site was transferred to the Kananaskis site. The $\delta^{34}S$ values in needles from these trees at the time of transfer and at the end of the experiment are shown in Figure 15. The $\delta^{34}S$ values of soil sulfur initially were lower than were those of needles (Fig. 15), but by the middle of August their values were similar. At the end of the experimental period the $\delta^{34}S$ value of soils was slightly higher than was that of the needles. The rate at which $\delta^{34}S$ values of soils and needles increases between October and November probably differs because of the physiological changes to trees which accompany the onset of winter. Hence, the rate of SO_2 accumulation by conifer needles declines late in the fall.

The soils of some of the potted trees collected from the lightly stressed Ram River site were covered with peat and transferred to the Balzac site where SO_2 stress was high. Peat absorbed SO_2 from the air and thereby prevented soils and tree roots from accumulating this pollutant. The needles of trees in pots without peat covers were considered to be experimental controls. The $\delta^{34}S$ values of needles from both

Figure 15. The isotopic changes for Pinus contorta needles (■) and uncovered soils (□) which were collected from the Ram River area and transferred to Kananaskis. Values represent a single analysis.


15
10
5
0
-5
-10
$\delta^{34}S$ Values
June
July
Aug
Sept
Oct
Nov

peat-covered and uncovered pots increased, but the values of the former group of needles increased more slowly and attained a lower final value than did the control plants (Fig. 16).

Mosses are known to accumulate airborne pollutants and this was confirmed by the use of stable sulfur isotopes. Three materials were tested as pot covers at the Balzac site during the 1976 growing season, namely peat, charcoal, or living moss (Tortula ruralis). The $\delta^{34}S$ values of all three increased during this experimental period (Table 13). Although each material reflects SO_2 accumulation, the final $\delta^{34}S$ value also is related to the initial value. The $\delta^{34}S$ value of Tortula ruralis at the end of the growing season nearly approached that of SO_2. Peat and charcoal, which started the experimental period with $\delta^{34}S$ values lower than those of Tortula ruralis, obtained final values which were proportionally less than were found for the living moss (Table 13).

Samples of feather mosses with a $\delta^{34}S$ value of +25 were fumigated with SO_2 which had a $\delta^{34}S$ value of -6.1 to determine if mosses showed isotopic selectivity during the process of SO_2 uptake. Feather moss samples which were not fumigated contained about 67% of the sulfur extracted from fumigated samples (Table 14). The increase in sulfur content due to the fumigations is similar for all feather moss species. Hence, these

Figure 16. The isotopic changes for *Pinus contorta* needles collected from the Ram River area and transferred to Balzac in pots which were either uncovered (●) or covered with charcoal (o). Values represent a single analysis.

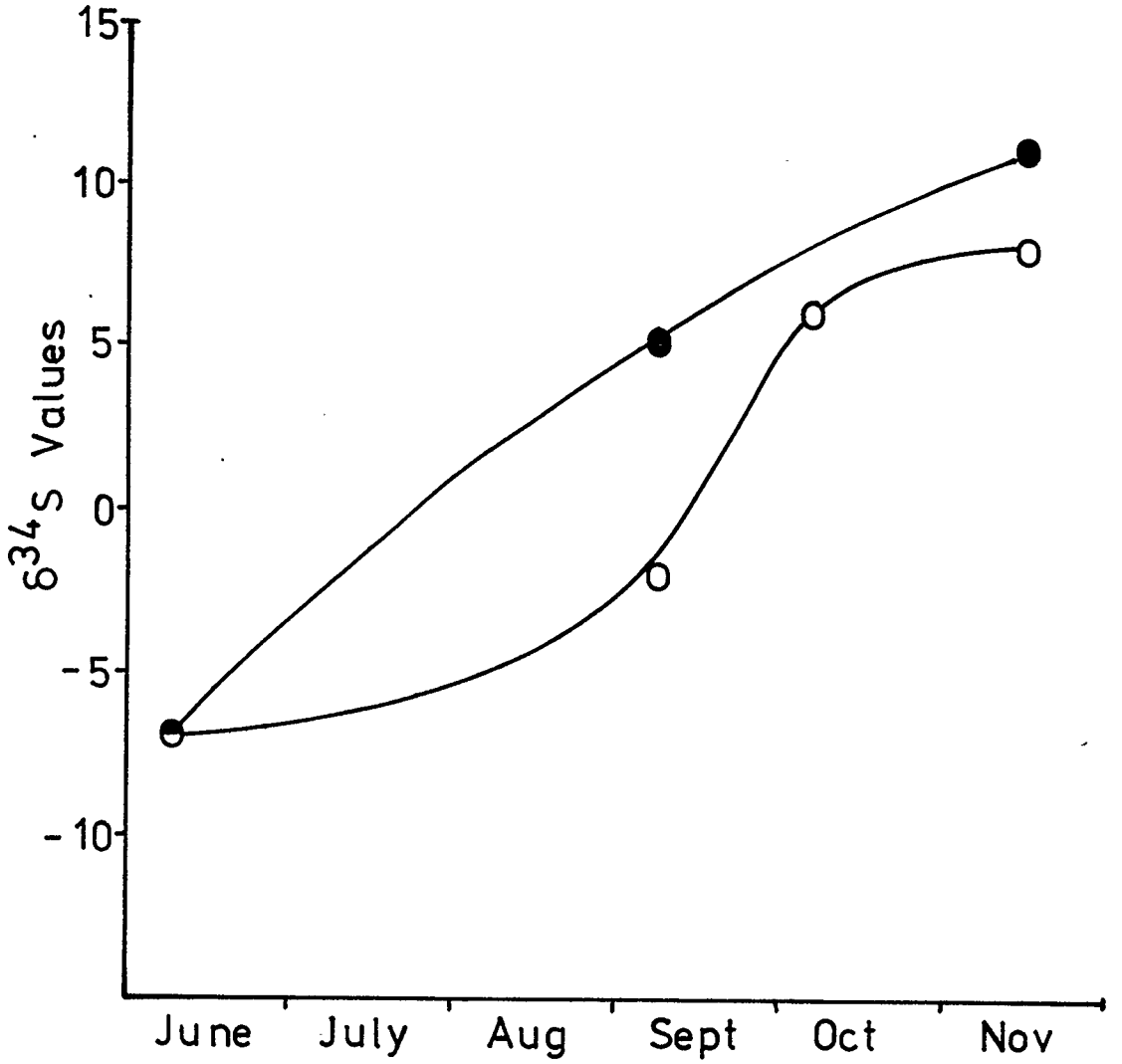

15
10
5
0
-5
-10
$\delta^{34}S$ Values
June
July
Aug
Sept
Oct
Nov

Table 13. The change in $\delta^{34}S$ values* of materials used as pot covers at the Balzac site during the 1976 growing season.

Material	$\delta^{34}S$ Values	
	June 7	November 15
Peat	-9.4	-3.3
Charcoal	-5.6	+6.1
Tortula ruralis	+10.1	+18.4

*representing analysis of one sample.

Table 14. The sulfur content and isotopic composition of the three feather moss species which were or were not fumigated with SO_2.

Moss species	Not fumigated		Fumigated	
	Mean Sulfur Content*	$\delta^{34}S$ Value**	Mean Sulfur Content*	$\delta^{34}S$ Value**
Pleurozium schreberi	0.41 ± 0.01		0.61 ± 0.05	
Hylocomium splendens	0.40 ± 0.01	+25	0.61 ± 0.05	+15
Ptilium crista-castrensis	0.41 ± 0.01		0.59 ± 0.04	

*Mean of triplicate samples, ± SE

**Determined by single analysis of all sulfur extracted from experimental mosses.

species did not differ in their uptake of SO_2. If 67% of the sulfur in fumigated plants has a $\delta^{34}S$ value of +25 and the value of the remaining 33% (which represents SO_2 uptake) is -6, then the expected $\delta^{34}S$ value for fumigated plants should be about +15 if no isotopic selectivity occurs during uptake. This is the value which was obtained (Table 14). Therefore, the change in $\delta^{34}S$ value of fumigated mosses is related to (1) the size and isotopic composition of the sulfur pool prior to fumigation, (2) the $\delta^{34}S$ value of SO_2, and (3) the extent to which sulfur from SO_2 can contribute to the total sulfur pool.

H_2S Emission

Fractionation of sulfur occurred during the reduction of oxidized sulfur salts which were applied to cucumber roots. The H_2S which resulted was emitted from the plant canopy and was captured for isotopic analysis. In a control experiment in which plants were not used it was found that traps placed in series captured H_2S (generated from FeS + HCl) which was identical in isotopic composition. Therefore, the H_2S trap of $AgNO_3$ did not appear to show isotopic selectivity.

The isotopic composition of the H_2S emitted from plants varied from the composition of the oxidized sulfur salt which was applied to their roots in solution

(Table 15). The fractionation for 0.1 M and 0.2 M HSO_3^- solutions was more extreme than it was for the 0.3 M solution. This may be due to the toxic effect of the ion at the highest concentration following uptake by the experimental plants.

The SO_4^{-2} solution showed less fractionation during H_2S emission than did the HSO_3^- solution (Table 15). Since sulfate ions are known to be less toxic than bisulfite ions (Guderian 1977) and the transport of sulfate ions requires a carrier molecule, the contrast in fractionation for these compounds may reflect differences in the metabolic process leading to H_2S emission.

The fractionation which occurs when the HSO_3^- ion and the SO_4^{-2} ion are applied together is similar to that of the HSO_3^- ion alone (Table 15). Thus, when both sulfur ions are present, the H_2S which results seems to be derived primarily from the HSO_3^- ion.

DISCUSSION

Ecological Analyses

The synecological impact of SO_2 is generally more severe on cryptogams than it is on vascular plants. The sensitivity of mosses and lichens is greater, in part, because these plants accumulate airborne compounds at a faster rate than do vascular plants (LeBlanc *et al.* 1974; Mattsson and Liden 1975; Olkkonen and Takala 1975).

Table 15. The $\delta^{34}S$ values of H_2S emitted from cucumbers treated with bisulfite or sulfate ions.

Treatment	$\delta^{34}S$ Value		$\Delta\delta^{34}S$ Value
	Solution*	Captured H_2S	
0.1M HSO_3^-	+2.2	-14.5	-16.7
0.2M HSO_3^-	+2.2	-14.6	-16.8
0.3M HSO_3^-	+2.2	- 3.6	- 5.8
0.2M $SO_4^=$	+4.0	- 5.7	- 9.7
0.1M HSO_3^-+0.1M $SO_4^=$	+3.1	-13.4	-16.5

*When 2 ions are combined, the $\delta^{34}S$ value which is given is the mean value of the ions.

The $\delta^{34}S$ data presented here verify that terrestrial mosses obtain proportionally more of their sulfur from atmospheric SO_2 than do vascular plants in the same habitat. Presumably this uptake phenomenon characteristic of lower plants is related to unique morphological features such as the lack of functional stomates, lack of a protective cuticle, and the absence of a root system.

Sulfur extracted from Pleurozium schreberi, the feather moss species which appears to be the most sensitive to SO_2 (Table 5), does not differ isotopically from that in Hylocomium splendens and Ptilium crista-castrensis. In addition, laboratory fumigations fail to reveal isotopic selectivity as these species accumulate SO_2. Thus, while variation in the ecological impact of SO_2 upon cryptogams and vascular plants may be due to differences in pollution accumulation, analysis of stable sulfur isotopes shows that this does not account for the variance in SO_2 tolerance exhibited by these feather mosses.

The $\delta^{34}S$ values of conifer needles vary only in relation to age and height and do not reflect the change in SO_2 stress along the two stress gradients in the Fox Creek study area. This appears to contradict the observation that the $\delta^{34}S$ values of conifer needles increase following the transfer of trees to a site of relatively high SO_2 stress. The difference in isotopic trends is related to the presence or absence of an

SO_2-absorbent layer above the soil. The SO_2-absorbent layers (such as a carpet of terrestrial mosses) accumulate pollutants, making them less available to the roots of vascular plants. When this cover is not present or not intact, soils accumulate SO_2 rapidly.

The patterns of synecological change for mosses along the distance-dependent SO_2-stress gradient differ from those along the angle-dependent gradient. It was suggested in the previous chapter that the vegetation reflects a different fumigation pattern in each gradient: the distance-dependent gradient may be the result of SO_2 dilution whereas the angle-dependent gradient may be due to variation in frequency of fumigation by relatively high concentrations of SO_2. Figure 13 supports this hypothesis. The relative percent coverage of mosses is highest in macroplots 15 and 5 of the two SO_2-stress gradients. However, isotopic analyses of sulfur extracted from mosses in these macroplots suggest that macroplot 15 is fumigated at SO_2 concentrations which are lower than those in macroplot 5.

$\underline{H_2S \text{ Emission}}$

No isotopic fractionation occurs during the process of SO_2 uptake by feather mosses. However, the process of H_2S emission by plants provides a possible mechanism whereby the $\delta^{34}S$ value of photosynthetic

tissues could have a value which is higher than the sulfur sources of the plant. If plants preferentially emit the ^{32}S atom as H_2S, then their leaves would accumulate the ^{34}S atom and the $\delta^{34}S$ value would become more positive. Therefore, the emission of H_2S gas by plants provides a mechanism whereby they may obtain $\delta^{34}S$ values which are higher than any sulfur source of the plant. This process thereby offers an explanation as to how plant materials which were collected from Fox Creek could attain $\delta^{34}S$ values which are higher than the mean value for SO_2 (Fig. 12). These studies could be extended by quantifying H_2S emission rates from vegetation in the Fox Creek study area.

area, (2) the thallus thickness, and (3) the presence or absence of a gelatinous cortex (Wirth and Türk 1974). Vascular plants also are known to vary in their capacity to avoid SO_2 stress due to differences in stomates (size, shape, abundance, and physiology) and leaf area.

The field studies at Fox Creek suggest that the feather moss species are poor SO_2 avoiders. This is so because mosses accumulate more SO_2 from air than do vascular plants in the same study area. In addition, stable sulfur isotope analyses and increases in sulfur content following fumigations in the laboratory show that these species do not differ in SO_2-uptake capacity.

If the feather mosses have similar avoidance as well as tolerance capacities, their responses to SO_2 stress should be similar. The distribution of these mosses at the Fox Creek study area suggests that this is not the case; canopy coverage analysis shows Pleurozium schreberi to be the feather moss species which is the most sensitive to SO_2 stress in the field.

The purpose of the study which follows is to expose the feather mosses to SO_2 stress under controlled laboratory conditions. Photosynthetic and respiratory rates are the criteria for determining the moss responses to SO_2 because these processes are known to be affected by pollutants and can be readily monitored. These analyses therefore should provide some indication of the

Chapter 3

INTRODUCTION TO FUMIGATION EXPERIMENTS

Air pollution bioindicators are used extensively to satisfy the premise that as air pollution increases, air pollution bioindicators become less prominent. Potential uses for air pollution bioindicators include correlating their abundance with (1) the impact of SO_2 on the synecological structure of a plant community, and (2) the geographical extent of covert SO_2 injury of plants which appear to be unaffected by SO_2. Air pollution bioindicators also could prove to be useful experimental organisms for determining the metabolic effects of SO_2. In order to develop these potential uses of air pollution bioindicators, attempts must be made to clarify the nature by which plants vary in capacity to accommodate SO_2 stress.

Levitt (1972) has suggested that the capacity for plants to accommodate environmental stress is related to both "avoidance" and "tolerance." This concept has been used to explain the variations in SO_2 sensitivity which has been observed for lichens (Türk, et al. 1974). Lichens vary in their capacity to absorb SO_2 and the poor absorbers better tolerate SO_2 fumigation because they are good SO_2 avoiders. Morphological features of lichens which may affect SO_2-uptake rates are (1) the surface

tolerances which these species exhibit for SO_2 stress as well as reveal whether SO_2 might account for the distribution of these species at Fox Creek.

METHODS AND PROCEDURES

Collections, Storage, and Preparation of Mosses

The effects of SO_2 on the photosynthetic and respiratory rates of four moss species were determined. The feather moss species--Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis--were collected from near the Eau Claire recreational area along the Kananaskis River (about 72 km west of Calgary). Samples of Tortula ruralis were collected from a site located about 6 km west of Calgary. Collecting at all sites was done in an area of about one km^2, and swatches of turf which were dominated by the desired species were gathered at random. These moss species are known to withstand desiccation and to resume metabolism upon rehydration after air-drying.

The feather moss species were maintained fully hydrated at 4^oC in diffuse light. Samples of Tortula ruralis were air-dried on a laboratory bench and were stored in darkness until required; the moss was then hydrated for 20-24 hours. The green, chlorophyllose portions of the experimental mosses were clipped from the turfs, washed in deionized water, blotted to remove excess moisture, and air-dried over silica gel. Air-dried

moss pieces lose little or no additional water when oven-dried, so I used them to obtain samples of plant material which had uniform weights of 0.2 g. These samples were rehydrated in deionized water for 20-24 hours and were blotted to an experimental weight of 1-2 g (mosses in the fully hydrated state) or to 0.8 g (mosses in the water-stressed state) prior to experimentation. After the moss pieces were heated at 100°C for at least six hours, their oven-dried weights were obtained.

The IRGA Fumigation System

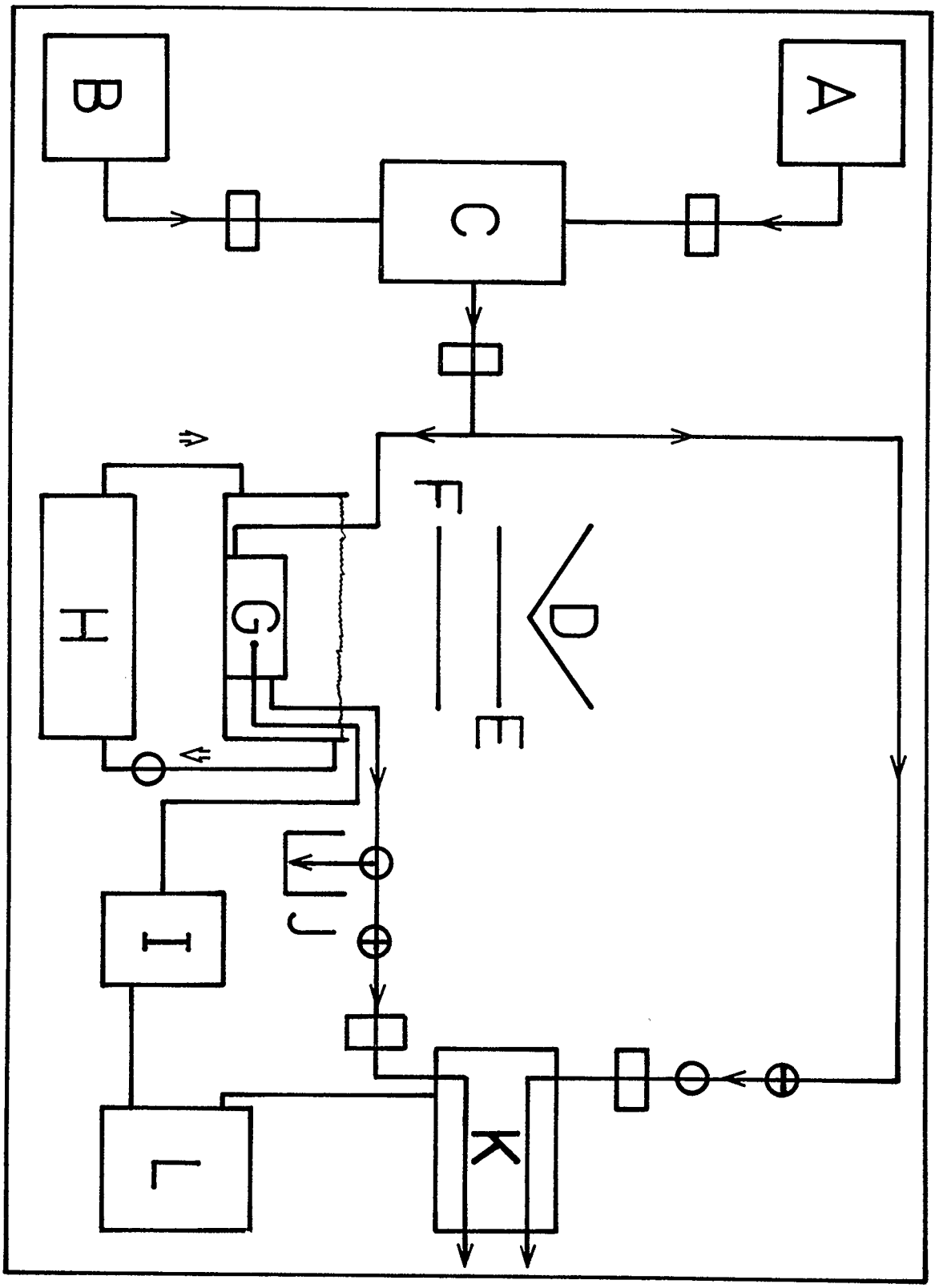
A system was designed to study the effect of SO_2 upon the photosynthetic and respiratory rates of mosses. SO_2 concentrations in Alberta are known to occur in excess of 10 ppm for 60 minutes, and far higher levels are thought to occur for shorter time periods. In these experiments, SO_2 usually was applied at a concentration of 9.2 ppm for 17 minutes. Although this fumigation is severe, comparable doses of SO_2 probably have occurred in Fox Creek stands which are heavily stressed.

Clipped moss portions were subjected to SO_2 stress while their photosynthetic and respiratory rates were monitored, as CO_2 exchange, by a Beckman model 865 infrared gas analyzer (IRGA). A flowthrough cuvette system was used so that SO_2 (at 92 ppm) in

air from a pressurized cylinder could be mixed with CO_2 (at 332 ppm) in air supplied from a second cylinder (Fig. 17). Both cylinders were supplied by Matheson Gas Company. The SO_2 and CO_2 in air were combined on the basis of flow rates which were monitored by flowmeters. Either Matheson series 7200 stainless steel and glass rotameters or Matheson series 7260 acrylic rotameters were used and they were calibrated by timing the displacement of water, by air, from a volumetric flask. SO_2 gas mixes were always handled in regulators, chambers, flowmeters, and tubing of glass, FEP teflon, or stainless steel.

The SO_2 and CO_2 in air were combined in a mixing chamber and moved from it, at a flow rate of 800 ml·minute^{-1}, to a junction where the gas flow was split and directed either to the reference channel of the IRGA or to the cuvette containing plants. Flow rate through the cuvette was 400 ml·minute^{-1}. Charcoal columns and silica gel + Drierite columns were positioned in the experimental and gas reference lines to remove both SO_2 and water vapor from the gases which entered the IRGA. Flow rates of gases in both the experimental and reference gas lines were monitored with flowmeters just prior to entering the IRGA and were equalized with an adjustable clamp attached to the reference line. Condensation of water vapor was not observed in any tubing or chamber during any experiment.

Figure 17: The IRGA fumigation system. SO_2 in air (A) was combined with 332 ppm CO_2 in air (B) in a mixing chamber (C). The gas stream was split such that gas was directed to the reference channel of the IRGA (K) or through the cuvette containing plants (G). Gas which exited the cuvette could be either bubbled into an absorbent for SO_2 assay (J) or directed into the IRGA. A single incandescent bulb (D) provided photosynthetically-active radiation which was transmitted through glass panes. Thus, infrared radiation was reflected (E) and the red and blue wavelengths were enhanced (F). Water was pumped from a water bath (H) into a cooling jacket which surrounded the cuvette. Data from the IRGA and a thermocouple connected to a telethermometer (I) were collected during experiments by a recorder. The positions of flowmeters (⧈), adjustable clamps (⧮), stopcocks (⊖), and columns of charcoal, silica gel, and Drierite (⊕) are shown along with the directions of gas (⇑) and water (⇈) flow.


B
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The cuvette containing plants was either irradiated or covered with black cloth; in this way CO_2 exchange during photosynthesis was monitored. Radiation was provided by a 300-watt General Electric all-glass, sealed beam PAR lamp which was suspended about 0.75 m above the cuvette. The lamp was connected to a rheostat which was used to control radiation intensity. Infrared radiation, which could heat the plants in the cuvette, was reflected by a pane of Corning infrared reflecting glass positioned above the cuvette. A second glass pane, a Corning 1-57 colored glass filter, also was used to enhance the photosynthetic qualities of the radiation. Photosynthetically active radiation in the cuvette was measured at 150 $\mu E \cdot m^{-2} \cdot second^{-1}$ with a Lambda Instruments LI 190S quantum sensor coupled with a LI 2190S millivolt adapter. This was the only radiation intensity which was used.

Water from a Gallenkamp Compenstat water bath was pumped with a Braun Melsungen model 1441 Thermomix pump into a cuvette coiling jacket (around and over the cuvette) and was allowed to drain back into the water bath. An adjustable clamp was used to restrict the drain rate and thereby to control the water level in the cuvette. The water temperature in the bath was $22^{o}C \pm 2^{o}C$. The circulating water helped to stabilize the temperature of the cuvette and to absorb some of the infrared radiation from the light. Temperatures of

the moss were monitored throughout all experiments with a thermocouple and a YSI telethermometer (model 425L). Temperatures were maintained at 22°C ± 2°C. Readings from the telethermometer and IRGA were recorded continuously with a Rikadenki 6-channel BP series multi-point recorder with a 6-channel millivolt attenuator.

The cuvette was opened by removing the ceiling of the chamber and was resealed with Dow Corning 3110 RTV encapsulent and RTV (F Fast) catalyst. The SO_2 in air mix entered the cuvette below a grid of stainless steel screen which served as a rack to support the mosses. The cuvette and moss support screen were about 9 cm in diameter and the chamber was about 9 cm in height.

Approximately 0.2 g dry weight samples of moss were used in each experiment. These samples were in either the fully hydrated (FH) or the water-stressed (WS) state. During the course of a 55-minute experiment, moss samples lost about 0.4 g of water. Therefore, samples of 1.2 g or 0.8 g at the beginning of an experiment had final weights of 0.8 g or 0.4 g respectively.

Standard mixes of CO_2 in air were used to calibrate the IRGA. When 300 ppm CO_2 was delivered to both IRGA channels, the IRGA output was 45 mV (0-100 mV=full span). An increase of 60 ppm CO_2 in the experimental IRGA channel resulted in an output of 95 mV, whereas a 260 ppm concentration of CO_2 gas fed to the same channel produced a current of 17 mV. From these

data it was calculated that a one mV change in output represented a 1.2 ppm CO_2 change in the experimental IRGA channel.

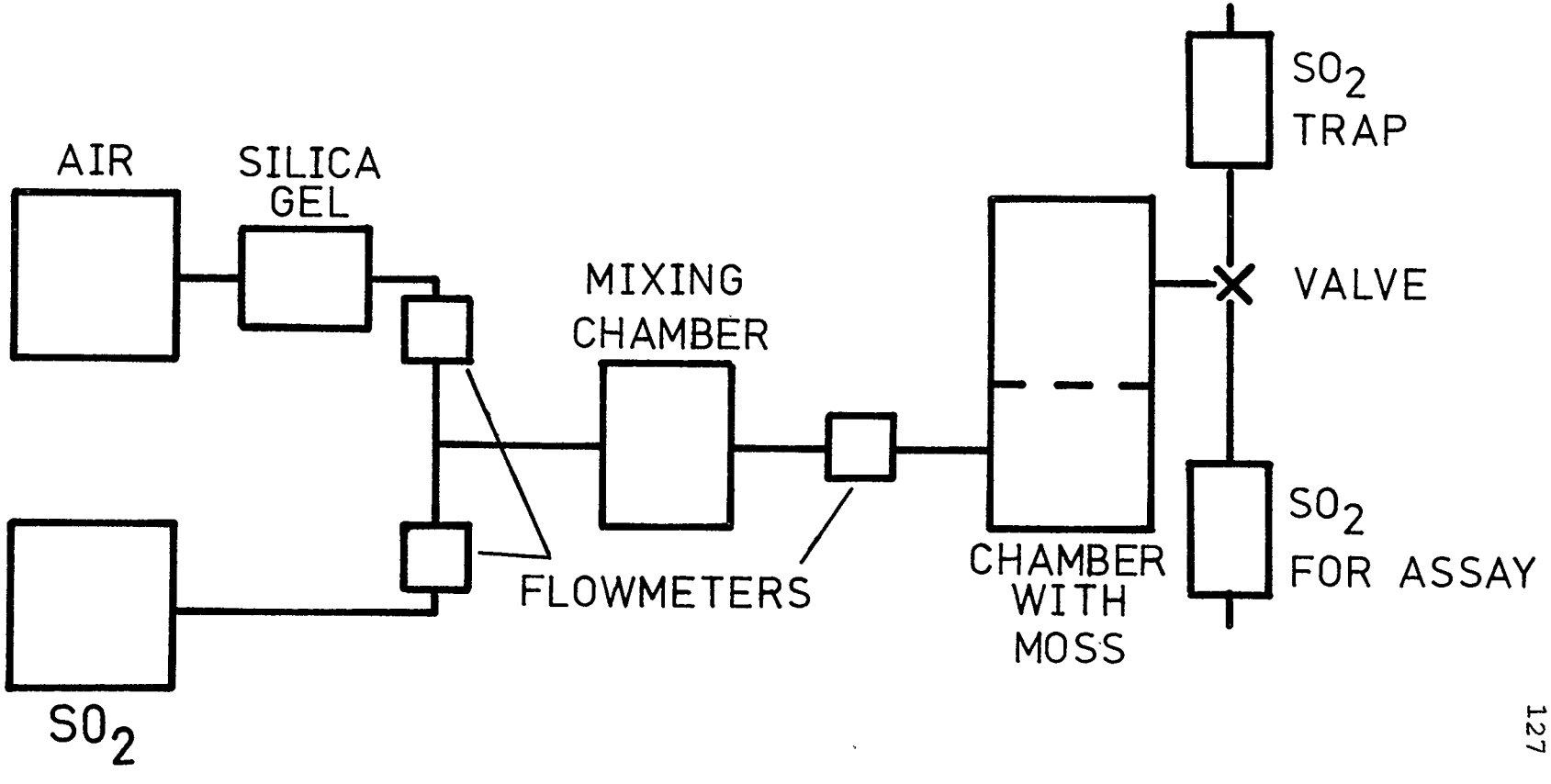
The CO_2 concentration of the gas used in all experiments was 332 ppm; when this gas was fed to both the reference and experimental IRGA channels, the IRGA output was 44 mV. A reading of more than 44 mV was recorded when moss respiration raised the CO_2 concentration. It follows that a reading of 44 mV represents the compensation point between respiration and photosynthesis. When mosses were in the cuvette, this and lower readings were obtained only in the light. The lag time between exposing plants to light and observing the IRGA response was about 30 seconds.

The Fumigation and Desiccation System

Moss pieces were fumigated as they dried in order to determine how changes in water content influenced SO_2 uptake during desiccation (Fig. 18). The SO_2 cylinder which was used in the IRGA fumigation system was diluted with cleaned and dried air from the tap at the laboratory bench. As with the IRGA fumigation system, the dilution of SO_2 to a desired concentration was made on the basis of gas flow rates which were monitored by the previously described rotameters. SO_2 gas was always handled in stainless steel, glass or FEP teflon tubing, rotameters, and chambers.

Figure 18. The fumigation and desiccation system.

FUMIGATION-DESICCATION SYSTEM



The mixed gases entered the fumigation chamber below a support stand and a grid of stainless steel screen. The stainless steel screen, which supported the moss pieces, and fumigation chamber were 12 cm in diameter and the chamber volume was 1.5 l. The gases passed by the mosses and exited via a vent near the chamber ceiling. The fumigation chamber rested upon a magnetic stirrer which caused a teflon-coated magnetic bar, suspended from the top of the chamber and over the moss pieces, to revolve. The mixing of gases which resulted was intended to distribute uniformly the SO_2 in the chamber. The SO_2, after passing through the fumigation chamber, was vented either to a charcoal trap or to an absorbent for assay. Fumigations were done under laboratory lights, the temperature was $22^{o}C \pm 2^{o}C$, and condensation of water vapor was not observed in any tubing or chambers during any experiment.

The open ends of a large, ground glass joint were fused with glass plates in order to make the fumigation chamber. The top of the container could be removed easily for changing moss samples. Samples of 0.2 g air-dry weight Tortula ruralis were rehydrated for 20-24 hours and blotted to 1.0 g fresh weight before fumigation-desiccation treatments. Air flow through the chamber was maintained at 5.0 $l \cdot minute^{-1}$, and dry air caused desiccation of the moss pieces within 70-80 minutes. Following the fumigation-desiccation treatments, the

mosses were divided into samples which weighed 0.02 g air-dry weight. These samples were rehydrated, blotted to 0.1 g fresh weight, and transferred to Gilson respirometry flasks for further study.

Gilson Respirometry

A 20-channel Gilson differential respirometer was used to measure O_2 consumption by 0.1 g fresh weight moss samples. All experiments were done at a water bath temperature of 21°C ± 1°C and a manometer temperature of 33°C ± 2°C. Single side-arm Gilson flasks (125 ml capacity) were used. A brass wire mesh shelf, supported by the center well, held moss pieces over (but never in contact with) 20 ml of Pardee buffer which maintained a constant atmosphere of 0.2% CO_2. Equilibration of CO_2 between buffer and atmosphere was facilitated by two filter paper wicks which adhered to the flask sides. O_2 consumption by mosses was determined by putting them into flasks which were either covered by tight-fitting black cloth bags or by exposing them to light from two Sylvania fluorescent Gro-lux tubes. Intensity of photosynthetically active radiation was measured by a Lambda Instruments LI 190S quantum sensor and was 60 $\mu E \cdot m^{-2} \cdot second^{-1}$. O_2 evolution was calculated by subtracting the volume exchanged in light from that consumed in darkness over a defined time period. Triplicate samples of each treatment were measured for

gas exchange. Hence, each data point is the mean of three values corrected for gas volumes at STP and for thermobarometer changes (readings taken from control flasks without moss) and expressed in units of dry weight and time.

SO_2 Concentration and Determinations

SO_2 gas, which exited chambers of the fumigation system, was periodically vented into an absorbent which could be assayed. The SO_2 determination method of West and Gaeke (1956) as modified by Huitt and Lodge (1964) was used. SO_2 was bubbled through a 0.1 M solution of sodium tetrachloromercurate (II), which is a specific absorbent for this gas and forms the stable disulfitomercurate (II) complex (West and Gaeke 1956). Appendix 4 contains the methods used to prepare the reagents necessary for the SO_2 assay. When a 0.2% formaldehyde solution and a 0.04% solution of pararosaline hydrochloride (bleached with HCl) was combined with the disulfitomercurate (II) complex, a red color resulted. The color formation is dependent upon the concentration of the disulfitomercurate complex and is enhanced by the addition of N,N-dimethyl formamide (DMF) (Huitt and Lodge 1964). This enhancement occurs because the bleached pararosaline hydrochloride-formaldehyde-tetrachloromercurate (II) complex can react with one, two, or three molecules of SO_2. The tri-substituted product forms the most stable,

colorful product and is presumably promoted by DMF.

A standardized solution of $Na_2S_2O_5$ is required in order that the SO_2 concentration absorbed by the tetrachloromercurate (II) solution can be determined. This is done by making a dilution series with this solution, by adding the reagents which are necessary to produce the red SO_2-indicator color, and by determining the color intensity by monitoring absorbence of light at 545 nm with a Perkin-Elmer Coleman 124 double beam spectrophotometer. The color intensities of the solutions obtained from the gas mixing systems also were measured. These unknown concentrations were determined by comparing their spectrophotometric readings with the readings obtained from the dilution series which were used as a standard curve. Once the SO_2 concentration of the experimental tetrachloromercurate (II) solution had been obtained, the SO_2 concentration of the air was calculated by considering the volume of the absorbent, the air-SO_2 flow rate, and the sampling time. Appendix 4 illustrates the calculations used for determining SO_2 gas concentrations.

The SO_2 concentration of the stock cylinder was determined to be 92 ppm. The procedure of mixing air and SO_2, based on flow rates, was found to be a satisfactory method of obtaining this air pollutant at desired

concentrations between 0-92 ppm (± 0.1 ppm). This is the range of concentrations which were assayed.

RESULTS

Moss Sample Weights

Upon completion of the IRGA experiments, moss samples were oven-dried and reweighed. The average sample weighed 0.2113 g (n=30 in the experiments presented), and the range of the 95% confidence limits for these samples was ± 2.5% of the mean value (Fig. 19). Hylocomium splendens was the feather moss species which showed the greatest variance in oven dry weight. This variance presumably was due to inaccuracy during weighing of the air-dried moss pieces. Even so, 95% confidence limits were ± 5% of the mean weight. The mean weights of the three feather moss species were within 0.001 g, and variances in weights of the moss samples are unlikely to account for the observed differences in metabolic responses between mosses.

CO_2 Exchange Rates of Feather Mosses

The general CO_2 exchange pattern of fully hydrated (FH) samples of Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis while subjected to periods of light and dark was similar (Fig. 20). When the plants were in the dark, CO_2 concentration in the experimental gas line (also referred to as cuvette) was enriched by

Figure 19. The mean oven dry weights of the feather moss species which were used in the IRGA fumigation system (± 95% confidence limits, n=10 for each species).

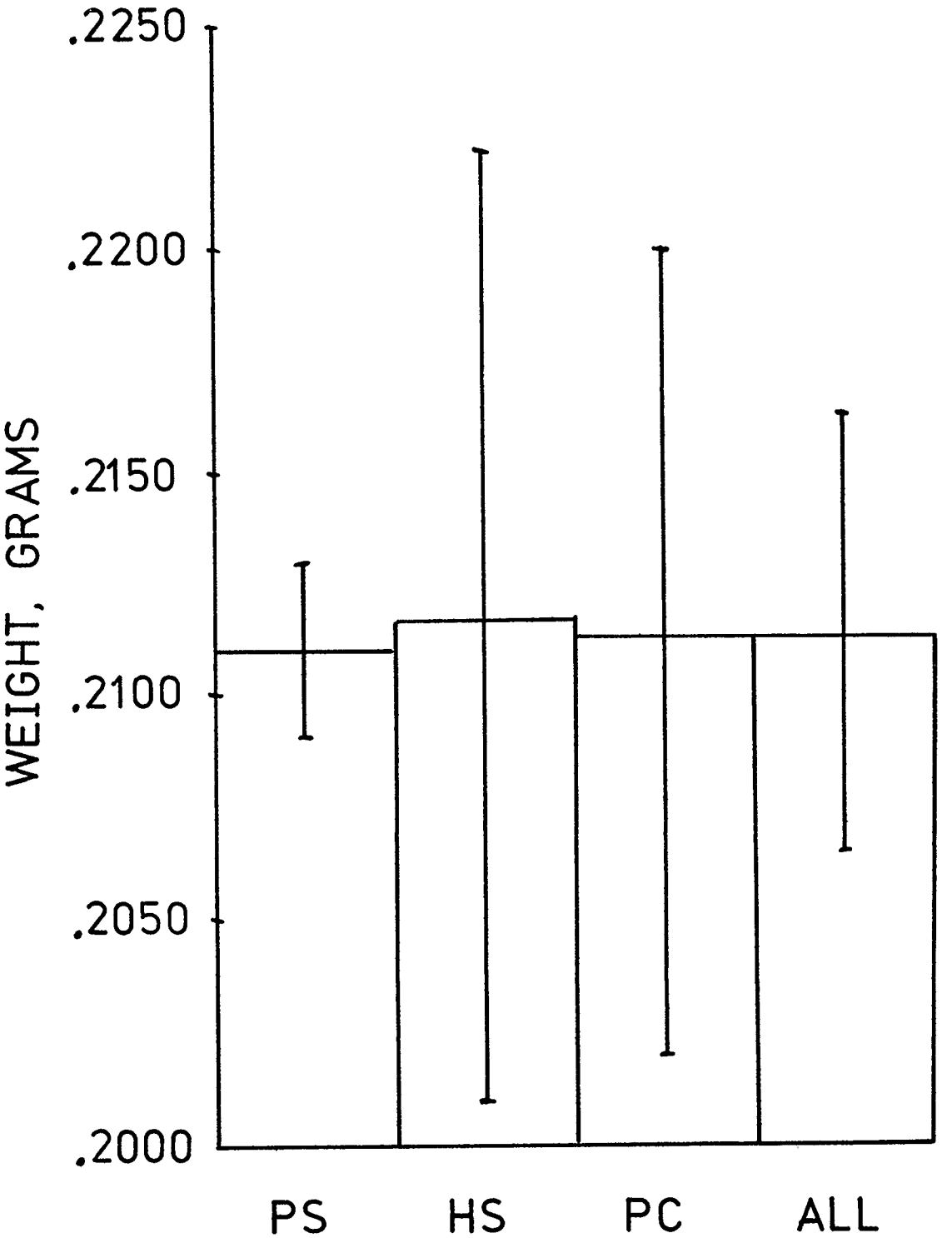
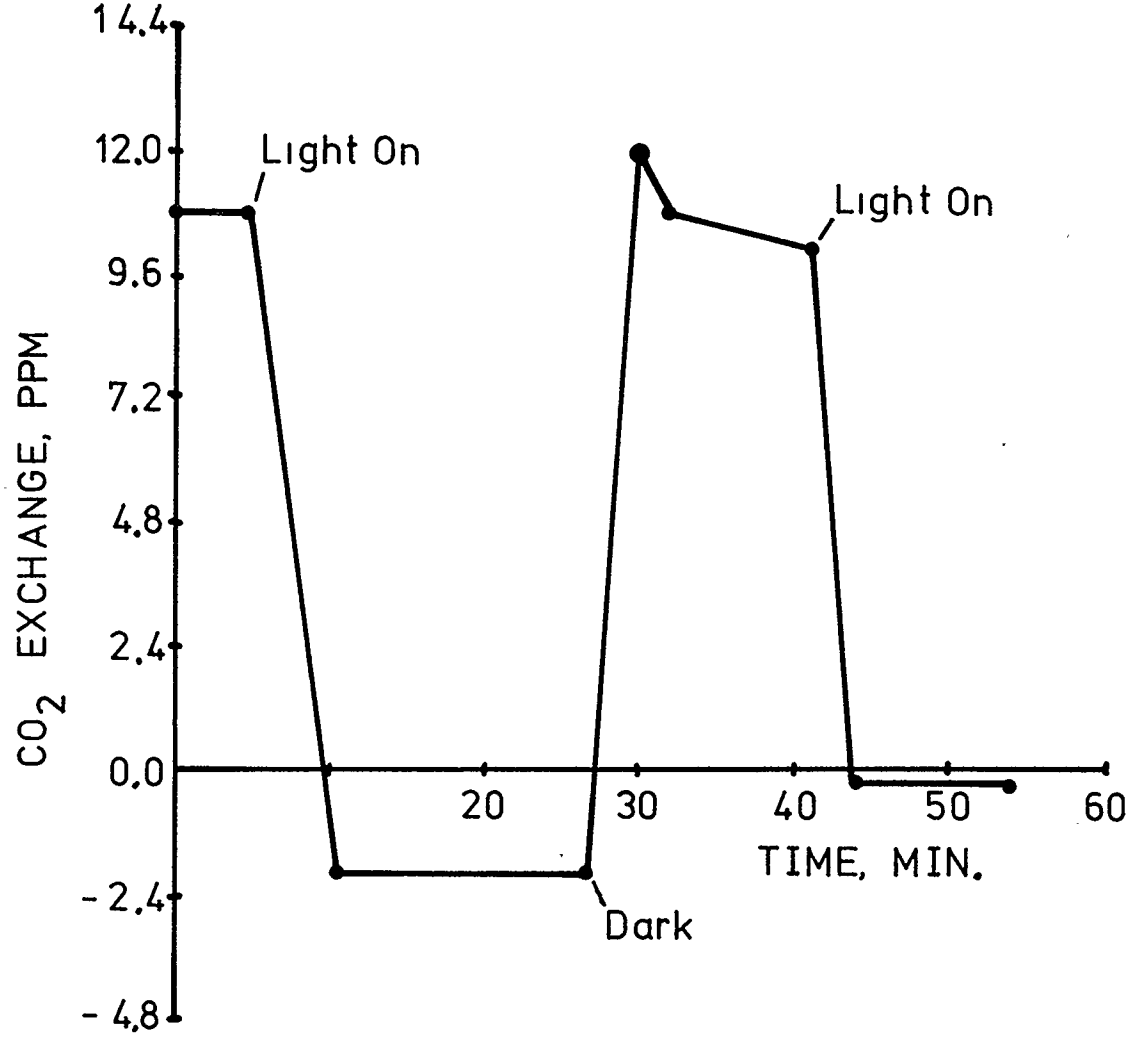

.2250
.2200
.2150
.2100
.2050
.2000
WEIGHT, GRAMS
PS
HS
PC
ALL

Figure 20. The CO_2 exchange by feather moss species in the fully hydrated (FH) state during exposure to light and dark. The units of the ordinate indicate the difference between the CO_2 concentration in the experimental and reference gas lines. The latter line is constantly at 332 ppm CO_2.


14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
Light On
Dark
20
30
40
50
60
TIME, MIN.

10.8 ppm. Within five minutes of turning on the light, the CO_2 concentration in the cuvette decreased by 12.6 ppm and remained as such for at least 17 minutes. Within three minutes of turning off the lights and covering the cuvette with black cloth, the CO_2 concentration increased to about 1 ppm greater than was originally observed for mosses in the dark. During the next two minutes of darkness, the CO_2 concentration declined to the original respiratory value. Over the next nine minutes this value dropped a further 0.6 ppm.

Moss photosynthesis, in response to a second exposure to light, reduced the CO_2 concentration in the cuvette to a value just below the compensation point (Fig. 20). The rates of photosynthesis and respiration towards the end of the experiment were lower than they were at the beginning because mosses had lost 0.4 g of water by this time.

The largest variation from mean CO_2 concentration values which were plotted in Figure 20 occurred at minute 30 of experimental time. This point indicates the mean CO_2 concentration $\pm$ the standard error (n=3). Where any standard error of any IRGA experiment was greater than this, two data points and curves are plotted.

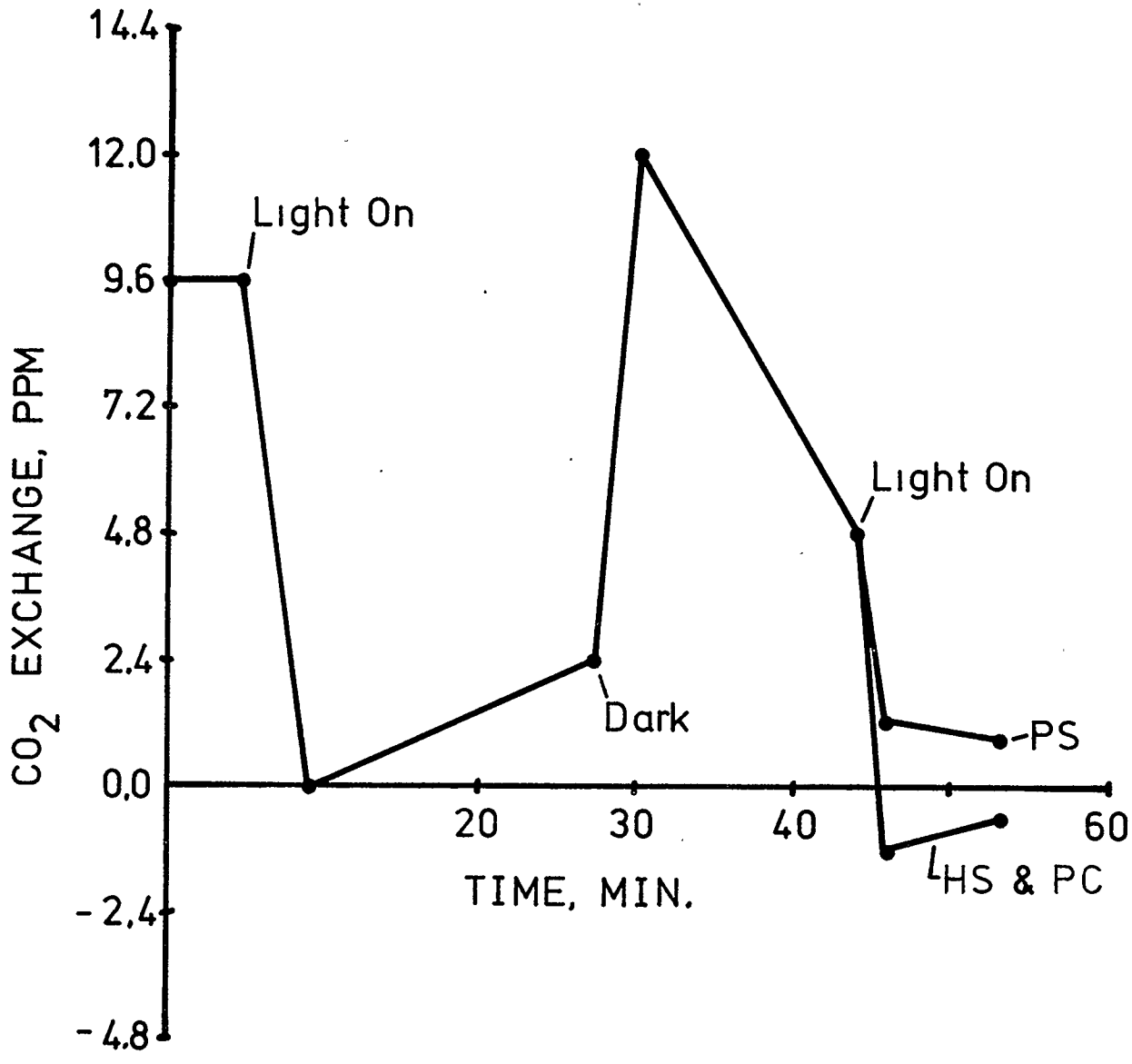
While in the FH state and in the dark, mosses added 10.8 ppm CO_2 to the gas which was fed to the cuvette (Fig. 20). Calculations (which involve the gas flow rate, the STP conversion factor, the constant value of 22.4 l =

1 M of gas, the molecular weight of CO_2, and the weight of the moss samples which were used) show that the CO_2 evolution rate during respiration was about 1.9 mg $CO_2 \cdot g$ dry weight$^{-1} \cdot$hour. (All subsequent CO_2 exchange and assimilation rates will be in these units.) Plants which were exposed to photosynthetically active radiation at 150 uE$\cdot m^{-2} \cdot$second^{-1} consumed 0.4 mg CO_2. It follows that total CO_2 assimilation in the light was about 2.3 mg CO_2.

Busby (1976), who used a similar radiation intensity, found Hylocomium splendens to have a CO_2 assimilation rate of about 3 mg and the other two feather moss species to have about 75% of this value. His experiments were completed at 15°C. This may partially account for the slight difference in CO_2 assimilation rates which exists between our studies. Other rates of CO_2 assimilation observed for feather mosses range from 3.2 mg CO_2 (Ståfelt 1937) to 1.1 mg CO_2 (Kallio and Karenlampi 1973).

The regime of exposures to light and dark periods was repeated for mosses which began the experiment at 0.7 g fresh weight and were considered to be water-stressed (WS). The rates of CO_2 exchange in both the light and dark were lower than they were for FH mosses (Figs. 20 and 21). Also, WS and FH mosses differed because the CO_2 exchange rates of the former group continually declined throughout the experimental period.

Figure 21. The CO_2 exchange by feather moss species in the water-stressed (WS) state during exposure to periods of light and dark.


14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
Dark
Light On
PS
HS & PC
20
30
40
60
TIME, MIN.

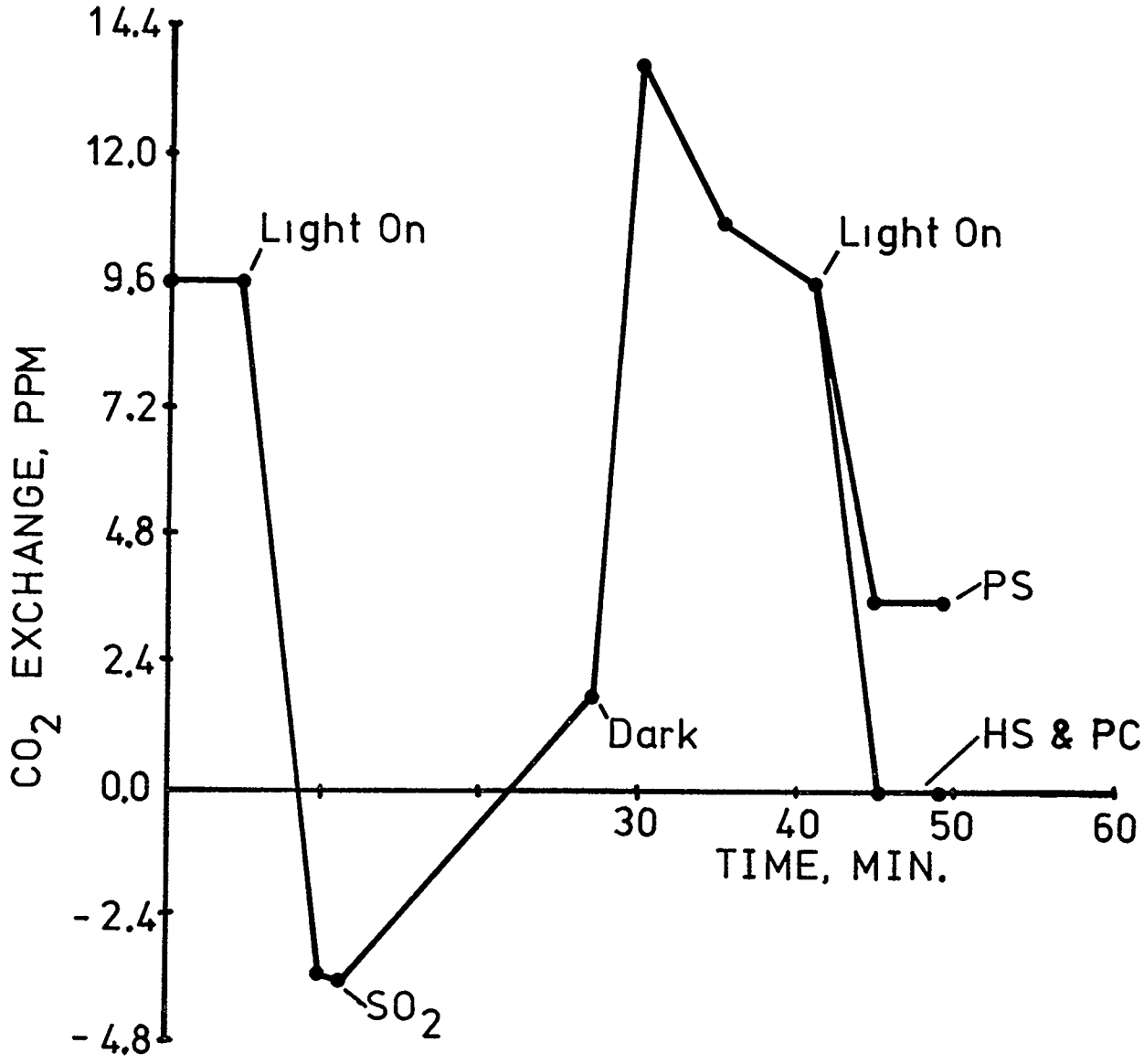
Forty-four minutes after the beginning of the experiment, and upon exposure to light, all species photosynthesized. This was indicated by the declining CO_2 concentration in the cuvette. However, in this second exposure to light, Pleurozium schreberi photosynthesized less than did either Hylocomium splendens or Ptilium crista-castrensis (Fig. 21). Hence, Pleurozium schreberi appears to be more sensitive to water stress than are the other two feather moss species.

Effects of SO_2 upon Moss CO_2 Exchange Rates

The experimental regime was repeated for the feather moss species in either the FH or WS state. However, in these experiments SO_2 at 9.2 ppm was applied for 17 minutes during the initial light period. The photosynthetic rate of the FH mosses initially was below the compensation point and steadily declined after SO_2 was added to the gases entering the cuvette (Fig. 22). In fact, the CO_2 concentration increased by nearly 2 ppm. The metabolic responses of all three feather mosses were similar until the end of the second dark period, during which the respiration rates exceeded and then returned to those which had been observed prior to fumigation.

Photosynthesis resumed in the mosses during the second light period, which was free of SO_2. The photosynthetic capacity of Hylocomium splendens and

Figure 22. The CO_2 exchange by feather mosses in the FH state following treatments with light, dark, and with SO_2 (at 9.2 ppm for 17 minutes) in the light.


14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
Light On
PS
Dark
HS & PC
SO_2
30
40
50
60
TIME, MIN.

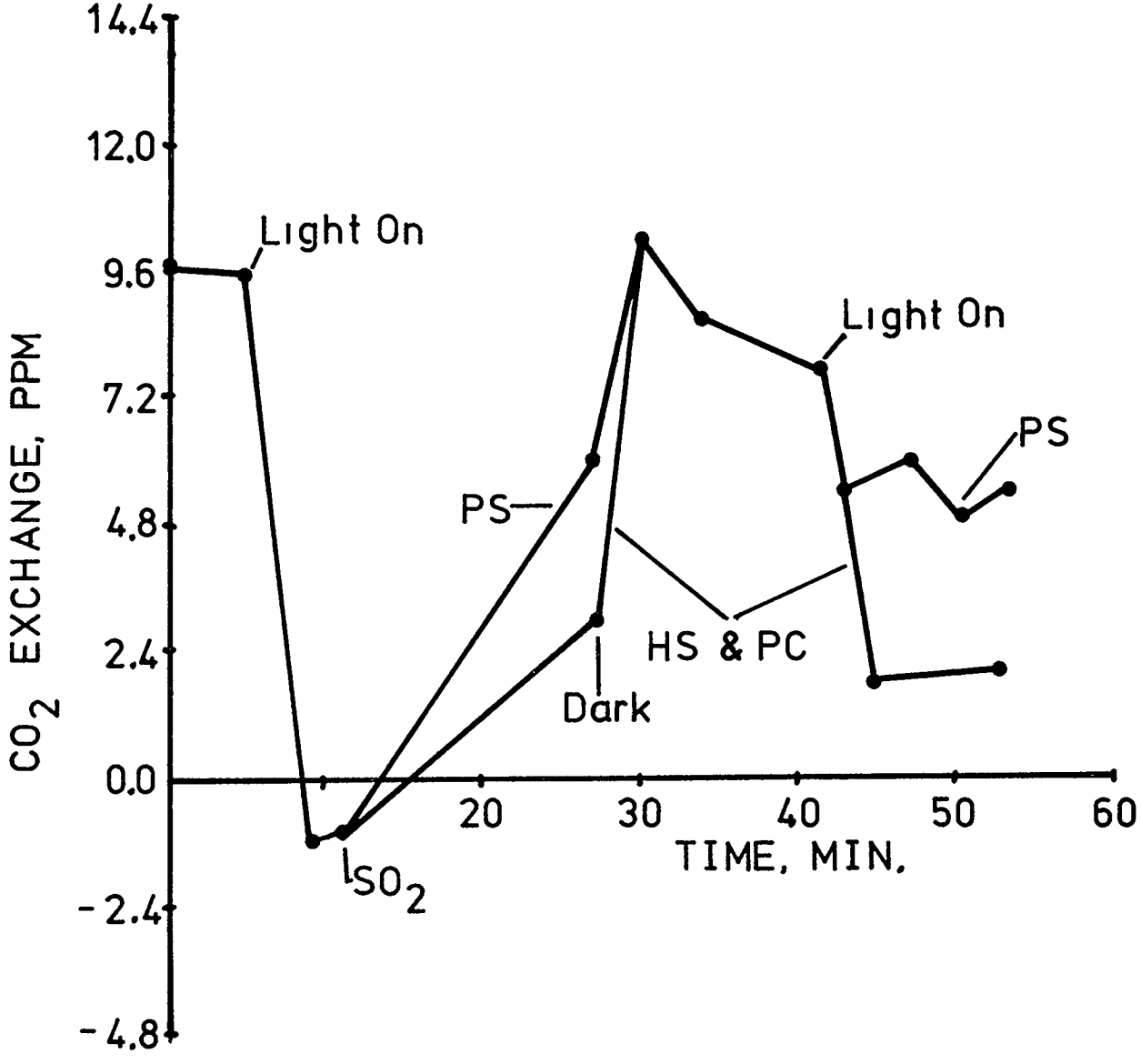
Ptilium crista-castrensis seemed to increase when the SO_2 was removed from the gas supply. This was not so for Pleurozium schreberi (Fig. 22).

WS mosses also declined in photosynthetic activity as SO_2 fumigation began (Fig. 23). Of the three feather moss species, Pleurozium schreberi showed the greatest sensitivity to the SO_2 exposure. The photosynthetic rate of this species declined by 66% at the end of the initial light period whereas that of the other two feather moss species declined by only 33%. In darkness and SO_2-free air, all three species exchanged CO_2 at a similar rate; this rate declined during the 14-minute dark period, as was the case for FH plants (Fig. 20).

A second exposure to light showed once again that stressed Pleurozium schreberi photosynthesized less than did the other moss species. The photosynthetic impairment observed for Pleurozium schreberi at the end of the second light phase appeared to be the sum of the impairments from (1) the SO_2 exposure (Fig. 23) and (2) the water stress observed at the end of the second light phase for the WS control plants (Fig. 21). Because the effects of both SO_2 and water stress were additive for Pleurozium schreberi, this moss appeared to be more sensitive to both stresses than did either Hylocomium splendens or Ptilium crista-castrensis.

For plants in the field SO_2 may not occur at a

Figure 23. The CO_2 exchange by feather mosses in the WS state following exposure to light, dark, and to SO_2 (at 9.2 ppm for 17 minutes) in the light.

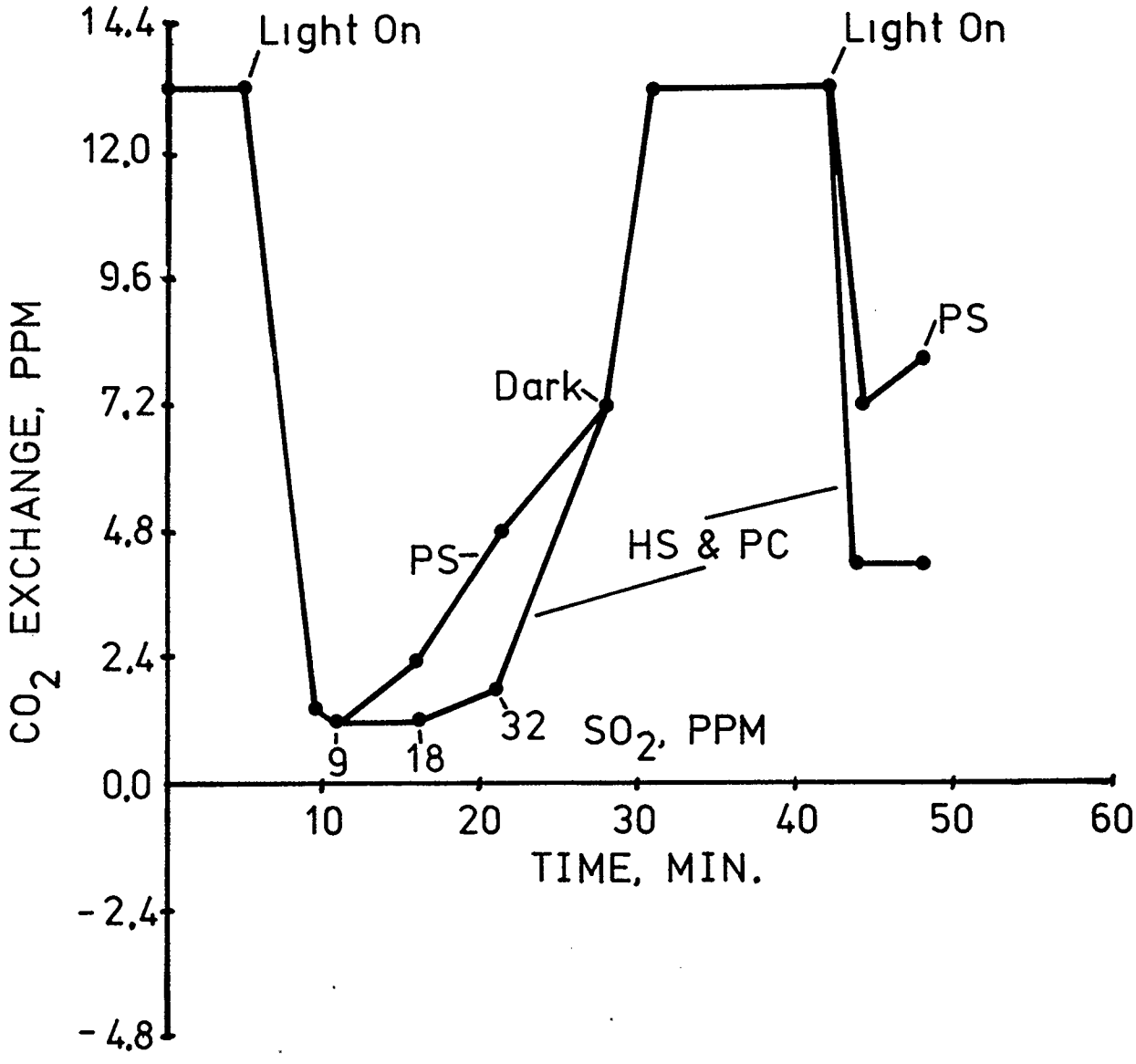

14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
Light On
PS
PS
HS & PC
Dark
SO_2
20
30
40
50
60
TIME, MIN.

constant concentration. To simulate an extreme fumigation episode, I exposed the feather mosses to the same light-dark regimes as were previously described. In these experiments, however, the SO_2 was given in consecutive doses of 9.2 ppm for 5 minutes, 18.4 ppm for 5 minutes, and 32.1 ppm for 7 minutes (Fig. 24).

As in the previous experiments, photosynthesis by the FH experimental plants was reduced by SO_2. Upon exposure to the two lower SO_2 concentrations, Pleurozium schreberi showed greater reduction in photosynthesis than did the other two feather moss species. However, the most severe fumigation resulted in all species showing similar degrees of photosynthetic impairment. Also, all moss species had similar CO_2 emission rates in the dark period following the fumigations.

During the second light period, the CO_2 exchange rates of Pleurozium schreberi were similar to those observed at the end of the first light-fumigation period. However, the other two species showed 50% more photosynthetic capacity (Fig. 24). In fact, Hylocomium splendens and Ptilium crista-castrensis consistently photosynthesized more during the second light period when they were exposed to SO_2-free air than they did at the end of the first light exposure when SO_2 was present (Figs. 22, 23, and 24). In these experiments, Pleurozium schreberi did not recover

Figure 24. The CO_2 exchange by feather mosses in the FH state following exposure to light, dark, and to increasing concentrations of SO_2 (9.2 ppm for 5 minutes, 18.4 ppm for 5 minutes, and 32.1 ppm for 7 minutes) in the light.


14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
Light On
PS
Dark
PS
HS & PC
9
18
32
SO_2, PPM
10
20
30
40
50
60
TIME, MIN.

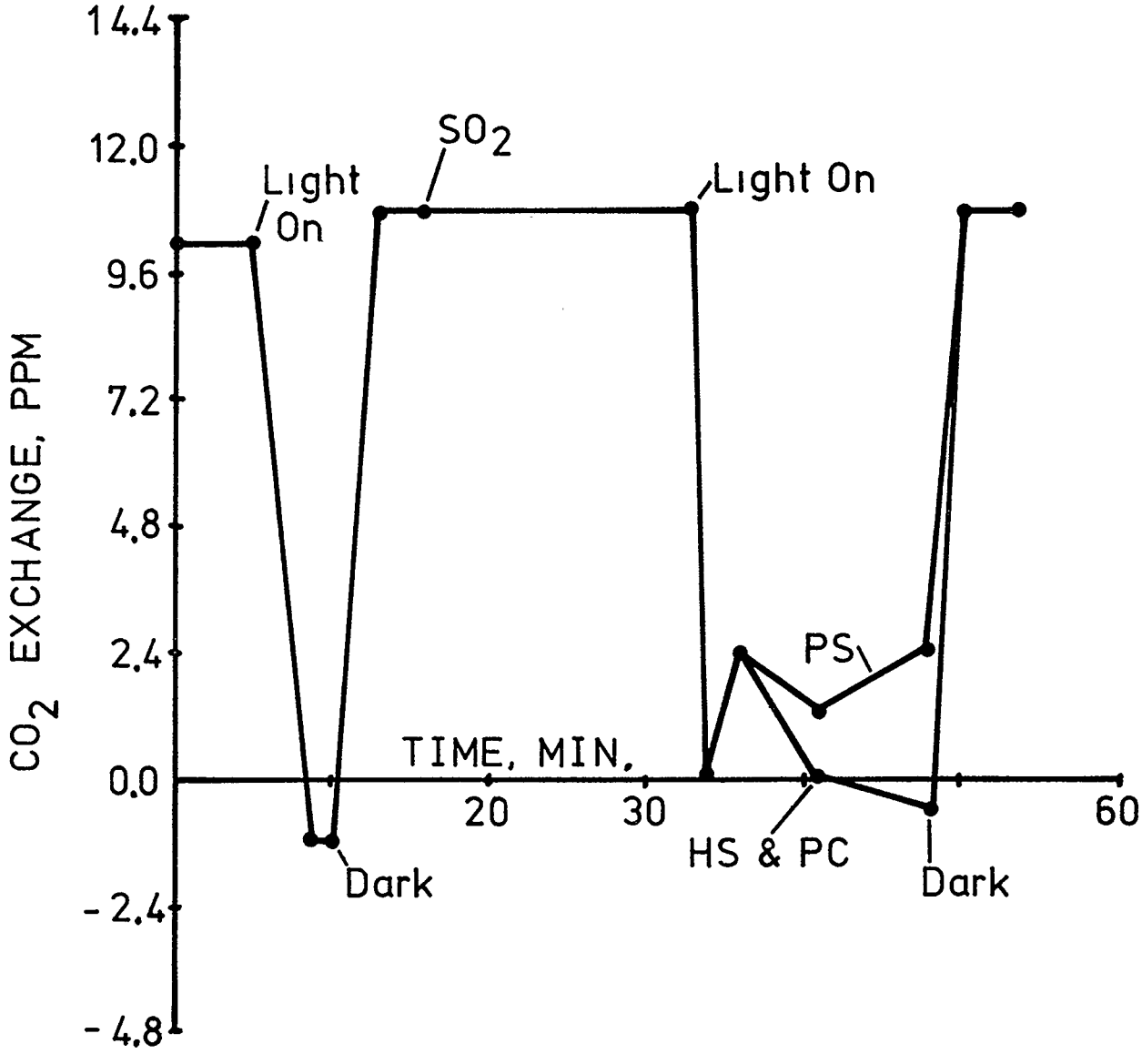
from SO_2-caused damage to photosynthesis, even after SO_2 was removed.

The feather mosses were treated such that the light-dark regime was reversed: 5 minutes in the light, 22 minutes in the dark (SO_2 was applied to mosses during the last 17 minutes of this dark period), 14 minutes in the light, and finally 6 minutes in the dark (Fig. 25). The purpose of this was to determine the SO_2 sensitivity of respiration in relation to that of photosynthesis and to simulate a night-time SO_2 fumigation.

The FH feather mosses showed CO_2 exchange patterns similar to those of control plants (Fig. 20). When SO_2 was applied to mosses in the dark, CO_2 exchange rates did not change (Fig. 25). This was not surprising, for even when the photosynthetic rates were dramatically altered, respiration rates were not affected by SO_2.

Upon exposure to SO_2-free air and a second light period, compensation rates of CO_2 exchange were attained within one minute. Photosynthetic rates for all three moss species sharply declined between 34-36 minutes from the start of the experiment. Hylocomium splendens and Ptilium crista-castrensis recovered from this seemingly temporary synthetic impairment, but Pleurozium schreberi did not (Fig. 25). At the end of the experiment all moss species exhibited respiration rates which were similar to those observed before SO_2

Figure 25. The CO_2 exchange by feather mosses in the FH state following exposure to light, dark, and to SO_2 (at 9.2 ppm for 17 minutes) in the dark.


14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
CO_2 EXCHANGE, PPM
Light On
SO_2
Light On
PS
TIME, MIN.
20
30
60
Dark
HS & PC
Dark

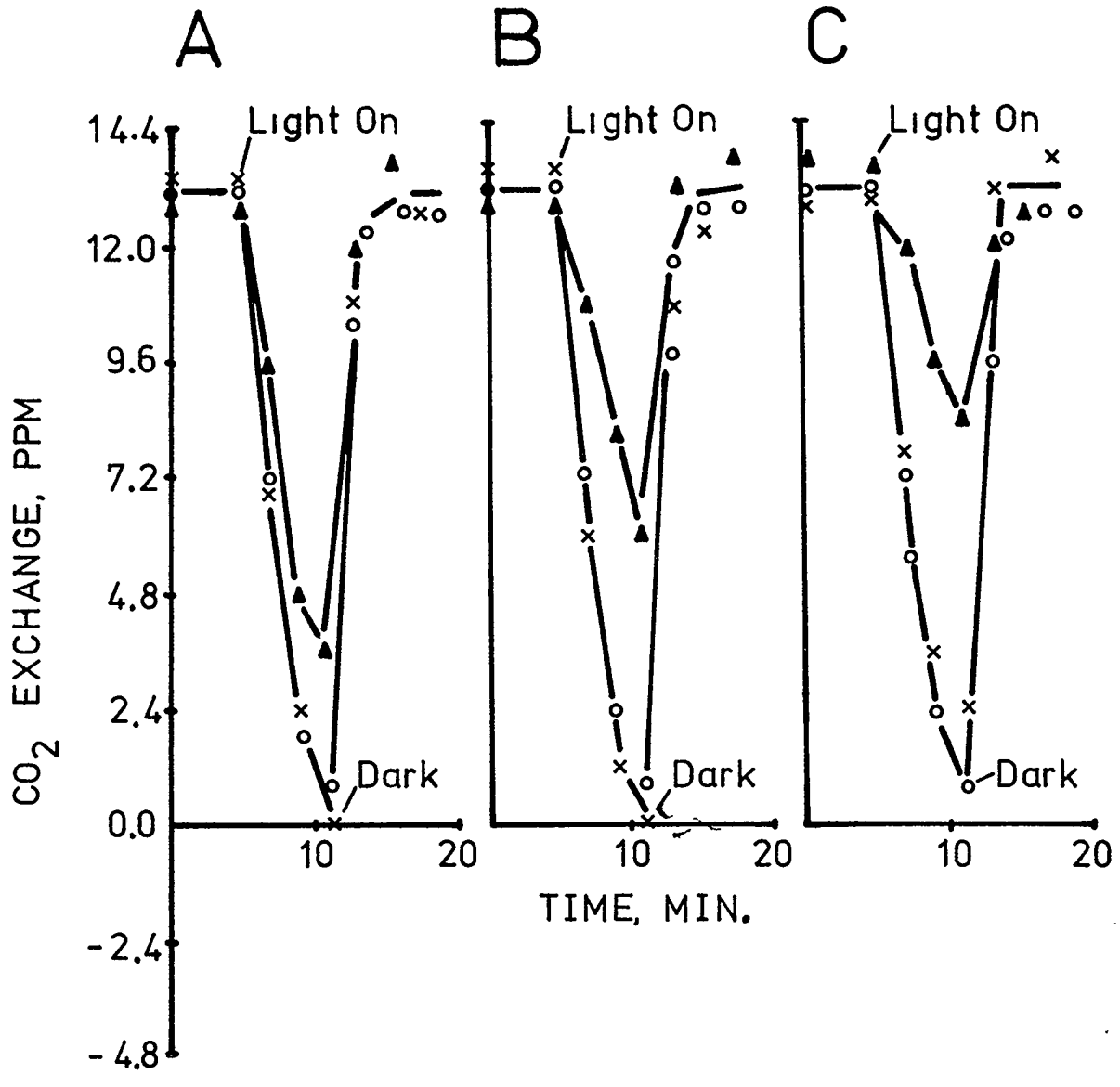
was applied.

Experiments were initiated to determine the impairments to moss photosynthesis 24 hours following SO_2 exposure. Moss was hydrated for 20-24 hours, clipped and washed, and exposed to a regime of light and dark treatments as is shown in Figures 22 and 23. They were exposed either to no SO_2 or to SO_2 in the first light period while moss plants were in the FH or WS state. The mosses were removed from the cuvette and kept in a Petri dish in the FH state for 24 hours. Then they were placed in the IRGA cuvette and CO_2 exchange was monitored for a 5-minute dark period, for an 8-9 minute light period, and for a second dark period. Mosses which were not exposed to SO_2 were considered as controls (Fig. 26).

Ptilium crista-castrensis did not attain the compensation point in the light and also had the lowest photosynthetic rate (Fig. 26). Twenty-four hours following fumigation in the WS state, respiration and photosynthesis of moss did not differ from those of control mosses (Fig. 26). However, less photosynthesis was apparent in mosses fumigated in the FH state.

Twenty-four hours after fumigation in the FH state, Pleurozium schreberi photosynthesized the most and Ptilium crista-castrensis the least (Fig. 26). Twenty-four hours after fumigation FH mosses had only 40%-70% of the photosynthetic capacity of control mosses.

Figure 26. The CO_2 exchange by Pleurozium schreberi (A), Hylocomium splendens (B), and Ptilium crista-castrensis (C) following exposure to either 0 ppm SO_2 (o), 9.2 ppm SO_2 for 17 minutes in the FH state (▲), or 9.2 ppm SO_2 for 17 minutes in the WS state (x), and then kept for 24 hours at 22°C in the FH state.


A
B
C
Light On
Light On
Light On
Dark
Dark
Dark
14.4
12.0
9.6
7.2
4.8
2.4
0.0
-2.4
-4.8
10
20
10
20
10
20
CO_2 EXCHANGE, PPM
TIME, MIN.

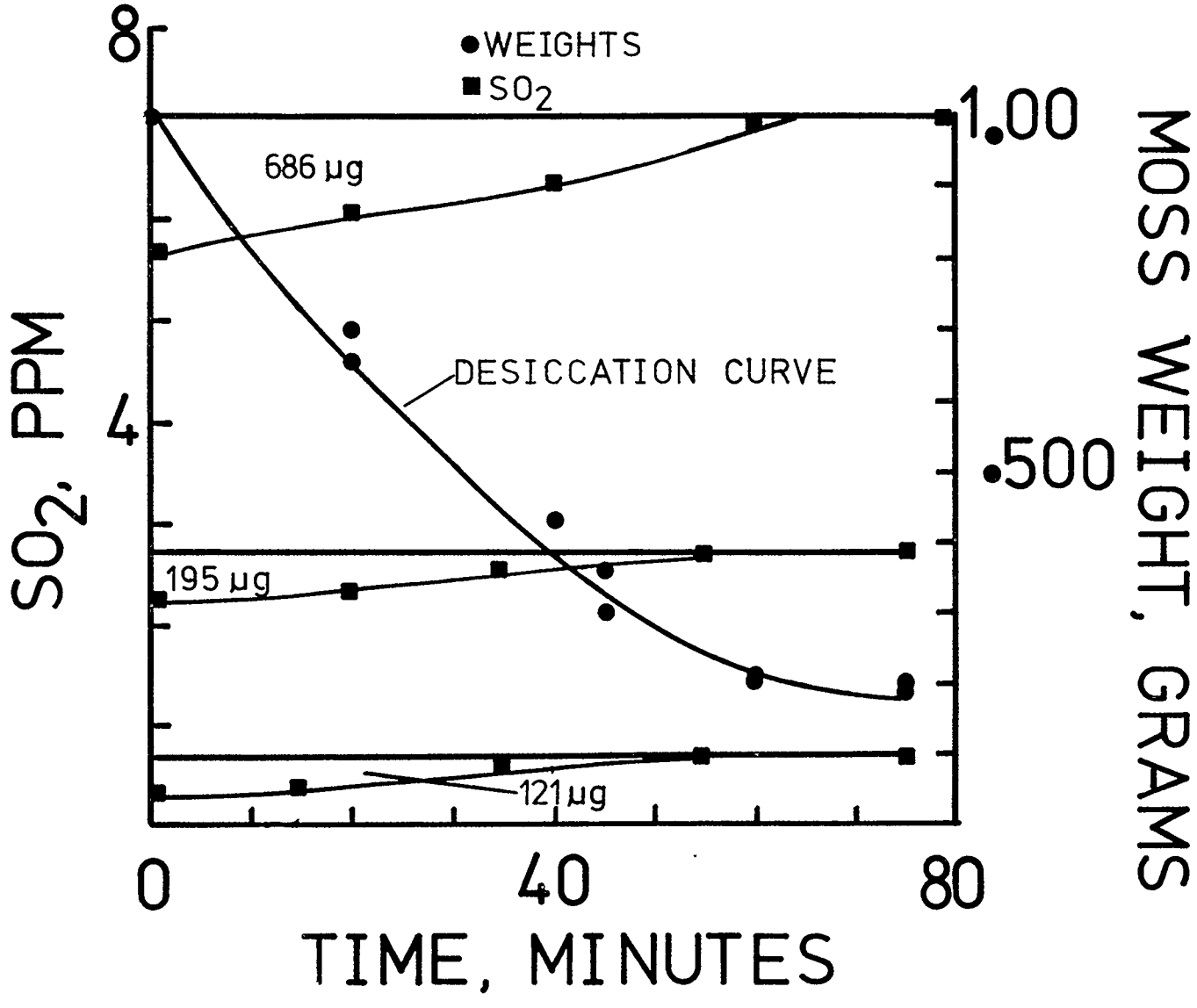
This was so even though they showed immediate impairment which was similar to that of WS mosses (Figs. 21 and 23). In addition, the apparent photosynthetic impairment which resulted when SO_2 was applied to WS mosses (Fig. 23) was not detected 24 hours later (Fig. 26).

In summary, these experiments show that the immediate depression of photosynthesis is greater for Pleurozium schreberi than it is for the other two feather moss species when SO_2 is applied (1) with or without water stress (Figs. 22 and 23), (2) in increasing concentrations (Fig. 24), or (3) in the dark (Fig. 25). The water content of the moss is related to SO_2 impact both immediately following a fumigation and 24 hours later (Fig. 26). It appears that the factors of water stress, SO_2 concentration, and light-dark regimes could be related to the effect of SO_2 on the distribution of mosses at Fox Creek.

SO_2 Uptake by Desiccating Mosses

Samples of Tortula ruralis which weighed 1.0 g fresh weight were placed in a fumigation chamber, and when SO_2 was supplied with dry air, the moss pieces attained an air-dry weight of 0.2 g within 80 minutes (Fig. 27). The gases which left the chamber were sampled at regular intervals (about 20 minutes) during the fumigation-desiccation process. The difference between

Figure 27. The SO_2 concentration of gas which leaves the fumigation chamber (■) in experiments where SO_2 concentrations of 0.7 ppm, 2.7 ppm, and 7.0 ppm are applied to desiccating mosses (●). When moss is not in the fumigation chamber the SO_2 concentrations entering and leaving the chamber are equal. The µg of SO_2 accumulated by 0.2 g dry weight of the moss during desiccation is indicated.


8
4
0
40
80
TIME, MINUTES
SO2, PPM
MOSS WEIGHT, GRAMS
1.00
.500
●WEIGHTS
■SO2
686 µg
195 µg
121 µg
DESICCATION CURVE

SO_2 concentrations which entered and exited the chamber was assumed to be due to SO_2 accumulation by the moss.

Mosses absorbed SO_2 only when they were hydrated, and they lost this capacity as desiccation progressed (Fig. 27). The moss pieces had similar uptake trends for SO_2 applied in concentrations of 0.7 ppm, 2.7 ppm, and 7.0 ppm. Also, uptake was proportional to the concentration which was applied (Fig. 27).

In order to determine whether SO_2 which was accumulated by mosses during the desiccation-fumigation process could influence them, I divided the samples into 17 mg air-dry weight portions, rehydrated them, and then transferred them to Gilson respirometry flasks. The CO_2 concentration in the SO_2-free atmosphere of the flasks was maintained constantly at 0.2% by Pardee buffer. O_2 exchange was measured in the dark (respiration) and light (photosynthesis) for 24 hours following rehydration. Upon completion of the experiment the moss portions were oven-dried and reweighed.

Mosses which were neither fumigated nor desiccated were considered as experimental controls; Figure 28 shows that in the dark they consumed 6 μl $O_2 \cdot 17$ mg dry weight$^{-1} \cdot$hour^{-1}. (All subsequent O_2 exchange rates will be in these units.) Moss fumigated at SO_2 concentrations of 2.7 ppm or 7.0 ppm during drying showed lower initial O_2 consumption rates upon

rehydration in the darkness. After 26 hours of rehydration, mosses which had received the most severe stress had higher rates of O_2 consumption than did controls (Fig. 28).

All mosses which received desiccation treatments had bursts in O_2 consumption when they were exposed to light (Fig. 29). Those fumigated at 0.7 ppm SO_2 returned to rates of moss which were desiccated but not fumigated. Tortula ruralis which was exposed to SO_2 at 7.0 ppm during desiccation showed extremely high rates of O_2 consumption 22-26 hours after rehydration (Figs. 28 and 29). This O_2 consumption rate was 300%-400% of the control rate and no photosynthesis was detected.

DISCUSSION

The terms "avoidance" and "tolerance" seem useful in clarifying the nature of variation in SO_2 tolerance which was observed in the feather mosses. Assuming that SO_2 uptake does not vary between feather moss species which are fumigated in the laboratory, these species can be considered to have similar degrees of SO_2 avoidance but dissimilar degrees of SO_2 tolerance. The results of the laboratory experiments also seemed to correlate well with the response of the feather moss species to SO_2 stress in the field. Pleurozium schreberi was the species which appeared to be most sensitive to

Figure 28. The mean O_2 exchange rates (± SE when larger than symbol size, n=3) by rehydrated Tortula ruralis samples in the dark following simultaneous exposure to desiccation and SO_2 concentrations of either 0.0 ppm (●), 0.7 ppm (o), 2.7 ppm (X), or 7.0 ppm (□). "A" indicates the O_2 exchange rate (± SE, n= at least 35) of samples subjected to neither SO_2 nor desiccation stress.

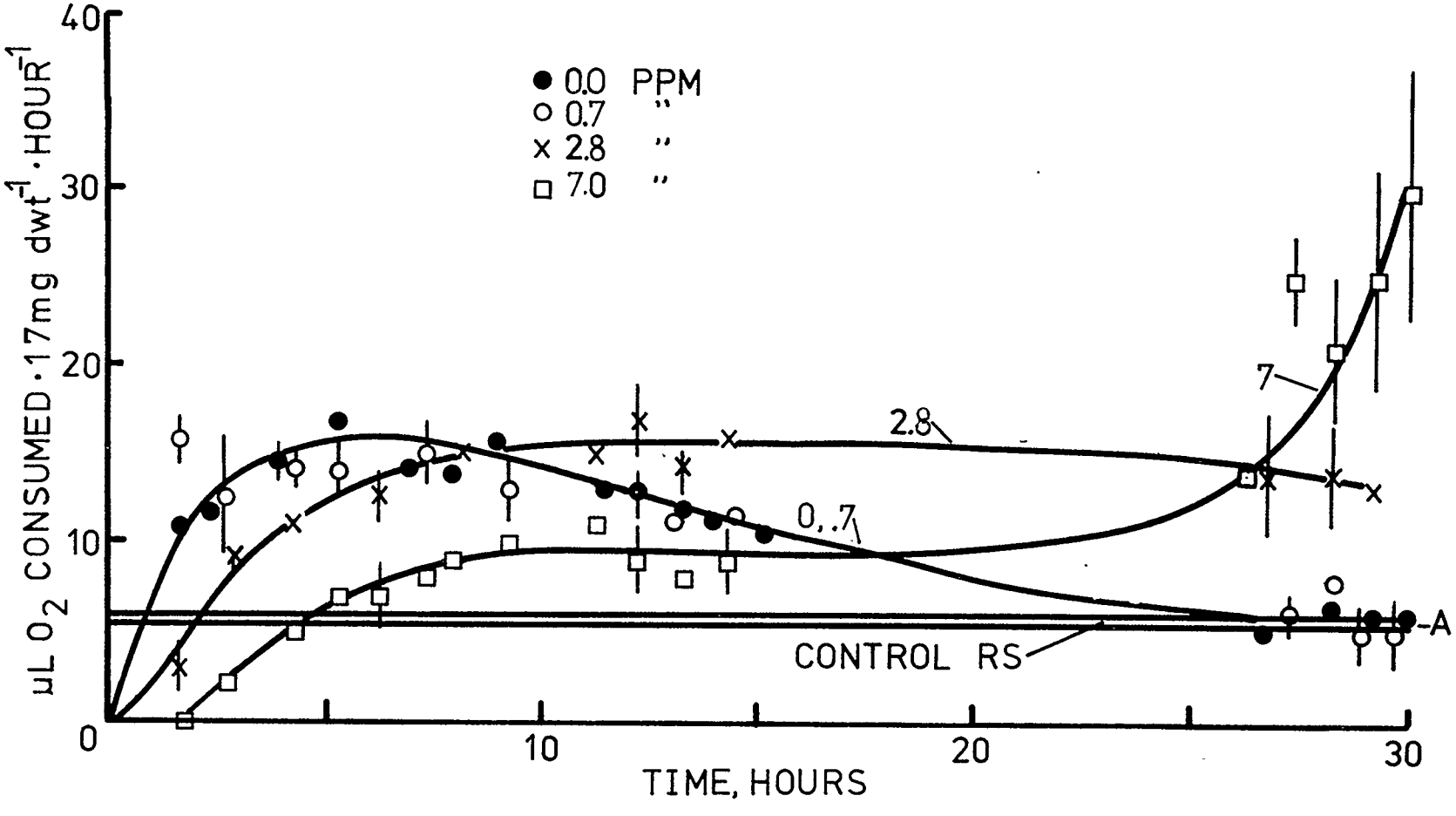
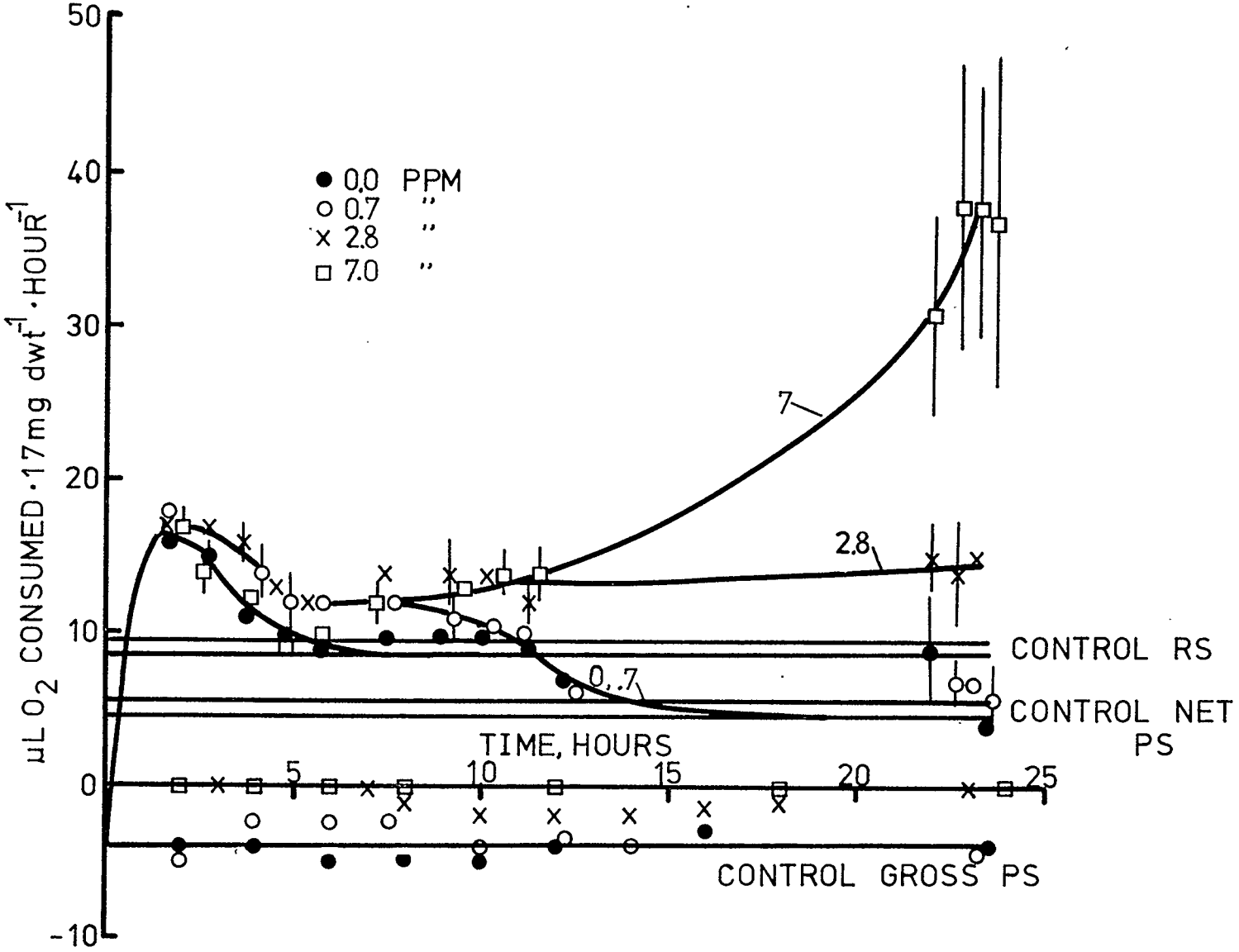

40
30
20
10
0
μL O2 CONSUMED · 17mg dwt-1 · HOUR-1
● 0.0 PPM
○ 0.7 "
× 2.8 "
□ 7.0 "
7
2.8
0,.7
CONTROL RS
A
10
20
30
TIME, HOURS

Figure 29. The mean O_2 exchange rates by rehydrated Tortula ruralis in the light. Symbols and calculations are as found in Figure 28. The gross O_2 exchange rate was calculated as the difference between O_2 exchange rates ($\pm$ SE, n= at least 35) of control samples in the dark and light and which are not stressed with either SO_2 or desiccation.


50
40
30
20
10
0
-10
● 0.0 PPM
○ 0.7 "
× 2.8 "
□ 7.0 "
µL O2 CONSUMED·17mg dwt-1·HOUR-1
7
2.8
0.7
CONTROL RS
CONTROL NET PS
CONTROL GROSS PS
TIME, HOURS
5
10
15
20
25

SO_2, while Hylocomium splendens and Ptilium crista-castrensis were less so. This held whether SO_2 was applied to FH or WS mosses or to FH mosses which were fumigated in the light or in the dark. At least this is the case when photosynthesis, measured as CO_2 exchange of mosses in the light, is the laboratory criterion for SO_2 sensitivity.

Although decreases in respiration rates of lichens which are immersed in sulfite solutions have been taken as symptoms of SO_2 injury (Baddeley, et al. 1971, 1972), this was not observed in fumigated mosses. In my experiments, respiration (CO_2 exchange in the dark) appeared to change very little as a consequence of exposing moss to SO_2.

Türk and Wirth (1975) also noticed that when SO_2 gas was applied to terrestrial mosses, photosynthetic rates were altered more dramatically than were respiratory rates. In their studies mosses were fumigated at an SO_2 concentration of 1.5 ppm for 14 hours. At this point respiration rates differed very little from those of control plants whereas photosynthetic rates were reduced by 50%-100%. In addition, lichens which were either fumigated with SO_2 or immersed in sulfite solutions and then subjected to IRGA analysis showed greater impairment to photosynthetic rates than they did to respiratory rates (Türk, et al. 1974; Wirth and Türk 1975).

The water content of mosses can influence the effect of an SO_2 fumigation upon their photosynthetic rates. This is apparent both during the fumigation process as well as 24 hours later. When mosses were fumigated with SO_2 at 9.2 ppm and photosynthesis was monitored 24 hours later, mosses fumigated in the FH state seemed more severely damaged than did mosses fumigated in the WS state. Presumably, the latter case reflects the trend for mosses to lose the capacity to absorb SO_2 as they dry. FH mosses would therefore accumulate more SO_2 during a fumigation than would those in the WS state.

The reduction in SO_2-uptake capacity associated with a reduction in plant water content has been demonstrated for other moss species. Syratt and Wanstall (1969) observed the degree of chlorophyll degradation of fumigated mosses to be proportional to the relative humidity of the atmosphere. Since the water content of mosses is also dependent upon the atmospheric humidity, their SO_2-uptake rates would be retarded in dry air. Lichens also lose the capacity to absorb SO_2 during desiccation (Türk and Wirth 1974).

If WS mosses absorb less SO_2 than FH mosses do, then the former group should show less photosynthetic impairment during a fumigation. However, this was not the case, for the impairment of photosynthesis in WS moss was as great as it was in FH moss. A possible

explanation for this could be that as mosses desiccate, the volume of the symplast decreases and the organelles and soluble enzymes become concentrated in the decreasing volume of cytoplasmic solution. Therefore, even though WS mosses absorb less SO_2 than FH mosses do, the SO_2 they absorb would be in closer proximity to an organelle or enzyme in WS cells than in FH cells. The reduced symplast volume therefore makes the photosynthetic membranes and enzymes more vulnerable to the toxic effects of the small amount of SO_2 absorbed by WS mosses. Ultimately, in spite of restricted SO_2 uptake, the effect upon WS moss photosynthesis could be detected.

Upon the addition of water, the small amount of SO_2 which is absorbed by WS moss would be diluted as the cells return to the FH state. Thus, upon rehydration, SO_2 damage may be somewhat reversible or repairable.

In addition to showing variations in SO_2 tolerance, the feather mosses also seemed to differ in response to water stress. Pleurozium schreberi showed less photosynthesis in the WS state than did the other feather moss species. This was so even though similar changes in water potential were observed for these species during the drying process (Busby 1976). Hence, as for SO_2, these species show similarities in water stress avoidance, but differences in water stress

tolerance. Therefore, if increases in water stress occur along with increases in SO_2 stress, then both factors may play a role in influencing the distributional patterns of feather mosses at Fox Creek.

Thus, the adaptations of both cryptogams (mosses and lichens) and vascular plants for accommodating water stress also act to restrict SO_2 uptake. Cryptogams have the physiological capacity to tolerate desiccation and SO_2-uptake rates are reduced during the drying process. On the other hand, the vascular plants have both morphological structures (cuticle and stomates) and physiological responses (regulation of stomatal apertures) which not only prevent water loss but also serve as barriers to SO_2 uptake. In a general sense, therefore, cryptogams which cannot tolerate desiccation and must maintain a high degree of water content may have higher SO_2-uptake rates than do species which can spend a great deal of time in the dried state. Similarly, vascular plants which readily lose water to dry air may have higher SO_2-uptake rates than do plants adapted to the desert. If this is so, then an analysis of water relations may offer insight into the SO_2-uptake capacity (and perhaps sensitivity) of plants.

SUMMARY

Canopy coverage analysis was used to examine the synecological changes exhibited by vascular plants and terrestrial mosses in a white spruce association exposed to SO_2 fumigation. Both these understory components declined in coverage as SO_2 stress increased, but mosses were more sensitive to SO_2 in the more heavily stressed areas. This was observed along both an angle-dependent and a distance-dependent gradient of pollution stress. Diversity steadily declined with increasing SO_2 stress along the angle-dependent gradient, but some localized increases in diversity occurred with increasing stress along the distance-dependent gradient. This was due to the invasion of openings which resulted from the attrition of SO_2-sensitive species by weedy angiosperms and by vegetative growth of moss species more tolerant of pollution stress. There were differences in the reproductive strategies of vascular plants and mosses growing in forest stands which were subjected to increasing concentrations of SO_2. These strategies also had consequences for community structure following the systematic removal of pollution-sensitive understory species from an otherwise stable vegetation unit.

I analyzed the turf structure of terrestrial

mosses in the understory of white spruce associations along two SO_2-stress gradients associated with a single SO_2 source. Changes in coverage roughly paralleled those in turf depth, biomass, and the percentage of living moss. SO_2 stress initially caused changes in turf structure in the vertical plane (green turf depth, percentage, and biomass) whereas changes in the horizontal plane (coverage) were most noticeable in areas of severe stress. The Index of Atmospheric Purity (IAP) reflected SO_2 stress only in these latter areas. The intrusion of weeds and the increase in prominence of subordinate species occurred in lightly stressed areas, but these changes were not reflected by the IAP. This was due to the interactions of Q_i, frequency, and coverage data in the IAP formula. Therefore, the criteria of the Index of Community Vigor, an index potentially more useful than the IAP, were proposed.

The $\delta^{34}S$ value of SO_2 emitted by natural gas refineries is about +25, which is higher than that for non-industrial sulfur sources in my study areas. Terrestrial mosses at the Fox Creek study area were found to absorb SO_2 from the atmosphere and they had $\delta^{34}S$ values which are directly related to the degree of SO_2 stress to which they are subjected. Stable sulfur isotope analyses and laboratory fumigation studies showed that the feather moss species which dominated

the white spruce bryoflora at Fox Creek had similar rates of SO_2 uptake. The $\delta^{34}S$ values for conifer needles were lower than they were for mosses at the same collection site, which indicates that trees obtain sulfur from both atmospheric and soil sources.

Potted conifers were transferred to sites differing in degree of SO_2 stress. This difference was reflected by the change in $\delta^{34}S$ values of their needles. SO_2-absorbent pot covers, such as charcoal and moss, reduced the amount of airborne sulfur which was available to trees. Moss also may reduce SO_2 absorbed by soils in forest stands. The $\delta^{34}S$ values were analyzed and were found to (1) help define SO_2-dispersion patterns, (2) reveal the rates at which plants accumulate this pollutant, and (3) associate suspected SO_2 injury more closely to an emission source.

The moss species Pleurozium schreberi, Hylocomium splendens, and Ptilium crista-castrensis were fumigated with SO_2 under controlled laboratory conditions while their photosynthetic and respiratory rates were monitored with an IRGA. SO_2 concentrations, light-dark regimes, and water content of mosses were all varied in order to simulate conditions at the Fox Creek study area where these moss species were found to grow.

Photosynthesis seemed more sensitive to SO_2 than did respiration. This was apparent both immediately

during and 24 hours following fumigation.

Pleurozium schreberi was the species which showed the greatest photosynthetic impairment during fumigations. This was the case regardless of whether fumigations were applied to mosses in the fully hydrated state or in the water-stressed state. SO_2 applied in the dark also resulted in photosynthetic impairment upon subsequent return to light and again showed the variation in sensitivity of the moss species. Since canopy coverage data from Fox Creek suggested that this species would be the most sensitive to SO_2, the observations made in both the field and laboratory correlated with one another.

Terrestrial moss species can generally survive desiccation-rehydration cycles, and the species Tortula ruralis was found to lose the capacity to absorb SO_2 as desiccation progressed. Yet the interrelations between drought and SO_2 stress must be more complex than this. Water-stressed mosses absorbed less SO_2 than did fully hydrated mosses, but in both cases the degree of photosynthetic impairment was similar.

Pleurozium schreberi was also the feather moss species which showed the greatest sensitivity to increases in water stress. Thus, if the gradients of increasing SO_2 stress at the Fox Creek study area also represented gradients of increasing xerophytism, it would be difficult to assess which stress ultimately

accounted for the distribution of the feather mosses which grew there.

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Appendix 1.

The actual number (N^a), the percent basal area coverage (C^b), and the ecological factor "Q" for the tree and erect shrubs taller than one meter encountered in the study area.

Species	Q	Macroplots 1 N/C	2 N/C	3 N/C	4 N/C	5 N/C	6 N/C	7 N/C	8 N/C	9 N/C	10 N/C	11 N/C	12 N/C	13 N/C	14 N/C	15 N/C
Picea glauca (Moench) Voss	36.7	27/.56	28/.42	71/.65	19/.47	24/.46	169/.40	510/.54	37/.50	64/.62	136/.49	33/.49	186/.53	110/.53	250/.55	119/.47
Abies balsamea (L.) Mill.	38.9		241/.10	37/.01	252/.08	77/.02	9/.07	176/.04	26/.01	176/.03	358/.08	47/.01	87/.02	185/.06	491/.22	474/.13
Betula papyrifera Marsh.	37.8		2/.01			4/.01			1/.01		1/*c				2/.01	1/.02
Pinus contorta Dougl.	36.5								10/*	3/.02						
Sorbus scopulina Greene	44.0										2/*					
Populus tremuloides Michx.	16.0		21/*													
Salix spp. L.	38.0												1/*			
Alnus spp. Hill	41.0				2/*											

a Number of trees in each macroplot

b Coverage, in percent, within each macroplot

c * refers to any value less than .005

Appendix 2. The percent cover (C), the percent frequency (F), and the ecological factor "Q" of the vascular plant species encountered within the understory (less than 1m tall) of the macroplots within the study area.

		Macroplots														
	Q	1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Cornus canadensis L.	36.7	.40/15	13/40	19/85	27/90	17/88	13/73	18/73	14/75	15/88	25/93	11/63	11/73	20/95	30/98	26/93
Linaea borealis L.	38.9		14/38	11/88	30/100	14/65	1.4/30	21/70	22/78	9.3/58	26/90	20/70	32/88	18/85	12/80	30/68
Mitella nuda L.	38.9		1.3/28	3.9/83	3.5/78	3.0/70	5.4/80	17/93	5.7/45	10/93	3.0/70	8.3/85	9.5/85	5.4/68	2.9/65	5.1/45
Pyrola asarifolia Michx.	39.5		.40/2.5	.40/5.0	.10/2.5	5.4/35	.20/7.5	.40/5.0		1.9/5.0	.10/2.5	2.8/23	2.4/20	2.4/20	3.3/30	8.3/35
Viburnum edule (Michx.) Ref.	39.3		.80/5.0	4.3/28	5.9/25	7.0/58	.80/5.0		.10/2.5	4.3/15	10/28	3.4/28	.10/2.5	5.8/30	.10/2.5	2.9/10
Rubus pubescens Raf.	40.8				.40/2.5	1.1/20	2.4/35	1.8/13	3.3/23	3.8/40	5.6/65	4.3/38	1.9/38	6.3/56	7.9/28	2.6/18
Rosa spp. L.	40.6			4.8/45	.40/2.5	3.0/13	.10/15	2.3/18	.50/7.5	1.7/7.5	3.5/20	1.5/13	3.1/25	2.3/28		10/30
Mertensia paniculata (Ait.) G. Don	40.4			1.9/28	.90/2.5	1.9/5.0		.40/2.5	.40/2.5	.10/2.5	4.4/10	4.3/28	2.1/13	6.1/50	3.9/25	5.1/25
Malanthemum canadense Desf.	40.6				7.9/50	1.8/28	4.3/60	.10/5.0	2.4/35	1.2/23	3.9/38	3.1/35	3.6/23	2.0/7.5		9.8/55
Viola spp. L.	41.5			.70/15	.20/7.5		.20/7.5	2.9/43		1.8/23	.70/15	2.1/20	2.7/23	1.9/18	.80/7.5	1.0/15

Appendix 2. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Mertensia lanceolata (Pursh) A. DC.	38.8	.10/2.5		2.6/25		.10/2.5	3.0/23	.50/7.5	.10/2.5	.10/2.5		2.6/21		5.5/48	.20/5.0	.50/7.5
Equisetum arvense L.	37.9	2.4/33		7.5/78			.20/7.5	7.1/53	.40/15	.20/10	.90/13		.10/5.0	3.0/58	1.6/13	1.3/15
Gramineae	37.9	4.2/38	.10/2.5	.20/10		2.3/40	.90/10			.10/2.5	.10/2.5	.80/18		.40/15		.80/18
Aralia nudicaulis L.	38.5		.90/2.5		4.9/15	2.2/30		8.8/28		23/75		5.4/28	4.8/30	3.5/23	12/58	25/58
Fragaria virginiana Duchesne	40.3			5.6/43	.10/2.5	2.0/18	.70/15	.40/5.0		.60/10	.40/5.0	4.8/43	.40/5.0	3.0/45		
Petasites palmatus (Ait.) Cronq.	42.3			2.8/38		1.4/20	2.9/43	3.4/25	1.3/5.0	.40/15	.50/7.5	4.2/23		6.1/50		.40/5.0
Dryopteris spinulosa (Muell.) Watt	43.0			.10/2.5		1.8/10	10/55		.50/7.5	1.0/15	14/63	9.6/50			.80/7.5	.10/2.5
Ribes spp. L.	43.0					.90/2.5		.10/2.5		.10/5.0	.40/2.5	.40/5.0	3.6/35	.10/2.5	.40/5.0	1.7/7.5
Carex spp. L.	40.3			1.9/18	.10/2.5	.10/2.5	3.3/23		.10/5.0		.20/7.5	1.6/28		.10/2.5		
Veronica alpina var. unalaschcensis C. & S.	39.8				.10/2.5			.40/15	.10/5.0	.90/13	.10/2.5	.50/7.5	1.6/5.0			.10/5.0

Appendix 2. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Streptopus amplexifolius (L.) DC.	44.1					.40 / 2.5	.10 / 2.5			.40 / 2.5		.10 / 2.5	.80 / 20		1.4 / 7.5	2.3 / 10
Lonicera involucrata (Rich.) Banks	34.5	.10 / 2.5		4.9 / 35			.20 / 7.5					.40 / 2.5		3.5 / 10	.40 / 2.5	
Vaccinium myrtilloides Michx.	42.5					.10 / 2.5	.10 / 5.0		2.1 / 13					4.4 / 23	.40 / 2.5	.10 / 2.5
Galium trifidum L.	41.0					1.3 / 13	.10 / 5.0	.60 / 10		.20 / 13	1.1 / 7.5				.10 / 10	
Galium boreale L.	41.6			2.1 / 33			1.6 / 28		1.2 / 10	.40 / 5.0		.40 / 5.0				
Lycopodium annotinum L.	46.0					2.2 / 25					.90 / 2.5	1.3 / 5.0			1.9 / 15	1.1 / 7.5
Lonicera dioica L.	44.8			.60 / 13			3.3 / 30					2.8 / 35		3.3 / 35		
Epilobium angustifolium L.	44.8					2.3 / 40								.50 / 7.5	.40 / 2.5	.90 / 2.5
Impatiens noli-tangere L.	42.0							2.7 / 13			.10 / 5.0		.10 / 5.0			.10 / 2.5

Appendix 2. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Potentilla norvegica L.	45.5										1.1/7.5			.10/2.5	.50/7.5	.30/13
Pyrola secunda L.	44.7						.80/2.5						.40/5.0			.40/5.0
Pyrola uniflora L.	42.3								.10/2.5			.90/13		1.9/15		
Adenocaulon bicolor Hook.	39.0								2.6/10	.50/7.5				1.3/15		
Osmorhiza occidentalis (Nutt.) Torr.	45.3									.10/2.5	.40/2.5	.40/2.5				
Amelanchier alnifolia Nutt.	42.0			1.6/2.5										.80/5.0		
Compositae	33.5				.10/2.5	3.3/20										
Vaccinium vitis-ideae var. minus L.	38.0						3.6/35		5.6/55							
Ledum groenlandicum Oeder	38.0						.80/5.0		.80/7.5							
Lonicera villosa (Michx.) R. & S.	38.0								.10/2.5		1.3/5.0					

Appendix 2. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Smilacina stellata (L.) Desf.	43.0										.10/5.0				1.6/2.5	
Rubus chamaemorus L.	44.5											.90/2.5	.40/2.5			
Achillea sibirica Ledeb.	40.0			.20/10												
Thallictrum occidentale Gray	40.0			.10/2.5												
Actaea rubra (Ait.) Willd.	44.0										.10/2.5					
Smilacina racemosa (L.) Desf.	41.0									.90/13						
Arnica cordifolia Hook.	51.0											3.1/28				
Rubus idaeus var. gracilipes Jones	51.0											.40/2.5				
Fragaria vesca L.	51.0											.10/2.5				
Gaultheria hispidula (L.) Muhl.	44.0													1.9/5.0		

Appendix 2. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Rubus parvifolia Nutt.	52.0															.40/2.5

Appendix 3. The percent cover (C), the percent frequency (F), and the ecological "Q" value of the terricolous moss species encountered within the macroplots of the study area.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Pleurozium schreberi (Brid.) Mitt.	38.9		5.6/88	9.5/75	9.8/100	9.7/69	4.8/81	16/94	11/94	23/100	20/88	9.1/100	22/100	46/100	25/88	40/100
Hylocomium splendens (Hedw.) B.S.G.	38.9		1.6/44	4.1/69	16/100	8.5/38	17/81	20/94	20/88	21/100	9.8/81	43/100	29/100	9.1/75	12/88	13/94
Ptilium crista-castrensis (Hedw.) De Not.	38.9		2.3/69	7.5/75	4.4/100	11/81	13/75	7.8/100	11/88	5.1/75	18/75	2.9/88	5.6/100	2.7/38	19/88	6.4/88
Brachythecium curtum (Lindb.) Limpr.	40.7			.28/31	.06/13	.31/63	.13/25	.50/75	.03/6.3	1.5/50	.56/38	.16/31	.19/38	.16/31	.41/81	1.2/69
Pohlia nutans (Hedw.) Lindb.	41.3			.75/25	.03/6.3	.19/13	.69/38		.16/6.3	.19/13	.16/6.3	.34/19	.06/13	.16/6.3	.28/31	.16/31
Dicranum fuscescens Turn.	42.8			.06/13		.16/6.3	3.4/31	.81/13		.69/13	.34/19	.06/13	2.2/38	.22/19	3.9/56	.94/38
Dicranum polysetum Sw.	41.7			.31/13		1.5/25	1.4/25	1.5/31	6.5/25	.03/6.3		.66/6.3	.06/13	.78/31	.06/13	.34/19
Brachythecium salebrosum (Web. & Mohr) B.S.G.	40.0		.03/6.3	.03/6.3		.03/6.3	.06/13	.06/13				.06/13	.22/44	.03/6.3		.09/19
Drepanocladus uncinatus (Hedw.) Warnst.	40.3		.06/13	.03/6.3			.03/6.3	.03/6.3			.03/6.3	.09/19	.03/6.3	.09/19		.16/31

Appendix 3. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Mnium spinulosum B.S.G.	42.3				.16/6.3		2.4/19			1.1/50	.03/6.3	.28/31	.88/50			1.3/19
Dicranum flagellare Hedw.	44.0			.16/6.3		.41/31						.09/19	.13/25	.88/25	.09/19	.22/19
Plagiomnium medium (B.S.G.) Kop.	43.0							.38/50		.03/6.3		.13/25	.16/6.3		.03/6.3	.03/6.3
Oncophorus wahlenbergii Brid.	45.0			.13/25			.03/6.3			.06/13		.03/6.3			.06/13	.03/6.3
Polytrichum commune Hedw.	38.7			.03/6.3	.03/6.3	.03/6.3	.03/6.3		1.9/13		5.4/19			1.3/13		
Plagiomnium drummondii (Bruch & Schimp.) Kop.	42.0							.59/94			1.8/44		1.0/25		.03/6.3	.19/13
Isopterygium pulchellum (Hedw.) Jaeg. & Sauerb.	41.0				.03/6.3		.03/6.3			.06/13					.06/13	.03/6.3
Plagiomnium ellipticum (Brid.) Kop.	45.5						.06/13							.16/6.3	2.6/56	.03/6.3
Mnium marginatum (With.) P. Beauv.	42.5					.09/19	.16/31	.03/6.3				.03/6.3				

Appendix 3. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Brachythecium nelsonii Grout	43.3					.03 / 6.3							.06 / 13		.09 / 19	.03 / 6.3
Herzogiella turfacea (Lindb.) Iwats.	47.3										.03 / 6.3	.06 / 13			.03 / 6.3	.06 / 13
Polytrichum juniperinum Hedw.	43.3					.16 / 6.3	.03 / 6.3				.66 / 6.3			.03 / 6.3		
Tetraphis pellucida Hedw.	46.3			.03 / 6.3								.06 / 13			.06 / 13	.06 / 13
Dicranum fragilifolium Lindb.	44.3			.16 / 6.3								.19 / 13			.31 / 13	
Aulacomnium palustre (Hedw.) Schwaegr.	40.7						.03 / 6.3	.66 / 6.3						.09 / 19		
Plagiomnium insigne (Mitt.) Kop.	40.0					.84 / 44				.31 / 13			.03 / 6.3			
Eurhynchium pulchellum (Hedw.) Jenn.	41.0							.06 / 13				.06 / 13	.06 / 13			
Rhizomnium pseudopunctatum (Bruch & Schimp.) Kop.	41.0					.66 / 6.3				.19 / 13						

Appendix 3. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Plagiothecium laetum B.S.G.	43.0										.03/6.3				.03/6.3	
Plagiomnium cuspidatum (Hedw.) Kop.	38.5				.03/6.3							.03/6.3				
Dicranum undulatum (Brid.)	52.0															.19/13
Dicranum acutifolium (Lindb. & Arnell) C. Jens. ex Weinm.	52.0															.03/6.3
Pylaisiella polyantha (Hedw.) Grout	52.0															.03/6.3
Dicranum scoparium Hedw.	44.0													.06/13		
Amblystegium serpens (Hedw.) B.S.G.	52.0															.03/6.3
Ceratodon purpureus (Hedw.) Brid.	44.0										.16/6.3					

Appendix 3. Continued.

Species	Q	Macroplots 1 C/F	2 C/F	3 C/F	4 C/F	5 C/F	6 C/F	7 C/F	8 C/F	9 C/F	10 C/F	11 C/F	12 C/F	13 C/F	14 C/F	15 C/F
Tomenthypnum nitens (Hedw.) Loeske	34.0							.03 / 6.3								
Rhizomnium gracile Kop.	40.0			.03 / 6.3												
Timmia austriaca Hedw.	40.0			.72 / 19												
Rhizomnium nudum (Britt. & Williams) Kop.	40.0			.06 / 13												

Appendix 4. Sample of an SO_2 assay problem.

I. Procedures to determine the concentration of a $Na_2S_2O_5$ standard solution.

1. Make these solutions

A. $K_2Cr_2O_7$

a. 1g $K_2Cr_2O_7$ at 120°C for 4 hours

b. cool over silica gel

c. 0.49g $K_2Cr_2O_7$ from above in 100 ml H_2O

d. 40 ml of this solution + 40 ml H_2O + 3g KI + 2g Na_2CO_3 + 5 ml concentrated HCl

B. $Na_2S_2O_3$

a. 25g $Na_2S_2O_3$ + 500 ml H_2O + 0.1g Na_2CO_3

C. IKI

a. 12.7g I_2 + 40g KI + 25 ml H_2O + 975 ml H_2O

b. 50 ml from above + 450 ml H_2O

2. Titrations

A. $K_2Cr_2O_7$	$Na_2S_2O_3$
5.0 ml	1.2 ml
5.0 ml	1.2 ml
5.0 ml	1.2 ml

$$N \text{ of } Na_2S_2O_3 = \frac{g\ K_2Cr_2O_7}{0.04904 \times ml\ Na_2S_2O_3} = \frac{0.01153}{0.04904 \times 1.2} = 0.1959$$

B. $Na_2S_2O_3$	IKI
0.5 ml	10.8 ml
0.5 ml	10.9 ml
0.5 ml	10.7 ml

Appendix 4. Continued

Titrate in presence of starch

$$N_1V_1 = N_2V_2$$

$$\text{N of IKI} = \frac{0.5 \times 0.1959}{10.8} = 0.00907$$

C.

IKI (0.00907N)	$Na_2S_2O_3$
5.0 ml	3.8 ml
5.0 ml	3.8 ml
5.0 ml	-

Titrate in presence of starch

$$\text{N of } Na_2S_2O_5 = \frac{0.00905 \times 5.0}{3.8} = 0.01193$$

3. Dilutions

A. $Na_2S_2O_5$ (0.0193N) diluted 100:1 to start series

B. 0.0193N = 0.003M; 0.0193N at 100:1 = 0.000 03M

C. Set up series as follows

A = 0.00003M

B 0.000015M

C 0.0000075M

D 0.00000375M

E 0.000001875M

F Blank

II. To do the assay

1. Make these solutions

A. Formaldehyde

a. 1 ml formaldehyde + 199 ml H_2O

Appendix 4. Continued.

B. Pararosaline Hydrochloride (indicator)

a. 0.2g pararosaline hydrochloride + 100 ml H_2O

b. 20 ml from above + 6 ml concentrated HCl + 74 ml H_2O

C. Tetrachloromercurate (II) (absorbent)

a. 27.2g $HgCl_2$ (II) + 11.7g NaCl + 1000ml H_2O

D. N,N-dimethyl formamide

a. Use concentrated

2. Combine these reagents

10 ml absorbent
2 ml $Na_2S_2O_5$ solution (A-F) } or 12 ml absorbent bubbled with SO_2
1 ml formaldehyde
1 ml indicator
1 ml N,N-dimethyl formamide

3. Let color develop for 30 minutes.

4. Spectrophotometry

Solution	Absorbence (Read at 545 nm)
A	0.48
B	0.23
C	0.10
D	0.04
E	0.015
F	0.000
Unknown (from fumigation)	0.20

Appendix 4. Continued.

5. Calculations

A. $Na_2S_2O_5$ (0.01193N) = $\frac{0.005965M\ HSO_3^-}{2}$ x 190 = 0.5667g $Na_2S_2O_5$/liter

B. 0.5667g $Na_2S_2O_5$/liter = 0.00597M $Na_2S_2O_5$ x 64 = 0.3821g SO_2/liter.

C. 0.3821g SO_2/liter at 100:1 dilution = 0.003821g SO_2/liter.

D. 0.003821g SO_2/liter = 0.00000764g SO_2/2ml (in assay)

E. $\frac{0.00000764}{0.48\ \text{absorbence}} = \frac{X}{0.200\ \text{absorbence}}$

X = 0.00000319g SO_2/2ml

F. 0.00000319g SO_2/2ml x 12.5 = 0.0000398g SO_2/25ml absorbent used.

G. (0.0000398 ÷ 64) x 22.4 = 0.0000139 liters of SO_2 captured in absorbent.

H. SO_2 + air flow rate = 400 ml/min.; 5 minutes of sampling = 1.5 l SO_2 + air at STP.

I. 0.0000139 liters SO_2/1.5 l air = 0.0000092 liters SO_2/1.0 liter air = 9.2 ppm.

III. Useful references

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Appendix 4. Continued.

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