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Rapid quantitative assessment of visible injury to vegetation and visual amenity effects of fluoride air pollution

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Abstract Quantitative measures of visible injury are proposed for the protection of the aesthetic acceptability and health of ecosystems. Visible indications of air pollutant injury symptoms can be assessed rapidly and economically over large areas of mixed species such as native ecosystems. Reliable indication requires close attention to the criteria for assessment, species selection, and the influence of other environmental conditions on plant response to a pollutant. The estimation of fluoride-induced visible injury in dicotyledonous species may require techniques that are more varied than the measurement of necrosis in linear-leaved monocotyledons and conifers. A scheme is described for quantitative estimates of necrosis, chlorosis and deformation of leaves using an approximately geometric series of injury categories that permits rapid and sufficiently consistent determination and recognises degrees of aesthetic offence associated with foliar injury to plants.

Keywords Air pollution · Biomonitoring · Visible injury · Quantitative indication · Visual amenity · Fluoride

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Introduction

Ecosystem health or environmental well-being is a desired condition that is established in much air pollution control legislation, and the concept has been advocated as being useful for communication between scientists and the non-scientific public (Costanza et al. 1992; Rapport et al. 1995). There are many components and indicators of ecosystem health; there is not a direct correspondence between a pollutant exposure and changes in each of these indicators, and they may all be influenced by environmental conditions other than the pollutant of interest (Ashmore 2005; Paoletti and Manning 2007). Neither is there a generally accepted procedure for detecting the minimal environmental change that may be used for testing the impacts of particular pollutants (Lindberg and McLaughlin 1986) or the responses of receptor ecosystems (Chen and Goldstein 1986; Rapport et al. 1995). In an attempt to identify suitable environmental monitors, Kratz et al. (1995) proposed that 'ecological signals in the structure of ecological variability observed in space and time' could indicate ecosystem health or response to stress. However, the limited information available meant that there were 'no general laws that allow us to predict the relative magnitude of temporal and spatial variability of different types of parameters across the full diversity of ecological systems' (Kratz et al. 1995).

In practice, detection of the minimal change in some plant attribute that can be attributed to a pollutant is difficult due to the variation in that parameter attributable to other causes that produce mimicking responses or symptoms (Weinstein et al. 1990).

Human well-being incorporates the notions of avoidance of injury to components of ecosystems other than humans (Tingey et al. 1990) and the avoidance of offensive or objectionable conditions or the creation of nuisance (e.g. United States Congress 1980), whereby the sensory perceptions of humans are used to prevent or limit the extent of olfactory, auditory or visual offence or discomfort. Whilst quantitative limits have been developed for odour and noise in some jurisdictions, there are few regulatory guidelines for preventing the impairment of visual amenity. Visual offence may be assessed only as the reduction in sight distance due to particulate matter (e.g. Queensland 1997). In contrast, the US Congress (1980) established that within specified areas (e.g. class 1 wilderness areas), air-quality-related values must be protected from deterioration. Such deterioration includes the occurrence of visible injury attributable to pollutants (Davis and Orendovici 2006). For wilderness areas in the Rocky Mountain region of the USA, Schoettle and Moir (1998) recommended that the acceptable extent of foliar lesions in coniferous and deciduous species should be less than 5% of the leaf area. In contrast, there is a lack of definition regarding the impairment of the visual quality of vegetation in general land use areas. Nevertheless, visible injury to vegetation is often a source of offence to citizens, and it needs to be quantified, especially where injury occurs at ambient pollutant concentrations that comply with air quality guidelines (Paoletti and Manning 2007).

The quantitative measurement of sensory offence is difficult (Gostelow et al. 2001), and varying degrees of offence may be taken by different observers to a given level of visual impact. Even though visible injury is accepted as a descriptor of pollutant effects (Smith et al. 2003; Weinstein and Davison 2003), a quantitative description does not appear to be available for the loss of visual amenity in vegetation. This paper describes a rapid method of vegetation injury assessment that takes account of visual amenity.

Biological indication

Although Horsfall and Cowling (1978) lamented that visible injury assessment had been dismissed by some plant pathologists as primitive, subjective and unscientific, the approach is an attractive tool for the indication of stress as it can be applied rapidly and cheaply at a large number of locations (Feder and Manning 1978; Manning and Feder 1980; Zonneveld 1982; Weinstein et al. 1990; Manning 2003; Weinstein and Davison 2003). Where the effects of pollutants are not severe and where large populations of plants occur over a suitable area, the assessment of injury may be based on the percentage of individuals or leaves that express visible injury (Chappelka et al. 2003; Davis and Orendovici 2006). If these conditions do not apply, attention may be directed to the quantitative assessment of injury within individual plants or individual leaves. The heterogeneity of field environments may greatly complicate the relationships between the extent of visible injury and the physiological responses to pollutant exposure (Hill et al. 1958; Weinstein et al. 1990; Schaub et al. 2005; Paoletti and Manning 2007), so any test must be applied with caution, and appropriate calibrations of assessments must be applied (Steubing 1982; Bussotti et al. 2006).

Statistical techniques, including before-after-control-impacted comparisons (Underwood 1994) and dose–response relationships (Weinstein and Davison 2004; Ashmore 2005), can be applied to enhance the analysis of complex environmental situations and to increase the confidence of environmental decision-making processes (Heck et al. 1988; Smith 1994; Michener 1997). While statistical analyses may be able to detect small mean changes in a large population (Underwood 1994; Michener 1997), changes predicted on the basis of individual estimates are much less precise (Smith 1994). Therefore, distinct changes are commonly required for reliable statistical indication, and it becomes critical to identify the most sensitive components and attributes of an ecosystem and to adopt assessment techniques that may not rely on the central statistics of a population.

The time scales over which different organizational scales should be assessed vary greatly (Osmond 1988; Huggett et al. 1992b), and this aspect must be considered carefully in the selection of the most appropriate biological monitoring technique (Michener 1997). Monitoring may be passive, where naturally occurring organisms are sampled (Weinstein and Davison 2003), or active, where plants are introduced into the environment in a pre-determined condition (Arndt et al. 1985, 1987; Weinstein et al. 1990; Franzaring et al. 2007). Passive indication using small samples of plants has the disadvantage of variability of genetic composition within taxa and of environmental situation, but it does not require investment in the maintenance of monitoring subjects (Weinstein and Davison 2003). It is also possible to focus attention on the most sensitive members of a population rather than attempt to determine the mean response for the population. As a result, passive monitoring is well suited to the evaluation of natural or extensively managed vegetation areas where variations in the responses of individual plants in a population to pollutant stress can be used as an indicator of response (Lacasse and Treshow 1976; Malhotra and Blauel 1980; Chappelka et al. 2003; Davis and Orendovici 2006).

Species attributes for biological monitoring

The major elements of species selection in relation to biological monitoring have been listed by Arndt (1982) and discussed in detail by Weinstein et al. (1990), Huggett et al. (1992a) and Mayer et al. (1992), but they can be regrouped and summarised as follows:

(1) The species response should be highly sensitive, responding to very low exposures of the pollutant being monitored.
(2) The species should respond quantitatively and precisely to pollutant exposure.
(3) The response of the species to a given pollutant should be readily distinguished from responses due to other pollutants and natural environmental stresses.
(4) The distribution of the species should encompass the area to be protected.
(5) The species should be responsive to pollutants throughout the growing season.

The species and attributes measured and the intensity of sampling will vary greatly, depending on the purpose of measurement of response to a pollutant (Smith 1994). Because it is often difficult to relate plant response directly to pollutant exposure (Ashmore 2005), even intensive sampling may not provide results with a high predictive value. On the other hand, repeated observations on a small sample of species or plants over a number of years may be sufficient for monitoring the effects of pollutants on plants.

If the detection of peak concentrations of a pollutant is important (Smith 1994), a desirable system is one that responds rapidly and sensitively but undergoes a permanent change. The functions of an organism with such properties are likely to be unstable in a fluctuating environment, the characteristics of the organism may change systematically with time, and the organism may not persist under extreme conditions, even those of natural origin. If an integrated record of exposure is required, then maintenance of structural and functional integrity of the organism in a fluctuating environment and a quantitative response to dose or exposure time are essential (Mayer et al. 1992). Clearly, a single organism is unlikely to simultaneously and satisfactorily record both peak and integrated pollutant exposures.

Sampling and analytical convenience is also a major practical determinant of both the species and locations selected, particularly where sampling must be frequent or access to the area of interest is limited. Consequently, the relatively few indicators of environmental stress, including pollution, that are in practical use tend to be gross and nonspecific (Mayer et al. 1992), such as the observation of visible injury symptoms in plants (Jacobson and Hill 1970; Bussotti et al. 2003; Weinstein and Davison2003).

The correspondence between a particular form or extent of injury and an ambient pollutant concentration will depend on the plant species being examined and also on the conditions of exposure, including the combination of concentration and exposure time (dose), temperature, humidity, light intensity, water availability and wind conditions (Weinstein and Davison 2004). Therefore, visible injury assessment should not be used to indicate ambient pollutant concentrations unless detailed information is available concerning the responses of the species or variety in question (Weinstein et al. 1990).

On the other hand, the responses of different species with similar sensitivity to a pollutant can be combined to provide a general indication of the extent of effects, and these patterns of effects can then be compared with measured or predicted ambient concentrations of the pollutant in question. In addition, species with different levels of sensitivity to a pollutant should show consistent differences in symptom expressions between locations where the ambient pollutant concentrations may vary substantially.

Injury assessment procedures

Quantitative assessment

Quantitative assessments of visible injury to plant leaves caused by pathogens, insects and herbicides are long-established (Cobb 1892) and have been used extensively to evaluate the severity of disorders and the efficacies of treatments (Large 1966; Horsfall and Cowling 1978). Quantitative descriptions of visible pollutant injury are less numerous but have been applied in Europe for general pollutant effects (Arndt et al. 1987) and for ozone in Europe (Ashmore et al. 1980; Karlsson et al. 1995; Lorenzini et al. 2000; Bussotti et al. 2003; Novak et al. 2003) and North America (Feder and Manning 1978; Smith et al. 2003). Estimates of fluoride injury to monocotyledon leaves have been based on estimates of the lineal extent (Feder and Manning 1978; Weinstein et al. 1990) or the percentage of leaf length affected (Klumpp et al. 1995, 1996). For dicotyledonous species, the area affected is a more relevant measure, but accurate measurement of the injured leaf area is slow or difficult, and injury expression may vary between species (Flagler 1998; Vollenweider et al. 2003; Weinstein and Davison 2004). As a result, injury may be estimated visually by counting the percentage of leaves showing injury (Moore 1943; Karlsson et al. 1995; Davis and Orendovici 2006) or by placing leaves in defined injury categories.

For general foliar pathology, Boone and Westwood (2006) used four categories, each spanning a 25% injury range, to assess the effects of power station emissions on vegetation. The US Forest Service (Miller et al. 1996) and Chappelka et al. (2007) described ozone injury by a variant of the Boone and Westwood (2006) approach with subdivision of the category of least injury. Injury increments of 20% were used by Moraes et al. (2002) to indicate pollutant responses in tropical tree species. Klumpp et al. (1994) used 5% increments of leaf area to describe necrotic injury due to ozone, peroxyacyl nitrate and organic compounds in *Nicotiana tabacum*, *Urtica urens* and *Petunia hybrida*, respectively.

Categories ‘based on equal ability to distinguish, not on equal disease’ were used by Horsfall and Barratt (1945) to describe disease injury. An injury scale of 100% was divided in geometric order around 50%, so that the upper limits for injury categories were 0%, 1%, 3%, 6%, 12%, 25%, 50%, 75%, 87%, 94%, 97%, 99% and 100%. Horsfall and Cowling (1978) pointed out that many injury assessment scales, developed independently over nearly a century, had a logarithmic base which was consistent with the mechanism of human perception. The Horsfall and Barratt injury scale was adopted for the estimation of ozone injury to dicotyledonous species in Europe by Bussotti et al. (2003), while an abbreviated injury scale was adopted for the assessment of insect injury (Tomkiewicz et al. 1993) and ozone injury in Europe (Lorenzini et al. 2000) and in North America (Innes et al. 2001; Smith et al. 2003). A disease assessment scale for describing the extent of foliar injury due to potato blight in the UK (Moore 1943) was identical in form to the Horsfall and Barratt scale except for an additional category at the lower injury end. The relationships between the extent of injury and recording categories for different approaches are compared in Fig. 1.

Horsfall and Cowling (1978) and Steubing (1982) stressed the importance of calibration of

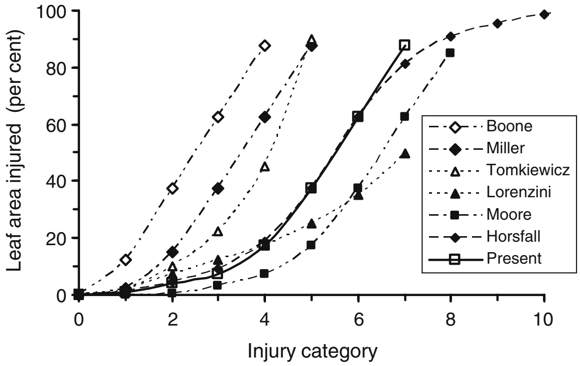


Fig. 1 Comparison of median foliar injury for assessment categories developed by Horsfall and Barratt (1945), Moore (1943), Tomkiewicz et al. (1993), US Forest Service Miller et al. (1996), Lorenzini et al. (2000), Boone and Westwood (2006) and in the present work

observers. Ashmore et al. (1980) reported that there was acceptable correspondence between observers in their estimates of percent leaf area injury due to ozone. For ozone injury affecting less than about 50% of foliage area, Lorenzini et al. (2000) and Bussotti et al. (2003) found that the correspondence between different assessors was close, but there was greater variation with more extensive injury and a tendency to overestimate the extent of injury. Novak et al. (2003) obtained satisfactory assessments by using the same assessor and by comparing assessments against a standard. These studies suggest that quantitative visual assessments can be made with acceptable precision, given the application of modest calibration measures.

A visual amenity scale

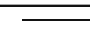
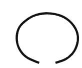
An injury scale was developed for Australian and New Zealand plant species, chiefly dicotyledonous, in situations where it became necessary to evaluate the degree of offence taken by different members of society. Environmental controls are commonly designed to protect the members of a population or the processes most sensitive to the environmental stress. Therefore, in order to develop a systematic approach to the occurrence of offence, it is useful to describe different types of observers.

With respect to visual effects, the most sensitive individuals may be those with a commercial or close personal interest in the appearance of vegetation. For example, professional observers should be expected to detect small changes in the appearance of vegetation, but they may not allow themselves to be offended even by extensive injury; they are skilled but disinterested observers. On the other hand, a commercial horticulturist may be affected materially and thereby is capable of being offended if injured plants or plant parts become unsuitable for sale. The manager of a conservation reserve is likely to be offended if the appearance of vegetation is impaired and also because this visible injury may reflect other changes that are much more difficult to measure. A domestic gardener may be offended if plants are unsuitable for exhibition or enjoyment either in situ or as cut flowers or foliage. It is also recognised that domestic gardeners may have a close attachment to vegetation that triggers sensitive responses to changes in plant condition.

Many people who do not have a personal or professional interest in plants do not notice injury until it is very obvious, or they do not associate the injury with an air pollutant. However, once alerted to the occurrence and extent of injury, they can recognise the form of injury and discern relatively small differences in its extent. These may be described as casual observers for their first introduction to injury or inexperienced observers if they have recently become acquainted with the injury. A further category of observers may be described as uninterested in that they express concern at the appearance of vegetation only when very extensive injury has occurred, and once the condition improves somewhat, their interest dissipates.

The boundaries between the levels of perception that may be associated with the various groups of observers are diffuse, and observers may change in their response to visible injury once they become aware that the injury may have been caused by an air pollutant. As a result, the classifications of observers must be very general. Environmental protection regulations are often designed to protect the interests of a typical member of society. For example, the general land use air quality guidelines for fluoride in Australia and New Zealand (ANZEC 1990) were developed with the intention that a typical domestic gardener

Table 1 Symptom code for visible injury to vegetation, with particular reference to fluoride

Category	Necrosis, chlorosis or anthocyanin accumulation % leaf length or area	Undulation or cupping of lamina degrees of arc	Leaf profile
0	0	Nil (0°)	
1	1–2%	Very slight (<30°)	
2	3–5	% Slight (30–60°)	
3	6–10%	Distinct (60–120°)	
4	11–25%	Marked (120–180°)	
5	26–50%	Severe (180–240°)	
6	51–75%	Very severe (240–360°)	
7	> 75%	Extreme (>360°)	

would not be offended by the extent of injury occurring in fluoride sensitive plant species at ambient fluoride conditions that conformed to the guideline value for general land use. Lower guideline concentrations were specified for fluoride sensitive commercial species, and lower concentrations again were specified for conservation areas where it was considered that any risk to the well-being or appearance of any organism was unacceptable.

Visible injury categories

Injury categories were selected to enable rapid assessment and to reflect the range of value judgments that may be associated with the concept of aesthetic environmental harm as it has been described here. For each category, the value in Table 1 indicates the range of injury expression in the assessed leaves associated with that category.

The resulting scale of injury (Table 1, Fig. 2) is almost identical to that proposed by Horsfall and Barratt (1945), except that the boundaries for the injury categories were based on percentages of leaf area that were judged to be convenient for both estimation and quantitative description, namely 0%, 2%, 5%, 10%, 25%, 50% and 75%

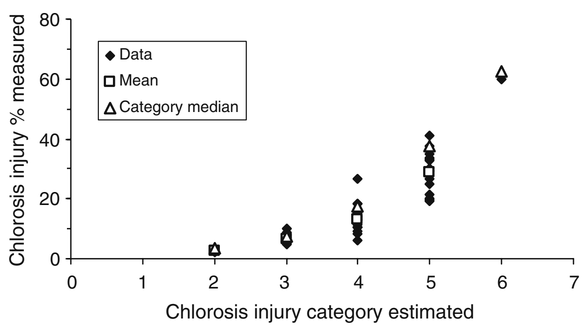


Fig. 2 Relationship between percentage of leaf area affected by chlorosis, measured by systematic dot grid counting, and injury category estimated by rapid visual inspection for individual leaves of *C. camphora*. *Solid diamonds* individual data points, *open squares* mean measured leaf area affected by chlorosis for the leaves assigned to the designated category, *open triangles* median leaf area affected by chlorosis for each injury category

of leaf length or area. Whereas the Horsfall and Barratt method establishes the average extent of injury throughout the plant, the greatest expression of injury within a cohort of leaves was selected in the present work. This approach was adopted for three reasons:

(1) The most-affected leaves on a shoot attract the attention of interested observers.
(2) In many situations, the distribution of injury within a seasonal cohort of leaves is not uniform, and the position of injured leaves within the seasonal growth often identifies relatively short periods that could be associated with natural stress events, or it could lead to an investigation of possible pollutant exposure events.
(3) Assessment of injury in the most prominently affected foliage in each cohort is much more rapid than the estimation of the total percentage of leaf area affected in the whole plant.

Table 2 relates the injury category (column 1) and the extent of injury in the portion of leaves giving rise to the assessment (column 2) to the extent of injury in the plant or the canopy as a whole (column 3), to indicators of the nature of recognition that may be associated with this injury (column 4), the degree of offence that may be taken by observers of the injury (column 5) and to possible commercial or ecological consequences of injury (column 6). The differences in values between columns 2 and 3 reflect the different proportions of leaves in a cohort that might express injury where pollutant exposure is episodic. These relationships are included because of the need to recognise that there is an aesthetic component to the effects of air pollutants on vegetation (Haddow et al. 1998). There is no assumed relationship between the different assessments of response, such as visible injury and loss of commercial yield, but it is useful to quantify the level of injury at which different observers might reasonably be expected to take offence at the occurrence of visible injury. Typical combinations of symptoms for *Eucalyptus citriodora* as presented in Table 3.

Symptom assessment

Necrosis

Where visible injury can be assessed rapidly and reliably and where leaf length is relatively constant, a direct quantitative scale of injury can be used. For example, the median length of the necrotic portion of leaves has been used to describe fluoride injury in *Gladiolus* species (Amaryllidaceae; Feder and Manning 1978; Weinstein et al. 1990) and tropical grasses (Oliva and de Figueiredo 2005), while Klumpp et al. (1995) estimated necrosis in *Hemerocallis* leaves to 1% of leaf length. In Australia, species of *Xanthorrhoea* carry narrow leaves that may be up to 1.5 m long, and they are generally very sensitive to fluoride (Doley 1986). Tip necrosis is characteristic of fluoride injury, although the soil-borne pathogen *Phytophthora cinnamomi* may also lead to extensive leaf necrosis in this genus. The New Zealand species, *Cordyline australis* and *Phormium tenax*, have leaves up to 1 and 2 m long, respectively. In *C. australis*, the boundary between necrotic and living tissue is relatively even, but in *P. tenax*, it is usually very irregular, so that the median length of necrosis is used to derive the extent of injury. Both of these species exhibit fluoride-induced tip necrosis at 90-day average ambient fluoride concentrations of less than 0.5 μg m^{-3}, the general land-use guideline (ANZEC 1990), making them useful indicator species (Weinstein and Davison 2003; Doley et al. 2004).

The patterns of occurrence of necrosis caused by fluoride are quite different from the dispersed necrosis associated with ozone (Flagler 1998). Dicotyledonous species may exhibit fluoride-induced necrosis at the leaf tip in species with predominantly linear venation (e.g. *Callistemon* spp.), uniformly along the margin (e.g. some *Eucalyptus* spp.) or irregularly around the margin (e.g. *Vitis vinifera*; Doley 1986). The pattern of development of necrosis depends on the leaf venation and the consequent pattern of accumulation of fluoride (Weinstein and Davison 2004). Uniform marginal necrosis can be quantified

Table 2 Plant visible injury categories, their expression, recognition, perception and range of effects

Category	% target leaf area affected	% total plant leaf area affected	Recognition	Perceptions of offence	Ecological or commercial effects
1	0–2	0–2	Recognised by skilled observers	Minimal offence to horticulturists and domestic gardeners	Very unlikely to affect plant growth or reproduction
2	3–5	1–5	Recognised by trained observers	May cause offence to persons with serious interest in plant condition	Unlikely to have a detectable effect on plant growth or reproduction
3	6–10	1–10	Obvious on careful viewing by inexperienced observers	Serious impairment of aesthetic quality for commercial horticulture	May reduce total plant growth, crop yield and reproduction
4	10–25	2–25	Obvious on brief viewing by inexperienced observers	Typical domestic gardener likely to be offended by appearance of plants	Likely to reduce total plant growth, crop yield and reproduction
5	26–50	5–50	Obvious on brief viewing by casual observers	Offensive to observers with no prior interest in vegetation	Very likely to reduce total plant growth, crop yield, premature senescence and failure of reproduction
6	51–75	10–75	Obvious to uninterested observers	Offensive to observers with no prior interest in vegetation	Very likely to result in premature death and loss of foliage, death of shoot tips, reduced plant growth and crop yield, and failure of reproduction
7	> 75	25 to >75	Very obvious from a distance to uninterested observers	Highly offensive to observers with no prior interest in vegetation	Very likely to result in rapid death and shedding of foliage, death of shoot tips and premature plant death if the injury recurs

Table 3 Symptom expression of fluoride injury in *E. citriodora* and its application to bioindication

Characteristics	Sensitivity[a]	Mimicking symptoms	Reliability of character	Period of indication	Quantitative expression
Marginal chlorosis	Very sensitive (young) intermediate (old)	Sulfur dioxide	High—with other information	Expanding leaves	Medium (visual assessment), high (computer assessment)
Cupping or buckling of the lamina	Very sensitive (young) tolerant (old)	Sap-sucking insect injury, calcium deficiency	High—with other information	Expanding leaves	Low (visual only)
Marginal and interveinal anthocyanin accumulation	Intermediate (young) sensitive (old)	Senescence, heat, cold	Low	Mature leaves	Low (visual assessment), high (computer assessment)
Tip and marginal necrosis	Very sensitive (young) sensitive (old)	Water deficit, heat	High—with other information	Young or mature leaves	Medium (visual assessment), high (computer assessment)

[a]Injury occurs after 7 days' exposure to: $<1.7\ \mu g\ m^{-3}$, very sensitive; $1.7–3.5\ \mu g\ m^{-3}$, sensitive; $3.5–7.0\ \mu g\ m^{-3}$, intermediate; $>7.0\ \mu g\ m^{-3}$, tolerant

relatively easily as the percentage of leaf area affected, but the estimation of irregular necrosis is more difficult and requires practice. Where they can be distinguished, tip and marginal necrosis in dicotyledonous species should usually be estimated independently.

Chlorosis

Foliar chlorosis is a common response of plants to pollutant exposure (Flagler 1998), but quantitative descriptions are uncommon, such as the assessment of ozone injury in white lupin leaves by an imaging chlorophyll fluorometer (Guidi et al. 2007). Pathological chlorosis has been assessed in a pasture legume by photography and image analysis (Tucker and Chakraborty 1997) and in wheat by chlorophyll meters and digital image analysis (Robert et al. 2005). Localised chlorophyll concentration and chlorophyll fluorescence parameters can indicate physiological stress, as in cadmium-affected oilseed rape leaves (Baryla et al. 2001). These methods provide detailed data for experimental studies or for species such as field crops with genetic and environmental uniformity, but instruments must be applied directly to the leaves, and the procedures are time-consuming and relatively expensive. The required effort renders detailed instrumental methods less suitable for large-scale field surveys that may encompass many wild species and varying site conditions.

Because the plants selected for quantitative indication of fluoride injury have mostly been monocotyledons such as gladiolus (Weinstein et al. 1990; Klumpp et al. 1996) or *Hemerocallis* (Klumpp et al. 1995), fluoride-induced chlorosis has been quantified less often, for example, in soybean (Bustamente et al. 1993) and tropical grasses in which the first response is chlorosis followed by tip necrosis (Oliva and de Figueiredo 2005). The scales of injury for chlorosis in these studies were coarse, indicating only slight and general chlorosis alone or in combination with necrosis. Four categories (0–20%, 21–40%, 41–60% and >60% of leaf area) were used by Moraes et al. (2002) to describe the development of chlorosis in the absence of necrosis in potted saplings of *Psidium guajava* and *Psidium cattleyanum* in Brazil after 16-week exposure periods.

In some species, including many from the Australian genera *Eucalyptus*, *Acacia* and numerous rainforest species, chlorosis may be a much more sensitive indicator of fluoride injury than necrosis (Doley 1986; Doley et al. 2004). As with necrosis, the patterns of distribution of chlorosis are closely related to the patterns of leaf venation and the distribution of fluoride within the tissues. One of the challenges with the estimation of chlorosis resulting from fluoride injury is that the distribution of chlorotic tissue may be much less regular than is common with necrosis. In addition, the degree of chlorosis varies with both the extent of injury and between species.

An injury scale for chlorosis was adopted with categories of chlorotic injury equal to those used for necrosis (Table 1). The fraction of the leaf area affected by chlorosis is assessed as the percentage of leaf area that would be occupied by completely chlorotic tissue if the leaf is divided into completely green and completely chlorotic tissues. The area of chlorotic tissue is estimated independently of that affected by necrosis. Three factors influence the estimation of chlorosis. First, the distribution of chlorosis may be relatively regular, at the leaf tip or along the leaf margins, or it may be distributed irregularly in the interveinal tissues; second, the intensity and demarcation of chlorosis may vary both with location in the leaf and between leaves; third, there are varying associations between the occurrence of chlorosis and photosynthetic responses or necrosis (Doley 1986; Baryla et al. 2001; Robert et al. 2005).

The precision of chlorosis assessment was investigated in a sample of 50 leaves from *Cinnamomum camphora* growing near a fluoride source and exhibiting a range of symptom severity. Each leaf was placed into an injury category as defined in Table 1, using a rapid visual estimate that was completed within 5 to 10 s. Subsequently, the area of each leaf affected by chlorosis was estimated by counting the elements of a systematic dot grid that lay over tissues judged to be chlorotic. The number of points over chlorotic tissues was then adjusted to take account of the degree of chlorosis at the different points, and this value was expressed as a percentage of the total area of the leaf. Depending on leaf size, each grid point represented between 0.3% and 1.0% of leaf area.

Figure 2 shows the percentage of chlorotic tissue in leaves allocated to injury categories by rapid assessment, the mean percentage chlorosis for the sampled leaves and the median injury percentage for the category for an operator with no training in the assessment of injury in this species. The difference between the sample mean and category median is an indication of the bias in the rapid estimation of injury for leaves allocated to each injury category. For injury categories 2 and 3, the differences between the means and category medians were small (1%), but they increased to 4% in category 4 and about 10% in category 5 (Fig. 2). That is, rapid estimation tends to overestimate the extent of chlorosis for leaves expressing injury to more than 25% of their area, but it is more accurate for injury between 2% and 10% of leaf area.

Individual observers showed consistent differences in the results of visual estimates of the extent of chlorosis (Fig. 3). In the test shown, an inexperienced observer (2) tended to overestimate the extent of chlorosis as compared with a more experienced observer (1), but the two observers maintained consistent differences in assessments over the range of injury tested.

When injury estimates were allocated to categories, the differences between operators were small over most of the range, and the errors in allocation were similar between an experienced and an inexperienced observer across the range of injury assessed (Fig. 4). Part of the difference

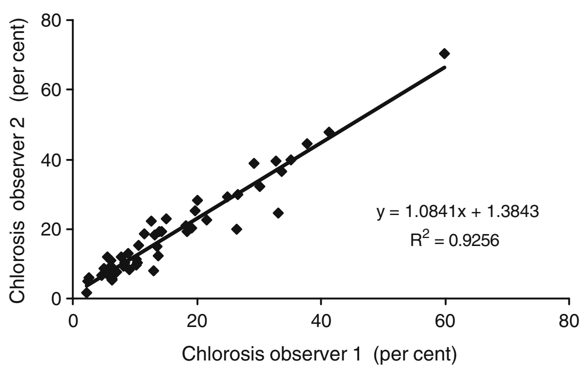


Fig. 3 Comparison of estimates of chlorosis in leaves of *C. camphora* made by an experienced observer (*observer 1*) and inexperienced observer (*observer 2*) using systematic dot grid counting

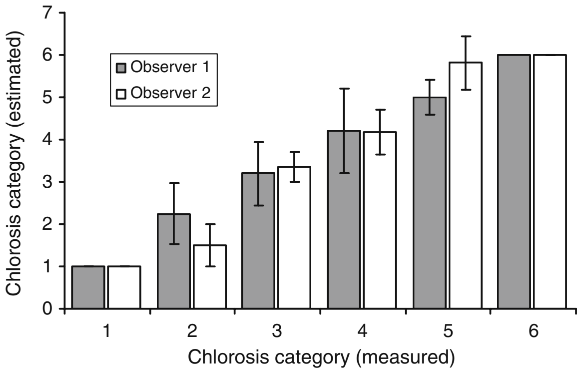


Fig. 4 Means and standard deviations of injury categories in *C. camphora* obtained by rapid visual estimates by an experienced observer (1) and an inexperienced observer (2) within injury categories measured by dot grind counting

in injury assessments may be associated with the differences in evaluation of chlorosis (Fig. 3).

Cupping or buckling

Deformation is one of the most sensitive expressions of fluoride injury in some species of *Eucalyptus* (including *Corymbia*) (Myrtaceae) and in some conditions may occur in the absence of either chlorosis or necrosis (Doley 1986). If cupping is due to fluoride, it may be expressed uniformly at the margins along most of the leaf length (e.g. in some species of *Eucalyptus*) or it may be concentrated at the tips of phyllodes in some *Acacia* species. Many other species, especially those with small, thick and flat leaves, may not exhibit cupping or buckling, while other species may have naturally cupped or undulated leaves.

The estimation of leaf deformation resulting from fluoride injury in susceptible species is not simple because the expression of the symptom is not always uniform over the length or width of the leaf, and it often varies substantially between adjacent leaves. As a result, judgments of deformation are more subjective than those for chlorosis, but the symptom is very characteristic and sensitive for some species, and its severity can be related to fluoride exposure during foliar expansion.

Expressions of fluoride injury in *Eucalyptus citriodora*, a species that is very sensitive to fluoride (Doley et al. 2004) are indicated in Table 2. If the pollutant concentration and exposure conditions are similar between successive growing seasons, there is usually an increase by about one injury category in the extent of necrosis, but not necessarily chlorosis, in one-year-old as compared with current season foliage. However, it is common for seasonal differences in the patterns of release of a pollutant and in plant growing conditions to influence the occurrence of injury.

Interpretation of injury symptoms

Field recording

In the application of the injury code in the field, the extent of a particular injury symptom is estimated for the leaves showing greatest injury on representative branches or plants, and this figure is applied to the species in question at that location. The fraction of leaves that is included in the assessment will vary, depending on the uniformity of distribution of symptoms throughout the cohort of leaves. For example, in *Pinus* species, fluoride injury appears typically as terminal necrosis of needles and its development tends to be uniform or sometimes progressive throughout a needle cohort because it depends on the accumulation of fluoride to a concentration that causes tissue death (Weinstein and Davison 2004). In many species of *Eucalyptus*, where the most sensitive fluoride injury symptoms are associated with the short period of leaf expansion and chlorophyll organization, adjacent leaves that are similar in age may show markedly different symptoms because expansion may be inhibited in a smaller leaf whereas chlorophyll synthesis may be most affected in a slightly larger and more mature leaf, and a fully mature leaf may show no symptoms at all (Doley 1986).

Selection of the maximum expression of injury avoids the difficulty of estimating the proportion of injured leaves in a plant canopy, and it assists in identifying pollutant exposure events. The extent of injury commonly varies even within leaves, and an average injury estimate is used. For example, marginal chlorosis and necrosis may be irregular in their occurrences throughout a leaf, and injury is assessed as an average percentage of the area of the most affected leaves. Depending on the species and exposure conditions, the leaves assessed may range from 10% to 100% of the cohort.

Expression of injury on a majority of branches or plants in a particular exposure situation is adopted because air pollutants would be expected to cause similar injury to all leaves of a similar age and exposure situation on one plant.

Where there are clear differences in the extent of injury to foliage at different positions on an annual shoot, the portions of seasonal growth may be recorded separately, together with the possible causes of injury. The actual ages of foliage at the time of an inspection will vary between species, depending on their major season of growth.

Injury due to pollutants

The injury ratings for necrosis, chlorosis, cupping, anthocyanin accumulation and insect or disease injury are applied independently in each determination, following a convention first used by Cobb (1892). An overall Injury Category is assigned to a species at a site on the basis of the highest injury category across all criteria. This is adopted in order to identify the extent of injury that could be attributed to all stresses, including non-pollutant stresses such as drought, storm winds, disease or insect attack.

An Emissions Injury Category is assigned for symptoms or for that portion of a symptom expression that is attributable to an air pollutant. The contribution of emissions to the total injury is estimated where there are considered to be clear differences in the amount of injury attributable to natural environmental stresses and those associated with the emission source. In addition, the combination and relative expression of different symptoms is of considerable assistance in diagnosing pollutant injury in different species. All these considerations may result in a moderation of the estimate of pollutant injury from that recorded in the field survey.

Factors affecting assessment

Foliage age

Foliage is classified as 0 (current season), 1 or 2 years of age if appropriate. Deciduous species have only a single leaf age class; in many evergreen species, previous season leaves are shed or may begin to deteriorate soon after the current season shoot has completed expansion but in some conifers and rainforest species, several cohorts of leaves may be retained. Where older foliage is judged to be senescent, assessment is usually restricted to current season foliage.

For evergreen species, including conifers, the injury code should be applied separately for foliage of different ages because of their differences in pollutant exposure and sometimes in injury expression. For example, current season leaves may be uninjured whilst one-year-old or older leaves may show injury or may show a different combination of symptoms from those in current season leaves. For species in which progressive fluoride accumulation leads to tip necrosis, there may be a progressive increase in injury with leaf age and one-year-old foliage often shows injury of one category higher than the current season foliage.

Position and orientation of foliage

Patterns of injury distribution at both large (hundreds of metres) and small scales (metres) should be consistent with the causal agent. For example, the large-scale pattern should show a reduction in the extent of injury that reflects the distance from the source of pollution, patterns of wind speed and the constancy of wind direction, particularly during the growing season. Small-scale patterns should also reflect the direction and speed of winds from the pollutant source, the density of foliage in the crown of the plant and the existence of obstacles to air movement. The directional pattern of pollutant injury distribution around a plant will be identical with that due to wind effects in the prevailing down-wind direction from the emission source, so it may be extremely difficult to separate pollutant and non-pollutant effects, such as salt spray or desiccation.

Therefore, a careful examination of the distribution of injury around a large plant, such as a tree, is essential, bearing in mind the effects of small-scale ground relief and the conformation of vegetation on the direction and speed of local winds. In these situations, relevant information on the location of foliage should be included. Where such information is not indicated, the injury records should relate to general estimates of condition for a complete plant crown or for a group of small plants.

Mimicking symptoms

The use of plants as biological indicators of pollution requires that the symptoms of pollutant injury can be distinguished from those of other environmental stresses. Several environmental conditions induce visible symptoms similar to those caused by pollutants, so the appearance of a particular category of injury does not necessarily mean that it is due to a pollutant (Flagler 1998; Weinstein and Davison 2004). In particular, the effects of drought and storm winds may be very similar to those of fluoride exposure, and chlorosis induced by fluoride may closely resemble symptoms of iron or magnesium deficiency.

Cupping or buckling of the leaf lamina may occur as a result of viral infections associated with leaf chewing and sap sucking insect attack, but viral deformation and chlorosis tends to be irregular in their distributions with respect to the pattern of venation and not confined to the leaf margins; Leaf-chewing insects that attack young expanding leaves may also interfere with normal expansion processes and these leaves may become cupped or buckled. In these situations, the deformation is usually associated with characteristic chewing injury and is not distributed regularly between leaves of a similar age.

Discussion

For many members of the public, the combined effects of all pollutants and all stresses may be more important than the effects of each constituent of a pollutant mixture, whereas for emitters, the effects of the pollutant for which they are responsible will be of principal concern. Regulators may be interested in biological monitoring as a supplement to other means of pollutant assessment. These three social groups are likely to have very different and possibly irreconcilable requirements of biological monitoring, so an acceptable technique or techniques must be developed for a clearly defined purpose.

The use of visible injury symptoms for indicating or monitoring pollutant stress may be criticised on the basis that changes in molecular, physiological and tissue functions may occur at exposure levels that are less than those resulting in visible injury, and that visible injury is regarded as a coarse and unscientific means of assessment (Hill et al. 1958; Horsfall and Cowling 1978). However, the benefits of rapid assessment have long been recognised (Croxall et al. 1952; Large 1966) and visible injury assessment has been accepted as a field survey technique for the estimation of the effects of regional ozone exposure on indicator plant species (e.g. Manning 2003; Smith et al. 2003). The extent of foliar necrosis or chlorosis has also been used for describing the distribution of pollutants in general (Moraes et al. 2002; Boone and Westwood 2006) or the more local effects of fluoride (Weinstein et al. 1990; Klumpp et al. 1994, 1995, 1996).

An extensive comparison of different combinations of assessors and tasks (Lorenzini et al. 2000) showed that the judgments of inexperienced assessors were acceptably accurate and precise at categories accounting for up to 20% of individual leaf areas. Allocation of leaves to correct categories for injury classes 5 and 6 (where injury affected 20–30% and 30–40% of leaf area respectively) was less certain. Very similar results were obtained in the present study when chlorosis was assessed in *C. camphora*.

It is relevant that Cobb (1892), Moore (1943), Horsfall and Barratt (1945) and the present scheme use a single injury class between 25 and 50% because of the difficulty of estimating percentage areas within that range. These calibration studies indicate that injury categories of different size are appropriate. Where aesthetic, commercial or ecological effects are the predominant concerns, there is little purpose in recording small differences in injury between 75 and 100% of the leaf area. For this reason, the present scheme is truncated as compared with that of Horsfall and Barratt (1945).

In many injury assessment procedures, one symptom is selected, for example, the leaf area affected by necrosis (Bussotti et al. 2003). In later work on the assessment of ozone injury in native vegetation in Europe, Bussotti et al. (2006) drew attention to the uncertainties associated with the variability in symptom manifestation between species. For the assessment of fluoride injury in Australian and New Zealand vegetation, it is necessary to use a range of symptoms because symptom expression varies so much between species (Doley 1986). In addition, the sequence of appearance of injury symptoms with exposure of leaves to fluoride varies between species and also between stages of leaf development within a species. Therefore, it was considered appropriate to construct a system of recording injury that could be applied independently for each symptom and which allowed the most severe expression of injury to be identified.

One difference between the present scheme for assessment of injury and those currently used is the confinement of attention to the leaves within an annual cohort that show the greatest injury. This recognition of a particular injury category may involve a variable proportion of the total leaf population, is indicated in Table 2. This procedure was adopted because it is easier for an observer to make a consistent estimate of the amount of injury in the most affected leaves than to estimate the percentage of total plant leaf area affected by a particular symptom. It is interesting that the early assessments of rust on wheat plants by Cobb (1892) were restricted to the flag leaf and the one below it in order to provide a more consistent sample.

Field application of this scheme of injury assessment permits the most sensitive expressions of different symptoms in different species to be combined in the construction of contours of injury distribution around a pollutant source. If these differing symptoms appear to the same extent at the same location, then they can each be associated with a particular ambient fluoride concentration. For example, the most sensitive expression of injury in one species (e.g. *Xanthorrhoea johnsonii*) may be tip necrosis, whilst in another species (e.g. *E. citriodora*) it may be marginal chlorosis and cupping of developing leaves (Doley 1986). Tip necrosis is likely to reflect the long term exposure as it is the result of redistribution of fluoride towards the leaf tip and the subsequent periodic death of tissues (Weinstein and Davison 2004). Therefore, tip necrosis may be most readily associated with long-term air quality guidelines, such as the 90-day average (ANZEC 1990). In *E. citriodora* marginal chlorosis and cupping reflect the ambient fluoride concentration over a period of a few days to two weeks during the expansion and greening of that leaf. As a result, the environmental sampling of developing *E. citriodora* leaves is more restricted and may reflect 7-day or even 1-day average fluoride concentrations. Once the leaves have matured, they are much less sensitive to fluoride injury and the symptoms expressed are tip and marginal necrosis associated with longer-term fluoride accumulation. Although *E. citriodora* may show tip and marginal necrosis at 30- and 90-day average fluoride concentrations lower than the air quality guidelines (Doley 1986), chlorosis and cupping may occur in the absence of tip necrosis, especially in current season leaves. An advantage of this method of recording symptoms is that fluoride exposure events can be identified, sometimes to within 2 or 3 weeks, even without the assistance of ambient fluoride measurements.

Assessment of the extent of injury must take account of leaf age as symptoms such as necrosis may increase in extent with age, whereas buckling and sometimes chlorosis may not vary once the leaf has reached maturity. In addition, a distinction must be made between chlorosis that is due to fluoride and that associated with senescence or nutrient deficiency. For many species, the detailed patterns of distribution of fluoride-induced chlorosis are characteristic in contrast to a more general distribution of chlorosis in senescing leaves.

Air quality guidelines for fluoride in Australia and New Zealand are expressed at four time intervals between 1 and 90 days (ANZEC 1990). It is convenient that a simple continuous relationship can be established between averaging time and guideline concentration (Doley 1986) so that intermediate exposure times and concentrations can be evaluated. As a result, the effect of fluoride exposure on plants can be expressed as a continuous response to these variables, even though the most effective symptom may vary with exposure time.

This means that different symptoms expressed in different species can be combined for identification of the extent of injury at a particular location.

Lorenzini et al. (2000) estimated that inexperienced observers could make an estimate of the extent of necrosis in individual leaves in about 11 s. In the present study, injury estimates on individual leaves of *C. camphora* could be completed in the laboratory within 5 to 10 s. Estimating injury in the most affected group of leaves in an annual cohort is slower in the field because of the need to select the sample and then make an estimate on several leaves. However, a determination for one species at a location can be completed within 1 min where there is relatively uniform symptom development within a plant. Where symptom expression varies with location around a plant crown or if the foliage is inaccessible, more time is required. Even where more than one estimate must be made for a species at a single location, visual estimation allows many more estimates to be collected and provides for variation in sensitivity within a species. Field experience shows that more than 300 injury estimates can be made at about 30 sites distributed over an area of approximately 2 km^2 in 1 day.

The collection of injury data from numerous sites provides valuable input for spatial databases (e.g. Bytnerowicz et al. 2002, 2007; Batzias and Siontorou 2006). When injury distribution patterns are plotted, contours can be drawn to enclose the extremities of occurrence of a particular grade of injury, and this outer limit represents the lower percentage of leaf area injured for that grade. For example, the outer limit of distribution of category 2 injury (2% to 5% affected) represents injury to 2% of the area of the selected leaves. Depending on the proportion of the leaf cohort that is injured, this may represent between about 1% and 5% of the total leaf area (Table 2). As a result, this expression of injury is conservative with respect to the likely effects of foliar injury on plant growth and reproduction.

Evaluation of the degree of offence taken by observers is more difficult than establishing the extent of injury. Professional observers should not register offence because their responsibility is to record the extent of injury. Casual observers can recognise severe injury, but they may require training in order to recognise the more subtle signs. However, once alerted to the occurrence of injury, former casual observers may become much more sensitive to offence, especially if they believe that the injury has not been made known to them at the appropriate time. It is considered to be useful that the extent of injury that attracts the attention of interested but untutored observers is category 4 or more than 10% of the area of affected leaves. This value equates approximately to the extent of injury that occurs when fluoride-sensitive plant species are exposed to environments that just meets the ANZEC (1990) air quality guidelines for fluoride.

The injury assessments described here can be combined with general forest health evaluations that assess foliage density, branch dieback, the vigour of vegetative growth and reproduction (e.g. Solberg and Strand 1999; Stolte 2001) to provide additional detail on the effects of air pollutants on ecosystems. While reproducible injury assessments can be made after a modest amount of practice, it is appropriate to echo the caution offered by Weinstein and Davison (2003) that it is often difficult to distinguish between pollutant and non-pollutant causes of injury to vegetation.

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References

ANZEC (1990). *Ambient air quality goals: Fluoride* (p. 9). Canberra: Australia and New Zealand Environment Council.

Arndt, U. (1982). Comparability and standardization of bioindication processes. In L. Steubing & H. J. Jager (Eds.), *Monitoring of air pollutants by plants. Methods and problems. Tasks for vegetation science* (Vol. 7, pp. 129–130). The Hague: Dr. W. Junk.

Arndt, U., Erhardt, W., Keitel, A., Michenfelder, K., Nobel, W., & Schluter, C. (1985). Standardisierte

Exposition von Pflanzlichen Reaktionsindikatoren. *Staub Reinhaltung der Luft, 45*, 481–483.

Arndt, U., Nobel, W., & Schweizer, B. (1987). *Bioindikatoren. Möglichkeiten, grenzen und neue erkenntnisse*. Stuttgart: Eugen Ulmer Verlag.

Ashmore, M. R. (2005). Assessing the future global impacts of ozone on vegetation. *Plant, Cell & Environment, 28*, 949–964. doi:10.1111/j.1365-3040.2005.01341.x.

Ashmore, M. R., Bell, J. N. B., & Reily, C. (1980). The distribution of phytotoxic ozone in the British Isles. *Environmental Pollution, B, 1*, 195–216.

Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C., & Havaux, M. (2001). Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: Causes and consequences for photosynthesis and growth. *Planta, 212*, 696–709. doi:10.1007/s004250000439.

Batzias, F. A., & Siontorou, C. G. (2006). A knowledge-based approach to environmental biomonitoring. *Environmental Monitoring and Assessment, 123*, 167–197. doi:10.1007/s10661-006-9190-0.

Boone, R., & Westwood, R. (2006). An assessment of tree health and trace element accumulation near a coal-fired generating station, Manitoba, Canada. *Environmental Monitoring and Assessment, 121*, 151–172. doi:10.1007/s10661-005-9112-6.

Bussotti, F., Schaub, M., Cozzi, A., Krauchi, N., Ferretti, M., Novak, K., & Skelly, J. M. (2003). Assessment of ozone visible symptoms in the field: Perspectives of quality control. *Environmental Pollution, 125*, 81–89. doi:10.1016/S0269-7491(03)00095-2.

Bussotti, F., Schaub, M., Cozzi, A., Gerosa, G., Novak, K., & Hug, C. (2006). Sources of error in assessing ozone visible injury symptoms on native vegetation. *Environmental Pollution, 140*, 257–268. doi:10.1016/j.envpol.2005.07.012.

Bustamente, M., Oliva, M. O., Sant'Anna, R., & Lopes, N. F. (1993). Sensibilidade da soja ao flúor. *Revista Brasileira de Fisiologia Vegetal, 5*, 151–157.

Bytnerowicz, A., Godzik, B., Fraczek, K., Grodzinska, K., Krywult, M., Badea, O., et al. (2002). Distribution of ozone and other air pollutants in forests of the Carpathian mountains in Central Europe. *Environmental Pollution, 116*, 3–25. doi:10.1016/S0269-7491(01)00187-7.

Bytnerowicz, A., Omasa, K., & Paoletti, E. (2007). Integrated effects of air pollution and climate change on forests: A northern hemisphere perspective. *Environment and Progress, 147*, 438–445.

Chappelka, A. H., Neufeld, H. S., Davison, A. W., Somers, G. L., & Renfro, J. R. (2003). Ozone injury on cutleaf coneflower (*Rudbeckia laciniata*) and crown-beard (*Verbesina occidentalis*) in Great Smoky Mountains National Park. *Environmental Pollution, 125*, 53–59. doi:10.1016/S0269-7491(03)00086-1.

Chappelka, A. H., Somers, G. L., & Renfro, J. R. (2007). Temporal patterns of foliar ozone symptoms on tall milkweed (*Asclepias exaltata L.*) in Great Smoky Mountains National Park. *Environmental Pollution, 149*, 358–365. doi:10.1016/j.envpol.2007.05.015.

Chen, C. W., & Goldstein, R. A. (1986). Techniques for assessing ecosystem impacts of air pollutants. In A. H. Legge & S. V. Krupa (Eds.), *Air pollutants and their effects on the terrestrial ecosystem* (pp. 603–630). Chichester: Wiley.

Cobb, N. A. (1892). Contribution to an economic knowledge of the Australian rusts (Uredineae). *Agricultural Gazette of New South Wales, 3*, 60–68.

Costanza, R., Norton, B. G., & Haskell, B. D. (Eds.) (1992). *Ecosystem health—new goals for environmental management*. Washington, DC: Island.

Croxall, H. E., Gwinne, D. C., & Jenkins, J. E. E. (1952). The rapid assessment of apple scab on leaves. *Plant Pathology, 1*, 39–41. doi:10.1111/j.1365-3059.1952.tb00022.x.

Davis, D. D., & Orendovici, T. (2006). Incidence of ozone symptoms on vegetation within a National Wildlife Refuge in New Jersey, USA. *Environmental Pollution, 143*, 555–564. doi:10.1016/j.envpol.2005.10.051.

Doley, D. (1986). *Plant-fluoride relationships*. Melbourne: Inkata.

Doley, D., Hill, R. J., & Riese, R. H. (2004). Environmental fluoride in Australasia: Ecological effects, regulation and management. *Clean Air and Environmental Quality, 38*(2), 35–55.

Feder, W. A., & Manning, W. J. (1978). Living plants as indicators and monitors. In W. W. Heck, S. V. Krupa, & S. N. Linzon (Eds.), *Handbook of methodology for the assessment of air pollution effects on vegetation*, TE-2, Informative Report No. 3, (pp. 9–1–9–14) Pittsburgh: Agricultural Committee, Air Pollution Control Association.

Flagler, R. B. (Ed.) (1998). *Recognition of air pollution injury to vegetation: A pictorial atlas*. Pittsburgh: Air and Waste Management Association.

Franzaring, J., Klumpp, A., & Fangmeier, A. (2007). Active biomonitoring of airborne fluoride near an HF producing factory using standardised grass cultures. *Atmospheric Environment, 41*, 4828–4840. doi:10.1016/j.atmosenv.2007.02.010.

Gostelow, P., Parsons, S. A., & Stuetz, R. M. (2001). Odour measurement for sewage treatment works. *Water Research, 35*, 579–597. doi:10.1016/S0043-1354(00)00313-4.

Guidi, L., Mori, S., Degl'Innocenti, E., & Pecchia, S. (2007). Effects of ozone exposure or fungal pathogen on white lupin leaves as determined by chlorophyll a fluorescence. *Plant Physiology and Biochemistry, 45*, 851–857. doi:10.1016/j.plaphy.2007.07.001.

Haddow, D., Musselman, R., Blett, T., & Fisher, R. (1998). *Guideline for evaluating air pollution impacts on wilderness within the Rocky Mountain Region: Report of a workshop, 1990* (p. 33). General Technical Report RMRS-GTR-4, Fort Collins: Rocky Mountains Research Station, Forest Service, United States Department of Agriculture.

Heck, W. W., Taylor, O. C., & Tingey, D. T. (Eds.) (1988). *Assessment of crop loss from air pollutants*. London: Elsevier Applied Science.

Hill, A. C., Tanstrum, L. G., Pack, M. R., & Winters, W. S. (1958). Air pollution with relation to agronomic crops:

VI. An investigation of the 'hidden injury' theory of fluoride damage to plants. *Agronomy Journal, 50*, 562–565.

Horsfall, J. G., & Barratt, R. W. (1945). An improved grading system for measuring plant diseases. *Phytopathology, 35*, 655.

Horsfall, J. G., & Cowling, E. B. (1978). Pathometry: The measurement of plant disease. In J. G. Horsfall & E. B. Cowling (Eds.), *Plant disease: An advanced treatise* (Vol. II, pp. 119–136). New York: Academic.

Huggett, R. J., Kimerle, R. A., Mehrle, P. M., & Bergman, H. L. (Eds.) (1992a). *Biomarkers: Biochemical, physiological, and histological markers of anthropogenetic stress*. Chelsea, Michigan: Lewis.

Huggett, R. J., Kimerle, R. A., Mehrle, P. M., Bergman, H. L., Dickson, K. L., Fava, J. A., et al. (1992b). Introduction. In R. J. Huggett, R. A. Kimerle, P. M. Mehrle, & H. L. Bergman (Eds.), *Biomarkers: Biochemical, physiological, and histological markers of anthropogenetic stress* (pp. 1–3). Chelsea, Michigan: Lewis.

Innes, J. L., Skelly, J. M., & Schaub, M. (2001). *Ozone and broadleaved species: A guide to the identification of ozone-induced foliar injury*. Berne: P. Haupt.

Jacobson, J. S., & Hill, A. C. (Eds.) (1970). *Recognition of air pollution injury to vegetation: A pictorial atlas*. Pittsburgh: Air Pollution Control Association.

Karlsson, G. P., Selldén, G., Skärby, L., & Pleijel, H. (1995). Clover as an indicator plant for phytotoxic ozone concentrations: Visible injury in relation to species, leaf age and exposure dynamics. *The New Phytologist, 129*, 355–365. doi:10.1111/j.1469-8137.1995.tb04306.x.

Klumpp, A., Klumpp, M., & Domingos, M. (1994). Plants as bioindicators of air pollution at the Serra do Mar near the industrial complex of Cubatão, Brazil. *Environmental Pollution, 85*, 109–116. doi:10.1016/0269-7491(94)90244-5.

Klumpp, A., Domingos, M., & Klumpp, G. (1996). Assessment of vegetation risk by fluoride emissions from fertiliser industries at Cubatão, Brazil. *The Science of the Total Environment, 192*, 219–228. doi:10.1016/S0048-9697(96)05298-9.

Klumpp, A., Klumpp, M., Domingos, M., & Guderian, R. (1995). Hemerocallis as bioindicators of fluoride pollution in tropical countries. *Environmental Monitoring and Assessment, 35*, 27–42.

Kratz, T. K., Magnuson, J. J., Bayley, P., Benson, B. J., Berish, C. W., Bledsoe, C. S., et al. (1995). Temporal and spatial variability as neglected ecosystem properties: Lessons learned from 12 North American ecosystems. In D. J. Rapport, C. L. Gaudet, & P. Calow (Eds.), *Evaluating and monitoring the health of large-scale ecosystems* (pp. 359–383). Berlin: Springer.

Lacasse, N. L., & Treshow, M. (Eds.) (1976). *Diagnosing vegetation injury caused by air pollution*. Research Triangle Park, North Carolina: Air Pollution Training Institute, U.S. Environmental Protection Agency.

Large, E. C. (1966). Measuring plant disease. *Annual Review of Phytopathology, 4*, 9–26. doi:10.1146/annurev.py.04.090166.000301.

Lindberg, S. E., & McLaughlin, S. B. (1986). Air pollutant interactions with vegetation: Research needs in data acquisition and interpretation. In A. H. Legge & S. V. Krupa (Eds.), *Air pollutants and their effects on the terrestrial ecosystem* (pp. 449–503). Chichester: Wiley.

Lorenzini, G., Nali, C., Dota, M. R., & Mafrtorana, F. (2000). Visual assessment of foliar injury induced by ozone on indicator tobacco plants: A data quality evaluation. *Environmental Monitoring and Assessment, 62*, 175–191. doi:10.1023/A:1006262603497.

Malhotra, S. S., & Blauel, R. A. (1980). Diagnosis of air-pollutant and natural stress symptoms on forest vegetation in western Canada. Information Report NOR-X-228. Edmonton, Alberta: Northern Forest Research Center, Canadian Forestry Service.

Manning, W. J. (2003). Detecting plant effects is necessary to give biological significance to ambient ozone monitoring data and predictive ozone standards. *Environmental Pollution, 126*, 375–379. doi:10.1016/S0269-7491(03)00240-9.

Manning, W. J., & Feder, W. A. (1980). *Biomonitoring air pollutants with plants*. London: Applied Science.

Mayer, F. L., Versteeg, D. J., McKee, M. J., Folmer, L. C., Graney, R. L., McCune, D. C., et al. (1992). Physiological and nonspecific biomarkers. In R. J. Huggett, R. A. Kimerle, P. M. Mehrle, & H. L. Bergman (Eds.), *Biomarkers: Biochemical, physiological, and histological markers of anthropogenetic stress* (pp. 5–85). Chelsea, Michigan: Lewis.

Michener, W. K. (1997). Quantitatively evaluating restoration experiments: Research design, statistical analysis and data management considerations. *Restoration Ecology, 5*, 324–337. doi:10.1046/j.1526-100X.1997.00546.x.

Miller, P. R., Stolte, K. W., Duriscoe, D. M., & Pronos, J. (1996). *Evaluating ozone air pollution effects on pines in the western United States*. Riverside, California: Pacific Southwest Research Station, USDA Forest Service.

Moore, W. C. (1943). The measurement of plant diseases in the field. *Transactions of the British Mycological Society, 26*, 28–35.

Moraes, R. M., Klumpp, A., Furlan, C. M., Klumpp, G., Domingos, M., Rinaldi, M. C. S., et al. (2002). Tropical fruit trees as bioindicators of industrial air pollution in southeast Brazil. *Environment International, 28*, 367–374. doi:10.1016/S0160-4120(02)00060-0.

Novak, K., Skelly, J. M., Schaub, M., Krauchi, N., Hug, C., Landolt, W., et al. (2003). Ozone air pollution and foliar injury development on native plants of Switzerland. *Environmental Pollution, 125*, 41–52. doi:10.1016/S0269-7491(03)00085-X.

Oliva, M. A., & de Figueiredo, J. G. (2005). Gramíneas bioindicadoras da presença de flúor em regiões tropicais. *Revista Brasileira de Botanica, 28*, 389–397.

Osmond, C. B. (1988). Ecology of photosynthesis in sun and shade: Summary and prognostications. *Australian Journal of Plant Physiology, 15*, 1–9.

Paoletti, E., & Manning, W. J. (2007). Toward a biologically significant and usable standard for ozone that will also protect plants. *Environmental Pollution, 150*, 85–95. doi:10.1016/j.envpol.200z7.06.037.

Queensland (1997). *Environmental protection (air) policy*. Brisbane: Queensland Government Parliamentary Counsel.

Rapport, D. J., Gaudet, C. L., & Calow, P. (Eds.) (1995). *Evaluating and monitoring the health of large-scale Ecosystems. NATO Series I: Global Environmental Change* (Vol. 28). Berlin: Springer.

Robert, C., Bancal, M.-O., Ney, B., & Lannou, C. (2005). Wheat leaf photosynthesis loss due to leaf rust, with respect to lesion development and leaf nitrogen status. *The New Phytologist, 165*, 227–241. doi:10.1111/j.1469-8137.2004.01237.x.

Schaub, M., Skelly, J. M., Zhang, J. W., Ferdinand, J. A., Savage, J. E., Stevenson, R. E., et al. (2005). Physiological and foliar symptom response in the crowns of Prunus serotina, Fraxinus americana and Acer rubrum canopy trees to ambient ozone in the field. *Environmental Pollution, 133*, 553–567. doi:10.1016/j.envpol.2004.06.012.

Schoettle, A., & Moir, W. (1998). Terrestrial ecosystems. In *Guideline for evaluating air pollution impacts on wilderness within the Rocky Mountain Region: Report of a workshop, 1990* (pp. 19–26). General Technical Report RMRS-GTR-4, Fort Collins: Rocky Mountains Research Station, Forest Service, United States Department of Agriculture.

Smith, E. P. (1994). Biological monitoring: Statistical issues and models. In G. P. Patil & C. R. Rao (Eds.), *Handbook of statistics* (Vol. 12, pp. 243–261). Amsterdam: North Holland.

Smith, G., Coulston, J., Jepsen, E., & Prichard, T. (2003). A national ozone biomonitoring program—results from field surveys of ozone sensitive plants in Northeastern forests (1994–2000). *Environmental Monitoring and Assessment, 87*, 271–291. doi:10.1023/A:1024879527764.

Solberg, S., & Strand, L. (1999). Crown density assessments, control surveys and reproducibility. *Environmental Monitoring and Assessment, 56*, 75–86. doi:10.1023/A:1005980326079.

Steubing, L. (1982). Problems of bioindication and the necessity for standardization. In L. Steubing & H. J. Jager (Eds.), Monitoring of air pollutants by plant. methods and problems. *Tasks for vegetation science 7* (pp. 19–24). The Hague: Dr. W. Junk.

Stolte, K. W. (2001). Forest health monitoring and forest inventory analysis programs monitor climate change effects in forest ecosystems. *Human and Ecological Risk Assessment, 7*, 1297–1316. doi:10.1080/20018091095014.

Tingey, D. T., Hogsett, W. E., & Henderson, S. (1990). Definition of adverse effects for the purpose of establishing secondary national ambient air quality standards. *Journal of Environmental Quality, 19*, 635–639.

Tomkiewicz, J., Skovgård, H., Nachman, G., & Münster-Swendsen, M. (1993). A rapid and non-destructive method to assess leaf injury caused by the cassava mite, Mononychellus tanajoa (Bondar) (Acarina: Tetranuchidae). *Experimental & Applied Acarology, 17*, 29–40.

Tucker, C. C., & Chakraborty, S. (1997). Quantitative assessment of lesion characteristics and disease severity using digital image processing. *Journal of Phytopathology, 145*, 273–278. doi:10.1111/j.1439-0434.1997.tb00400.x.

Underwood, A. J. (1994). Beyond BACI: Sampling designs that might reliably detect environmental disturbance. *Ecological Applications, 4*, 3–15. doi:10.2307/1942110.

United States Congress (1980). *Clean Air Act Amendments of 1977*. House Committee on Interstate and Foreign Commerce. 96th Congress: No. 96–110. (pp. 856). Washington: United States Government Printing Office.

Vollenweider, P., Ottiger, M., & Gunthard-Goerg, M. S. (2003). Validation of leaf ozone symptoms in natural vegetation using microscopical methods. *Environmental Pollution, 124*, 101–118. doi:10.1016/S0269-7491(02)00412-8.

Weinstein, L. H., & Davison, A. W. (2003). Native plant species suitable as bioindicators and biomonitors for airborne fluoride. *Environmental Pollution, 125*, 3–11. doi:10.1016/S0269-7491(03)00090-3.

Weinstein, L. H., & Davison, A. (2004). *Fluorides in the environment*. Wallingford: CABI.

Weinstein, L. H., Laurence, J. A., Mandl, R. H., & Walti, K. (1990). Use of native and cultivated plants as bioindicators and biomonitors of pollution damage. In W. Wang, J. W. Gorsuch, & W. R. Lower (Eds.), *Plants for toxicity assessment ASTM STP1091* (pp. 117–126). Philadelphia: American Society for Testing and Materials.

Zonneveld, I. S. (1982). Principles of indication of environment through vegetation. In L. Steubing & H. J. Jager (Eds.), *Monitoring of air pollutants by plants. Methods and problems, tasks for vegetation science 7* (pp. 3–17). The Hague: Dr. W. Junk.