Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Measurement of Effects of Environmental and Industrial Chemicals on Terrestrial Plants

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ABSTRACT

Growth of plants and composition of terrestrial ecosystems are adversely affected by a wide variety of environmental and industrial chemicals in the soil and air. Assessment of effects of chemicals is difficult because of the complex nature of chemical stresses, the influence of environmental factors on the impact of chemicals, and variations in composition, stability, and productivity of ecosystems. Chemicals sequentially influence plants at the cellular, plant, species, and ecosystem level. Whereas physiological responses occur rapidly there may be a very long time lag in growth responses of individual plants and of ecosystems to chemicals. Methods selected to evaluate effects of chemicals will vary with the objectives of the investigator, accuracy desired, and available labor, facilities and time. Methods should be selected to yield data that can be analyzed statistically and compared among investigators, locations, and time. Among the factors modifying responses of individual plants and ecosystems to environmental chemicals are types of chemical substances and dosage; species, clones, and cultivars; variations in inherent plant growth patterns and developmental stage; environmental regimens; and plant composition. Principles of methods of assessment of plant responses to environmental chemicals and references to details of methods are given for biochemical and physiological responses, growth responses (biomass, height growth, leaf area, cambial growth, root growth, reproductive growth, plant composition, etc.), and injury. Advantages and disadvantages of evaluation of plant responses in controlled environments and in the field are discussed.

1 INTRODUCTION

Terrestrial plants are exposed to a wide variety of chemical substances in the soil

and air including allelopathic chemicals (Rice, 1984), agricultural chemicals such as fungicides, insecticides, herbicides, and antitranspirants (Kramer and Kozlowski, 1979) and air pollutants, including sulfur dioxide (SO_2), ozone (O_3), fluorides, peroxyacyl nitrates (PAN), oxides of nitrogen and various particulates, such as cement kiln dusts, soot, lead particles, magnesium oxide, iron oxide, foundry dusts, and sulfuric acid aerosols (Mudd and Kozlowski, 1975).

Assessment of the effects of environmental chemicals on plants is difficult because of the complex nature of chemical stresses, the influence of environmental factors on the impact of chemicals, and variations in composition, stability, and productivity of ecosystems. Methods selected to evaluate effects of chemicals on individual plants or plant communities will depend on the objectives and accuracy desired as well as available resources and time. Procedures should be selected to produce data that can be statistically analyzed and compared among investigators, location, and time.

A wide variety of methods has been used for assessing physiological and biochemical plant responses, growth and yield, visual injury, mortality, and changes in composition of ecosystems. Physiological techniques are used to provide a basis for explaining development of signs of injury and account for growth reduction, characterize 'hidden injury', study the action of chemicals on living cells, and rapidly identify plant responses because growth reduction may not be evident for a long time. Techniques for growth and yield assessment are used to appraise economic impacts of chemicals while visual assessments are used to determine growth abnormalities (Tingey *et al.*, 1979).

1.1 Types of Chemicals and Dosage

Signs of injury vary with the chemicals to which plants are exposed. For example, SO_2 injury is characterized by areas of injured leaf tissue located between the healthy tissue and the veins. Signs of ozone injury appear as flecks or stipples of dead tissue, usually only on the upper leaf surface. When ozone injury is severe, the flecks usually coalesce into larger lesions. In the field, however, plants are often exposed to several different pollutants at the same time and it is difficult to ascertain which pollutant is exerting the greatest effect.

In conifers, several pollutants, including SO_2, O_3, and fluoride cause tipburn depending on exposure and plant species. Tipburn of conifers is also caused by some herbicides, deicing salts, and excess fertilizers, making it difficult to ascertain the specific cause.

Even tolerable levels of a single pollutant may injure plants when present with another pollutant at an equally low level. Growth of *Ulmus americana* seedlings was reduced most by joint exposure to SO_2 and O_3, intermediately by SO_2, and least by O_3 (Constantinidou and Kozlowski, 1979a). The effects of combined pollutants may be synergistic, additive, or antagonistic.

Variations in plant responses to dosage (concentration × duration of ex-

posure) of environmental chemicals have been well documented. They are illustrated by differences in amount of injury sustained by plants at various distances from point sources of pollution and studies showing reduced growth and yield and increased injury of plants with increase in dosage of chemicals (Mudd and Kozlowski, 1975). With high dosages of SO_2, cells of leaves of broadleaved plants collapse soon after exposure and necrotic patterns subsequently appear. With low dosages signs are usually characterized by slow development of chlorosis and early leaf senescence (Kozlowski, 1980b). Exposure of *Pinus strobus* seedlings to high concentrations of SO_2 caused direct disruption of mesophyll tissues, and direct or indirect injury to dermal or vascular tissues (Costonis, 1970). Under long-term exposure to 132 μg SO_2/m^3, cellular injury was hidden and restricted to mesophyll parenchyma. Although needle ontogeny was slowed there were no macroscopic signs of SO_2 injury (Percy and Riding, 1981).

Guderian and Kueppers (1980) discussed the effects of dosage of air pollutants on forest ecosystems. High dosages lead to breakdown of community structure, with a change in species composition toward simplification of the system. Degradation of the ecosystem is characterized by rapid changes in structure, including composition, and is accompanied by secondary succession. Direct and chronic injury, especially to leaves, first affects the most sensitive species of the tree stratum, often leading to total destruction of the canopy. With an intermediate pollution dosage various subtle direct and indirect effects lead to reduced vigor of individuals from certain species. Trees slowly develop thin crowns, in turn influencing the supply of light and precipitation for understory plants. McClenahan (1978) described subtle changes in structure and composition of mixed mesophytic forest communities along an industrial air pollution gradient that included an intermediate pollution dosage. Under low pollution dosages the effects may vary from increases to decreases in biomass. Change in structure of a relatively stable forest because of low-level pollution is subtle and gradual at first. However, the structure of delicately balanced forest ecosystems often depends on a few initial species. During slow, early degradation, the natural balance of a forest may reach a critical point beyond which changes leading to deterioration are accelerated. For example, the effect of disease may become suddenly greater when air pollution lowers disease resistance of trees (Treshow, 1975).

1.2 Species, Clones and Cultivars

Wide variations occur in responses of different species of plants to a given air pollutant (Ormrod *et al.*, 1976; Kozlowski, 1980a,b). However, published lists of pollution-sensitive and pollution-resistant species often differ with the criteria used for ranking, such as leaf injury, physiological responses, or changes in species composition. Nevertheless, such lists are very useful. Rankings of species

at extremes (very tolerant or very sensitive to a pollutant) usually are more useful than those for species in an intermediate class (Davis and Gerhold, 1976).

A major problem with rankings of pollution tolerance of plant species is that there is considerable variation in tolerance of different clones and cultivars. For example, variations in sensitivity to ozone have been found among cultivars of petunia, tomato, corn, and spinach (Ormrod *et al.*, 1976). The high tolerance of certain clones of forest trees to air pollution may reflect pollution avoidance because of low stomatal conductance (Kimmerer and Kozlowski, 1981) or biochemical tolerance of pollutants (Braun, 1977).

1.3 Environmental Factors

Interactions between environmental factors and sensitivity of plants to air pollutants are complex, with plant response influenced by environmental regimens before, during and after a pollution episode (Norby and Kozlowski, 1981a,b,c, 1982). Absorption of pollutants is greater when soil moisture availability is high because of high turgor of guard cells and open stomata (Noland and Kozlowski, 1979). A pollution episode in the morning may be more harmful than one in the afternoon because leaf turgor and stomatal aperture are greater in the morning. After an air pollutant is absorbed by leaves there may be direct effects of environmental regimens on tissue sensitivity to the pollutant or on the rate of its detoxication. There may also be indirect effects on sensitivity as a result of effects on metabolism (Guderian, 1977). Post-fumigation environmental regimens that favor rapid growth often allow for rapid recovery from a pollution episode (Norby and Kozlowski, 1981a,b). A further complication is that some environmental factors affect mechanisms of, say, SO_2 avoidance and SO_2 tolerance, or both, to various degrees in different species (Norby and Kozlowski, 1981c).

1.4 Growth Pattern and Developmental Stage

Air pollution affects growth of shoots by inhibiting internode elongation as well as initiation and expansion of leaves. The amount of growth reduction often depends on inherent patterns of shoot growth. In species with 'fixed' growth all the leaves that will be on the expanded shoot already are present in the winter bud (e.g., *Pinus resinosa*). Height growth and elongation of lateral shoots are completed early in the growing season, for example, in Wisconsin by the end of June (Kozlowski, 1978). Hence, a late-summer pollution episode does not affect internode elongation or the number of needles during the current year. It may, however, inhibit growth of needles which continue elongating late into the summer. It will also decrease the size of the bud forming during late summer and,

hence, will decrease shoot growth in the subsequent year. A large bud produces a long shoot; a small bud a short shoot (Kozlowski *et al.*, 1973). By comparison, species with 'free growth' (e.g., *Populus* spp., *Betula* spp.) continue to elongate internodes and add new leaves throughout the summer, as do recurrently flushing species such as tropical pines. Hence, shoot extension of such species, as well as leaf production and expansion, are reduced much more by a late-summer pollution episode. The response of individual leaves to air pollutants also varies with developmental stage. The younger, fully expanded leaves and those near full expansion are most sensitive to pollutants. Old leaves are less sensitive and small expanding leaves are least sensitive (Kozlowski, 1980b).

Young plants are more susceptible than old plants to a given dosage of air pollutant, with seedlings in the cotyledon stage of development especially sensitive. For example, when seedlings of *Pinus resinosa* in the cotyledon stage of development were exposed to four concentrations of SO_2 (0.5, 1.0, 3.0 or 4.0 ppm) at four exposure times (15, 30, 60 or 120 min), subsequent seedling growth was inhibited. The adverse effects were proportional to SO_2 concentrations and duration of exposure. Fumigations induced chlorosis, slowed expansion of primary needles, inhibited dry weight increase of seedlings and caused death of leaf tips. Great sensitivity of young seedlings was shown by significant growth inhibition following exposure to SO_2 for only 15 min (Constantinidou *et al.*, 1976). Despite the high susceptibility of young trees to air pollution, as shown in controlled environments, adult trees in forests often are eliminated by pollution faster than young trees because the canopy serves as a filter. Hence, the young seedlings are exposed to less of the polluting substance.

1.5 Plant Competition

The response of various components of a forest ecosystem in a polluted atmosphere is an integrated one both as regards pollutants and other stresses as well, particularly the effects of competition among trees for light, water, and minerals. A young forest stand may contain several thousand trees per hectare but only a few hundred will be present in the mature forest, others having been eliminated by competition. As trees increase in size they need more space to accommodate their increasing photosynthetic surface and a larger soil volume to supply the increasing need for water and minerals. The trees that survive are most efficient physiologically. Eventually many of the slow growing suppressed trees die from deficiencies of carbohydrates, hormones, water and minerals. Diameter growth of trees is much more sensitive to competition than height growth (Kramer and Kozlowski, 1979).

Competition between trees of the same species does not influence species succession. However, competition between species results in succession until finally a mature climax forest is produced with a group of species capable of

tolerating the stresses of competition. However, mature forest ecosystems are not really stable but exist in an oscillating steady state, with suppressed and old trees continuously eliminated while new trees are added. Catastrophic disturbances in climax forests cause reversion to pioneer stages of succession whereas mild disturbances tend to maintain mature forest in a relative steady state (Kozlowski, 1980b).

The response of individual species in a polluted forest stand may differ greatly from results predicted on the basis of responses determined without plant competition. Growth of some species in a forest ecosystem may be increased, despite pollution stress, because they may gain a competitive advantage as a result of greater impacts on other species with which they interact in the successional process. Growth of certain other species may be reduced by pollution more than expected because of reduced competitive potential (Kozlowski, 1980b).

The remainder of this paper will present an overview of methods used in assessing responses of terrestrial plants at the cellular, plant, and ecosystem level to environmental chemicals. Specific methods will not be outlined in detail but principles and sources of error will be mentioned and references will be given (Tables 1 and 2) to a variety of methods.

Table 1 Literature sources on methods of studying physiological and biochemical responses of plants to chemicals

Plant pigments	Strain and Svec, 1966; Goodwin, 1976
Photosynthesis	Strain, 1965, 1970, 1975; Hill and Powell, 1968; Mooney *et al.*, 1971; Mooney, 1972; Sestak *et al.*, 1971
Proteins, amino acids, and enzymes	Glick, 1954–1981; Colwick and Kaplan, 1955–1981; Lowry *et al.*, 1951; Guilbault, 1973; Haschemeyer, 1973; Bergmeyer, 1974; Needleman, 1975; Catsimpoolas, 1975, 1976; Nairn, 1976; Mayer and Walker, 1980
Plant growth hormones	Zweig, 1963–1980; Hillman, 1978
Water relations	
General	Kozlowski, 1968–1983; Slavik, 1974; Kramer and Kozlowski, 1979
Stomatal aperture	Waisel *et al.*, 1969; Hsiao and Fischer, 1975; Jarvis and Mansfield, 1981; Meidner, 1981
Mineral relations	Epstein, 1972; Walsh and Beaton, 1973; Reisenauer, 1976
Non-structural carbohydrates	Priestley, 1965; Smith, 1973, 1981; McRae *et al.*, 1974
Lipids	Radin, 1969; Tomlinson and Rich, 1969

Table 2 Literature sources on methods of studying plant growth responses to chemicals

Biomass	
General	Neubould, 1967; Whittaker and Woodwell, 1971b; Whittaker and Marks, 1975
Mean-tree approach	Ovington, 1962, 1965
Undergrowth and production rates	Whittaker, 1961, 1962, 1963, 1966; Whittaker *et al.*, 1963
Dimension analysis	Whittaker, 1962; Kira and Shidei, 1967; Whittaker and Woodwell, 1967, 1968, 1969, 1971a; Zavitkovski, 1971; Zavitkovski and Stevens, 1972
Shoot growth	
Height growth and internode elongation	Liming, 1946; Worrall, 1973; Cremer, 1976; Garthwaite, 1981
Leaf growth	*Angiosperms*: Kvet and Marshall, 1971; Anderson, 1971; *Gymnosperms*: Kozlowski and Schumacher, 1943; Cable, 1958; Waring *et al.*, 1978
Cambial growth	McCully, 1952; Young, 1952; Phillips *et al.*, 1962; Bormann and Kozlowski, 1962; Grosenbaugh, 1963; Fritts and Fritts, 1955; Smith, 1967; Kozlowski, 1967, 1968, 1972; Polge, 1969; Splinter, 1970; Nash *et al.*, 1975
Root growth	
General	Whittaker and Marks, 1975; Böhm, 1979
Excavation methods	Beard, 1943; Coupland and Johnson, 1965; Lichtenegger, 1976; Böhm, 1979, pp. 5–19
Monolith methods	Rogers and Vyvyan, 1934; Kirby and Rackham, 1971; Armson, 1972; Walker *et al.*, 1976; Böhm, 1979, pp. 20–38
Auger methods	Kelly *et al.*, 1947; Weller, 1971; Schuurman and Goedenwaagen, 1971; Böhm, 1979, pp. 39–47
Profile wall methods	Garin, 1942; Böhm, 1976, 1979, pp. 48–60; Böhm and Köpke, 1977
Glass wall methods	Bilan, 1964; Lyr and Hoffmann, 1967; Rogers, 1969; Pereira and Kozlowski, 1976; Böhm, 1979, pp. 61–76
Neutron methods	Stone *et al.*, 1976; Cahoon and Stolzy, 1959; Böhm, 1979, pp. 70–79
Staining methods	Gurr, 1965; Böhm, 1979, p. 79
Radioactive tracer methods	Hall *et al.*, 1953; Boogie and Knight, 1960; Woods and Brock, 1964; Böhm, 1979, pp. 80–87
Container methods	Bilan, 1960; Reicosky *et al.*, 1972; Billings *et al.*, 1976; Pereira and Kozlowski, 1976; Böhm, 1979, pp. 95–114

Table 2 (*Contd.*)

Reproductive growth	
Flowers	Brewer *et al.*, 1966; Craker and Feder, 1972; Adedipe *et al.*, 1972
Fruits and seeds	Todd and Garber, 1958; Brewer *et al.*, 1969; Thompson and Taylor, 1969; Pack, 1971; Heagle *et al.*, 1974
Simulation modeling	Kickert and Miller, 1979; Luxmoore, 1980; West *et al.*, 1980
Controlled environments	
General	Evans, 1963; Downs, 1975; Downs and Hellmers, 1975; Tibbitts and Kozlowski, 1979
Exposure chambers	Thompson and Taylor, 1966; Hill, 1967; Heagle *et al.*, 1972, 1973, 1974, 1979; Heagle and Philbeck, 1979; Miller and Yoshiyama, 1973; Mandl *et al.*, 1973; Heck *et al.*, 1978; Bennett, 1979; Drummond and Pearson, 1979
Experimental design and analysis	Steele and Torrie, 1960; Snedecor and Cochran, 1967; Oshima and Bennett, 1979

2 BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES

Environmental chemicals have numerous sites of action and rapidly influence many physiological processes, leading to changes in plant constituents. Responses include release of volatile hydrocarbons (Kimmerer and Kozlowski, 1982), changes in plant pigments (Constantinidou *et al.*, 1976; Suwannapinunt and Kozlowski, 1980), photosynthesis (Mudd and Kozlowski, 1975), reserve carbohydrates (Constantinidou and Kozlowski, 1979b), amino acids (Tingey, 1974), proteins (Constantinidou and Kozlowski, 1979b), enzymes (Keller, 1974), minerals, lipids (Malhotra and Khan, 1978) and water relations (particularly stomatal aperture) (Kozlowski, 1968–1983; Jarvis and Mansfield, 1981). For references see Table 1.

2.1 Plant Pigments

Decrease in chlorophyll content in response to environmental chemicals is often used as an index of injury. For example, chlorophyll content around point sources of air pollution has been related to the degree of injury. When using chlorophyll measurements, care should be taken to be sure that test and control leaves are comparable with respect to leaf age and exposure (Tingey *et al.*, 1979).

2.2 Stomatal Aperture

Investigators have been interested in studying stomatal responses to air pollutants for two main reasons: stomatal aperture controls absorption of gaseous pollutants, and stomatal responses to pollutants modify loss of water vapor and absorption of CO_2 by leaves.

Exposure to ozone usually leads to stomatal closure (Heath, 1975) whereas SO_2 at low concentrations induces stomatal opening, with the specific effect modified by air humidity (Jarvis and Mansfield, 1981). By comparison, high SO_2 concentration leads to stomatal closure, presumably because of direct effects of SO_2 on turgor of guard cells and indirectly from increased CO_2 content of intercellular spaces in leaves (Noland and Kozlowski, 1979). Stomatal aperture is also variously influenced by mixtures of air pollutants (Unsworth and Black, 1981).

2.2.1 Microscopic Measurements

Stomatal aperture can be measured by observing stomata on strips of epidermis or their replicas under a microscope. Because stomatal aperture may change with variations in environmental conditions, stomatal apertures are usually fixed by immersing epidermal strips in immersion oil or alcohol (Kanemasu *et al.*, 1975). Because of difficulty in obtaining good epidermal strips, investigators often prefer replicas (peels) of collodion, silicone, rubber, cellulose acetate or other materials that are applied to leaf surfaces and later stripped from them. Excellent impressions of leaf surfaces can be obtained by this method (Waisel *et al.*, 1969). Stomatal apertures are measured with calibrated eye-piece micrometers. Microscopic measurements are reliable only if the stomata are on leaf surfaces and not sunken as in many xerophytes, gymnosperms, and some mesophytic angiosperms. For example, *Populus* leaves have cuticular ledges surrounding the guard cells and ledge apertures are not closely related to pore apertures of guard cells. Because stomatal pore and ledge apertures are independent, leaf surface impressions, which reproduce ledge apertures only, should not be used to estimate stomatal aperture (Pallardy and Kozlowski, 1980b).

2.2.2 Porometry

Various porometers have been devised to measure the capacity of a leaf or epidermis to conduct gases by mass flow or diffusion. The main advantage of porometers is that they measure conductance of a large number of stomata at one time.

In mass flow porometry air is forced under pressure through a leaf and the rate of flow or resistance to flow is used as an index of porosity. Mass flow porometers can be used with amphistomatous leaves. Air under pressure is applied to one

side of a leaf. It enters the stomata, traverses the mesophyll, and exits through stomata on the other side of the leaf. Hence, flow depends on stomatal resistance on both leaf surfaces as well as the resistance of the mesophyll, which usually is small. If stomatal resistance on one side of a leaf is very high the porometer readings largely reflect the openings on that side of the leaf. Mass flow porometers can also be used, although much less effectively, on plants with hypostomatous leaves (which includes many species of woody plants). Leakage is a common source of error with mass flow porometers.

In the last decade a variety of diffusion porometers have been extensively used in controlled environments and in the field (Pereira and Kozlowski, 1977a,b; Kozlowski and Pallardy, 1979; Sena Gomes and Kozlowski, 1980). These instruments measure diffusion of water vapor from substomatal cavities through the stomata and can be used on either leaf surface independently. Most diffusion porometers use a humidity sensor connected to an electronic circuit and measure changes in a cup placed over a leaf for a few seconds (Kanemasu *et al.*, 1969). Recently steady-state porometers have been introduced. Dry gas is passed over an enclosed leaf at a measured rate and the humidity of the exhaust gas is measured. Excellent versions of gas flow porometers are commercially available.

2.3 Photosynthesis

Because of the need for carbohydrates for plant growth, much attention has been given to effects of chemicals in the environment on photosynthesis. Over a period of hours or days the amount of photosynthate produced can be determined from increase in dry weight in a sample of tissue carrying on photosynthesis. The increase in dry weight of the sample during the experimental period is considered a measure of net photosynthesis.

For very short periods of time, pulse labeling with $^{14}CO_2$ has often been used. Leaf samples or shoots are exposed in chambers to $^{14}CO_2$ for periods of 1 min to 15–20 seconds. It generally is desirable to use the shortest exposure that can be accurately timed if the objective is to estimate the rate of photosynthesis under near natural conditions. During exposure for periods up to a few minutes, translocation of ^{14}C-labeled photosynthetic products is very small. As soon as possible after plant tissue is exposed to $^{14}CO_2$, leaf discs can be taken with a cork borer from the exposed leaf. The amounts of ^{14}C present can be determined directly on the leaf discs by Geiger counting or by extraction and use of liquid scintillation counting (Dickmann and Kozlowski, 1968, 1970; Marshall and Kozlowski, 1974).

The most widely used method for measuring photosynthesis involves determining the rate of CO_2 absorption by plant tissues. The photosynthesizing tissue is enclosed in a transparent enclosure (cuvette) and the concentration of CO_2 in the air before and after it passes over the plant tissue is determined with an infrared gas analyzer (IRGA). Either an open or closed system may be used. In a

closed system both the air input and output from the plant chamber (cuvette) are connected to the gas analyzer. A closed system is relatively simple but does not permit steady-state observations. Additionally, photosynthesis is not linearly related to CO_2 concentration except in the region of 100–200 μl/l. Therefore a closed system should be used over a small CO_2 concentration range and when the concentration is changing slowly (Sestak *et al.*, 1971). An open system is especially useful for measurements of photosynthesis by individual leaves, parts of plants, or small plants. In an open system air is passed through the plant enclosure at a measured and constant rate. All or some of it is passed through the infrared gas analyzer (IRGA). Usually air is brought to known temperature, humidity and CO_2 concentration before it enters the plant enclosure. IRGAs are popular because they are accurate and simple to operate. They continuously and directly measure CO_2 concentrations and can be used with many types of recorders.

The size of the plant enclosure is arbitrary and depends on the objectives of the investigator. Such chambers vary from small cuvettes clamped over a portion of a leaf to cuvettes containing a fascicle of needles or whole leaves (Kramer and Clark, 1947), to portions of communities of small plants (Billings *et al.*, 1966), to portions of tree stands (Ordway, 1969).

Early attempts to measure photosynthesis in the field were not wholly successful because of overheating of plant chambers. However it is now possible to control temperature and humidity in field cuvettes with considerable precision (Mooney, 1972). The long-term influence of the chamber microenvironment can be reduced if the plant or leaf is only intermittently enclosed in a cuvette. To that end some investigators have designed chambers that open and close periodically, thus making it possible for the photosynthesizing tissues to exchange radiation, CO_2 and water vapor with their surroundings. The large amount of equipment required for measurement of CO_2 uptake by plants in the field has led to the development of mobile laboratories (Strain, 1965, 1970, 1975; Mooney *et al.*, 1971).

Rates of CO_2 uptake by plant tissues are usually expressed in terms of some unit amount or dimension of plant material. For leaves and stems the most common unit is surface area but dry weight is also used. Although fresh weight has also been used it generally is unsatisfactory because it varies from leaf to leaf and in the same leaf over relatively short periods because of changing environmental conditions. The rate of CO_2 uptake has also been expressed in terms of chlorophyll content, number and volume of cells, number of chloroplasts, and activity of carboxylation enzymes.

With hypostomatous leaves the area of one surface usually is used; with amphistomatous leaves both surfaces are used. However, there often are problems because the number of stomata on the abaxial and adaxial leaf surface of some plants often varies widely (Pallardy and Kozlowski, 1980a).

In planning measurements of photosynthesis considerable thought should be

given to location of leaves sampled. Tree leaves are at different stem heights and usually are in dissimilar environments. The investigator should also consider that the rate of photosynthesis varies greatly during the day and it varies seasonally. It also varies with a host of environmental factors including light, temperature, CO_2 content of the air, water supply, soil fertility, insects and diseases, as well as such cultural treatments as thinning of stands, pruning, irrigation, and application of fertilizers. Important plant factors affecting photosynthesis include past history of the plants (for example, environmental preconditioning); age and structure of leaves; size, number, and responses of stomata; chlorophyll content; water deficits; and carbohydrate utilization and accumulation (Kozlowski and Keller, 1966; Kramer and Kozlowski, 1979).

Although photosynthetic products are required for growth of plants, short-time measurements of photosynthesis are not always good indices of growth potential. In addition to photosynthetic rate three other physiological considerations determine growth potential. These include the seasonal pattern of photosynthesis, partitioning of photosynthate within the plant, and the relation of photosynthesis to respiration. Low correlations between the rate of photosynthesis and growth often are the result of sampling inadequacy of CO_2 uptake. Growth represents an integrated response to many fluctuating environmental factors. Measurements of photosynthesis, however, often are made over very short time periods and, often, under very favorable environmental conditions. Hence, the values of CO_2 uptake are not necessarily representative of rates averaged over a long period of time under normally fluctuating environmental conditions in the field.

3 GROWTH RESPONSES

Investigators have assessed plant responses by a variety of growth indices (Table 2), making it difficult to compare studies. Methods of measuring various growth parameters will be discussed briefly.

3.1 Biomass

In selecting methods for estimating dry weight increment of individual plants and communities, different aspects of biomass accumulation should be recognized. Net primary production (NPP) is the sum of net production by all plants in a unit area. NPP is influenced by death and loss of plants and loss of tissues from living plants. The actual increase of plant mass in a study plot is net community growth or net ecosystem production (NEP). NPP and NEP often are very different. In a young forest some 30–60% of NPP may accumulate annually as NEP; in a mature climax forest NPP may be similar in amount to death and loss of tissues and plants. Hence, in the latter NPP may be high and NEP near zero (Whittaker and Marks, 1975).

Primary productivity may be variously estimated, the best method depending on the type of ecosystem. In many annual crop plants biomass and net production are similar. Growth of communities of small plants (annual crop plants, old fields, grasslands, tundra, bogs and shrubs) may be estimated by harvesting sample plants or plant parts only once during the growing season. In more complex perennial ecosystems several harvests may be necessary. In most woody successional communities detailed dimensional analysis has been most useful. For stands more than a few years old it often is best to examine in detail the growth relations of a small number of sample trees.

Most studies of NPP of forests involve measurements of sizes and weights of plants and various tissues and organs. These measurements can be converted to estimates of production by a mean-tree approach, for example, for single-age plantations. If trees are consistent in size the mass of an average tree × the number of trees per unit is used to obtain biomass of a stand. However, when these conditions do not hold, and when different dimensional components are used (for example, diameter and height, basal area, foliage area, stem volume) it is difficult to establish the average tree, leading to different estimates of biomass. Problems with the mean-tree approach can be avoided by measuring stand characteristics that best express production rate and by use of ratios to estimate NPP. For example, for forest undergrowth clipping of current twigs of shrubs is useful. Production of larger trees can be measured through estimated volume increments (EVI), defined as 1/2 annual wood area increment at breast height × plant height. Production estimates from ratios on EVI can be readily applied to mixed-age forests. Better yet, most investigators dealing with mixed-age forests have used logarithmic regressions by which growth and dimensional relationships are well expressed. Sample trees are harvested and measured intensively so biomass production and other dimensions can be related to diameter or other independent variables. The Brookhaven system of dimension analysis, described by Whittaker and Woodwell (1971a), involves use of regressions to calculate for each tree in a sample quadrat its probable biomass, production, volume, and surface dimensions.

Because dimension analysis depends on such indicators as xylem increments (annual rings) and bud scale scars, it cannot be used to estimate productivity of old or climax, mixed-age tropical forests. In tropical forests it often is necessary to use:

(1) sample quadrats in which growth and death of trees are followed, together with increase in diameter and height,
(2) dimension analysis of trees for biomass relations to diameter and height,
(3) calculation, using regressions of stem and branch biomass of sample trees at the beginning and end of a study period (to obtain stem production and increase in branch mass),
(4) estimation of root production, and
(5) estimation of foliage production (Whittaker and Marks, 1975).

3.2 Height Growth

It is much easier to measure height growth than cambial growth of trees. Height growth of seedlings and small trees can be measured with various growth gauges, rules and graduated poles (Garthwaite, 1981). Cremer (1976) used a growth recorder to continuously measure height growth of seedlings. Heights of large trees can be measured with hypsometers or a surveyor's transit (Husch *et al.*, 1972; Worrall, 1973).

3.3 Leaf Area

Several methods are available for measuring leaf areas of individual plants or stands (Table 2). These involve tracing leaf outlines on paper and counting squares or dots obscured by leaves, or weighing cut-out leaf outlines made on light-sensitive paper, and use of photoelectric planimeters. In the latter method the intensity of light emitted from a constant source and reaching a detector is considered proportional to the area of leaves placed between the light source and detector (Marshall, 1968). Photoelectric planimeters may be selective or non-selective with respect to differences in light transmission by leaves. Non-selective instruments are preferable but if only a selective type is available a correction factor can be obtained by measuring output of the light source for comparable areas of leaf tissue and black paper. Leaf areas also can be estimated from linear measurements of leaf length and width or from leaf area–leaf weight ratios of representative samples of leaves.

Leaf areas of trees or stands are usually estimated from careful observations on a few felled sample trees. Relationships are established between total leaf area and any of several easily measured characteristics that are related to leaf area, for example, tree height, tree diameter, crown diameter or crown volume (Kvet and Marshall, 1971).

A common measure of leaf production is leaf area index (LAI), the ratio of the leaf surface of a plant or stand to ground surface area (Anderson, 1971). LAI depends on plant size, age, spacing and other factors influencing plant size. Many deciduous forests have LAIs of 3–6, evergreen communities up to 8 (conifer needles up to 16). Lower values occur in desert shrubs and higher values in some ecosystems such as fast-growing poplar plantations.

The surface area of a population of gymnosperm needles has been calculated through correlation with needle volume, a value more easily determined by displacement (Kozlowski and Schumacher, 1943). By using equations developed by the method of least squares, Cable (1958) determined surface areas of individual pine needles from needle weight. He then used the relation between surface area and weight of individual fascicles with an estimate of the number of fascicles on a tree to compute the surface area of needles on whole trees. Estimates of total number of fascicles on a tree were derived from the relation

between total weight of foliage and tree diameter and from the relation between weight of the average fascicles and tree diameter. Thompson and Leyton (1971) coated gymnosperm shoots with a pressure-sensitive adhesive and covered the coated surface with a uniform monolayer of glass beads. Shoots were weighed before and after bead application. Bead weight was related to surface area calibrated by use of paper squares of known area. Gholz *et al.* (1976) and Waring *et al.* (1978) determined leaf areas of communities of gymnosperms by using mensurational techniques to estimate biomass and converting to leaf area.

3.4 Cambial Growth

Cambial growth and wood production are usually determined by periodically measuring change in stem radius, diameter or girth with dendrometers, calipers, or tapes. Measurements have traditionally been made at breast height (1.3 m above ground). Various types of optical dendrometers are used to measure diameters in the upper stem (Grosenbaugh, 1963).

Dendrometers either measure changes in stem circumference by a vernier band or changes of a single stem radius by gauging the distance between a fixed plane anchored in the wood and a point on the surface of the bark. Dial gauge dendrometers have been very useful (Bormann and Kozlowski, 1962). Sensitive recording dendrometers (dendrographs) provide a continuous and permanent record of changes in stem radius (Fritts and Fritts, 1955; Phipps and Gilbert, 1960). Dendrographs, originally designed for use on large trees, have been adapted to continuously measure diameter changes in tree seedlings (Kozlowski, 1967, 1968). Diameter changes of tree seedlings have also been measured with caliper micrometers (McCully, 1952) and recording micrometers (Splinter, 1970).

Dial gauge dendrometers have greater mechanical precision than vernier tree bands but dendrometer measurements taken at only one radial position often give misleading data on average tree growth (Young, 1952). Although tree bands underestimate the amount of stem growth (Braekke and Kozlowski, 1975) they are useful for many types of long-term growth studies (Winget and Kozlowski, 1965). Dial gauge dendrometers detect the beginning of seasonal cambial growth and periodic stem shrinkage more accurately than tree bands (Bormann and Kozlowski, 1962).

Cambial growth varies greatly at different stem heights. The annual xylem increment is narrow near the tree apex. Ring width then increases downward and usually is thickest at the height of the stem where there is maximum leaf volume. Below the crown the amount of xylem increment varies with crown size. In dominant trees (large crowns), the width of the annual ring narrows below the crown but thickens again near the base of the stem. In suppressed trees the annual ring not only is thinner at all stem heights but also narrows rapidly below the crown and often no wood is produced near the base of the stem. In contrast to

dominant and suppressed trees, open-grown trees show a progressive increase in ring thickness from the top to the bottom of the tree. For these reasons stems of open-grown trees are much more tapered than stems of dominant trees in a stand, and very suppressed trees have almost cylindrical stems (Kramer and Kozlowski, 1979). It should be obvious that measurements of cambial growth taken at lower stem positions only do not provide reliable estimates of cambial growth in the upper stem.

Reversible changes in stem diameter caused by periodic hydration and dehydration sometimes are appreciable and are possible sources of error in determining the amount of cambial growth by change in stem diameter. Tree stems usually shrink during the day because of high transpirational water loss and they rehydrate and expand at night (Kozlowski, 1972, 1982). Sometimes daily stem shrinkage exceeds the amount of cambial growth increment over a several-day period (Holmes and Shim, 1968; Braekke and Kozlowski, 1975; Braekke *et al.*, 1978). In addition, during rainless periods tree stems may shrink progressively for periods of days,-weeks or months. Even stems of rain forest trees may shrink over a period of weeks (Dawkins, 1956).

3.4.1 Microscopy

Most of the reliable information on time of seasonal beginning and ending of cambial growth and on types and structure of cambial derivaties produced has been obtained by light microscopy of sections taken at various times across the cambial zone. Useful methods of growth ring assessment involve linear microscopic measurement of the width of growth rings and accumulation of measurements (Kozlowski, 1970). Linear cross-sectional dimensions of cambial derivatives can be determined by (1) direct comparison with a graduated scale by use of traversing microscopes, (2) micrometer eyepieces, or (3) measurement with a graduated scale on projected images (Smith, 1967). Both scanning and transmission electron microscopy have yielded much information on fine structure of cambial derivatives (Coté, 1967).

3.4.2 Increment Cores

Cores of wood extracted from stems with increment borers have been widely used to determine growth of trees and forest stands. Measurements of growth increment in cores can be made with or without magnification, depending on objectives. Extracted cores are usually placed in protective holders for transport and often require additional treatment in the laboratory (Kozlowski, 1970).

3.4.3 Scanning Methods

Physical characteristics of xylem cells in parts of an annual ring and in different rings have been studied by various 'scanning' methods. For example, density of

cells has been determined by measuring absorption by wood of a collimated beam of beta particles (Phillips, 1960); additional techniques include scanning with a densitometer of X-ray photographs of wood (Polge, 1969).

3.5 Root Growth

Root growth and distribution have been expressed in number, weight, surface, volume, diameter, length, and number of root tips (Böhm, 1979). Root weights are useful for characterizing the total mass of roots in a soil, but not the amount of absorbing roots. Root surfaces can be determined from root diameters and total length, photoelectric methods, dye adsorption, titration values, and gravimetric methods. Root volumes can be obtained from average diameters and root length by water displacement. Root length has been determined by direct measurement, intersection methods, and with root counting machines. Many different methods have been used to study roots in the field and laboratory (Table 2).

3.6 Reproductive Growth

Yield and quality of flowers, fruits, and seeds have been variously determined. The number of flower buds or flowers, percent of plants producing flowers and time of floral initiation have been of particular interest. Yield of fruits and seeds have been assessed by numbers produced, diameter and weight. Fresh weight often has been determined for fleshy fruits and some vegetable crops. Some attempts have also been made to assess quality of reproductive structures (Tingey *et al.*, 1979).

4 INJURY

Methods vary for determination of injury to individual cells, tissues or whole plants. Records of injury as a percent of plants or leaves affected enable comparisons among different studies. Plants may simply be rated as sensitive, intermediate or tolerant (Berry, 1974), or injury assessed in as many as ten categories (Middleton, 1956; MacDowell and Cole, 1971). For greater precision percentages are used to indicate the extent of foliar injury (Berry, 1973). Miller (1973) evaluated oxidant injury to conifers by a more complex numerical system based on needle retention and condition, needle length and branch mortality. Some investigators determined incidence of injury by exposing a population of plants to pollutants and averaging the number of injured leaves (Menser *et al.*, 1963). For uniformity in rating air pollutant injury, comparisons with visual standards are desirable. However, standardization of methods is difficult because of variations in leaf morphology, differences in susceptibility in various environmental regimens and physiological impacts of different injury symptoms (Tingey *et al.*, 1979).

4.1 Cell Ultrastructure

Several air pollutants induce alterations in cell ultrastructure, often prior to appearance of visible signs (Thomson, 1975). For example, early responses of palisade parenchyma cells of *Phaseolus* to ozone included increase in granulation and electron density of the chloroplast stroma and in some chloroplasts, appearance of clusters and ordered arrays of fibrils. Next the plasmalemma, tonoplast and chloroplast envelope broke down. Subsequently, the mitochondria swelled and accumulated electron-dense material. Finally, the cellular contents collapsed in a mass in the center of the cell. These changes resembled those induced by peroxyacetyl nitrate (Thomson *et al.*, 1965). Sequential ultrastructural changes induced by SO_2 in chloroplasts included granulation of the stroma and swelling of fret membranes (Fisher *et al.*, 1973).

4.2 Membrane Permeability

Injury to cellular membranes leads to altered cell metabolism and necrosis. The speed with which ozone alters membrane functions has led to wide acceptance of the view that cell membranes are primary sites of ozone action (Heath, 1975). There is some evidence that SO_2 also affects membrane permeability (Puckett *et al.*, 1977). In addition to inducing changes in whole cells, ozone alters the permeability of organelles, for example, mitochondria (Lee, 1968) and chloroplast membranes (Coulson and Heath, 1974).

Alterations in membrane permeability can be studied by changes in metabolite uptake (Perchorowitz and Ting, 1974), solute leakage (Evans and Ting, 1974; Beckerson and Hofstra, 1980) or water uptake. Evans and Ting (1973) studied ozone-induced water permeability changes using leaf discs by (1) changes in net weight determined gravimetrically and (2) change in refractive index of the suspending media measured refractometrically.

5 CONTROLLED ENVIRONMENTS VERSUS FIELD STUDIES

Objectives and available facilities often dictate whether controlled environments, field experiments, or both are best used to assess effects of environmental chemicals on plants. Controlled-environment experiments often are more informative than field experiments because close control over the environment is maintained, and a wide range of conditions is available simultaneously, allowing for comparisons of effects of environmental chemicals in different environmental regimens. Also it often is impossible in field situations to establish meaningful control plots (for example, near pollution sources). A major advantage of environment-controlled research facilities is that they decrease variability and increase reproducibility of data (Tibbitts and Kozlowski, 1979).

5.1 Exposure Chambers

In studies of air pollution effects, plants should be exposed to chemicals under conditions that will yield results which would be obtained under normal growing conditions. Although this may best be accomplished in the open, pollutant dosages cannot be controlled and comparison often cannot be made between fumigated and unfumigated plants. Hence, exposure chambers are necessary. A multitude of exposure chambers, varying in size, shape and complexity have been used. The important requirements of all chambers include:

(1) uniform distribution of the pollutant between and within chambers,
(2) uniform environment (radiation, temperature, relative humidity and air movement) between and within chambers,
(3) nonreactive chamber surfaces,
(4) close control of pollutant concentrations, and
(5) an environment that resembles ambient conditions.

Desirable but not always essential characteristics include a single pass system, negative chamber pressure, portability, low cost, and ease of construction and cleaning (Heagle and Philbeck, 1979). A useful system for greenhouses and phytotrons is the continuous stirred tank reactor (CSTR) system described by Heck *et al.* (1978). In field chambers with closed tops, temperature is greater and light intensity lower than ambient. There has been a trend toward use of open-top chambers because they do not have these problems (Heagle and Philbeck, 1979).

Important requirements for dispensing chemical pollutants into test chambers include:

(1) close control of delivery of desired concentrations of chemicals,
(2) freedom from concentration drift once desired concentrations are reached in chambers,
(3) lack of leakage and
(4) construction with materials that do not react with the polluting chemical.

A useful dispensing system for SO_2, O_3, and NO_2, alone or in combination, was described by Heck *et al.* (1978). A variety of pollutant control systems for delivering a single set concentration of a pollutant, several set concentrations varying with time or gradually changing concentrations are described by Heagle and Philbeck (1979). A single pollution monitoring instrument can be used to determine concentrations of pollutant at several locations.

5.2 Design of Field Studies

Numerous field studies have been conducted to assess the character, magnitude, and effects of air pollution on plants. Although many early studies lacked suitable control plots to assess prepollution conditions, some efforts have been made in recent years to obtain such information. Factors influencing design of

field studies include the type and characteristics of the pollutant source, meteorological conditions, type of pollutant(s) evaluated, type of vegetation and objectives of the study, for example, short- or long-term, acute or chronic effects, etc. The following recommendations are based on those of Skelly *et al.* (1979) for various pollutant sources.

5.2.1 Single Event Point Sources

Accidental spills A typical design consists of radial transects from the center of the source, with upwind transects shortest. Plants should be located along the transects at geometric and logarithmic intervals out from the source (Figure 1).

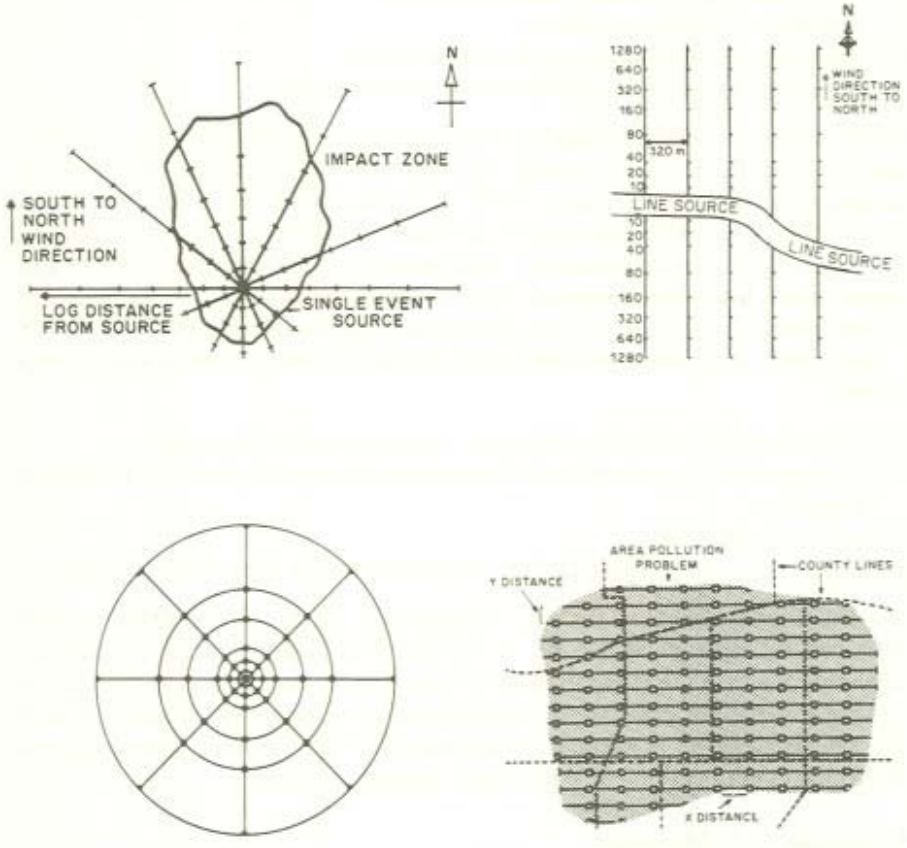

Figure 1 Some examples of plot distribution for studying effects of air pollution on vegetation. Reproduced by permission of the Air Pollution Control Association from Skelly *et al.* (1979). Upper left: single event source of pollution; upper right: line source of pollution; lower left: single point source of pollution; lower right: area-wide source of pollution

5.2.2 Line Sources

Highways Pollutants which are generated continuously usually consist of coarse particles, fine particles and gases. Typical design consists of multiple transects located perpendicular to the source at set distances. The distance between plots along transects increases with greater distance from the roadway. All plots should contain the same species under observation (Figure 1).

5.2.3 Continuous or Long-term Point Sources

Smoke stacks of power generating plants, smelters, refiners, etc. Pollutants include coarse particles, fine particles and gases. Design for *ex post facto* studies consists of radial transects (for perhaps up to 35 miles) that predominate in the direction of the affected area. Sampling sites are established on transects and concentrated in the impacted zone. Control plants should be established in an upwind direction, if possible (Figure 1).

5.2.4 Preoperational and Operation Studies

New or changing point source Design is similar to that of *ex post facto* studies except that sample plots should be distributed in all directions so that plots not subject to predominant wind direction can serve as background sites after the polluting source is operational.

5.2.5 Area and Regional Sources

These are more difficult to assess than those discussed above. Area source problems are the result of pollutant emissions from many sources that eventually lose their point, mobile or line source character, or from widespread use of agricultural chemicals. Regional source problems involve those associated with large urban areas and long distance pollutant transport into other areas that do not emit pollutants (Figure 1).

Design in the field will depend greatly on predetermined and clearly outlined specific objectives and on available time, funds and other resources. Methods will differ for (1) single crop responses to specific pollutants (Menser, 1969), (2) multiple crop responses (Oshima, 1973), and (3) for studies of crop loss estimates by governmental boundaries (Skelly and Hayes, 1977; Williams *et al.*, 1977).

6 PLANT COMMUNITY STRUCTURE

There is widespread interest in assessing the effects of environmental chemicals on plant community structure, especially that of forest ecosystems. In normal

succession in an unpolluted atmosphere the number of species, productivity, biomass, community height and structural complexity of an ecosystem increase. Environmental chemicals such as air pollutants induce retrogression characterized by reduction in productivity, biomass, coverage and species diversity (Linzon, 1978).

Various approaches have been used to assess the effects of air pollution on plant community structure. Most commonly biomass, productivity, species diversity, frequency, percent cover and injury have been determined at various distances from the pollution source (Gordon and Gorham, 1963; Winner and Bewley, 1978). Details on methods are given in Table 1 and in Oosting (1956).

6.1 Simulation Modelling and Computer Analysis

Traditionally, studies of responses of forest trees to air pollution stress centered on species level responses, seedlings or physiological processes. However, to assess pollution effects on trees within forest stands requires combining autecological and synecological approaches by simulation modelling. A few examples will be given.

West *et al.* (1980) used a forest growth and succession model to evaluate the time-integrated effects of air pollution on responses of 32 species of forest trees growing in forest stands. Their approach addressed several important questions, including:

(1) what level of pollution stress significantly alters forest growth and development,
(2) how are stress effects integrated over time,
(3) how important is competition in influencing induced stresses on various species, and
(4) how are species responses integrated into responses of forest systems.

A basic assumption of the model was that the relative sensitivity of species to reduced physiological activity under chronic stress regimens paralleled their relative sensitivity to foliar injury as reported in the literature. They applied various degrees of growth inhibition to trees in different pollution sensitivity classes to simulate changes in biomass of both individual species and an entire forest stand. *Liriodendron tulipifera*, a rapidly growing, early successional species (intermediately intolerant to pollution) showed a rapid growth increase under chronic pollution as a result of the greater inhibitory effect on other species in the stand. By comparison, *Quercus velutina* (also intermediately tolerant to pollution) exhibited greater growth inhibition, reflecting largely its inability to compete with other species (for example, *Liriodendron*) under the added stress of air pollution. *Quercus alba* (tolerant to pollution) showed increased growth in a

polluted stand which became evident only as the stand matured. Pollution dramatically decreased growth of *Prunus serotina* (very sensitive to pollution), particularly in stands more than 100 years old and resulted in its almost total elimination.

The effect of competition on the impact of pollution on herbaceous plants was demonstrated by Bennett and Runeckles (1977) who compared the effect of ozone on growth of rye grass (*Lolium multiflorum*) and clover (*Trifolium incarnatum*) when these were grown singly or together. The data indicated increased competitive potential of rye grass over clover when ozone stress was applied.

Kercher *et al.* (1980) developed a simulater for the mixed conifer forest type of the Sierra Nevada. Their model calculated environmental parameters of the stand and initialized numbers and sizes of trees from environmental and control data, respectively. A table of good and bad seed years and a list of fire years were generated. The effect of air pollution on trees was calculated. The number of new seedlings for that year, growth of each tree and mortality were then determined for each year. Growth was modelled in terms of differences in tree diameter and as a function of environmental variables. Mortality was determined stochastically depending on its probability as determined by ecological risk, lack of growth and fire injury. The dynamics of accumulation of litter and brush were also modelled. For a 10 % reduction in growth of *Pinus ponderosa* from pollution stress, and with growth reductions in other species as determined by their relative sensitivities, standing crops of *Pinus ponderosa* were reduced and those of *Abies concolor* increased.

Another example of effective simulation modelling of effects of pollution stress on plant processes and community dynamics is that of Luxmoore (1980).

6.2 Effects of Chemicals on Lichens and Bryophytes

Recognizing that many lower plants are much more susceptible than vascular plants to air pollution, several investigators studied the frequency and density of lichens and mosses as an index of the impact of pollutants on plant community structure.

Zone maps of plant distribution have been commonly used, with some studies delineating 3 or 4 zones and others as many as 10 (Rao and LeBlanc, 1967; LeBlanc *et al.*, 1972a; Rose, 1973). Some investigators collected data for only one widely distributed species (Granger, 1972; Hawksworth, 1973). Vegetation maps based on a mathematically derived 'index of atmospheric purity' to express the long-term effect of air pollution on lichens and bryophytes were used by DeSloover and LeBlanc (1968) and LeBlanc *et al.* (1972b). Brodo (1961, 1966), Pyatt (1970), and Hawksworth (1971) studied growth of transplanted lichens and

bryophytes, together with their substrates, from unpolluted to polluted sites.

7 SOIL POLLUTION

There has been a rapidly growing interest in soil pollution as an index of accumulation of potentially toxic chemicals in the environment around point- and area-sources of pollution. The soil pollutants of greatest interest include metals such as mercury, metalloids, halides, sulfur, boron, various agricultural chemicals (e.g. herbicides, insecticides, fungicides), and allelopathic chemicals.

Soil surveys for the presence of polluting chemicals fall into two general categories: (1) those in which natural soil horizons are sampled and (2) those in which the soil is sampled at uniform depths. The former are appropriate in areas where the soil profile has not been disturbed; the latter where soil disturbance is evident, as in urban, industrial or agricultural areas.

Methods of analysis for soil pollutants largely involve chemical analyses of soil samples. Usually the concentration of pollutants in soil samples is compared with unpolluted samples or with samples considered to contain normal or background concentrations. For metals, atomic absorption spectroscopy is most commonly used (Slavin, 1968) but flame emission is preferable for alkali metals. For simultaneous determination of many elements atomic fluorescence and spark emission spectroscopy are used (Jones, 1976; Fassel, 1978). Both colorimetry and ion selective electrodes have been used for determination of halides (Cook *et al.*, 1976; Jacobson and Weinstein, 1977). Methods for sulfur determination are usually based on colorimetry and turbidimetry (Chan, 1975; McQuaker and Fung, 1975; Norby and Kozlowski, 1982). Various bioassays involving growth responses of sensitive test plants in polluted and unpolluted soils have also been useful (Kozlowski and Torrie, 1965; Kozlowski *et al.*, 1967). Methods for analyzing allelopathic chemicals are described in papers cited by Rice (1984).

Considerable caution is advised in interpreting data on concentrations of chemicals in soils. Actual toxicity of chemicals to plants cannot be precisely determined by applying them to the soil because such chemicals are lost to variable degrees by evaporation, leaching, microbial or chemical decomposition, and adsorption on the soil (Kramer and Kozlowski, 1979). A useful apparatus for studying the effects of precipitation and other environmental factors on the fate, movement, potential bioaccumulation and interactions of chemicals applied to soils is the compartmentalized microcosm described by Lichtenstein *et al.* (1978). The apparatus consists of terrestrial and aquatic components that can be maintained separately under a variety of environmental conditions. For a good discussion of problems in collection and preservation of soil samples, sample analysis and interpretation of data the reader is referred to Temple and Wills (1979).

8 REMOTE SENSING

Some investigators have shown that, to some degree, pollution damage to vegetation can be assessed by remote sensing from satellites or aircraft. To date most work has concentrated on aerial photography and airborne multispectral scanning. Although there are many problems with remote sensing techniques now available (Thorley, 1975), they have many potential advantages over other methods, including use of many parts of the electromagnetic spectrum; saving of time, money and manpower; ability to cover large areas; and use of successive remote sensing surveys to follow the extent of injury to vegetation by air pollutants.

Using color aerial photography Heller (1969) studied the effects of oxidant air pollution on *Pinus ponderosa* trees in California. The most useful signs for identifying pollutant-affected trees were color, low density and shortness of needles, and high frequency of bare branches. Whereas healthy pines retained needles for 5 years, pollutant-affected trees retained only current-year needles. Similar remote sensing techniques have been used in Canada (Murtha, 1972) and Europe with variable success (Thorley, 1975). For a good discussion of problems with currently available remote sensing techniques for assessment of pollution damage to vegetation the reader is referred to Thorley (1975), van Genderen (1974), and Sapp (1978).

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