Monitoring Ground-Level Ozone and Nitrogen Dioxide in Parts of KwaZulu-Natal and Mpumalanga (South Africa) by Means of Chemical and Biological Techniques

Sharon Ann Blair

Submitted in fulfilment of the academic requirements for the degree of masters in the Department of Biology, University of Natal, Durban.

15 December 1998

PREFACE

The experimental work described in this dissertation was carried out in the Department of Biology, University of Natal, Durban, from January 1996 to December 1998, under the supervision of Professor John A. Cooke.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

8 - 4 - 99

ACKNOWLEDGEMENTS

I would like thank my supervisor, Professor J.A. Cooke for the support, guidance and advice he provided throughout this study. I would also like to thank the Foundation for Research and Development for the financial support they gave to myself and this project.

I am very grateful to Tim McLurg , the Botanic Gardens and Treverton School for allowing me to leave plants and sampling equipment with them, and for watering these plants and taking care of the equipment. Thanks also go to Frank Sokolic of the Geography Department for supplying me with Dasibi data promptly whenever I requested it. I also thank Roland David at the CSIR for his assistance with the chemical analysis of some of the samples collected.

I thank my family for all the love and support they gave me during my years of study, and I thank my friends, especially those in the lab, for their encouragement, assistance, advice and great sense of humour. I also thank Jason for his love and support during the last months of the writing up of my thesis.

ABSTRACT

Surface ozone (O_3) is one of the most toxic and abundant air pollutants. It has deleterious effects on human and animal respiration processes, and adversely affects plants. Four sites were selected for monitoring ambient O_3 in the Durban metropolitan area: Botanic Gardens, University of Natal (UND), top of Kloof Gorge, and Mooi River. At each site tobacco Bel-W3 bioindicators, and NO_2 and O_3 passive diffusion tubes were placed. An O_3 analyser (Dasibi 1108) was situated at the UND site. Monitoring was carried out for four weeks during summer, autumn and winter at each site, and during spring at the UND site. Two weeks of data from the diffusion tubes were collected during spring, from the Nelspruit area, Mpumalanga. Ozone concentrations measured with the Dasibi at the UND site were low in comparison to other urban-industrial areas in the world, with hourly values falling between 5ppb and 10 ppb. The highest hourly mean maximum recorded was 40ppb. A general spring/winter maximum and summer minimum was observed. This is typical of subtropical locations, where subsidence in prevailing anticyclonic circulation occurs. Diurnal characteristics included early morning minima and maxima at 12h00 in spring and summer, and maxima approximately two hours later in autumn and winter. This pattern was typical of that found in polluted environments, the magnitude, however, being lower. An unusual secondary nocturnal peak occurred during autumn, winter and spring. This could have been due to the long-range transport of relatively O_3-rich air from a non-urban, high altitude inland area. Ozone concentrations were not strongly influenced by meteorological variables.

Diffusion tube data indicated low O_3, however, the coefficients of variation were high, implying a lack of precision in this technique. This technique would have to be improved before data obtained could be regarded as valid. Nitrogen dioxide, NO_2, one of the precursors

to O_3, was monitored using diffusion tubes at the same sites. Concentrations were highest closer to the city centre, the highest concentration being 31ppb in autumn. In the Mpumalanga study, NO_2 concentrations were higher in the city of Nelspruit than the surrounding areas. No significant differences were found in the O_3 concentrations between the Mpumalanga sites.

The tobacco plants showed the highest visible leaf injury in winter, corresponding with the higher Dasibi values, but there were no significant differences between the sites, and no significant differences in chlorophyll contents between the sites. In this study, O_3-induced injury occurred below the previously established threshold of 40ppb.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Sources and atmospheric chemistry of ozone and nitrogen dioxide

Ozone (O_3) is a highly reactive gas composed of three atoms of oxygen (McKee, 1994a). In the earth's atmosphere, O_3 plays a dual role in its effects on the environment. In the stratosphere, it performs a critical task of absorbing some of the ultraviolet radiation of the sun (Heagle, 1989). Paradoxically, O_3 in the troposphere is a component of photochemical air pollution and causes more damage to vegetation than all other air pollutants combined (Heagle, 1989). Ozone, which is a major component of photochemical air pollution (smog), is one of the most toxic and abundant air contaminants. Not only can it have deleterious effects on plants, but it can also adversely affect human and animal respiration processes, and manufactured products such as rubber, cotton, paints, plastics, nylon and fabric dyes (Lorenzini, 1994; Wellburn, 1994). Essentially anything that is susceptible to oxidation can be affected adversely by O_3 (McKee, 1994a). Any double bond in a hydrocarbon is highly sensitive to chain-breaking and cross-linking reactions initiated by O_3 (Wellburn, 1994). Atmospheric visibility can also be affected (Lorenzini, 1994).

It has been estimated that about half the O_3 at the surface of the earth is due to downwind transport of the stratospheric O_3 (Annegarn *et al.*, 1996b). Within the troposphere, because O_3 is not emitted directly into the air, it is described as a secondary or transformation pollutant rather than a primary pollutant (McKee, 1994a). The precursor pollutants of O_3 are nitrogen oxides, which include nitrogen dioxide (NO_2) and nitric oxide (NO) (this mixture will be subsequently referred to as NO_X), volatile hydrocarbons (VOCs), and atmospheric oxygen (O_2)

in the presence of sunlight (McKee, 1994a). The principal contributions to the precursor pollutants, NO_X and VOCs, come from motor vehicles (UK Department of Health, 1993), both these being produced typically at higher rates in urban areas (Lorenzini, 1994).

The presence of NO_X, VOCs and O_2 alone, however, are not sufficient to produce elevated levels of O_3. High ambient O_3 levels generally occur when ambient air temperatures exceed 25^0C (McKee, 1994a). An exception to this, however was found in cities at high elevations such as Mexico City (3047m) where very high levels of O_3 ($\geq$ 300 ppb) have been observed even when air temperatures were lower (about 20^0C) (McKee, 1994a). This is most probably due to high solar radiation which pushes the chemical equilibrium toward higher levels of O_3 production (McKee, 1994a). Therefore, on hot, sunny, windless days the production of O_3 is promoted more readily than the destruction of O_3, often resulting in elevated O_3 levels, whereas the opposite occurs on cold cloudy days with high wind speeds (Bronnimann & Neu, 1997), and so O_3 concentrations are generally higher in summer and lower in winter (McKee, 1994a). Also, O_3 concentrations are higher during the day than at night.

The chemical reactions involved in the formation and destruction of O_3 are as follows (McKee, 1994a): a) Firstly the photodissociation of NO_2 by near ultraviolet solar radiation, where $h\nu$ is a photon of energy, h being Planck's constant and ν being the frequency of the radiation (Preston-Whyte & Tyson, 1988);

$$NO_2 + h\nu\ (295 \leq \lambda < 430nm) \rightarrow NO + O \qquad (I)$$

b) Secondly, there is the reaction of O and O_2 in the presence of M, where M is a molecule (e.g. nitrogen) which removes excess energy of the reaction;

$$O + O_2 + M \rightarrow O_3 + M \qquad \text{(II)}$$

c) Thirdly, if no competing reactions are occurring, NO reacts rapidly with O_3

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad \text{(III)}$$

The equilibrium which results between the above chemical reactions (I, II and III) can be expressed by the following:

$$[O_3] = K[NO_2]/[NO] \qquad \text{(IV)}$$

where K is the equilibrium constant, dependent on sunlight intensity (McKee, 1994a). From this equation it can be seen that O_3 is directly proportional to the sunlight intensity, as well as the $[NO_2]/[NO]$ ratio (Niki *et al.*, 1972).

Ozone, being an unstable compound, is rapidly destroyed by contact with ground surfaces, which can result in highly variable concentrations near the ground (Annegarn *et al.*, 1996b). This type of 'scavenging' on surfaces that are in contact with ambient O_3 usually occurs within a few metres of the ground and involves organic and non-organic surfaces, for example vegetation, soil, and man-made polymers (McKee, 1994a).

Ozone formation tends to be downwind of pollution centres. It is usual for the highest ozone levels to be in peri-urban or rural locations, rather than in the urban and industrial centres (AEA

Technology, 1996). The explanation for this is that, although urban NO_2 levels are generally higher, in such environments there is a dynamic trend for O_3 concentration to be continually lowered by reaction (III). That is because of the continuous injection of NO from petrol powered vehicles and stationary combustion sources.

The opposite conditions can be found in the rural environment where O_3 levels are often quite high and NO_2 concentrations are low (Ljunstrom & Hallquist, 1996). It has also been observed that average O_3 concentrations are higher on weekends than on weekdays for mean values and for maxima. The lower NO concentrations on weekends are considered to be responsible for this effect, since O_3 concentrations depend on the NO/NO_2 ratio. Decreased NO leads to increasing O_3 concentration as shown in equations I, II and III (Bronniman & Neu, 1997).

The term nitrogen oxides includes NO_2 and NO, as discussed above also includes N_2O, N_2O_3 and N_2O_5. NO_2 and NO are not the predominant nitrogen oxides of the atmosphere but they are the ones which appear to give the most pollution problems in the troposphere (Wellburn, 1994). In the last decade, there has been an increasing interest in the measurement of NO_2 concentrations in the atmosphere because this molecule is strongly involved in the formation-destruction processes of O_3. Nitrogen dioxide is directly involved in the O_3 production at ground level and in the O_3 depletion at the higher altitudes (Merienne *et al.*, 1995). Nitrogen dioxide is produced by stationary heating or power generation sources and from motor vehicle emission. In combustion processes, nitrogen oxides are formed through the oxidation of atmospheric nitrogen (UK Department of Health, 1993). The main product is nitric oxide (NO) constituting 90 to 95% of NO_X emissions, and a small proportion of NO_2. Nitric oxide is,

however, rapidly oxidised by O_3 to form NO_2 (refer to reaction III page 3). In fact, NO is regarded as one of the most potent O_3 'scavengers' (McKee, 1994a). To control O_3 pollution, strategies need to be developed to reduce these precursor emissions (Badiani *et al.*, 1996).

There can be episodes of relatively high hourly NO_2 concentrations occurring at any time of the year (UK Department of Health, 1993). There are two mechanisms responsible for what can be termed 'summer' and 'winter' NO_2 episodes. During summer periods of increased photochemical activity, the mechanisms responsible for elevated O_3 levels can also lead to elevated levels of NO_2. This can occur as a result of the oxidation of NO to NO_2 by the organic peroxy radicals produced in the so-called photochemical smog reactions (Bronnimann & Neu, 1997), and also via the direct reaction of NO with elevated levels of O_3 (UK Department of Health, 1993). These episodes are characterised by high NO_2/NO_X ratios, since much of the NO in the total NO_X is converted to NO_2 (UK Department of Health, 1993).

Winter episodes of elevated NO_2 concentration can occur during cold stagnant anticyclones when dispersion is poor (UK Department of Health, 1993). The exact method of formation of NO_2 in this situation is not completely clear. The reaction between NO and oxygen (O_2) probably makes a large contribution. At normal ambient levels of NO this reaction is very slow and makes no contribution to NO_2 formation. However, in the high concentrations found during winter stagnation episodes, the opposite may occur. The rate of the reaction between NO and O_2 is also faster at lower temperatures (UK Department of Health, 1993).

1.2 The effects of ozone on vegetation

The effects of O_3 on vegetation were first noticed in the Los Angeles area in 1944 when new types of foliar injury developed on the vegetation (Krupa & Manning, 1988). Weather fleck of cigar-wrapper tobacco was reported in Connecticut as a disease of unknown cause in 1952 (Rich *et al.*, 1969). In 1958, elevated O_3 was reported to cause damage to grapes in southern California (Richards *et al.*, 1958). Since these early studies injury to vegetation induced by exposure to air pollutants has become an important factor in evaluating the impact of mans' activities on the environment (Jacobson & Hill, 1970).

Injury to vegetation occurs when O_3 enters the plant via open stomata during the normal process of gas exchange between a leaf and the atmosphere (Krupa & Manning, 1988). Once inside the leaf, O_3, or some intermediate, for example an OH radical, can oxidise cell membranes which can lead to changes in membrane permeability's (Krupa & Manning, 1988). As a result of these reactions with cell membrane lipids and proteins, chain reactions are initiated, giving rise to more free radicals (Wolfenden *et al.*, 1992). Cellular disturbances that are not repaired or compensated for, by the plant detoxifying or metabolising the O_3, are expressed as leaf injury, which is observed as visible symptoms on leaf surfaces, reduced plant growth, decreased yield and changes in quality of crop plants, alterations in susceptibility to abiotic and biotic stress, and decreased reproduction (Tingey *et al.*, 1994). Protective mechanisms within plants include carotenoids (e.g. β-carotene), phenolic compounds such as vitamin E (α-tocopherol), peptides (e.g. glutathione), enzyme systems (e.g. superoxide dismutase), polyamines, and organic buffering systems (Wolfenden *et al.*, 1992). These form part of the internal resistance of the plant to atmospheric pollutants. Rates of deposition to

leaves will be affected by the various resistances to O_3 uptake by a leaf, for example boundary layer resistance, epidermal resistance, cuticular resistance, and stomatal resistance. Thus, environmental parameters should always be considered when assessing leaf injury by O_3. Any conditions leading to full stomatal opening predisposes plants to greater uptake and subsequent injury (Treshow *et al.*, 1989). For example, when relative humidity is high, stomata are more open and the O_3 uptake is increased. Increasing relative humidity from 35% to 73% can increase O_3 uptake four times (MacLauglin *et al.*, 1981). However, if the relative humidity exceeds 90%, sensitivity to O_3 may decrease (Dunning & Heck, 1977). Previous studies have also indicated that conditions favourable to high plant growth rates, such as warm temperatures (Heck, *et al.*, 1965), sunlight, high relative humidity, good nutrition and adequate soil moisture, also tend to be most conducive to pollutant injury (Krupa & Manning, 1988). Krupa *et al.* (1993) found that Bel-W3 tobacco sensitivity to O_3 increased as temperature increased. As metabolic rate increases with temperature, so does sensitivity to O_3, therefore, plants are most sensitive during conditions that are optimal for growth (Treshow & Anderson., 1989). Also, it must be taken into account that since environmental conditions may change during exposure, the actual cellular exposure and resultant visible injury does not necessarily reflect the ambient dose to which the plant has been exposed (Treshow & Anderson, 1989).

Ozone can also influence plant reproduction by: reducing pollen production and germination through influences on the flowers; reducing seed germination and restricting seedling growth; reducing flower or cone initiation (Cox, 1992). It is the process of pollination which may be the most affected by air pollutants, since pollination requires transport of the dehydrated pollen grains to the stigmatic surface which must be well exposed to wind or insects, for the success

of the pollination process, and therefore, will be exposed to air pollution (Cox, 1992). During transport, the pollen may be exposed to O_3 which can affect membrane integrity, which will then affect its ability to withstand water stresses (Cox, 1992).

There is evidence suggesting that previous exposures to oxidants (O_3 predominantly, as well as NO, NO_2, PAN, and SO_2) predisposes tobacco plants to greater foliar injury during subsequent exposures (Heagle & Heck, 1974). In relation to this it is important to note that plants are likely to be exposed to some very low concentration of O_3 most of the time, except during periods of rain (Krupa *et al.*, 1993). It has also been shown under experimental conditions that the combination of SO_2 and O_3 has a greater effect on biomass yield of plants, for example *Pinus halepensis*, than either gas alone, suggesting a synergistic interaction between these gaseous pollutants (Diaz *et al.*, 1996). Synergism occurs when two, or more, gases increase each other's action, whereas antagonism suggests that there is competition between the gases for an active site (Ormrod, 1982). If the effects are not equal to the additive effects of the single gases, this indicates that the plant response to one gas is dependent on the presence, and possibly the amount, of the second gas (Ormrod, 1982). Strong synergistic responses are of concern in agricultural and horticultural plant production, because very substantial crop losses can occur when gases are present in concentrations that, on their own, would not cause as much damage (Ormrod, 1982). In spruce and fir trees, the production of α-tocopherol and glutathione showed a more than additive response to a mixture of SO_2 and O_3 (Melhorn *et al.*, 1986). It has been observed in studies performed on white bean leaves, that O_3 by itself caused early development of bronzing, while a mixture of O_3 and SO_2 caused yellow interveinal chlorosis on corresponding leaves, after several days additional exposure (Ormrod, 1982).

Soybean has been shown to develop the characteristic symptoms of O_3 injury in an SO_2 and O_3 mixture, but required a longer exposure than that needed for the development of corresponding injuries with O_3 alone (Ormrod, 1982). This also indicates the importance of the effect of duration on exposure. While the mechanisms of action of SO_2 and O_3 separately are well understood, those of the two pollutants combined are unclear (Diaz *et al.*, 1996). A combination of SO_2, NO_2 and O_3 has been shown to have a synergistic effect resulting in the decrease in pollen tube growth (Wellburn, 1988).

Ozone concentrations in most agricultural areas of the United States have been estimated to be about two to three times higher than they would have been without human influence (Heagle, 1989), and it would appear that ozone causes more damage to vegetation than any other air pollutant in the United States (Hill *et al.*, 1970). Plants are not selective in gas uptake so agriculture is confronted with a class of 'diseases' caused by air pollutants that are very difficult to recognise and can affect the productivity of most crops. Concentrations high enough to injure sensitive species have been observed more than 70 miles from large metropolitan areas such as Philadelphia, New York and Los Angeles (Hill *et al.*, 1970). Injury to leaves, where they themselves are the marketable products (e.g. tobacco and spinach), can result in reduced yield or quality or loss of the crop (Hill *et al.*, 1970). It has been estimated that there could be a benefit of $2 800 million from a 40% reduction in O_3 levels (Bell, 1992). This is equivalent to 2.8% of the United States total agricultural revenue.

The impact of O_3 on plants in the USA and the fear of considerable loss of yields led to the extensive National Crop Loss Assessment Network (NCLAN) project (Heck *et al.*, 1987) and

the Unites States National Acid Precipitation Assessment Program (NAPAP) (Tanner, 1990) to assess the potential economic impacts of O_3 as well as other air pollutants. A similar project was established in Europe. This was the European Open Top Chamber Programme (Bonte & Mathy, 1989).

The NCLAN project was initiated and sponsored by the U.S. Environmental Protection Agency (EPA) to evaluate the effects of O_3 on the productivity of major regional crops under field conditions (Lefohn, 1997). The primary objectives of NCLAN were to: define the exposure-response relationships for yields of crops and O_3 exposure; assess the national economic consequence resulting from the exposure of crops to O_3; and advance understanding of the cause and effect relationships that determine crop responses to exposure (Tingey *et al.*, 1994). The initial O_3 standard in the USA had been set to prevent adverse effects on human health, this being a maximum hourly average of 120 ppb (Lefohn, 1997). This standard was not necessarily appropriate for protecting vegetation from O_3 exposures (Lefohn & Foley, 1992). Lefohn & Foley (1992) have summarised some of the findings that were then applied to the standard-setting process. The NCLAN experimental data showed that the repeated occurrences of hourly average of O_3 concentrations of 100 ppb and higher resulted in adverse effects on vegetation. Further analysis of the data carried out by Lefohn & Foley (1992), showed that the characterised distribution reflected the importance of the higher hourly mean concentrations in affecting crop yield reductions. It was evident that the initial standard in the USA did not adequately protect vegetation from O_3 exposure (Lefohn & Foley, 1992). On July 16, 1997, the United States EPA Administrator passed regulations that resulted in a new standard (Lefohn, 1997). The EPA will be replacing the one-hour initial standard mentioned above (i.e. a

maximum hourly average of 120 ppb) with a new 8-hour standard, which will be based on measurements over three-year periods (Lefohn, 1997). The fourth highest 8-hour average daily maximum O_3 concentration will be calculated for each year and averaged across an annually-rolling three-year period, then rounded to the nearest 10 ppb. If this value exceeds 80 ppb, then it is in violation of the new standard (Lefohn, 1997). This standard will, thus, take into account and protect against longer exposure periods.

The NAPAP studies concluded that air pollutants could be ranked in the following order of importance, in terms of their potential to negatively impact the growth, yield and/or quality of agricultural crops (Shriner *et al.*, 1990 *loc cit* Scholes *et al.*, 1996).

$$O_3 > SO_2 > \text{acidic deposition} > NO_2$$

Ambient O_3 concentrations of 60 ppb were shown to cause yield reductions of up to 56%, depending on the species, location, and duration of exposure. Ambient SO_2 (3-30 ppb) and NO_2 (50-300 ppb) concentrations were not found to be responsible for regional-scale crop losses, although some vegetation injury may occur in the vicinity of point sources (Shriner *et al.*, 1990 *loc cit* Scholes *et al.*, 1996).

The NAPAP ranked the regional pollutants, in terms of their potential to impact forest growth and species composition as follows (Shriner *et al.*, 1990 *loc cit* Scholes *et al.*, 1996).

$$O_3 > \text{acidic deposition} > SO_2 > NO_2$$

In general, trees are less sensitive to O_3 than annual crops, and monocotyledonous species (maize, sorghum and tall fescue) are less sensitive to O_3 than dicotyledonous (dry bean, soybean and tobacco) species (Shriner *et al.*, 1990 *loc cit* Scholes *et al.*, 1996). According to Heagle (1989), visible symptoms of O_3 injury occur at concentrations of 20 ppb to 400 ppb for sensitive species. Stomatal closure has been observed to occur at O_3 concentrations of 200 ppb, leading to a reduction in photosynthetic rate and increased respiration rates (Heagle, 1989).

Ozone can result in acute injury, chronic injury, or it may affect growth, with or without visual symptoms (Krupa & Manning, 1988). Acute injury involves cell death and occurs with exposure to high concentrations of O_3 for short periods of time. Chronic injury results from long-term exposure of plants to low concentrations of O_3. Both types of symptoms may occur on the same plant, but at different times in their development, due to the fluctuating nature of ambient O_3 concentrations (Krupa & Manning, 1988). Disturbances caused by air pollution, through stress on the individual sensitive plants, may alter ecosystems and lead to the dominance of the more tolerant species, which could lead to a decrease in species richness (due to acute exposures). Chronic exposures may bring about gradual modifications of ecosystem structure (Treshow & Anderson, 1989). Therefore, there is increasing concern and interest in developing techniques to monitor the effects of O_3 on ecosystem health (Heagle *et al.*, 1994). For this purpose, a useful tool would be a plant system that responds to ambient levels of O_3 and can be calibrated to estimate O_3 concentrations as well as the effects on other plant species (Heagle *et al.*, 1994).

Plants have different thresholds for foliar O_3 injury and yield losses (Krupa & Manning, 1988). Davison and Barnes (1998) have warned that making assessments on visible symptoms alone may be of little use. They suggest that these symptoms do indicate an effect at the biochemical level but that this does not necessarily imply that the plant growth is affected or that the plant is ecologically disadvantaged. Although research has been done on the effects of O_3 pollution on crops, very little research has been done on wild species or natural ecosystems (Davison & Barnes, 1998). Davison and Barnes (1998) state the importance of identifying sensitive taxa, processes and ecosystems, if the ecological risk posed by O_3 is to be properly evaluated.

1.3 The effects of nitrogen dioxide on vegetation

The nitrogen compounds that are of concern, with regard to the impacts they may have on vegetation, include NH_Y as well as NO_X, since both result in an increase in nitrogen in the environment (Bobbink *et al.*, 1992). The impacts of increased nitrogen deposition upon plants and vegetation are many, but the most important are: direct toxicity to plant species; soil-mediated effects on plants species; increased susceptibility to other stress factors; and changes in competitive relationships between plants species, resulting in changes and/or loss of biodiversity (Bobbink *et al.*, 1992).

Generally, the movement of NO_X into leaves is subject to similar diffusive resistances as found for O_3 but the stomata are not the only path of entry for NO_X (Wellburn, 1994). Cuticular resistances for NO_X are much lower than those for O_3 (Wellburn, 1994). Therefore, even when stomata are closed, significant amounts of NO_X may enter a leaf through the epidermal layer (Wellburn, 1988). This means that plants with closed stomata in bright sunlight responding, for

example, to water deprivation could be especially vulnerable to damage by NO_X (Wellburn, 1988). Within the leaf, the cell walls of the spongy mesophyll are surrounded by extracellular water and provide a very large surface area for the absorption of NO_X (Wellburn, 1988). Once inside the leaf, NO is only slightly soluble in this water, but the solubility of NO_2 is greatly increased by reacting with the water to produce nitric acid (Wellburn, 1988).

Following exposures to typical atmospheric levels of NO_X, instances of visible injury are rare and may be confused with visible damage cause by SO_2 (Wellburn, 1994). The main sources of high levels of NO_2 that would affect vegetation are localized, accidental releases which cause relatively short periods of high exposure which can result in necrotic lesions and excessive defoliation (Taylor, 1970). Symptoms of NO_2 exposure are often divided into invisible and visible injury (Wellburn, 1988). Invisible injury, where there is an overall reduction in growth but no obvious signs of damage, is often associated with decreases in photosynthesis and transpiration (Wellburn, 1988).The visible injury is characterised by bleached (chlorotic) areas on the leaves associated with the damaged or necrotic areas which occur when plants are exposed to very high concentrations of NO_2 (e.g. 2500 ppb for 8 hours) (Wellburn, 1988). Collapsed and dying bleached tissue occurs mostly at the apex of the leaves and along the margins, most severely on older leaves (Wellburn, 1994).

Plant species vary greatly in their susceptibility to NO_2 and the threshold dosage required to produce injury on a plant may be affected significantly by the environmental conditions under which the plant was growing when exposed. It has been observed (Taylor, 1970) that sensitive species such as pinto bean, tomato and cucumber may be injured by a two-hour exposure to

about 6000 ppb NO_2 when they are growing under light intensity equivalent to full sunlight, but under low light intensity equivalent to a very cloudy day these plants display injury when exposed for two hours to 2500 - 3000 ppb.

Long-term exposures to low concentrations of NO_2 do not result in the typical foliar lesions associated with high concentration, short-term exposure. Significant effects have, however, been observed (Taylor, 1970). Continuous exposure of pinto bean and tomato plants for ten to twenty-two days at less than 500 ppb reduced growth by 25% without producing any foliar damage. There has been no report of visible symptoms of leaf injury from nitric oxide. During one study, controlled fumigations failed to produce foliage injury if NO_2 was excluded from the system, but when exposed to very high concentrations of NO (400 to 1000 ppb) growth suppression was detected (Taylor, 1970).

Pollutant mixtures containing both SO_2 and NO_2 have been shown to be more harmful to plants than either gas alone (Tingey *et al.*, 1971), resulting in depressions of growth and reductions in leaf area (Wellburn, 1982). Together, SO_2 and NO_2 have a synergistic effect on plants (Wellburn, 1982). This synergistic effect occurs partly by failure to induce additional nitrite reductase activity and partly by the combined effects of sulphite and nitrite (Wellburn, 1982). These may act by the intermediate formation of free radicals damaging the chloroplasts (Wellburn & Higginson, 1981) and preventing sufficient proton gradients, which would normally have allowed extra ATP to be formed. This extra ATP is required to counteract the negative effects of the two pollutants on the stroma and cytoplasm of plant cells (Wellburn, 1982).

Another way that NO_X can affect vegetation is through acid deposition or acid precipitation (Wellburn, 1994). The term acid deposition refers to deposition from the atmosphere of pollutants, which when dissolved in water, alter the solution concentrations of H^+ or acid anions (Hendrey, 1985). Oxides of nitrogen (NO and NO_2), as well as SO_2, are some of the most important of these pollutants (Hendrey, 1985). The main paths of atmospheric acidification are reactions among pollutants in the gas phase and in the aqueous phase (Durham & Kenneth, 1985). Ecosystem acidification occurs through the wet and dry deposition of these acids (Hendrey, 1985). The acidification reactions are as follows:

$$NO_2 + HO \rightarrow HNO_3 \qquad \text{(V)}$$

$$O_2 \bullet H_2O \leftrightarrow H^+ + HSO_3^- \qquad \text{(VI)}$$

$$HSO_3^- + O_3 \leftrightarrow H^+ + SO_4^{2-} \qquad \text{(VII)}$$

$$HSO_3^- + NO_2^- \leftrightarrow H^+ + SO_4^{2-} \qquad \text{(VIII)}$$

Reaction (V) is a gas-phase reaction and reactions (VI) to (VIII) are aqueous-phase reactions (Durham & Kenneth, 1985). A second method of nitric acid formation is now thought to be important at night. This involves oxidation of NO_2 by O_3 to form nitrate, NO_3 (Tanner, 1990). This NO_3 is not stable during the day due to photolysis and/or reaction in the presence of NO, but at night these decompositions become slow, and other NO_3 chemistry can become important, namely, reactions either with NO_2 and H_2O, or with gaseous aldehydes forming nitric acid in both cases (Tanner, 1990).

Acid deposition can potentially affect plants directly and indirectly (Evans, 1989). Direct effects include injury to aboveground tissues, foliar leaching and uptake of ions, and changes in metabolic processes (Haines & Caelson, 1989). With the direct effects, the process of pollen

germination and pollen tube growth would be a particular sensitive time in of the plant life cycle (Haines & Caelson, 1989). Leaves may also be sensitive because they are exposed for long periods to acid deposition. Visible damage to leaves of sensitive crops like radishes, beets, soybeans and kidney beans has been observed when exposed to artificial rain of pH 3.4 or lower (Wellburn, 1994). The degree of injury by acid deposition depends on dosage, dosage being a function of concentration and time period of contact of acidic precipitation on leaf surfaces (Wellburn, 1994). Factors such as temperature, humidity, wind turbulence, leaf surface 'wettability', and leaf morphology (leaf size, shape, or attachment angle) all influence the period of contact and, therefore, the degree of surface injury (Shriner & Johnston, 1985). The morphological characteristics will vary between species and this may, therefore, determine relative species-sensitivity to acid deposition (Evans, 1989). Strong acids hydrolyse the waxy esters of leaf cuticles to release long fatty acids. This then changes the hydrophobic characteristics of leaf cuticles and increases the leaf 'wettability' (Wellburn, 1994), which in turn makes them more susceptible to further injury, and results in alterations in leaf structure or function (Shriner & Johnston, 1985). Plant roots would be considerably less affected by the direct effects of acid deposition, during the short-term, because they exist in the soil, which is buffered against rapid pH change (Haines & Caelson, 1989).

Indirect effects include altered plant growth and nutrition due to changes in soil chemistry, including pH and nutrient availability, changes in mycorrhizal development and growth, and interactions with other stresses such as O_3, drought, insects, plant pathogens, and frost (Haines & Caelson, 1989). The possible interactions of acid deposition with these other stress factors could enhance the vulnerability of plants to nutrient losses (Fernandez, 1989). The potential for

acid deposition to acidify soils was the earliest concern identified for detrimental effects that may indirectly affect plant health via changes in the soil chemical environment (Fernandez, 1989). Increased soil acidity can result in increased solubility of aluminium and heavy metals (mercury, lead, cadmium and copper), increasing their availability to plants (Fernandez, 1985; Haines & Caelson, 1989). In areas receiving acid deposition, aluminium concentrations may remain high throughout the soil profile, instead of precipitating out (Haines & Caelson, 1989), since acid deposition in the soil solution induces the mobilization of aluminium cations (Evans, 1989). Aluminium can reduce nutrient availability to plant roots (Evans, 1989) and, therefore, is one of the most important growth-limiting factors on acidic soils (Taylor, 1989). The most important effects of aluminium toxicity are reduced root growth, with root elongation being inhibited, leading to roots which become thickened, brittle and occasionally necrotic (Taylor, 1989).

Initially there was concern over the impacts of acid deposition on forest systems, but now the effects on agricultural systems are also being observed (Fernandez, 1985). The impacts on agricultural systems are thought not to be as severe because of the possible overriding effects of fertilizer and lime on soil chemistry. It is thought that these soil amendments control soil acidity in farmlands and outweigh the effects of the increase in acidity from deposition (Fernandez, 1985).

1.4 Effects of ozone and nitrogen dioxide on human health

Air pollutants can affect human beings directly as a consequence of inhalation or contact, and indirectly by affecting the environment which ultimately affects our well being (Terblanche &

Sithole, 1996). Ozone and NO_X are both oxidants, causing the oxidation of cell membranes and death of cells, and are thus regarded as chemical irritants. It has been noted that many more biologically reactive species of NO_X occur (e.g. N_2O, NO, NO_2, NO_3, N_2O_4, HNO_3) in comparison with O_3 (UK Department of Health, 1993). However, of the major photochemical oxidants in ambient air, O_3 is of greater concern with regard to human health because of the very high concentrations that can occur (Horvath & McKee, 1994).

Ozone enters the human body through the respiratory system. The rate, amount, and sites of O_3 uptake and removal largely determine the extent to which exposure of an individual to a particular concentration of O_3 will evoke adverse health effects (Horvath & McKee, 1994). The biologically important functional groups which will react relatively rapidly with O_3 include the alkenic (carbon-carbon double bonds), amino, and sulphydryl groups (Horvath & McKee, 1994). The alkenic bonds occur in the essential fatty acids and in the polyunsaturated fatty acids, which are both important components of the lipids in cell membranes. Oxidation of amino and sulphydryl groups can result in protein denaturation with accompanying loss of structural and functional integrity for the affected protein (Horvath & McKee, 1994).

Acute exposure to O_3 will result in inflammation of the respiratory system and cause conditions such as rhinitis, bronchitis, laryngitis, coughing and chest pain (Horvath & McKee, 1994). It can also change respiratory function without the development of symptoms (Terblanche & Sithole, 1996). These acute effects can become severe as exposure concentrations increase, as exercise levels increase, as the exposure duration increases, and if there is an increase in the frequency of repeated peak exposures (McCurdy, 1994). The chronic effects of O_3 exposure

are more serious for people living in cities with generally elevated O_3 levels, which can induce inflammation in the lungs, which in turn acts as a precursor to irreversible lung damage (Horvath & McKee, 1994).

According to Utell (1989), the most important concern with O_3 is the potential for irreversible damage to the lung, through oxidation of the cells, caused by repeated exposures to O_3 over time. The two most common respiratory diseases are chronic obstructive pulmonary disease (COPD) and asthma. In 1989 in the United States, 10 million people were suffering from COPD, a term that includes pulmonary emphysema and chronic bronchitis. At this time COPD was the fifth leading cause of death. Evidence has suggested that bronchial hyperreactivity may be the predisposing factor to the development of COPD, and any alterations in the airway responses after acute but regular exposures to pollutants may prove to be an important risk factor in the development of COPD. It has been predicted that people with asthma would have an increased sensitivity to the effects of O_3.

It has been suggested that repeated exposure to NO_2 from gas-burning appliances and/or tobacco smoke may aggravate existing lung disorders, particularly in children (UK Department of Health, 1993). Children are often more susceptible to the effects of air pollution because of their higher intake relative to their body surface area, their enhanced absorption of pollutants, their complete defense systems and their behaviour which may increase their exposure to some pollutants (Terblanche & Sithole, 1996). Studies on laboratory animals have shown them to be more susceptible to both bacterial and viral infections following exposure to NO_2. Whether this also occurs in humans is not clear, but in the epidemiological studies related to indoor

environments, increased respiratory illnesses have been reported among children in homes with sources of NO_2 at concentrations within the range occurring in the outdoor air (Terblanche & Sithole, 1996). There are, however, few reports of controlled human studies on the effects of NO_2 on the defense mechanisms of the immune system (UK Department of Health, 1993). Threshold Limit Values (TLV) established by the American Conference of Government Industrial Hygienists, have been set for the industrial exposure of humans to NO and NO_2 (Wellburn, 1988). The European Economic Community (EEC) also uses these TLVs. The TLV for NO is 25 000 ppb and the TLV for NO_2 is 2000 ppb lasting for 8 hours at a time (Wellburn, 1994). These levels are well above those which the general public usually experience, but some individuals occasionally experience high concentrations of nitrous fumes (Wellburn, 1994). Those industries involved in the nitration of various aromatics to form nitrocellulose (the basis of lacquers, films and celluloid) or with nitrophenols used in the drug and dye industries, are prone to the effects of NO_X fumes (Wellburn, 1994). If someone is exposed to high levels of these fumes, above the TLV, such a lack of warning symptoms means that only after 1 to 24 hours of exposure, symptoms such as coughing, headaches and chest tightness develop (Wellburn, 1988). If particularly severe they are followed by either, sudden circulatory collapse or, congestion and water accumulation in the lungs about 36 hours after exposure (Wellburn, 1988). Nitric oxide can cause the haem of red blood cells to form a type of methaemoglobin by chelation mechanisms and excess blood nitrate may cause reduced blood pressure (Wellburn, 1988). This then causes enhanced destruction of blood cells, liver and kidney defects (Wellburn, 1988). With regards to industrial exposure, the World Health Organisation (WHO) recommended to member countries of the United Nations a guideline of 210 ppb of NO_2 not to

be exceeded for a period of one hour, and over a period of 24 hours this should be reduced to below 80 ppb in order to protect human health (Wellburn, 1988).

The National Ambient Air Quality Standards (NAAQS) developed in the USA in the 1960s, and the O_3 standards developed in Europe during the 1960s and 1970s are based on safe levels for air pollution whereby sensitive individuals in the population exposed to these levels for prolonged periods of time should not be affected (WHO, 1987; McKee, 1994b). The US NAAQS have been set for criteria pollutants which are the most common pollutants in the atmosphere and for which relatively sufficient information has been obtained to determine safe levels (Terblanche & Sithole, 1996). The NAAQS for these common pollutants for the USA, together with exposure limits given by the WHO and South African guidelines are given in Table 1.1 (Ferris 1978; WHO, 1987; and DNHPD, 1991).

Table 1.1: Comparison of air pollution exposure limits.

Pollutant	Air Pollution Exposure Limits		
	USA	WHO	RSA
Sulphur dioxide	140 ppb (24-hr ave.)	50 ppb (24-hr ave.)	100 ppb (24-hr ave.)
Carbon monoxide	$35x10^3$ ppb (1-hr ave.)	$25x10^3$ ppb (1-hr ave.)	$35x10^3$ ppb (1-hr ave.)
Ozone	120 ppb (1-hr ave.)	60 ppb (24-hr ave.)	120 ppb (1-hr ave.)
Nitrogen dioxide	50 ppb (annual ave.)	75 ppb (8-hr ave.)	50 ppb (annual ave.)
PM_{10}*	150 $ug.m^{-3}$ (24-hr ave.)	70 $ug.m^{-3}$ (24-hr ave.)	150 $ug.m^{-3}$ (24-hr ave.)
TSP**	260 $ug.m^{-3}$ (24-hr ave)	120 $ug.m^{-3}$ (24-hr ave.)	300 $ug.m^{-3}$ (24-hr ave.)

*PM_{10}=Respirable particulate matter (particle diameter less than μ10 m)
**TSP=Total suspended particulates (particle diameters 0-20 μm)

The South African standards are very similar to those established in the USA. The limits set by the WHO are much lower however. The current USA NAAQS and the South African standard for O_3 is 120 ppb (1-hr average) (Table 1.1), based on the health effects due to acute exposures of 1 hour as measured by continuous monitors. However, exposures to O_3 for up to 8 hour at less than 120 ppb have been shown to result in progressive and significant changes in respiratory function in exercising individuals (Hortsman *et al.*, 1990). Previous work has indicated that large decrements in lung functions, moderate to severe respiratory symptoms, and a doubling in nonspecific airway reactivity resulted when young men performed exercise equivalent to a day of moderate to heavy work or play while exposed to 120 ppb O_3 (Folinsbee *et al.*, 1988). Hortsman *et al.*, (1990) found that some individuals experience substantial pulmonary distress if they exercise for long periods of time at low O_3 concentrations, but others will not experience any effect from such exposures. They also observed that lung function was significantly reduced after only three hours at 120 ppb, 4.6 hours at 100 ppb, and 5.6 hours at 80 ppb, indicating that there is an interaction between duration of exposure and O_3 concentration. These findings would suggest that the current O_3 standard is too high to sufficiently protect public health (Liu *et al.*, 1997). Therefore, an alternative O_3 standard is being considered by the US Environmental Protection Agency (EPA) (Lefohn, 1997). This standard involves taking into account how many days have an integrated 8-hour exposure greater than an O_3 concentration level thought to cause adverse human health effects (Lioy *et al.*, 1985). The adverse agricultural effects (Lefohn, 1997) were discussed in section 1.2.

1.5 The South African situation

In South Africa the most common air pollutants are: sulphur dioxide, NO_X, volatile hydrocarbons, carbon monoxide, carbon dioxide and chlorinated fluoro-hydrocarbons, as well as particulates (Department of Environmental Affairs and Tourism & Department of Water Affairs and Forestry, 1997). The highest levels of air pollutants found at ground level are in the townships where there is no electricity. The use of coal stoves for cooking and heating in these areas results in air pollution levels well above the safety levels. The Highveld areas of the Mpumalanga Province (situated inland on the eastern side of the South African plateau) are affected by the electricity industry with approximately 64% of Eskom's total generating capacity concentrated in this area (Department of Environmental Affairs and Tourism & Dept. of Water Affairs and Forestry, 1997).

Air pollution control in South Africa is regulated by the Air Pollution Prevention Act 45 of 1965. This Act regulates the control of noxious and offensive gases emanating from industrial processes, the control of smoke and wind borne dust pollution, and pollution from diesel vehicles (Annegarn *et al.*, 1996a). The Act is now, after 30 years, in the process of being updated. Discussions towards a White Paper on integrated pollution control and waste management began in May 1997 (SA Department of Environmental Affairs and Tourism & Department of Water Affairs and Forestry, 1997). It was decided then that air pollution control would include pollution at the local, regional and global scales, and the policy would include atmospheric odour generation and control, and indoor pollution apart from occupational health exposures. The formation of secondary air pollution was also regarded as an important issue.

The possibility of negative impacts of air pollution on the environment in South Africa was highlighted at a workshop in October 1987 (Olbrich, 1994). This workshop identified commercial forestry in the Eastern and South-eastern Transvaal regions (now Mpumalanga Province) as one of the major resources at possible risk from air pollution (Tyson *et al.*, 1988).

In South Africa very few measurements of rural O_3 concentrations were available until 1988 (Tyson *et al.*, 1988). Since then, monitoring has provided insight into the behaviour of O_3 over the highveld region. Measurements of O_3 were taken in the Highveld region on a continuous basis over several years (Rorich 1991a; 1992; 1993 *loc cit* Annegarn *et al.*, 1996b). The following distinct characteristics were observed. The annual mean concentrations ranged between 20 ppb and 38 ppb, and hourly mean concentrations rarely exceeded 120 ppb, the level set by the South African Department of Environmental Affairs and Tourism as the threshold for damage to sensitive ecosystems (Table 1.1). Concentrations at the rural sites showed the same diurnal pattern as observed in urban sites, that is the broad maximum during the mid-afternoon. Therefore, the same mechanisms of O_3 production probably operate in both rural and urban areas, whatever the source of the precursors. Most sites showed maximum monthly mean concentrations in spring. This was thought to be due to precursor emission from biomass fires throughout the subcontinent during this period (Annegarn *et al.*, 1996b). It was also observed that there was an increase in concentration with an increase in elevation of the site above sea level. This could be due to increase fluxes of ultraviolet radiation experienced at higher elevations, or due to enhanced exposure due to downward movement of stratospheric O_3 (Annegarn *et al.*, 1996b).

In South Africa the climate would seem favourable for O_3 production, however, little is known about the effects of O_3 on crops or the natural ecosystems in South Africa and so the true economic effects, if any, are not known. What is essential in South Africa is that air quality objectives need to be established where these objectives are related to critical atmospheric levels.

1.6 Establishing critical levels and critical loads

In order to control O_3 pollution, strategies are necessary to reduce precursor emissions of NO_X and VOCs. These strategies need to take into account potential effects on sensitive receptors. The critical levels and critical loads concepts are part of a strategy adopted by the Convention on Long Range Transboundary Air Pollution of the United Nations Economic Commission for Europe (UN-ECE) (Stadelmann & Roch., 1994). These concepts are based on the principle that these sensitive receptors will be protected when there is no longer an exceedance of the critical levels or loads for particular air pollutants (Stadelmann & Roch, 1994). Critical levels or critical loads of atmospheric pollutants are tools for developing emission abatement strategies which should provide a better scientific basis for future gaseous pollutant emission controls (Bull, 1992).

The critical load for a particular receptor-pollutant combination is defined as the highest deposition load that the receptor can withstand without long term damage occurring (Bull, 1992; Smith *et al.*, 1995). A critical level is similarly defined, but in terms of air concentrations of pollutant gases, that is critical levels are established for gaseous pollutants acting on specific sensitive receptors (Bull, 1992). A critical load or level can be used by policy makers to

determine what restrictions should be enforced (Pardo & Driscoll, 1993), and, as stated by Badiani *et al.*, (1996) these critical loads or levels need to be re-assessed on a regular basis as new information becomes available. Research on the effects of air pollution on terrestrial ecosystems often yields results which are not quantitative. As a result, this information may be of little value to policy makers and resource managers. It is in order to facilitate the transfer of scientific information for policy decisions concerning emissions of air pollutants, which the concepts of critical loads and levels have been developed (Pardo & Driscoll, 1996).

The definition of a critical level for O_3 has to consider the following: air concentration; length of exposure; timing of exposure; and the nature or sensitivity of the receptor. The issue of time scale is quite crucial to obtaining and interpreting meaningful results when applying critical levels (Pardo & Driscoll, 1993). The time period over which exposures are integrated, must be clearly defined in order to make initial calculations and especially to compare the critical levels of different regions (Pardo & Driscoll, 1993). In terms of defined ecosystems, time scale can become somewhat conceptually problematic. It is important for critical level calculations to consider the time period in which damage could occur to an ecosystem: the change from an undamaged (healthy) to a damaged (unhealthy) state (Pardo & Driscoll, 1993). It is also important to consider what length of time is reasonable to expect an ecosystem to remain in 'the same' healthy state functionally and structurally (Pardo & Driscoll, 1993). At an ecosystem level, this should be 100 to 200 years (Grennfelt & Thornelof, 1992).

Much work has been done on critical levels throughout Europe and in parts of Canada and the United States (e.g. Ashmore, 1994; Skarby, 1994; Braun & Fluckiger, 1994). The goal is to

discover 'threshold levels' above which some adverse effect is measurable. Such threshold values are often highly controversial. Even if an air quality standard could be agreed on, the next step of attaining this standard, for example through the control of emissions, remains difficult (Treshow & Anderson, 1989). At a workshop held in Switzerland in 1993, critical levels of O_3 were revised for agricultural crops, forest trees and natural/semi-natural plant communities on the basis of the recent scientific findings (Fuhrer & Achermann, 1994). The proposed long-term critical level for O_3 is proposed as accumulative exposure over the threshold concentration of 40 ppb for both agricultural crops and forest trees. This is consistent with that being used in the United States. This exposure index is referred to as AOT40 (accumulated exposure over a threshold of 40 ppb). One must take into account however, that although visible injury may not be present, other long-term effects, for example effects on reproduction, may occur below an O_3 concentration of 40 ppb (Hogsett *et al.*, 1997). Measurements of AOT40 will vary spatially and temporally. Smith *et al.* (1995) have developed a statistical model which takes into account the spatial variability of AOT40 in terms of known covariates such as geographical location and site altitude, taking the remaining unexplained variability to be explained by a spatially coherent random process. The temporal variability is similarly modelled as partly a function of known covariates with the remaining unexplained variability as a year to year random effect. An important problem is that critical levels cannot be applied in an additive manner for a mixed pollutant situation (Wellburn, 1994). As mentioned above, interactions between pollutants do occur (both synergistic and antagonistic) and, consequently, individual critical levels are difficult to apply. However, Wellburn (1994) suggests that new combined critical levels need to be established for mixed pollution scenarios.

The present UN-ECE critical level for NO_2 is 30 ugm^{-3} (which is about 15 mlL^{-1}), as an annual mean for adverse ecophysiological effects on plants (Ashmore *et al.*, 1994).

1.7 Monitoring techniques

When measuring air pollutants there are three common means of obtaining data, these being continuous monitors (ultraviolet photometric analysers or chemiluminescent analysers), passive monitors, and biomonitors or bioindicators. These methods and approaches are described and evaluated in the following sections.

1.7.1 Continuous ozone monitoring

The continuous monitor used in this project was the Dasibi, a self-contained instrument which measures the concentration of O_3 in ambient air. This is accomplished by measuring the absorption of ultraviolet light in the sample of gas flowing through an absorption cell contained in the instrument. The instrument is designed to provide a highly stabilised measurement capability for extended periods without repeated adjustments, and it operates over a wide range of ambient conditions without adverse effects on measurement accuracy (Dasibi Environmental Co., 1989). The advantages of the continuous monitor are that it provides continual accurate data, and according to Wellburn (1994) it is more adaptable to unattended operation. The disadvantage of using a continuous monitor over the other methods of O_3 monitoring is the expense involved in acquiring and maintaining it. A continuous monitor requires expert maintenance and calibration. Continuous monitors can also be difficult to transport, and cannot be used in remote areas where there is no supply of electricity.

1.7.2 Passive diffusion tube monitors

The most common form of passive monitoring is by the use of diffusion tubes. The diffusion tubes used in this study were first developed by Palmes *et al.* (1973) for the measurement of sulphur dioxide and water vapour and subsequently for nitrogen dioxide (NO_2), and were described and referred to as Palmes tubes (Palmes *et al.*, 1976). The sampling reagent that was used for the NO_2 tubes was based on that established by Levaggi *et al.* (1973). Grosjean *et al.*(1992) have described a passive sampler which is suitable for measuring parts per billion (ppb) levels of O_3. A passive sampler for hydrogen sulphide (H_2S) has also been described (Shooter *et al.*, 1995).

The basic components of a diffusion-based passive sampler are: an inert barrier to convective air; an air gap between the barrier and the trap; and a trap to collect the chemical of interest, which diffuses in the air gap at a constant rate given by,

$$S = 60DA/L$$

where S is the diffusion rate, or sampling rate, (cm^3min^{-1}), D is the diffusion coefficient (cm^2 sec^{-1}), A is the trap sampling area (cm^2), and L is the length of air gap between the diffusion barrier and trap (cm). Passive sampling assumes that the mean concentration of the trace gas under consideration is proportional to the deposited amount of the respective gas (Dammgen *et al.*, 1996).

The tube is exposed for periods of at least a week in urban areas and longer (up to a month) in less polluted situations, as sampling time is inversely proportional to the concentration of the

pollutant being measured (UK Department of Health, 1993). Information on short term peaks is therefore impossible to obtain and diffusion tubes are usually used to obtain long term average patterns in air concentrations. They can be useful for defining spatial patterns in concentrations over a large area; this is expensive using automatic analysers (UK Department of Health, 1993).

Passive samplers offer a simple, cost-effective means of measuring air pollutants in the many situations where integrated measurements (for example dose, exposure, time-averaged values) are more useful than short-time resolution (Grosjean *et al.*, 1992). Measurements of the concentrations of air pollutants are normally restricted to those places which have the electric mains needed to operate sampling equipment, and as a consequence such measurements are performed at relatively few locations (Dammgen *et al.*, 1996).

1.7.3 Plant bioindication

Ambiguous use of the terms bioindication and biomonitoring has occurred. A bioindicator can be defined as an organism (part of an organism or a community of organisms) containing one or several items of information about the quality of the environment (or part of it); whereas a biomonitor is defined as an organism (part of an organism or a community of organisms) containing one or several items of information about the quantity of the quality of the environment, or part of it (Markert, 1991; 1993 *loc cit* Markert, 1994). Passive bioindicators or biomonitors are organisms which already exist in the area under investigation. Active bioindicators or biomonitors are organisms which are exposed (e.g. brought from a laboratory) to the area under investigation for a defined period of time (Markert, 1994).

The development of foliar injury on tobacco (*Nicotiana tabacum* L.) cultivars Bel-W3 (O_3 sensitive) and Bel B (O_3 tolerant) has been used for over 25 years as a differential bioindicator of ambient O_3 pollution (Heggestad, 1991). Bel-W3 is highly sensitive to O_3 and will produce easily recognisable symptoms on the new, fully expanded leaves or newly matured leaves (Plate 1.1). The O_3 threshold for visible injury is about 30 ppb to 50 ppb (Lorenzini, 1994). The tobacco indicator system was improved in some studies by comparing the response of Bel-W3 to that of Bel-B (which is relatively tolerant to O_3) and Bel-C (which has intermediate sensitivity to O_3) (Heggestad, 1991). This tobacco system can be used to indicate the occurrence of phytotoxic levels of tropospheric O_3 and it can give an estimate of relative seasonal changes in concentrations of O_3 in different regions (Heagle *et al.*, 1994).

The visible symptoms of Bel-W3 are bifacial lesions which are often referred to as weather fleck. Injury to leaves can be caused by other oxidants (especially peroxyacetylnitrate) but according to Darley (1960) a distinction can be made between O_3 and other oxidant injury to plants. Oxidant injury has been characterized as a silver, bronze or otherwise metallic sheen on the undersurface of affected leaves. Ozone injury was described as stippling, mottling or bleaching of the upper leaf surface (Darley, 1960).

There are however, limitations to bioindicator systems that depend solely on visual estimates of O_3 leaf injury, because the symptom type and intensity can depend on other factors (Heagle *et al.*, 1994). Other stresses may also cause visible symptoms similar to those caused by O_3, making it difficult to separate them from O_3 effects. As mentioned above, previous O_3 exposures can also affect the degree of plant response to O_3 (Heagle *et al.*, 1994). Therefore, a

Plate 1.1 *Nicotiana tabaccum* (L.) variety Bel-W3, showing O_3-induced injury. These plants were used in this study.

given amount of O_3 does not necessarily result in a given amount of foliar injury. There is not always a simple relationship between the atmospheric concentration of O_3 and a given degree of response and all the above factors must be taken into consideration when the Bel-W3 system of bioindication is being used.

More recently research has used white clover, *Trifolium repens* L., as an alternative to the tobacco Bel-W3 system (Heagle *et al.*, 1994; Heagle *et al.*, 1995; Heagle, *et al.*, 1996; Heagle). Two clones of white clover were used - a clone that was highly resistant to O_3 (NC-R) and one that was highly sensitive (NC-S). The findings of Heagle's laboratory indicated that the white clover system was an improvement over previous plant bioindicator systems (Heagle *et al.*, 1994). The white clover system utilizes measurements of chlorophyll and biomass in addition to the subjective estimates of leaf damage (Heagle *et al.*, 1994). Another advantage is that the use of clones reduces variability between plants when the plants are grown from seeds. White clover also grows over a wider range of weather conditions than many other plant species (Heagle *et al.*, 1994). For instance tobacco is sensitive to low temperatures and to wind, and for these reasons tobacco does not grow in northern Europe, whereas clover does grow successfully in these parts (Karlsson *et al.*, 1995). Experiments have shown that ambient levels of O_3 at one site routinely caused significant effects on the NC-S/NC-R ratios for chlorophyll contents and biomass that were relatively stable over a wide range of environmental conditions (Heagle *et al.*, 1994).

1.8 Aims and objectives of this study

The occurrence of a regional scale effect in photochemical smog distribution in areas downwind from urban centres in many parts of the world is of much concern because of the potential hazard to vegetation (Krupa & Manning, 1988; Goren & Donagi, 1979). The knowledge of the distribution of O_3 at ground level, as well as the precursors to O_3, is therefore essential in the monitoring of O_3 and satisfactory environmental management on a geographical scale. Little is known of the geographical distribution of O_3 and NO_2 around the Greater Durban area in the KwaZulu-Natal province of South Africa. One of the objectives of this project was therefore, to measure ambient O_3 and NO_2 concentrations at selected sites in the Greater Durban area by means of the three monitoring techniques described above: the continuous ozone monitor; the passive diffusion tube monitors; and plant bioindicators.

Since high levels of O_3 had previously been reported in the Mpumalanga province of South Africa (Annegarn *et al.*, 1996a), a second objective was to monitor O_3 and NO_2 in selected sites in Mpumalanga, using the same monitoring techniques as mentioned above.

White clover has been described as an improvement over tobacco Bel-W3 in terms of bioindication, the reason being that changes in chlorophyll contents of the leaves, caused by high concentrations of O_3, can be used to establish the effects of O_3 on these plants (Heagle *et al.*, 1994). The third objective then was to establish whether this approach could be applied to the tobacco Bel-W3.

Chapter 2 contains a description of the selected sites in the Greater Durban area and Mpumalanga, the materials and methods that were used to perform this study, and a description of the statistics used to analyse the data collected. Chapter 3 contains the results obtained during this study, and Chapter 4 is a discussion of these results.

2. MATERIALS AND METHODS

2.1 Experimental sites

For the Greater Durban area study the following research sites were selected: Botanic Gardens (BG) ($29^0$50.76S $31^0$50.10E), University of Natal (Durban) (UND) ($29^0$52.10S $30^0$58.53E), Kloof (K) ($29^0$45.87S $30^0$50.10E), and Mooi River (MR) ($29^0$11.84S $30^0$00.19E) (Figure 2.1). Sites were selected which represented a transition from the urban industrial areas within the city of Durban, through peri-urban to rural areas inland. The sites were not immediately adjacent to industrial areas as these would exhibit low levels of O_3 since it is scavenged by NO, due to the continuous production of NO from petrol-powered vehicles as well as stationary combustion sources in the urban areas (Ljunstrom & Hallquist, 1996).

For the Mpumalanga study, the selected sites were: Council for Scientific and Industrial Research (CSIR) ($25^0$29.38S $30^0$58.06E), Agricultural Research Council (ARC) ($25^0$27.01S $30^0$58.15S) (both in Nelspruit), Mpumalanga Provincial Administration Offices (MPA) (outside Nelspruit) ($25^0$24.74S $30^0$58.93E), and Long Tom Pass (LT) (an area of high elevation) ($25^0$10.60S $29^0$11.84E) (Figure 2.2).

2.2 Continuous ozone monitoring

In both the Greater Durban area study and the study performed in Mpumalanga, the continuous monitor used was the Dasibi photometric ozone analyser Model 1108. During the Mpumalanga study, measurements were taken simultaneously at the UND site using the continuous O_3 monitor, so that comparisons could be made. The Dasibi measured the concentration of

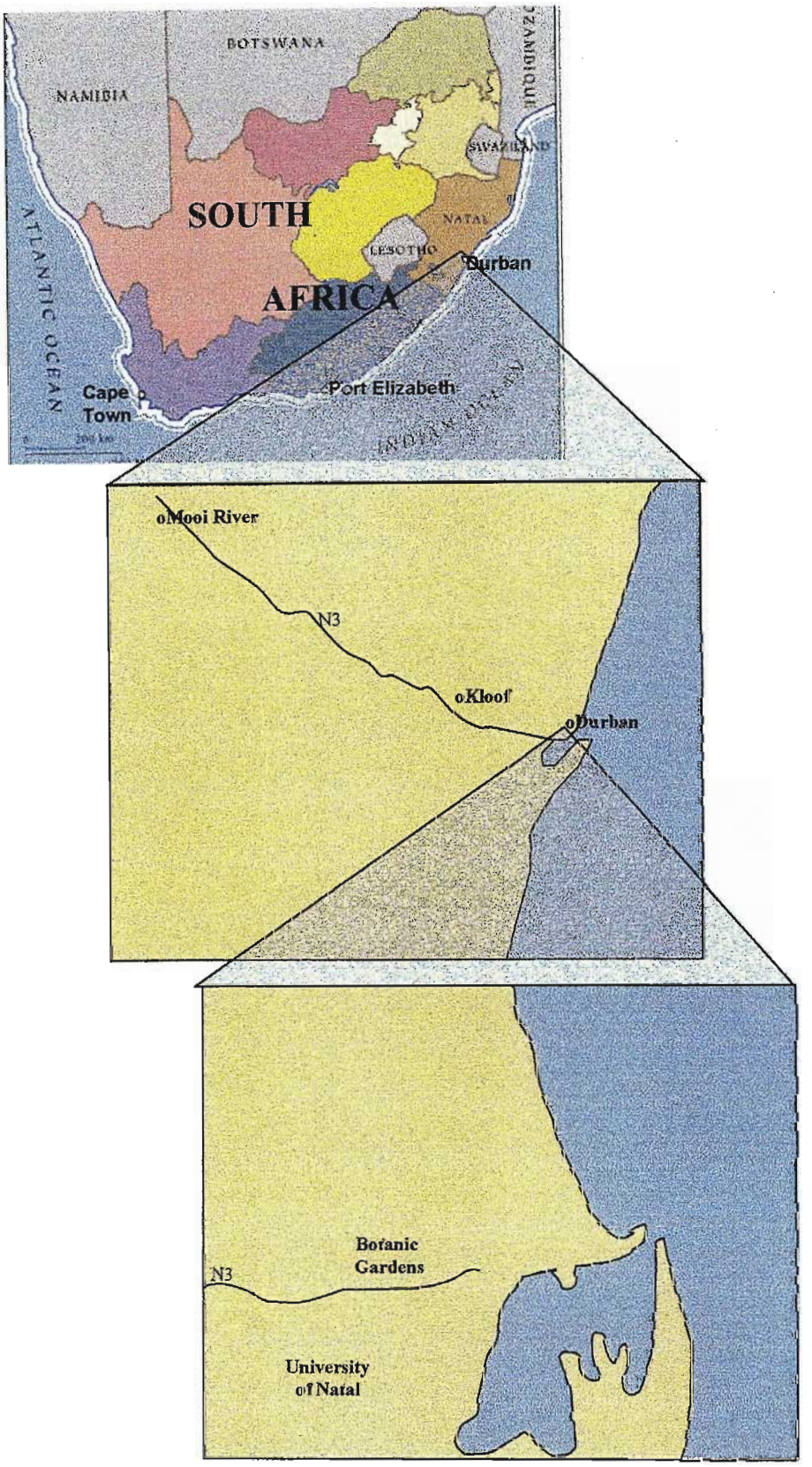


Figure 2.1: Map showing the selected sites for Greater Durban area study.

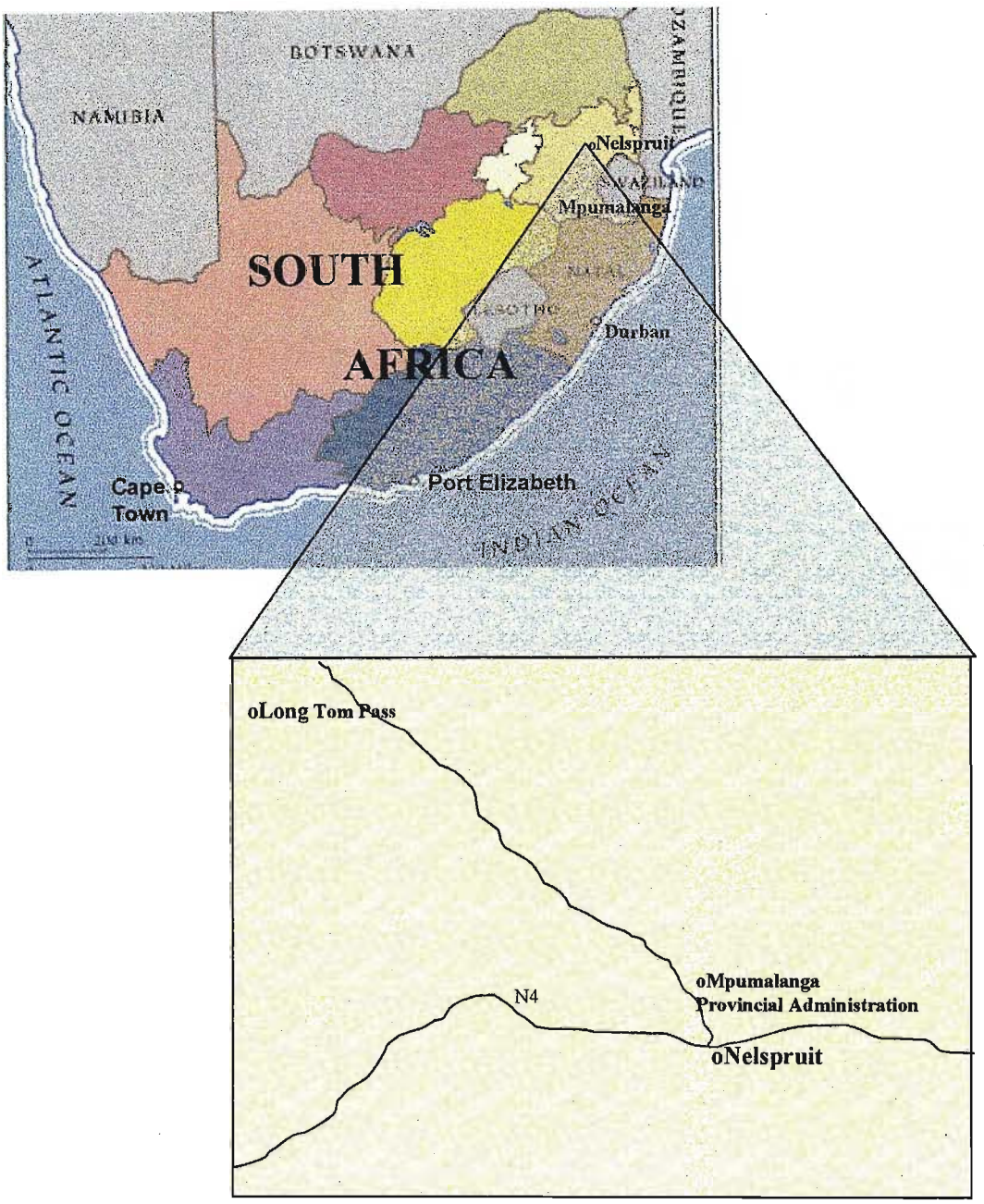


Figure 2.2: Map showing the selected sites for the Mpumalanga study.

ambient O_3 in the air at the UND site every ten minutes. This instrument has a precision of approximately 1 ppb (Dasibi Environmental Co., 1989). The Dasibi is located on the roof of one of the buildings on the university campus, 20 m above ground level and 150 m above sea level. The UND site is to the west of the city centre and to the north-west of the main industrial area of the city (Figure 2.1). The weather data (maximum and minimum temperatures, rainfall, and cloud cover) for this site, corresponding to the sampling periods that measurements were taken, were obtained from South African Weather Bureau located at the Durban International Airport (about 10 km from the University), in order to investigate what relationship such meteorological data may have on the O_3 concentrations measured with the Dasibi.

2.3 Passive diffusion tubes

The passive monitors used were acrylic tubes, 71 mm in length and 10 mm in diameter and had caps at either end (Plate 2.1). Preparation of the tubes involved coating pairs of stainless steel mesh discs with a relevant coating solution (see below) and fitting the discs into the cap at one end of the tube. When in position in the field, the cap at the opposite end to where the discs were positioned was removed and the tubes were orientated with the open end pointing downwards. When exposing them, the tubes were attached to dowel rods and these were then placed in the vertical position in weighted pots. The tubes were placed approximately 0.5 m above the ground.

2.3.1 Preparation of ozone diffusion tubes

The stainless steel mesh discs were coated with 50 µl solution of 1% w/v $NaNO_2$, 1% w/v KCO_3 and 1% w/v glycerol dissolved in 30% methanol (Koutrakis *et al.*, 1993). The discs were

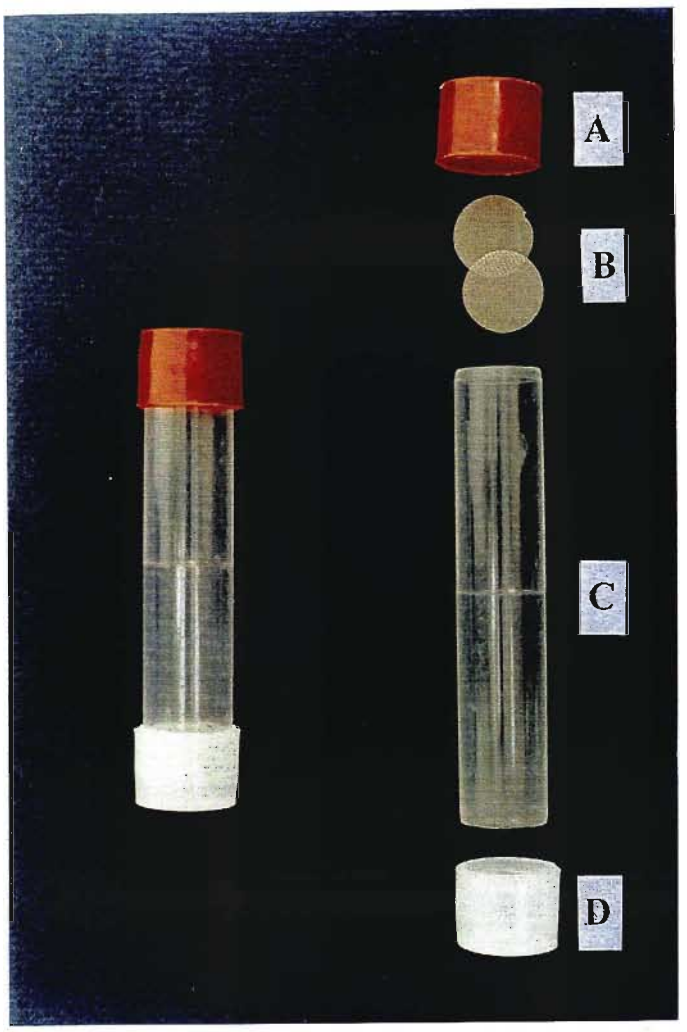


Plate 2.1 The passive diffusion tube sampler used for monitoring O_3 and NO_2 in this study, where A= a polythene cap (fixed during sampling), B= stainless steel mesh discs, C= acrylic tube (71mm long and 10mm wide), and D= a polythene cap (removed during sampling).

cleaned in detergent overnight, with agitation in an ultrasonic bath, to remove the grease introduced during manufacture. They were then rinsed three times with tap water, three times with distilled water, and twice with acetone and dried on Whatman 40 paper in an oven at 125^0C (Gradko International, 1995).

2.3.2 Analysis of ozone diffusion tubes

Analysis of the O_3 diffusion tubes was performed at the CSIR (Durban). The sealed tubes were stored at approximately 5^0C (Koutrakis *et al.*, 1993) until they were analysed. The discs used in the O_3 diffusion tubes were placed in 9 cm^3 of deionised water to allow the coating solution to become dissolved in the water. The solution was then analysed for nitrates using an AutoAnalyser (Perstop Analytical Flow Solution III System). This AutoAnalyser gives readings of nitrate and nitrite concentrations in parts per million (ppm). The NO_3 concentration read is equivalent to the O_3 concentration since NO_2 coating on the discs reacts with O_3, in a 1:1 ratio, to produce NO_3 and O_2 . The O_3 concentration in parts per billion (ppb) could therefore be calculated.

2.3.3 Preparation of nitrogen dioxide diffusion tubes

Stainless steel discs were also used in NO_2 tubes for the application of the coating solution. The absorbent (or coating solution) used was composed of 50% v/v triethanolamine (TEA) in deionised water and 0.5 cm3 Brij 35 (Gradko International, 1995). In each tube, 50 µl of the absorbent was applied to the discs (Levaggi *et al.*, 1973).

2.3.4 Analysis of nitrogen dioxide diffusion tubes

To analyse the diffusion tubes, the protective cap was removed and 3.15 cm^3 of the combined reagent was added directly into the sampler. To make the combined reagent the following was added to one part water (1.5 cm^3): one part (1.5 cm^3) sulfanilamide reagent (2 g sulfanilamide and 5 cm^3 concentrated H_3PO_4 diluted to 100 cm^3 with water); and one tenth part (0.15 cm^3) N-1-Naphthylene-diamine-dihydrochloride (NEDA) reagent (70 mg NEDA dissolved in 50 cm^3 water) (Levaggi *et al.*, 1973). Following the addition of sulfanilamide, it was necessary to add the NEDA very promptly since the diazotized sulfanilamide is very unstable (Palmes *et al.*, 1976).

The solution was then read spectrophotometrically at 540 nm. The amount of NO_2 in nanomoles was determined using a standard curve. A standard curve of sodium nitrite was established so that the concentrations ranged from 0 to 14 nm NO_2^- per 3.15 cm^3 of the final combined solution. This was done by replacing the water used to make up the combined reagent with appropriate dilution's of standard NO_2 solutions. The concentration of NO_2 per hour was then calculated using the following equation (Palmes *et al.*, 1976):

$$NO_2 \text{ ppb.hr}^{-1} = \frac{(NO_2(\text{nmoles})) \times 10^3}{2.3 \times \text{hours exposed}}$$

2.4 Bioindicators

2.4.1 Visible injury

To determine the phytotoxicity effects of O_3, the tobacco cultivar Bel-W3 (which is sensitive to O_3) was used (refer to Figure 1.1). Initially plants were grown to maturity in the greenhouse,

and seeds to be used for the bioindicating selected from those plants exhibiting the most O_3 damage.

For the various studies, seedlings were germinated and grown in seedling trays in seedling soil mix (Grovida) under greenhouse conditions (18^0C-25^0C; watered once daily). Small plants (approximately two to three weeks old), consisting of the two cotyledons and the first emerging leaves, were placed into individual pots and exposed at the various sites. At each site the plants were placed under shadecloth since it has been shown that their sensitivity is increased when they are shade grown (Ashmore *et al.*, 1994). The cotyledons and leaflets exhibit the typical flecking indicative of cell damage caused by ozone. Ten seedlings were placed at each site. Seeds were germinated at each site as well and these plants were left at the respective sites throughout each sampling period. The results obtained from these plants were compared to those of the plants that were two weeks old when monitoring exposure started. After seven days exposure at the site, visible injury was noted and scored according to the scores shown in Table 2.1.

Table 2.1 Scheme of numerical injury scores and the corresponding values of percent leaf area showing injury symptoms.

Numerical Score	% Area Leaf Injury
1	0
2	1-3
3	4-10
4	11-25
5	26-50
6	51-75
7	76-100

From these scores, a damage index (DI) for each plant was calculated according to the following equation:

$$DI = \frac{\Sigma(\text{no. of leaves with numerical score 1} \times 1) + \ldots + (\text{no. of leaves with numerical score 7} \times 7)}{\text{Total number of leaves}}$$

In previous studies, assessments have been of the number of leaves falling into each of the numerical scores shown above and expressed as a percent of the total leaves per plant (Ashmore *et al.*, 1994). In this study, however, damage index values were calculated since statistical analysis could not be performed on percentages.

2.4.2 Chlorophyll analysis

The method used to analyse chlorophyll contents of the biomonitors was based on previous studies where the effects of O_3 on the chlorophyll contents of *Phaseolus vulgaris* (L. cv. Pinto) (Knudson *et al.*, 1977) and *Trifolium repens* (L.) (Heagle, 1996) were measured. After the foliar injury was visibly estimated (Table 2.1) as mean percentage damage, the leaves were then placed in 10 cm^3 of 95% ethanol (one plant sample per container). The previous study had used 30 cm^3 of the 95% ethanol to extract the chlorophyll (Heagle, 1996). It was established, however, that 10 cm^3 was sufficient for this study. An experiment was performed in which six tobacco plants were placed in 30 cm^3 of the 95% ethanol and six plants were placed in 10 cm^3 of the 95% ethanol. The plants had been grown under the same conditions. An analysis of variance showed that there were no significant differences ($p>0.05$) in the chlorophyll contents for the two different treatments.

The containers used for the chlorophyll extraction were brown glass bottles and these were stored in the dark at room temperature and agitated once each day for seven days to extract the chlorophyll. The extract was then measured spectrophotometrically at 665 nm, 649 nm and 435 nm. To calculate dry weights, the plantlets were placed in paper bags and dried at 55^0C in an oven until the weight was constant and then the dry weight measured in milligrams. To convert these readings to chlorophyll a and chlorophyll b content per unit dry weight, the following equations were used:

$$\mu g \text{ Chla/mg} = (13.70)(A_{665\,nm}) - (5.76)(A_{649nm})$$

$$\mu g \text{ Chlb/mg} = (25.80)(A_{649nm}) - (7.60)(A_{665nm})$$

To calculate the carotenoid:chlorophyll a ratio, the extract absorbance at 435 nm was divided by the extract absorbance at 665 nm. This was computed as a spectrophotometric index of the ratio of carotenoids to chlorophyll a (Penuelas *et al.*, 1995).

2.5 Sampling and measurement

At each site four plants, four O_3 tubes and four NO_2 tubes were placed. The tubes were prepared the day before and refrigerated overnight. The sampling period for each was fourteen days, and then the tubes replaced. A period of fourteen days was decided upon for the passive monitors since previous studies had shown that a two week period of sampling was optimal (Dodd, 1996). The results obtained for both the biomonitors and the diffusion tubes were a measure of the average concentration for that period of exposure. Total time for each

measurement period was four weeks (i.e. two consecutive periods of two weeks). The Dasibi Model 1108 was situated at the UND site. Ozone measurements using the Dasibi were collected for the same sampling period and so could be compared with the results obtained from the bioindicators and the O_3 passive diffusion tubes.

Sampling at all the Greater Durban area sites was carried out at three different periods. These were 8/1/1997 to 5/2/1997 (summer), 23/4/1997 to 28/5/1997 (autumn), and 23/7/1997 to 20/8/1997 (winter). Sampling in Mpumalanga was carried for two weeks only (22/9/1997 to 6/10/1997). During this two week sampling period, sampling was carried out simultaneously at the UND site, in order for comparisons to be made. A total of four weeks of measurements were obtained from the Dasibi over this spring period for seasonal comparisons in the Greater Durban study.

2.6 Statistical analysis

All the data were analysed using Statgraphics Plus version 7.0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. The data were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data were then analysed using analysis of variance (ANOVA). Where significant differences in the data were indicated, a Scheffe multiple range test was carried out, which constructs intervals for pair-wise differences of means to determine significant differences ($p<0.05$). Data that were not normally distributed were analysed using the Kruskal-Wallis non-parametric test. Where the Kruskal-Wallis test indicated significant differences in the data, the Mann-Whitney U test (for unpaired data) was used to test whether the central tendency in the independent samples was the same or not. Correlation

analyses were also performed using the Pearson's Product-Moment parametric correlation analysis.

3. RESULTS

3.1 The Greater Durban Area study

3.1.1 Continuous ozone monitoring

Ozone concentrations measured with the photometric analyser (Dasibi 1108) at the UND site during the four sampling periods, summer, autumn, winter and spring, are presented in Tables 3.1a, b, c and d. The sampling periods were approximately one month, with the data in spring also used for comparison with results from the Mpumalanga study. Weather conditions during most of the summer (8/01/1997 to 5/02/1997) sampling period (Table 3.1a), were not generally conducive to photochemical oxidation formation, that is substantial rain and frequent cloudiness, although temperatures were relatively high (daily maximums ranging from 20.8^{0}C to 31.3^{0}C). The O_3 concentrations measured with the Dasibi were very low at the UND site throughout the duration of this period, the highest one-hour mean value ranging from 0.7 ppb to 26.3 ppb and the highest 24-hour mean value ranging from 0.2 ppb to 4.6 ppb.

The weather conditions during the autumn (23/04/1997 to 28/05/1997) and winter (23/07/1997 to 20/08/1997) sampling periods (Tables 3.1b and 3.1c respectively) were also not conducive to O_3 formation, that is frequent rain and cloudiness, and the temperatures were lower than during the first sampling period. The O_3 concentrations during the autumn sampling period (Table 3.1b), measured with the Dasibi at the UND site were quite low, the highest one-hour mean ranging from 0 ppb to 20.6 ppb and the highest 24-hour mean value ranging from 0 ppb to 18.9 ppb. The highest 24-hour mean O_3 concentration during the winter sampling period was low, ranging from 0 ppb to 9.2 ppb (Table 3.1c). The highest one-hour mean measurements

Table 3.1a : Summary of continuous photometric O_3 analyser data and weather conditions for the area around the University of Natal (UND) site in summer (8 January to 5 February 1997).

Date	*Weather Conditions	Lowest 1 hour daily mean ozone	Highest 1 hour daily mean ozone	24 hour mean ozone
8 Jan	partly cloudy 23.2 - 30.5°C	0	12.8	2.6
9 Jan	cloudy/rain(0.7mm) 21.7 - 29.4°C	0	14.1	3.3
10 Jan	cloudy/rain(15.1mm) 18.8 - 24.7°C	0	4.5	1.4
11 Jan	cloudy/rain(19.0mm) 18.9 - 20.8°C	0	0.7	0.2
12 Jan	cloudy/rain(0.2mm) 18.8 - 25.2°C	0	15.0	2.3
13 Jan	partly cloudy 19.2 - 27.9°C	0	6.3	2.6
14 Jan	partly cloudy 19.9 - 29.1°C	0	10.4	3.4
15 Jan	sunny 23.4 - 30.4°C	0.2	8.8	3.2
16 Jan	partly cloudy/rain(0.1mm) 23.3 - 27.8°C	0	5.5	2.3
17 Jan	cloudy/rain(17.4mm) 21.0 - 23.2°C	0	2.0	0.5
18 Jan	partly cloudy/rain(0.1mm) 19.4 - 25.8°C	0	8.8	2.0
19 Jan	sunny 19.7 - 30.9°C	0	10.4	3.2
20 Jan	partly cloudy 22.0 - 28.1°C	0	26.3	4.6
21 Jan	partly cloudy 23.1 - 29.0°C	0	18.4	4.4
22 Jan	cloudy 23.9 - 31.0°C	0	8.3	3.0
23 Jan	sunny 22.0 - 28.0°C	0	8.4	1.7
24 Jan	sunny 20.1 - 31.3°C	0	10.5	3.3
25 Jan	sunny 23.6 - 31.0°C	0	8.3	3.0
26 Jan	partly cloudy/rain(40mm) 23.6 - 31.2°C	0	8.2	3.1
27 Jan	cloudy/rain(40.5mm) 20.1 - 23.1°C	0.3	3.4	2.0
28 Jan	cloudy 19.2 - 26.6°C	0.3	5.0	2.4
29 Jan	partly cloudy 19.0 - 27.2°C	0	3.1	1.0
30 Jan	partly cloudy 19.1 - 27.2°C	0	5.4	1.52
31 Jan	partly cloudy 20.3 - 27.3°C	0	7.5	2.6
Feb	cloudy/rain(7.4mm) 22.5 - 27.6°C	0	4.3	1.1

Table 3.1a continued

2 Feb	cloudy/rain(5.6mm) 20.8 - 26.9°C	0	9.3	1.9
3 Feb	partly cloudy 22.2 - 29.3°C	0	6.2	3.0
4 Feb	sunny 22.2 - 29.7°C	0.2	10.8	4.4
5 Feb	partly cloudy 23.4 - 28.9°C	0	10.4	2.4

*Weather conditions include amount of cloud cover (cloudy, partly cloudy and sunny), amount of rainfall, and minimum and maximum temperatures.

Table 3.1b: Summary of continuous photometric O_3 analyser data and weather conditions for the area around the University of Natal (UND) site in autumn (23 April to 28 May 1997).

Date	**Weather Conditions	Lowest 1 hour daily mean ozone	Highest 1 hour daily mean ozone	24 hour mean ozone
23 Apr	cloudy/rain(1.5mm) 19.3-24.0°C	0	5.8	16.7
24 Apr	*/* 12.9-24.0°C	0	8.4	18.9
25 Apr	*/* 12.8-22.6°C	0	14.6	3.8
26 Apr	*/* 14.5-26.6°C	0	17.4	5.6
27 Apr	cloudy/rain(4mm) 15.5-25.5°C	0.2	16.2	7.6
28 Apr	cloudy/rain(0.2mm) 17.3-21.5°C	1.0	9.5	5.3
29 Apr	*/* 13.5-21.6°C	0	8.9	5.5
30 Apr	*/* 13.3-22.1°C	0	7.7	2.9
1 May	sunny 12.6-24.3°C	0	18.7	6.8
2 May	partly cloudy/ rain(4.2mm) 12.1-24.4°C	0	14.6	6.7
3 May	partly cloudy/ rain(1.6mm) 14.1-22.9°C	0.2	6.8	3.4
4 May	partly cloudy 15.8-22.5°C	0.2	8.2	2.3
5 May	cloudy 13.8-20.9°C	0	4.9	2.3
6 May	partly cloudy 12.9-23.1°C	0	12.8	5.8
7 May	partly cloudy/ rain(0.2mm) 12.8-21.6°C	0	8.8	2.0
8 May	p.cloudy 15.8-23.6°C	0	7.6	2.6

Table 3.1b continued

9 May	partly cloudy/rain(6mm) 13.7-23.6°C	0	11.9	1.7
10 May	partly cloudy/ rain(0.2mm) 14.6-23.3°C	0	1.7	0.1
11 May	partly cloudy/ rain(0.3mm) 14.8-23.6°C	0	0	0
12 May	sunny 15.3-23.0°C	0	8.9	1.9
13 May	sunny 15.8-22.6°C	0	6.3	2.0
14 May	sunny 12.3-22.6°C	0	10.1	1.9
15 May	partly cloudy 12.6-23.8°C	0	10.9	4.2
16 May	sunny 12.7-22.7°C	0	8.8	3.7
17 May	sunny 11.4-26.8°C	0.8	19.7	6.4
18 May	sunny 11.7-26.7°C	0	15.2	5.0
19 May	sunny 11.9-27.4°C	0	22.2	8.8
20 May	cloudy 13.0-24.5°C	0.3	14.4	4.9
21 May	partly cloudy 15.5-25.0°C	0	11.7	3.3
22 May	partly cloudy 13.5-24.0°C	0	8.9	2.6
23 May	sunny 14.5-25.7°C	0	17.5	6.5
24 May	partly cloudy 18.2-26.9°C	0	20.6	4.6
25 May	cloudy/rain(3.7mm) 17.2-23.4°C	0	20.5	4.2
26 May	cloudy/rain(0.5mm) 17.5-22.2°C	0	11.3	1.8
27 May	partly cloudy/ rain(2.8mm) 11.2-22.4°C	0	10.9	5.0
28 May	cloudy/rain(12mm) 11.5-16.0°C	1.0	11.3	6.8

*Data unavailable from the Weather Bureau

**Weather conditions include amount of cloud cover (cloudy, partly cloudy and sunny), amount of rainfall, and minimum and maximum temperatures.

Table 3.1c: Summary of continuous photometric O_3 analyser data and weather conditions for the area around the University of Natal (UND) site in winter (23 July to 20 August 1997).

Date	*Weather Conditions	Lowest 1 hour daily mean ozone	Highest 1 hour daily mean ozone	24 hour mean ozone
23 Jul	sunny 11.96-27.5^{0}C	0	13.3	5.9
24 Jul	partly cloudy 13.4-22.5^{0}C	0	8.8	1.4
25 Jul	cloudy/rain (0.8mm) 12.8-21.9^{0}C	0	10.9	4.3
26 Jul	partly cloudy 13.8-20.3^{0}C	0.5	17.9	7.5
27 Jul	partly cloudy/ rain(17.6mm) 9.7-21.4^{0}C	0	14.4	5.2
28 Jul	cloudy/rain (9.2mm) 13.8-16.2^{0}C	0	11.1	4.9
29 Jul	partly cloudy 12.6-19.8^{0}C	0	13.0	4.7
30 Jul	partly cloudy/ rain(0.1mm) 12.8-21.8^{0}C	0	12.7	6.8
31 Jul	partly cloudy/ rain(44.2mm) 12.1-21.5^{0}C	0	9.7	3.4
1 Aug	partly cloudy 15.0-21.3^{0}C	0	17.5	3.4
2 Aug	cloudy/rain(0.6mm) 13.4-21.3^{0}C	0	13.9	6.1
3 Aug	sunny 9.6-21.2^{0}C	1.1	15.3	9.2
4 Aug	sunny 8.7-22.2^{0}C	0	15.7	6.4
5 Aug	cloudy/rain(3.2mm) 12.9-20.3^{0}C	0.7	7.1	4.4
6 Aug	partly cloudy 14.3-20.6^{0}C	0	11.0	5.0
7 Aug	partly cloudy/ rain0.7mm) 8.3-20.3^{0}C	0.8	13.9	6.0
8 Aug	sunny 11.6-23.1^{0}C	0.2	13.7	4.5
9 Aug	cloudy 8.9-23.1^{0}C	0.5	15.5	7.1
10 Aug	partly cloudy 13.3-24.9^{0}C	0.9	21.2	6.4
11 Aug	partly cloudy 15.4-21.5^{0}C	0	9.4	2.9
12 Aug	partly cloudy 9.6-25.2^{0}C	0	11.2	4.1
13 Aug	sunny 11.3-23.2^{0}C	0	3.8	1.2
14 Aug	partly cloudy 12.6-22.8^{0}C	0	2.8	0.9
15 Aug	sunny 13.4-23.8^{0}C	0	1.4	0.2

Table 3.1c continued

16 Aug	sunny 13.7-32.8°C	0	0.9	0.1
17 Aug	partly cloudy 18.3-23.1°C	0	0.3	0.1
18 Aug	partly cloudy 13.7-24.1°C	0	0.2	0
19 Aug	sunny 13.8-36.1°C	0	40.0	5.5
20 Aug	cloudy/rain(10.4mm) 14.0-21.3°C	0	12.9	5.4

*Weather conditions include amount of cloud cover (cloudy, partly cloudy and sunny), amount of rainfall, and minimum and maximum temperatures.

Table 3.1d: Summary of continuous photometric O_3 analyser data and weather conditions for the area around the University of Natal (UND) site in spring (22 September to 6 October 1997).

Date	*Weather Conditions	Lowest 1 hour daily mean ozone	Highest 1 hour daily mean ozone	24 hour mean ozone
22 Sep	cloudy 17.4-22.9°C	0	15.1	2.9
23 Sep	cloudy/rain(5mm) 19.8-24.8°C	0	13.5	4.5
24 Sep	cloudy 18.0-23.7°C	0	15.3	7.4
25 Sep	partly cloudy 16.2-24.7°C	0	14.3	6.2
26 Sep	partly cloudy 16.2-25.1°C	0	19.8	8.9
27 Sep	partly cloudy 18.0-25.1°C	0	16.1	6.3
28 Sep	partly cloudy 17.5-24.3°C	0	21.6	4.4
29 Sep	cloudy/rain(38.5mm) 18.5-25.4°C	2.1	16.7	7.6
30 Sep	cloudy/rain(7.6mm) 16.2-22.6°C	0	11.9	6.3
1 Oct	partly cloudy 13.9-24.3°C	0	13.8	5.6
2 Oct	partly cloudy 17.8-26.9°C	0	15.2	6.6
3 Oct	partly cloudy 20.3-33.2°C	0	32.0	10.8
4 Oct	partly cloudy 18.1-32.7°C	0	36.7	14.3
5 Oct	partly cloudy 20.1-27.3°C	3.6	25.5	10.4
6 Oct	partly cloudy 17.8-25.4°C	0.5	17.0	8.0
7 Oct	cloudy/rain(39.2mm) 16.8-19.7°C	0	8.5	3.0
8 Oct	cloudy/rain(16.6mm) 13.2-15.8°C	0	6.3	3.2

Table 3.1d continued

9 Oct	cloudy 13.5-21.2°C	0.5	9.3	5.1
10 Oct	sunny 15.3-24.1°C	0..6	14.5	8.5
11 Oct	cloudy/rain(3.2mm) 17.6-22.5°C	0	11.6	5.8
12 Oct	cloudy/rain(13.7mm) 16.0-19.9°C	0	7.2	4.3
13 Oct	cloudy/rain(3.2mm) 16.3-21.9°C	0	9.1	4.7
14 Oct	cloudy/rain(19.3mm) 18.2-23.3°C	0.1	11.5	6.1
15 Oct	cloudy/rain(45.6mm) 17.1-19.3°C	0	9.7	3.7
16 Oct	cloudy/rain(0.9mm) 14.9-19.8°C	0	9.9	4.9
17 Oct	partly cloudy 14.7-22.3°C	0	12.1	5.6
18 Oct	sunny 15.1-23.8°C	0.8	14.0	7.1
19 Oct	sunny 14.7-24.8°C	0.9	15.8	7.4
20 Oct	sunny 14.2-27.8°C	0	17.8	5

*Weather conditions include amount of cloud cover (cloudy, partly cloudy and sunny), amount of rainfall, and minimum and maximum temperatures.

were mostly low ranging from 0.2 ppb to 21.2 ppb, except on the 19 August where the highest one-hour mean was 40.0 ppb. The weather on this day was sunny and the maximum temperature was high (36.1^{0}C) compared to the most of this sampling period. However, similar weather conditions were experienced on 16 August (sunny with a maximum temperature of 32.8^{0}C) but the highest one-hour O_3 concentration was only 0.9 ppb.

The concentrations during the spring (22/9/1997 to 6/10/1997) sampling period were relatively higher than during the other three sampling periods. The weather conditions during this period appeared to be quite conducive to O_3 formation. The rainfall was low, although partly cloudy conditions were experienced throughout this sampling period. The temperatures were relatively high, with the maximum temperatures ranging from 22.6^{0}C to 33.2^{0}C. The highest daily one-hour mean concentrations ranged from 11.9 ppb to 36.6 ppb, and the 24-hour daily means ranged from 2.9 ppb to 14.3 ppb.

The data in Table 3.1 were summarised and are shown in Table 3.2. This summary table relates to the statistical analysis performed. Kruskal-Wallis tests, performed on the data in Table 3.1, indicated that there were no significant differences ($p>0.05$) in the lowest daily one-hour mean concentrations of O_3 between the four different sampling periods. An ANOVA showed however, that there were significant differences in the highest daily one-hour mean concentrations. A Scheffe multiple range test further indicated that the highest daily one-hour mean O_3 concentrations during the spring sampling period were significantly higher ($p<0.001$) than the other three sampling periods. This difference is evident in Table 3.2. There were no significant differences between the other three sampling periods. An ANOVA indicated that

there were significant differences ($p<0.01$) in the 24-hour mean concentrations between the sampling periods. A Scheffe multiple range test showed that the daily 24-hour mean O_3 concentrations were higher during the autumn sampling period than during the summer sampling period. Such an increase in concentration from summer to autumn is surprising since temperatures were lower in autumn and, therefore, less conducive to O_3 formation. There were, however, no significant differences ($p>0.05$) in the 24-hour means measured between the summer and winter sampling periods, or between the autumn and winter sampling periods. The 24-hour daily mean during the spring sampling period was significantly higher ($p<0.001$) than the other three sampling periods (Table 3.2).

Table 3.2: Ozone concentrations for the four sampling periods, summer, autumn, winter, and spring, displayed as the means of the lowest one-hour mean monthly O_3, highest one-hour mean monthly O_3, and the 24 hour monthly mean O_3. The ranges are displayed in parentheses beneath the mean values.

Sampling Period	Mean of the lowest 1-hour O_3 concentration for each day of sampling period	Mean of the highest 1-hour O_3 concentration for each day of sampling period	Mean of the 24 hour O_3 concentration for each sampling period
Summer	0.04	8.7	2.5
(n=29)*	(0-0.3)	(0.7-26.3)	(0.2-4.6)
Autumn	0.1	11.5	4.8
(n=36)	(0-1.0)	(0-20.6)	(0-18.9)
Winter	0.2	11.7	4.3
(n=29)	(0-1.1)	(0.2-40.0)	(0-9.2)
Spring	0.4	19.0	7.3
(n=15)	(0-3.6)	(11.9-36.7)	(2.9-14.3)

* n is the number of days in the sampling period.

Correlation analysis was carried out in order to relate the O_3 levels obtained from the continuous monitor and the recorded weather conditions. The actual mean daily cloud cover values, measured as oktas (amount of sky covered by clouds measured in eighths) were

calculated to use in this correlation. Table 3.3a and b show the correlation values obtained. There were significant positive correlations with maximum daily temperature for the highest O_3 concentrations expressed as one hour daily means in summer, autumn and spring (Table 3.3a). This correlation was highly significant in spring ($p<0.001$). The 24 hour daily mean O_3 concentrations were also positively significantly correlated with maximum daily temperature in summer ($p<0.001$) and spring ($p<0.01$) (Table 3.3b). Rainfall was of little significance except for the one hour daily mean value in summer when a barely significant negative correlation coefficient of -0.37 ($p=0.5$) was obtained. Cloud cover also only reached a significant negative correlation coefficient in summer for the 24 hour daily mean values. There were no observable significant correlations between the mean daily O_3 values and the recorded weather conditions during the winter sampling period.

Table 3.3a: Correlation values calculated for the highest daily one hour mean O_3 values and the recorded weather conditions (maximum temperature, the amount of rain, and cloud cover), for each of the four sampling periods. Significant differences are indicated as follows: * represents $p\leq0.05$, ** represents $p\leq0.01$, and *** represents $p\leq0.001$.

Sampling period	Maximum temperature	Rainfall	Cloud cover
Summer (n=29)	0.44*	-0.37*	-0.15
Autumn (n=36)	0.52**	0.10	-0.12
Winter (n=28)	0.28	0.04	-0.23
Spring (n=15)	0.90***	-0.17	-0.40

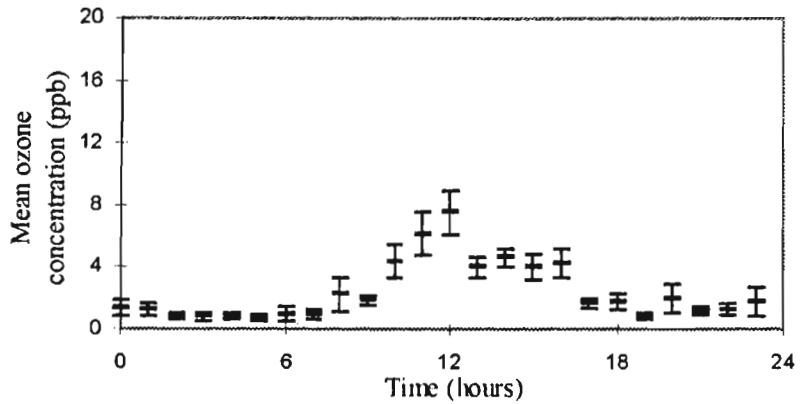
It would appear that maximum daily temperature had the greatest influence of the three measured meteorological variables especially in spring and summer when the O_3 concentrations were the lowest, in summer, and highest, in spring, (Table 3.2). During winter, when the mean

values were intermediate between summer and spring, meteorological conditions seemed to have little direct influence on the daily O_3 concentrations.

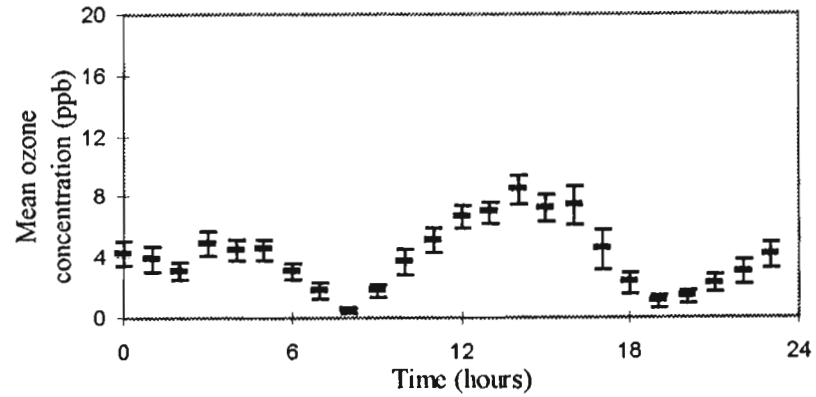
Table 3.3b: Correlation values calculated for the daily 24 hour mean O_3 values and the recorded weather conditions (maximum temperature, the amount of rain, and cloud cover), for each of the four sampling periods. Significant differences are indicated as follows: * represents $p \leq 0.05$, ** represents $p \leq 0.01$, and *** represents $p \leq 0.001$.

Sampling period	Maximum temperature	Rainfall	Cloud cover
Summer (n=29)	0.70***	-0.31	-0.41*
Autumn (n=36)	0.23	0.20	0.01
Winter (n=28)	-0.23	0.13	-0.05
Spring (n=15)	0.82***	-0.04	-0.42

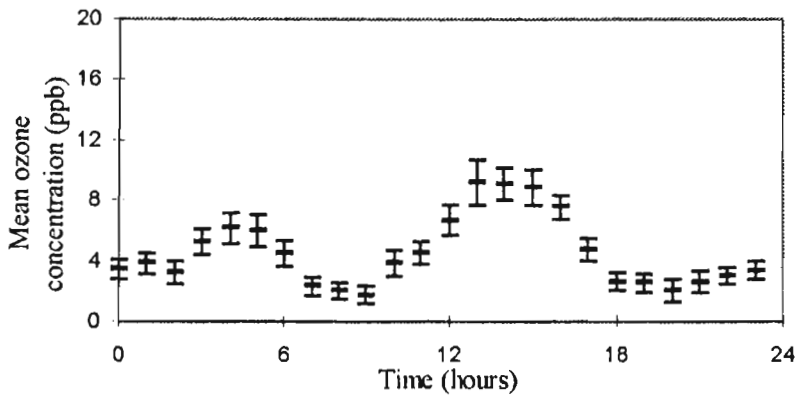
The mean diurnal O_3 concentrations for each of the four sampling periods were calculated from the Dasibi data (Figure 3.1). The summer data (Figure 3.1a) showed the expected simple pattern of a single maximum peak at midday of a mean value of 7.8 ppb and low night concentrations (0.1 ppb). The autumn (Figure 3.1b) and winter (Figure 3.1c) data showed relatively high night time peaks (4.9 ppb and 6.3 ppb respectively) with a sharp decline in the early morning, increasing again during the day to 8.8 ppb in the autumn sampling period and 11 ppb in the winter sampling period. In autumn and winter, the day time peak occurred at approximately one to two hours after midday, whereas during the summer and spring sampling period this peak occurred at midday. During the spring sampling period there was the highest observed midday peak of 18.2 ppb (higher than during the other sampling periods) and no significant early morning peaks as in autumn and winter.



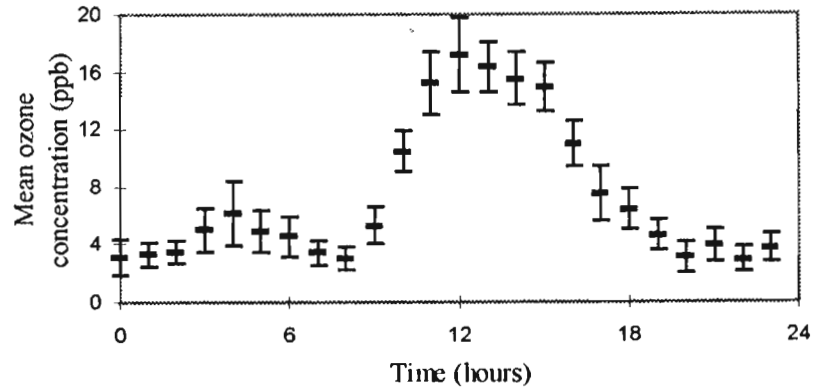
a. 8 January to 5 February (summer)



b. 23 April to 28 May (autumn)



c. 23 July to 6 August (winter)



d. 22 August to 6 October (spring)

Figure 3.1: Mean diurnal ozone concentrations (ppb) for each period of sampling, summer, autumn, winter and spring, measured with the continuous monitor. Standard errors shown as vertical bars.

Day:night ratios of O_3 concentration were also calculated from the Dasibi data for each of the four sampling periods. These ratios are shown in Table 3.4. It was observed that the day:night ratios were highest during summer and spring and lowest during autumn and winter.

Table 3.4: Ratios between the daytime (6h00 to 18h00) O_3 concentrations and night (18h00 to 6h00) O_3 concentrations for each of the sampling periods, summer, winter, autumn and spring.

Sampling period	Day:night ratio
summer	3.45
autumn	1.46
winter	1.43
spring	2.60

It is interesting to note that the positive correlation between the maximum daily temperatures and 24 hour mean O_3 concentrations seemed to be important in summer and spring (Table 3.3) when the pre-dawn early morning peaks were absent (Figure 3.1a and d) and the day:night ratios (Table 3.4) in mean O_3 concentration were greater.

3.1.2 Passive diffusion tube measurements

3.1.2.1 Ozone

Passive diffusion tubes were used to measure O_3 concentrations at each of the four sites (University of Natal (Durban), Botanic Gardens, Kloof, and Mooi River). The mean O_3 concentrations and standard errors, measured at the four sites during the three sampling periods are shown in Figure 3.2. Within each sampling period, measurements were taken fortnightly, therefore there are two mean measurements for each sampling period and six in total. An analysis of variance of the data obtained for O_3 diffusion tubes indicated that there were no significant differences ($p>0.05$) in O_3 concentrations between the four sites. These results can

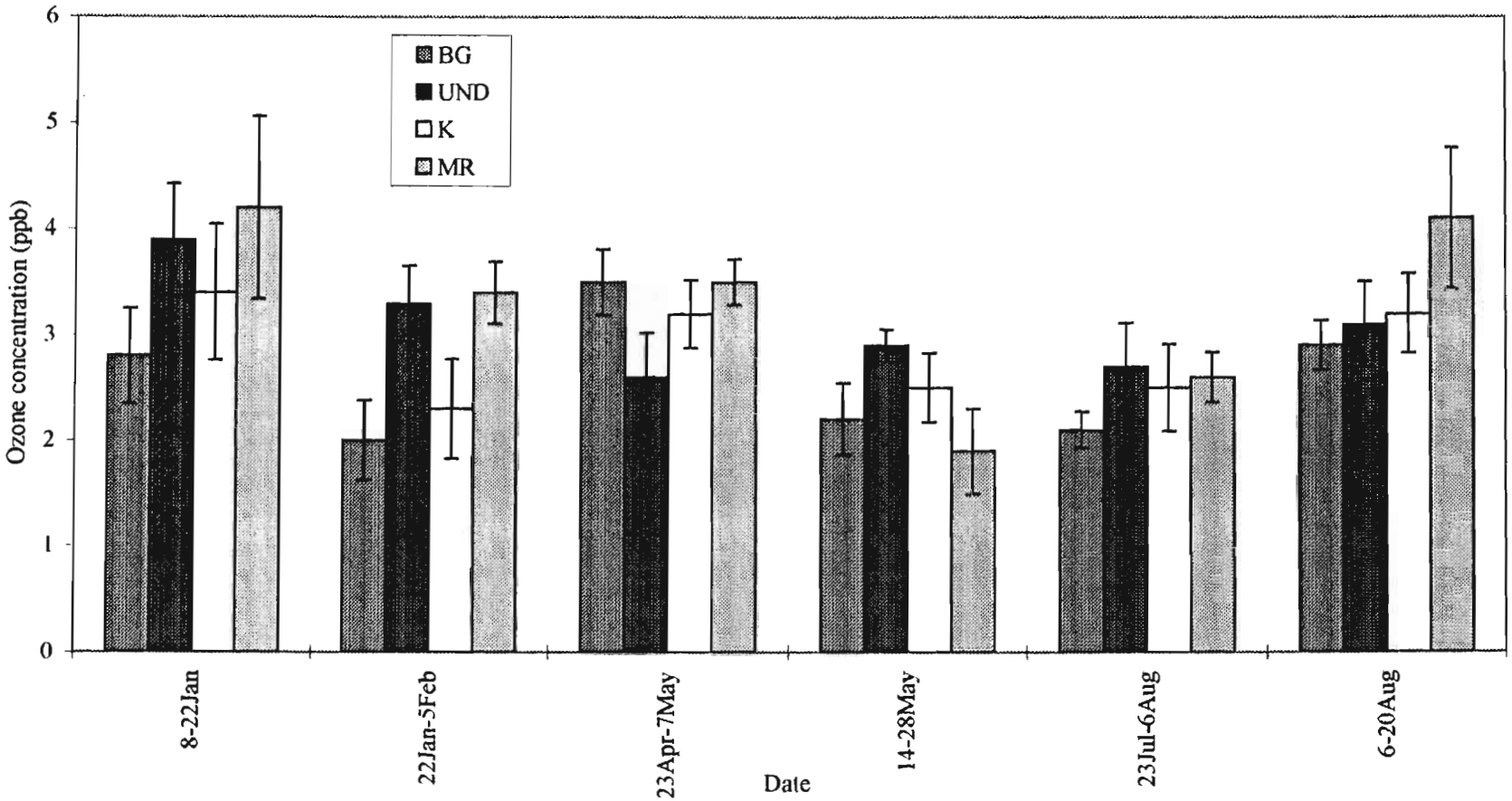


Figure 3.2: Mean ozone concentrations (ppb) measured in the KwaZulu-Natal region using passive diffusion tubes, standard errors shown as vertical bars (n=4). BG=Botanic Gardens site, UND=University of Natal Site, K=Kloof site, and MR=Mooi River site.

also be inferred from Figure 3.2 where there is overlap in the standard errors of the mean values. There was also no significant difference ($p>0.05$) in O_3 concentration between the sampling periods.

During the Mpumalanga study, measurements were taken simultaneously at the UND site in Durban to use as a comparison with the Mpumalanga data (Figure 3.11). The O_3 concentrations measured with the passive diffusion tubes during the Mpumalanga study were also compared to the O_3 concentrations measured at the UND site during the other sampling periods (summer, autumn and winter). Analysis of variance showed that there were no significant differences ($p>0.05$) between the O_3 concentrations in the spring sampling period and the other three sampling periods.

The results obtained using the passive diffusion tubes at the UND site were compared to those obtained using the continuous O_3 monitor. Figure 3.3 shows the mean O_3 concentration data obtained from the continuous O_3 monitor as well as that obtained from the diffusion tubes at the UND site, for each two week period of sampling within the sampling months. The mean ratio of continuous monitor/passive sampler values was calculated as being 1.3, but this was not a constant error (Figure 3.3). Interestingly a correlation analysis showed that there was a significant negative correlation between the two data sets ($r= -0.9$; $p<0.01$). Figure 3.4 is a scatter plot which clearly shows this negative correlation. It might be expected for there to be a 'saturation effect' in the diffusion tube data set and thus lower accuracy at higher atmospheric O_3 concentrations. However, this does not explain the decline in O_3 values measured with the diffusion tubes, as the continuous monitor values increased. The diffusion tubes recorded

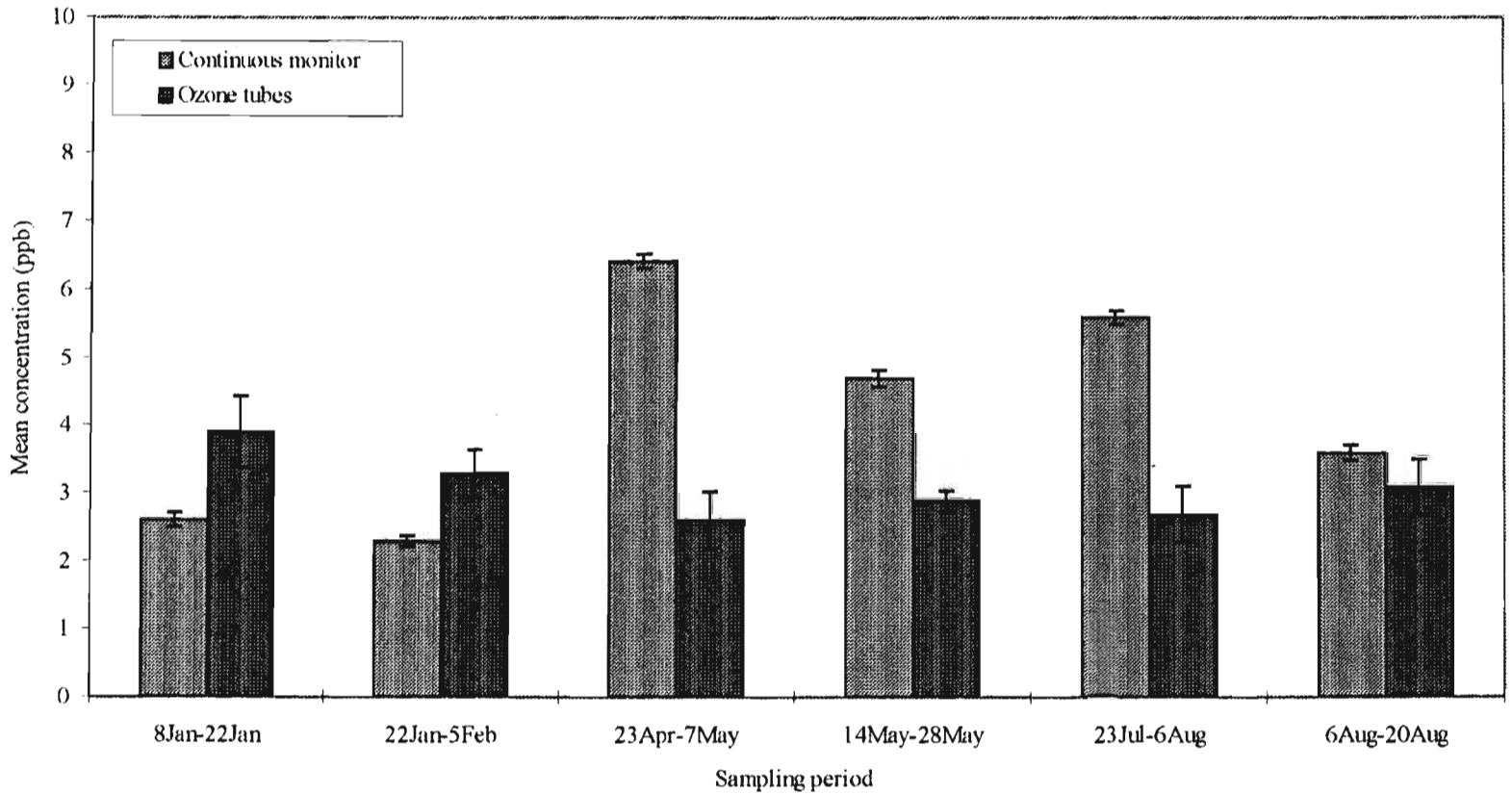


Figure 3.3 :Mean ozone concentrations obtained from the continuous monitor and the passive diffusion tubes at the UND site. Data expressed as mean daily ozone concentrations and standard errors represented as vertical bars.

relatively higher values during summer and lower values during autumn and winter. This simply may be an effect of the little variation in the diffusion tube mean values (Figure 3.3) and that they were inefficient at higher O_3 concentrations. It could also indicate that summer conditions, for example very high humidity, could also have an effect. Thus there is considerable discrepancy between the data obtained for the two different methods of O_3 sampling.

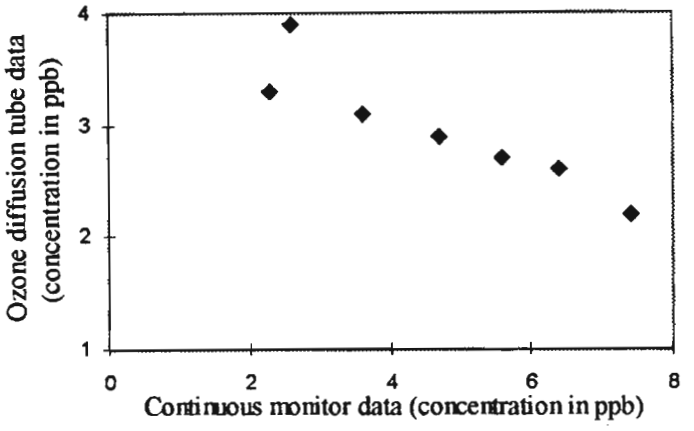


Figure 3.4: Scatter plot of continuous ozone monitor data versus ozone diffusion tube data. Concentrations expressed as mean ozone concentration per day for each of the two week sampling periods.

Coefficients of variation were calculated for the data obtained from the O_3 diffusion tubes (Table 3.5). The coefficients of variation were highest during the first week of sampling (ranging from 22.7% to 42.9%). These high coefficients of variation are probably due to some exposure of the discs of the passive monitors during preparation of the discs, since O_3-free chambers were not available in which to prepare the samplers. For the rest of the sampling periods the coefficients of variation were relatively lower, ranging from 7.8% to 33%. Thus

precision was improved even though there was a lack of O_3-free chambers during preparation and analysis of all the samplers.

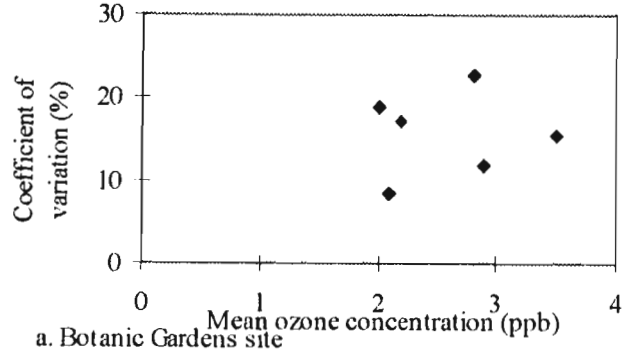
Table 3.5: Coefficients of variation (%) for the mean O_3 concentrations calculated for each sampling period at each site (n=4).

Date	BG	UND	K	MR
8Jan-22 Jan	22.7	26.4	32.2	42.9
22Jan-5Feb	18.8	17.6	23.6	14.6
23Apr-7May	15.5	21.1	16.7	10.7
14May-28May	17.1	7.8	16.4	20.0
23Jul-6Aug	8.6	20.8	20.6	11.8
6Aug-20Aug	11.8	20.4	18.9	33.0

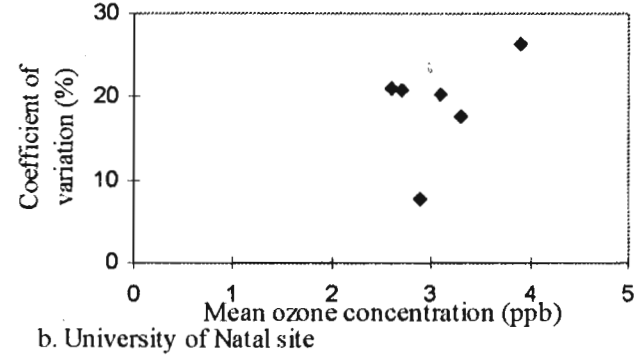
The coefficients of variation, calculated above, were plotted against the mean O_3 concentrations (Figure 3.5), to observe whether or not there was an increase in the coefficient of variation as the mean concentration increased. An increase in the coefficient of variation with increasing mean O_3 concentration would imply a lack of precision in this method. It was observed that there was an increase in the coefficient of variation as the mean concentration increased for all the sites except the Botanic Gardens site. It can, therefore, be concluded that the diffusion tubes probably lacked acceptable precision and accuracy.

3.1.2.2 NO_2

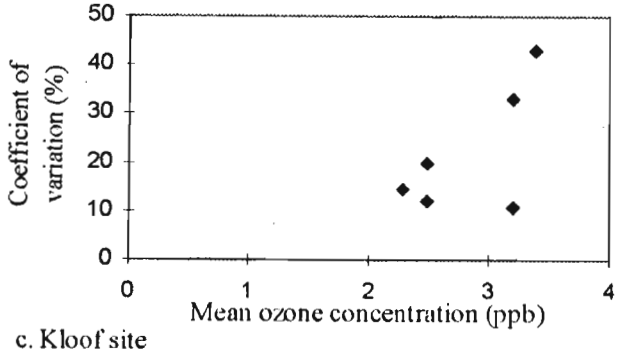
The mean concentrations of NO_2 as well as the standard errors of these concentrations, measured at the four sites during the three sampling periods, are shown in Figure 3.6. Within each sampling period, measurements were taken fortnightly, therefore, there are two mean



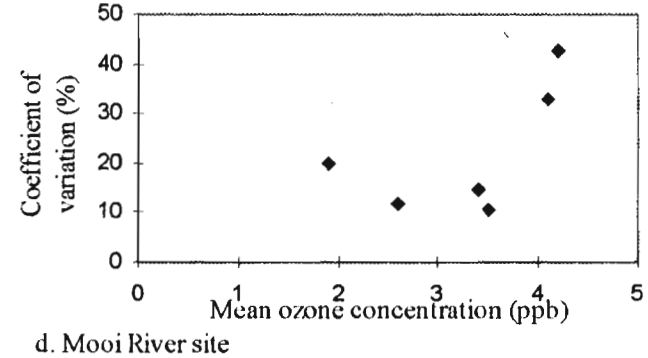
a. Botanic Gardens site



b. University of Natal site



c. Kloof site



d. Mooi River site

Figure 3.5: Coefficients of variation plotted against the means of the O_3 concentrations measured with the passive diffusion tubes, for each site during each sampling period, summer, autumn, and winter.

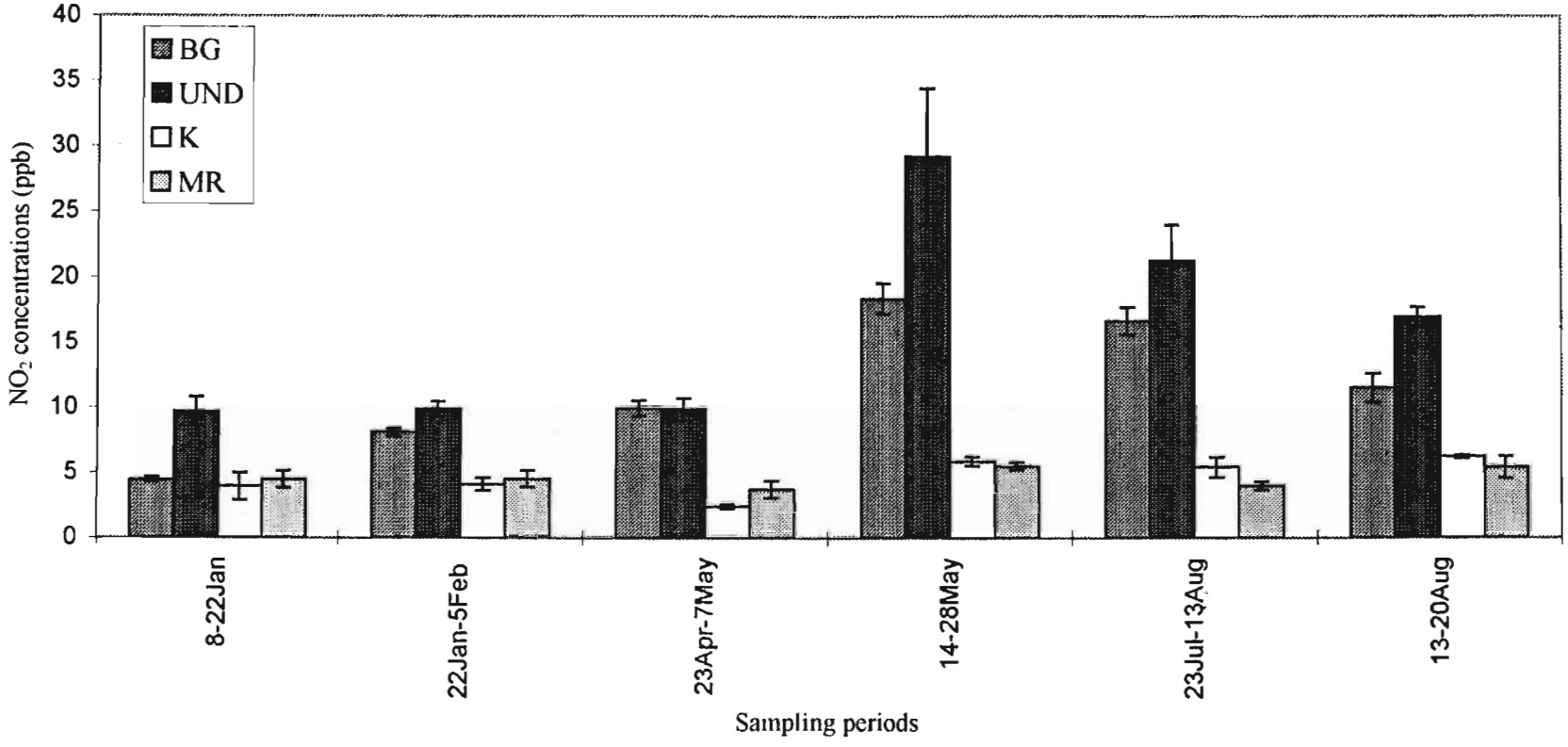


Figure 3.6 Mean NO_2 concentrations (ppb) measured in the KwaZulu-Natal region using passive diffusion tubes, standard errors shown as vertical bars (n=4). BG=Botanic Gardens, UND=University of Natal, K=Kloof, amd MR=Mooi River.

measurements for each sampling period and six in total. A multiple range test of the data showed that the mean NO_2 levels at the UND site were significantly higher ($p<0.001$) than those at the Botanic Gardens, Kloof and Mooi River sites, and that the NO_2 levels at the Botanic Gardens site were significantly different ($p<0.001$) to the UND, Kloof and Mooi River sites, being less than those at the UND site but higher than the mean NO_2 concentrations at the Kloof and Mooi River sites. There were no significant differences ($p>0.05$) between the NO_2 levels at the Kloof and Mooi River sites. Therefore, the levels of NO_2 were highest at the two urban sites, that is at the Botanic Gardens and UND sites. It would, therefore, appear that there were spatial variations in the NO_2 concentrations in the Greater Durban area, with the higher concentrations being measured closer to the city centre.

There were no significant differences ($p>0.05$) in NO_2 concentration between the different sampling periods. There was also no significant differences between the NO_2 concentration data, obtained at the UND site during spring (Figure 3.12), and the data obtained at this site during the other three sampling periods (summer, autumn and winter).

Correlation analysis indicated that there was a significant positive correlation in the NO_2 concentration data obtained for the Botanic Garden and UND sites ($r=0.92$, $p<0.01$), these sites both being situated near to the city centre. Table 3.6 is a correlation matrix for the data obtained from all the sites, for all the six sampling periods.

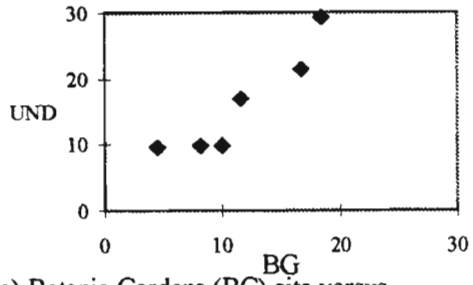
Table 3.6: Correlation matrix of the mean NO_2 concentrations observed at each of the four sites over the three sampling periods (n=6), where a significant correlation value ($p<0.05$) is indicated by * .

	Botanic Gdns	UND	Kloof	Mooi River
Botanic Gdns		0.92*	0.60	0.30
UND			0.75	0.56
Kloof				0.77

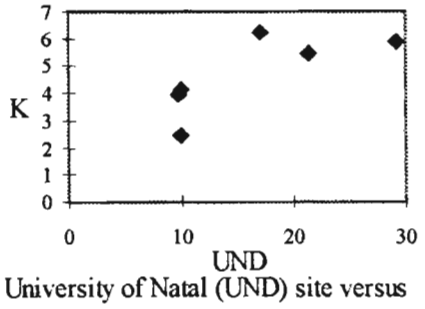
Scatter plots (Figure 3.7a-f) of these data for paired comparison between these four sites show general positive relationships between the sites but more data points are required to make conclusions about any relationships between NO_2 concentrations at the four sites.

Analysis of variance (performed on all the measured data for all four sites) indicated that NO_2 concentrations differed significantly ($p<0.001$) between the summer sampling period and the other sampling periods. A multiple range test showed that the mean concentration during the summer (8/01/1997 to 5/02/1997) sampling period was lower than the concentrations during the autumn (23/04/1997 to 28/05/1997) and winter (23/07/1997 to 20/08/1997) sampling periods. Thus there appeared to be a seasonal difference in the NO_2 concentrations, with the summer concentrations being significantly lower than the autumn and winter concentrations.

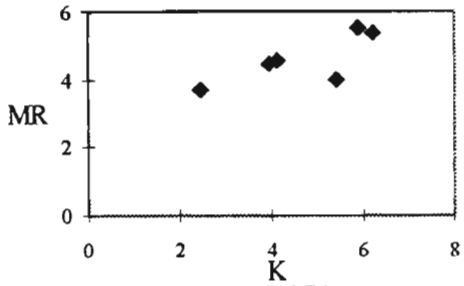
Coefficients of variation were calculated for each site during each sampling period (Table 3.7). The coefficients of variation were larger in the NO_2 diffusion tubes than they were in the O_3 diffusion tubes, ranging from 5% to 261.5%, with two exceptionally high values being calculated for the UND site (261.5% and 134.4%). The relatively large standard errors



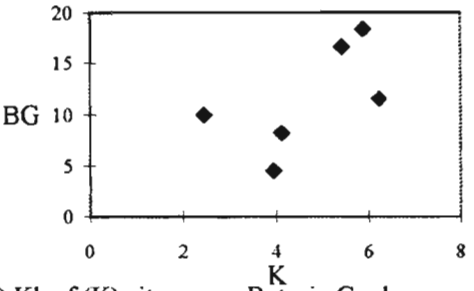
a) Botanic Gardens (BG) site versus University of Natal (UND) site.



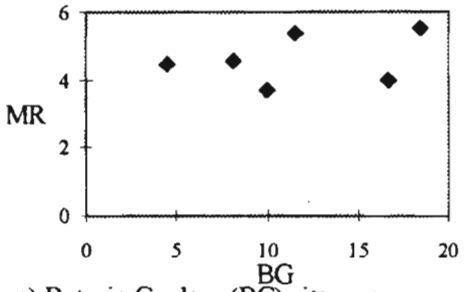
b) University of Natal (UND) site versus Kloof (K) site.



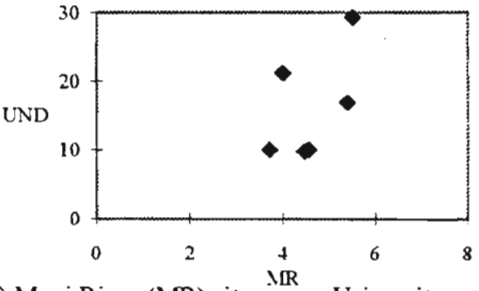
c) Kloof (K) site versus Mooi River (MR) site.



d) Kloof (K) site versus Botanic Gardens (BG) site.



e) Botanic Gardens (BG) site versus Mooi River (MR) site.



f) Mooi River (MR) site versus University of Natal (UND) site.

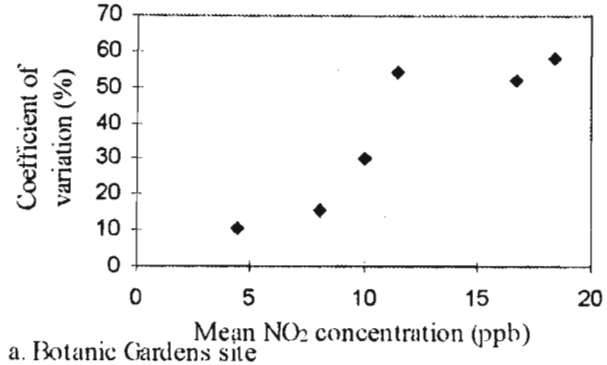
Figure 3.7: Scatter plots of the mean daily NO_2 concentrations, measured with the passive diffusion tubes, for paired comparison between the four sites.

calculated for the data as well as the large coefficients of variation would imply a lack in precision in this method.

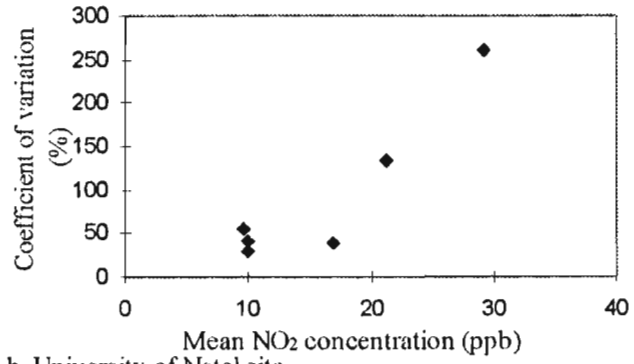
Table 3.7: Coefficients of variation (%) for the mean NO_2 concentrations calculated for each sampling period at each site (n=4).

Date	BG	UND	K	MR
8Jan-22 Jan	10.4	56.5	52.7	33.3
22Jan-5Feb	15.5	29.9	23.9	30.8
23Apr-7May	30.2	41.6	8.5	33.3
14May-28May	58.5	261.5	17.6	15.4
23Jul-6Aug	52.2	134.4	37.8	16.9
6Aug-20Aug	54.3	38.6	5.0	41.6

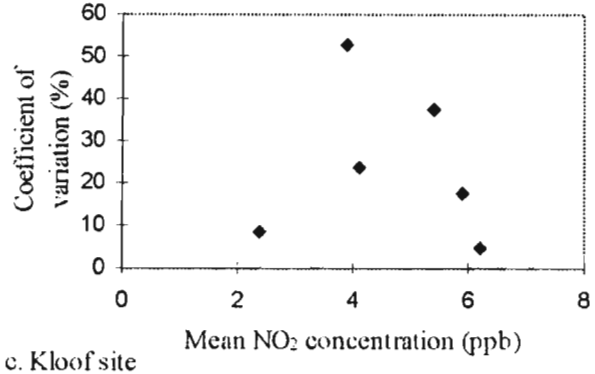
The coefficients of variation were plotted against the mean NO_2 concentrations (Figure 3.8). It was observed at the Botanic Gardens site and the UND site that as the mean NO_2 concentration increased so the coefficients of variation increased. There was, however, no increase in the coefficients of variation as the concentration increased at the Kloof and Mooi River sites. This could be explained by the fact that the measured concentrations were very low at the Kloof and the Mooi River sites (ranging between 5 and 7 ppb), whereas they were relatively high at the Botanic Gardens site and the UND site (ranging between 17 and 30 ppb). Thus, when the concentrations were high the coefficients of variation increased implying a lack of precision when concentrations were high.



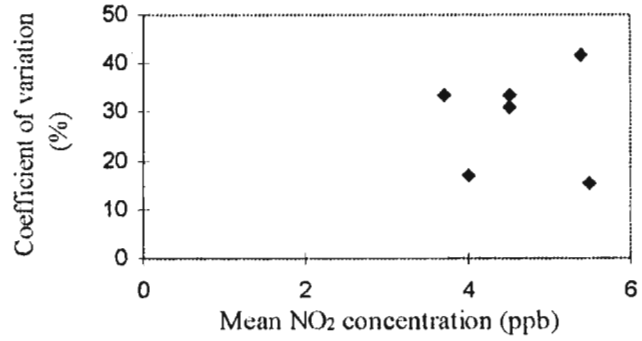
a. Botanic Gardens site



b. University of Natal site



c. Kloof site



d. Mooi River site

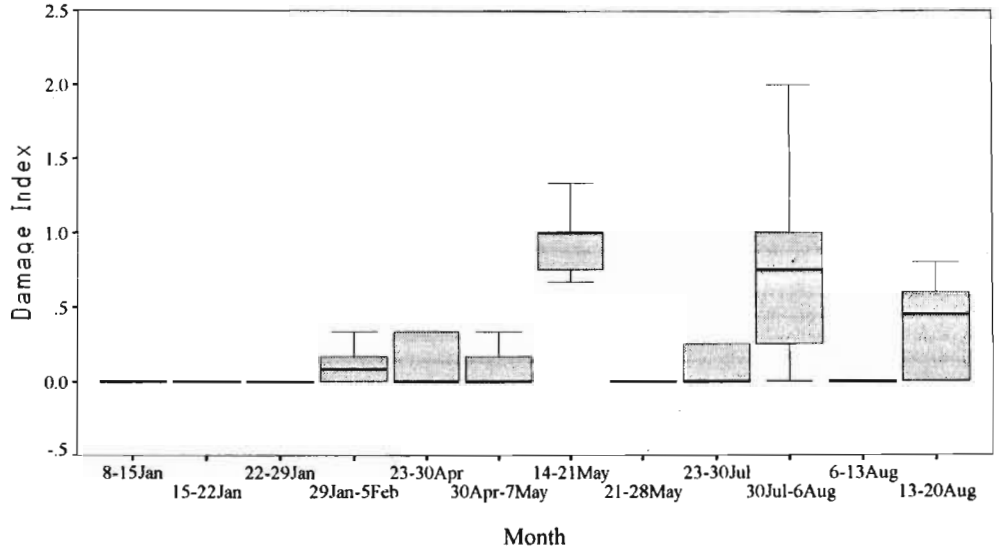
Figure 3.8: Coefficients of variation plotted against the mean concentrations of NO_2 measured with the passive diffusion tubes at each of the sites during each sampling period, summer, autumn, and winter.

3.1.3 Bioindicators

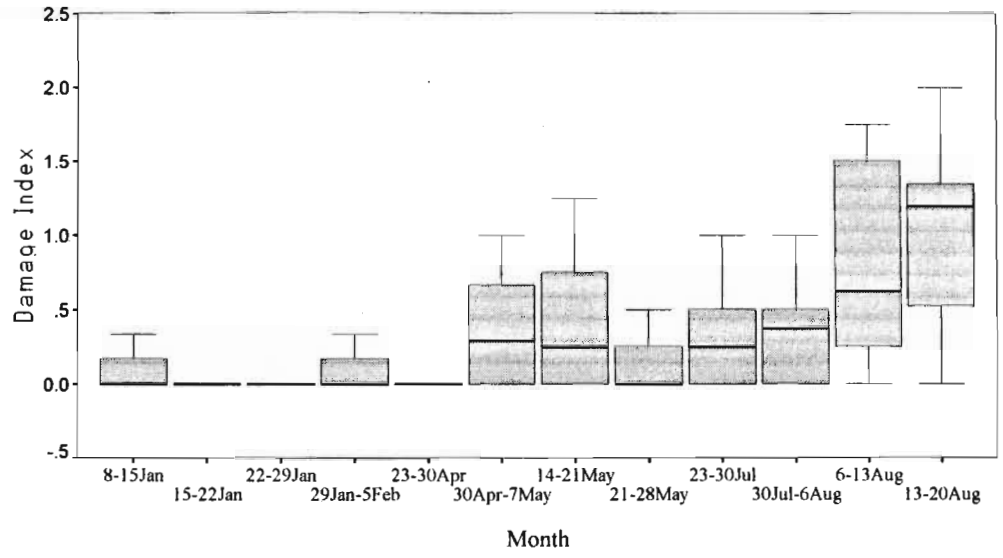
3.1.3.1 Ozone phytotoxicity

The leaf injury data were initially expressed as the percentage of leaves in each damage category, obtained for both the plants that were placed at each of the sites when two weeks old (subsequently called 'two week old plants') and those that were germinated at each of the sites for the three sampling periods. These data were converted to damage index values (refer to Table 2.1 and Section 2.4.1). The mean damage index values are shown in Figure 3.9 a-d for the two week old plants and Figure 3.10 a-d for the plants germinated at the exposure site. In Figures 3.9 and 3.10 it can be observed that the highest damage index values were obtained at the UND and Kloof sites, the damage index values were relatively lower at the Botanic Gardens site and were the lowest at the Mooi River site. The increase in the damage index value is a multiplicative one, with the scale increasing geometrically and not linearly. This means that the actual visible damage occurring on plants with a mean damage index of 2 is much greater than twice the visible damage occurring on plants with a mean damage index of 1.

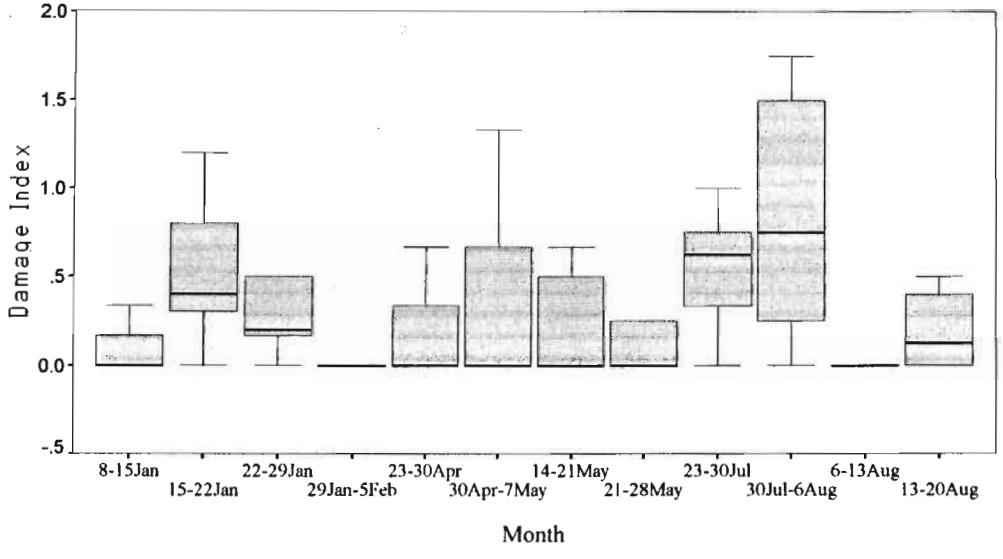
The calculated damage index values obtained from the two week old plants and those germinated at each site were analysed separately. A Kruskal-Wallis test showed that there were no significant differences ($p>0.05$) in the damage index values between the four sites for the two week old plants within the sampling periods. A Kruskal-Wallis test showed, however, that there were a significant differences ($p<0.05$) in those plants germinated at the sites. A Mann-Whitney U test showed that the plants at the UND and Kloof sites had the highest mean damage, the plants at the Botanic Gardens site had less damage, and the plants at the Mooi River site had the least damage.



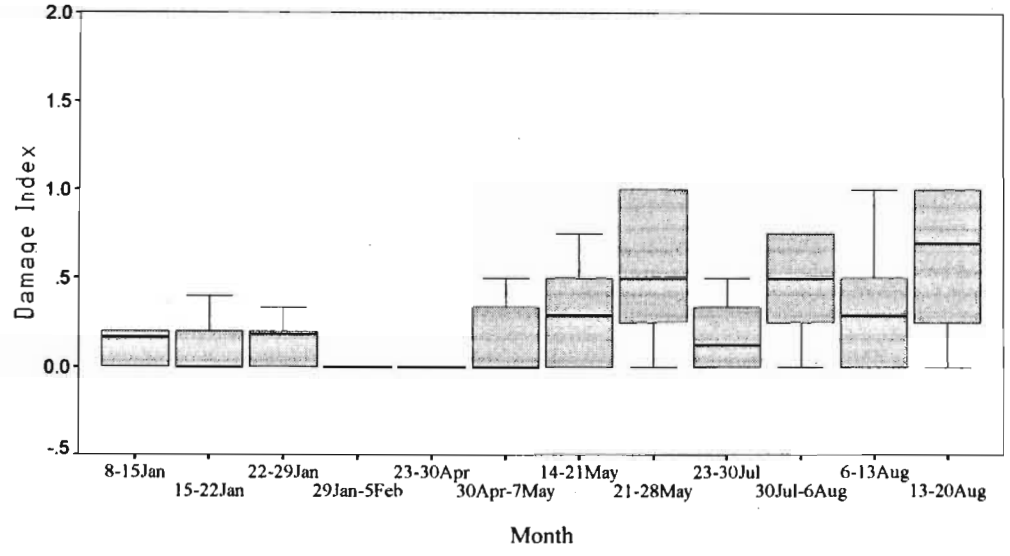
a. Botanic Gardens site



b. University of Natal site

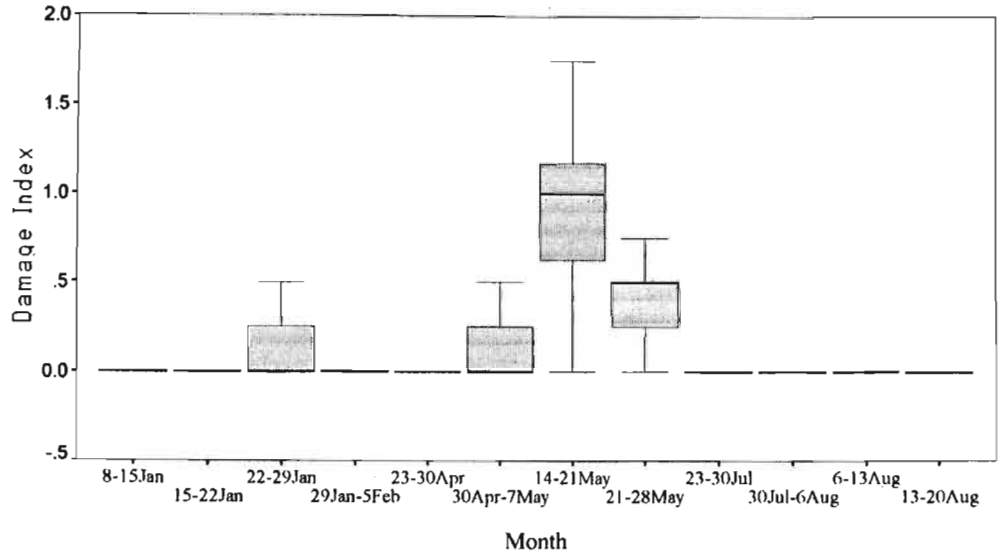


c. Kloof site

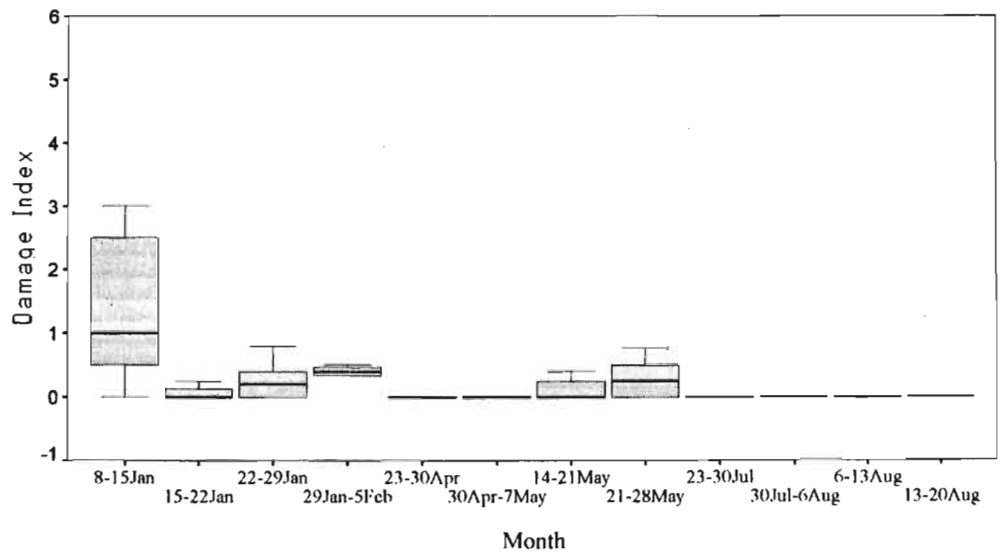


d. Mooi River site

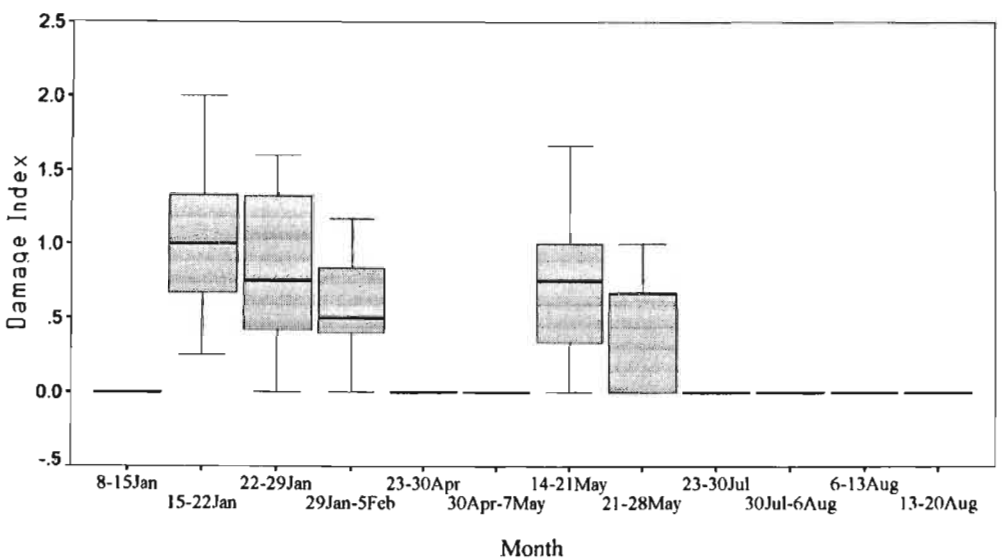
Figure 3.9: Box-and-whisker plots of the damage index values for the two week old plants, calculated for each site during each sampling period.



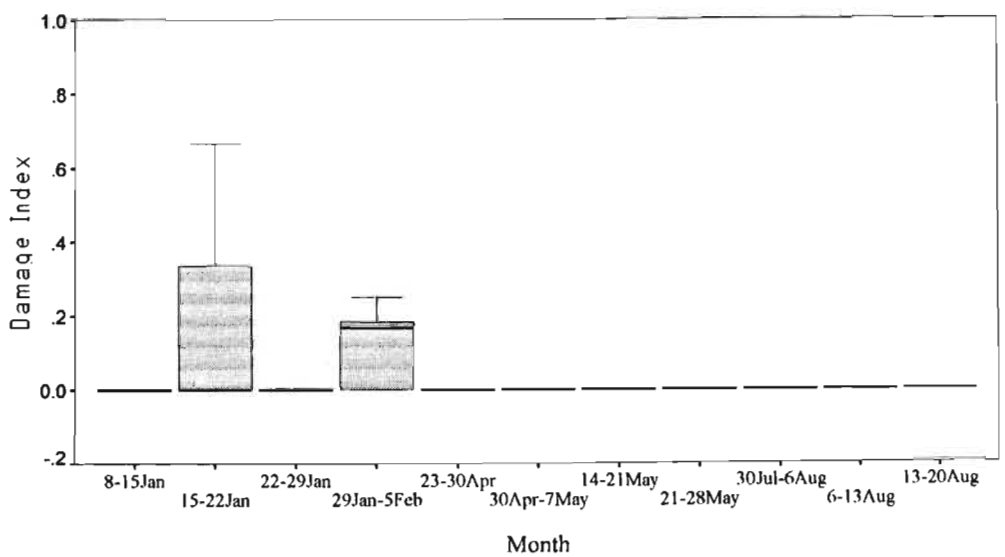
a. Botanic Gardens site



b. University of Natal site



c. Kloof site



d. Mooi River site

Figure 3.10: Box-and-whisker plot of the damage index values for the plants germinated on site, calculated for each site during each sampling period.

Since the results obtained from the plants germinated on site indicate that there was a significant variation in O_3 levels between the four sites, but the results obtained from the two week old plants implied that there were no differences, caution should be taken when interpreting these data as to which way the bioindicating should be performed. It can also be noted that the continuous O_3 monitor at the UND site was recording relatively low levels of O_3, that is below 30 ppb which is regarded as the threshold concentration before O_3 damage occurs on this variety of tobacco. However, damage was recorded on the plants placed at the UND site. Therefore, the threshold may be lower than has been usual for this genotype of Bel-W3, or there is some kind of accumulative effect, or there may be one or more other environmental or biotic factors influencing the levels at which injury occurs.

A Kruskal-Wallis test indicated that there were significant differences ($p<0.05$) in the observed damage between the three sampling periods for both the two week old plants and those germinated on site. A Mann-Whitney U test showed that for the two week old plants, there was a significant difference between each of the three sampling periods, the highest mean damage being observed in the winter sampling period and the lowest mean damage being observed during the summer sampling period. For those plants germinated on site, the summer sampling period damage index values were significantly higher than the other two, but there was no significant difference ($p>0.05$) in the observed damage between the autumn and winter sampling periods. Therefore, the plant damage data implied that there was a seasonal difference in the levels of O_3 between the sites. The plant damage data also indicated that there was a significant difference in O_3 concentration between the various sites, whereas the O_3 diffusion tubes measured no significant differences in O_3 between the four sites (Figure 3.2).

Various correlations were carried out for each of the sampling periods, between the damage index values of the plants at the UND site and the Dasibi data. This correlation analysis was performed between the damage index values and a) the weekly means of all the Dasibi data, b) the highest one-hour daily means c) the highest one-hour daily means above 10 ppb, d) the highest one-hour mean values above 15 ppb, and e) the time the plants were exposed to O_3 concentrations above 10 ppb, 15 ppb, 20 ppb, and 30 ppb. The results of these correlations are shown in Table 3.8. These correlation analyses revealed a significant positive correlation between the damage index values of the two week old plants and the highest one-hour daily mean values above 10 ppb ($p<0.05$; correlation coefficient=0.67) and above 15 ppb ($p\leq0.01$; correlation coefficient=0.69). There was also significant but negative correlation ($p<0.05$; correlation coefficient=-0.63) between the damage index values of the plants germinated on site and the weekly mean of all the Dasibi values. There were no significant correlations observed for the other measured variables. It would therefore appear that there was little correlation between the damage index values and the Dasibi values.

Correlation analysis performed between the damage index values of the two week old plants and the plants germinated on site for each of the sites showed that there were no significant correlations between the damage index values for the two week old plants and those germinated on site for the UND site, the Kloof site or the Mooi River site. There was, however, a significant positive correlation between the two week old plants and those germinated on site at the Botanic Gardens site ($p<0.05$; correlation value=0.61).

Table 3.8: Correlation analyses between O_3 concentrations measured with the continuous monitor (the Dasibi) and the damage index values of the two week old plants as well as the plants germinated on site (n=12).

		correlation value
DI of the two week old plants versus...	weekly means of all values	0.12
	highest 1-hr daily means	0.14
	highest 1-hr daily means above 10ppb	0.67*
	highest 1-hr daily means above 15ppb	0.69*
	duration of exposure above 10ppb	0.30
	duration of exposure above 15ppb	0.08
	duration of exposure above 20ppb	-0.35
	duration of exposure above 30ppb	-0.14
DI of the plants germinated on site versus...	weekly means of all values	-0.63*
	highest 1-hr daily means	-0.46
	highest 1-hr daily means above 10ppb	0.47
	highest 1-hr daily means above 15ppb	0.41
	duration of exposure above 10ppb	-0.48
	duration of exposure above 15ppb	-0.64*
	duration of exposure above 20ppb	-0.10
	duration of exposure above 30ppb	0.09

* Significant at $p<0.05$

There were no significant correlations ($p>0.05$) between the calculated mean damage index values for the two week old plants, or the plants germinated on site, and the mean O_3 concentrations measured with the passive diffusion tubes.

3.1.3.2 Chlorophyll analysis

The mean dry weights, the mean chlorophyll a:b ratios, and the mean carotenoid:chlorophyll a ratios of the bioindicators for the three sampling periods are shown in Table 3.9a-c. The data obtained for the two week old plants and the plants germinated on site were analysed separately. Using the Kolmogorov-Smirnov test, it was found that the data was non-parametric, so all the data was analysed using the Kruskal-Wallis and the Mann-Whitney U tests.

Analysis of the data for both the two week old plants and those germinated on site indicated that there were no significant differences ($p>0.05$) in the chlorophyll a:b ratios, the carotenoid:chlorophyll a ratios, or the dry weights between the four sites. This can also seen in Tables 3.9 a-c where it can be observed that the values obtained do not vary much between the various sites.

A Kruskal-Wallis analysis of the data from the two week old plants revealed that there were no significant ($p>0.05$) differences in the carotenoid:chlorophyll a ratios between the three sampling periods. A Kruskal-Wallis test showed, however, that there were significant differences ($p<0.01$) in the chlorophyll a:b ratios and the dry weights between the three different sampling periods. Mann-Whitney U tests showed where these differences occurred. The chlorophyll a:b ratios were significantly different between each of the sampling periods,

Table 3.9a: Mean dry weight, chlorophyll a:carotenoid ratio and chlorophyll a:b ratio and the respective standard errors, measured for plants exposed in the summer sampling period (8 January to 5 February 1997). s=plants germinated on site and p=plants put at the site when they were two weeks old. n=9 for the plants germinated on site, n=5 for the two week old plants.

Date	Site	Dry weight(mg)	Chla:carotenoid ratio	Chla:chlb ratio
8 - 15Jan	BG(s)	0.05±0.01	0.5±0.1	5.6±1
	UND(s)	0.1±0.02	0.6±0.1	7.9±1.4
	K(s)	0.1±0.01	1.0±0.1	7.3±6.2
	MR(s)	0.1±0.01	0.5±0.1	3.5±6.7
	BG(p)	2.8±0.4	1.6±0.02	3.8±0.2
	UND(p)	3.1±0.5	1.7±0.01	4.3±0.2
	K(p)	3.4±0.5	1.6±0.05	4.1±0.2
	MR(p)	3.0±0.4	1.7±0.02	4.3±0.2
15 - 22Jan	BG(s)	0.2±0.01	2.1±0.04	6.7±0.8
	UND(s)	0.5±0.05	1.9±0.03	3.5±0.2
	K(s)	0.4±0.06	1.8±0.02	3.6±0.2
	MR(s)	0.3±0.02	1.8±0.01	3.1±0.2
	BG(p)	1.2±0.2	1.7±0.01	3.7±0.1
	UND(p)	1.2±0.2	1.7±0.01	4.1±0.2
	K(p)	2.3±0.4	1.7±0.01	4.5±0.04
	MR(p)	1.4±0.2	1.7±0.01	4.5±0.1
22 - 29 Jan	BG(s)	0.5±0.04	1.8±0.01	5.3±0.4
	UND(s)	1.2±0.3	1.8±0.01	5.9±0.6
	K(s)	1.0±0.1	1.8±0.01	5.4±0.2
	MR(s)	0.6±0.1	1.9±0.04	8.4±1.4
	BG(p)	2.9±0.5	1.7±0.01	4.7±0.1
	UND(p)	4.2±0.5	1.7±0.01	5.0±0.1
	K(p)	5.0±0.3	1.7±0.02	5.2±0.1
	MR(p)	2.5±0.3	1.7±0.02	5.2±0.2
29Jan-5Feb	BG(s)	1.9±0.3	1.8±0.02	4.3±0.1
	UND(s)	2.7±0.4	1.9±0.05	3.7±0.2
	K(s)	1.9±0.3	1.8±0.01	4.6±0.2
	MR(s)	2.0±0.4	1.8±0.02	4.3±0.2
	BG(p)	2.6±0.4	1.8±0.03	4.3±0.2
	UND(p)	3.2±0.5	1.8±0.04	4.3±0.1
	K(p)	4.7±0.6	1.8±0.02	4.2±0.1
	MR(p)	3.3±0.6	1.8±0.02	4.1±0.1

Table 3.9b: Mean dry weight, chlorophyll a:carotenoid ratio and chlorophyll a:b ratio and the respective standard errors, measured for plants exposed in the autumn sampling period (23 April to 28 May 1997). s=plants germinated on site and p=plants put at the site when they were two weeks old. n=9 for the plants germinated on site, n=5 for the two week old plants.

Date	Site	Dry weight(mg)	Chla:carotenoid ratio	Chla:chlb ratio
23 - 30 Apr	BG(s)	0.03±0	1.3±0.1	4.8±1.4
	UND(s)	0.04±0	1.4±0.1	4.2±1.1
	K(s)	0.05±0	1.4±0.1	4.5±1.7
	MR(s)	0.04±0.01	1.20.2	3.3±1.2
	BG(p)	0.1±0.01	1.8±0.1	2.0±0.5
	UND(p)	0.2±0.02	1.6±0.1	2.6±0.9
	K(p)	0.1±0.01	1.5±0.05	3.0±0.9
	MR(p)	0.1±0.01	1.3±0.1	4.4±2.3
30 Apr-7May	BG(s)	0.1±0.01	2.0±0.2	7.0±1.6
	UND(s)	0.1±0.01	2.1±0.3	4.7±1.2
	K(s)	0.1±0.01	1.7±0.1	5.5±1.2
	MR(s)	0.04±0	1.8±0.1	4.4±1.1
	BG(p)	0.1±0.01	1.9±0.1	6.1±2.2
	UND(p)	0.1±0.01	1.6±0.1	4.3±0.9
	K(p)	0.1±0.01	1.9±0.2	6.6±1.6
	MR(p)	0.1±0.01	1.7±0.1	6.7±2.0
14 - 21 May	BG(s)	0.3±0.04	2.3±0.1	63.1±0.5
	UND(s)	0.3±0.04	2.0±0.1	4.6±1.0
	K(s)	0.2±0.02	2.2±0.1	4.5±1.2
	MR(s)	0.1±0.01	2.0±0.1	5.5±1.2
	BG(p)	0.1±0.02	2.2±0.1	2.4±0.3
	UND(p)	0.2±0.01	1.9±0.04	4.2±0.6
	K(p)	0.2±0.01	1.8±0.1	3.9±0.6
	MR(p)	0.2±0.02	2.0±0.2	5.6±3.5
21 - 28 May	BG(s)	0.2±0.05	1.7±0.1	9.1±1.2
	UND(s)	0.3±0.03	1.7±0.03	12.3±3.9
	K(s)	0.2±0.03	1.6±0.1	9.0±2.1
	MR(s)	0.1±0.02	1.6±0.1	6.6±2.0
	BG(p)	0.4±0.03	1.7±0.01	3.7±0.5
	UND(p)	0.4±0.1	1.7±0.01	5.4±1.9
	K(p)	0.3±0.03	1.7±0.01	5.8±1.2
	MR(p)	0.4±0.1	1.7±0.02	5.5±0.8

Table 3.9c: Mean dry weight, chlorophyll a:carotenoid ratio and chlorophyll a:b ratio and the respective standard errors, measured for plants exposed in the winter sampling period (23 July to 20 August 1997). s=plants germinated on site and p=plants put at the site when they were two weeks old. n=9 for the plants germinated on site, n=5 for the two week old plants.

Date	Site	Dry weight	Chla:carotenoid ratio	Chla:chlb ratio
23 - 30 July	BG(s)	0.1±0.01	1.9±0.1	2.9±0.4
	UND(s)	0.1±0.01	2.4±0.3	4.4±2.6
	K(s)	0.04±0	2.1±0.2	3.6±2.1
	MR(s)	-	-	-
	BG(p)	0.4±0.1	1.8±0.02	4.5±0.7
	UND(p)	0.3±0.04	1.9±0.03	4.4±0.5
	K(p)	0.2±0.02	1.9±0.02	5.3±0.3
	MR(p)	0.2±0.01	2.0±0.02	3.7±0.5
30July-6Aug	BG(s)	0.1±0.03	1.9±0.05	9.7±3.6
	UND(s)	0.1±0.02	1.7±0.03	6.5±1.2
	K(s)	0.1±0.01	1.9±0.07	6.5±1.4
	MR(s)	0.03±0	2.0±0.1	2.7±0.2
	BG(p)	0.4±0.04	1.7±0.02	4.4±0.3
	UND(p)	0.4±0.1	1.7±0.01	4.9±0.4
	K(p)	0.3±0.03	1.7±0.01	3.7±0.4
	MR(p)	0.2±0.03	1.8±0.06	4.5±0.6
6 - 13 Aug	BG(s)	0.3±0.1	1.8±0.02	6.0±0.5
	UND(s)	0.3±0.04	1.9±0.1	5.6±0.6
	K(s)	0.3±0.04	1.9±0.04	11.8±3.7
	MR(s)	0.1±0.02	2.1±0.06	7.5±1.5
	BG(p)	0.5±0.1	1.7±0.02	5.8±0.3
	UND(p)	0.6±0.1	1.7±0.01	7.2±1.0
	K(p)	0.4±0.03	1.7±0.01	6.8±0.2
	MR(p)	0.3±0.03	1.7±0.01	8.5±3.4
13 - 20 Aug	BG(s)	1.3±0.2	1.7±0.01	5.0±0.04
	UND(s)	0.8±0.1	1.8±0.02	4.6±0.2
	K(s)	0.5±0.1	1.8±0.04	4.3±0.3
	MR(s)	0.2±0.01	2.0±0.04	3.8±0.5
	BG(p)	0.7±0.1	1.7±0.01	4.8±0.1
	UND(p)	0.6±0.1	1.7±0.02	4.6±0.1
	K(p)	0.6±0.2	1.8±0.1	4.1±0.4
	MR(p)	0.4±0.04	1.8±0.02	4.4±0.2

with the highest mean occurring in the winter sampling period and the lowest mean occurring in the autumn sampling period, with ratios ranging from 3.7 to 5.2 in the summer sampling period (Table 3.9a), 2 to 6.7 in the autumn sampling period (Table 3.9b), and 3.7 to 8.5 in the winter sampling period (Table 3.9c). The dry weights also differed significantly ($p<0.05$) between each of the sampling periods, the highest mean dry weight occurring in the summer sampling period and the lowest mean dry weight occurring in the autumn sampling period. Therefore, the chlorophyll a:b ratios and the dry weights measured for the two week old plants showed a seasonal variation but no variation between the four sites.

A Kruskal-Wallis test of the plants germinated on site showed that there were no significant differences ($p>0.05$) between chlorophyll a:b ratios and the three sampling periods. There were, however, significant differences ($p<0.01$) in the chlorophyll a:carotenoid ratios as well as the dry weights between the three sampling periods. The Mann-Whitney U test revealed that the mean chlorophyll a:carotenoid ratios were greater during the winter sampling period, and there were no significant differences ($p>0.05$) between the summer and autumn sampling months. The mean dry weights were greatest in the winter sampling period and lowest in the autumn sampling period.

Correlation analysis was performed between the data obtained from the continuous monitor and the chlorophyll contents as well as the dry weights for the two week old plants at the UND site and the plants germinated at the UND site. It was found that there were no significant correlations ($p>0.05$). It was also established that there were no significant correlations between the chlorophyll contents or the dry weights and the mean damage index values,

calculated for the two week old plants as well as the plants germinated on site, for each of the four sites.

It would appear then that variations in the chlorophyll contents were occurring due to seasonal differences, but there were no variations occurring between the different sites. The results also varied between the different aged plants used for the bioindicating.

3.2 The Mpumalanga study

3.2.1 Continuous ozone monitoring

The data obtained from the continuous O_3 monitor positioned at the UND site in Durban (Table 3.1d) indicated that the O_3 levels in the Durban area, specifically at the UND site, at the time of the Mpumalanga study, were relatively higher than during the previous sampling periods in the study in the Greater Durban area. The highest one-hour mean concentration was 36.7 ppb and the highest 24-hour mean concentration was 14.3 ppb. As mentioned above (Section 3.1.1), the 24-hour daily mean as well as the highest daily one-hour mean O_3 concentrations measured with the continuous monitor at the UND site were significantly higher ($p<0.001$) during this spring sampling period (22/9/97 to 6/10/1997) than during the other seasonal sampling periods. There was, however, no significant difference ($p>0.05$) in the lowest daily one-hour mean measured O_3 concentration between the different sampling periods. It was also observed that there was a significant positive correlation between the daily maximum temperatures and the highest one-hour daily mean O_3 concentrations as well as the 24-hour daily means ($p<0.001$) (Table 3.2).

3.2.2 Passive diffusion tube measurements

3.2.2.1 Ozone

The mean O_3 concentrations, measured with the passive diffusion tubes, and the calculated standard errors for these means are displayed in Figure 3.11. The highest concentration measured was at the Long Tom Pass where the mean for the two week period was 3.6 ppb. Analysis of variance indicated, however, that there were no significant differences in the observed O_3 concentrations ($p>0.05$) between the four selected sites in Mpumalanga or the UND site in Durban. Therefore, it would appear that during this study, O_3 concentration was not affected by proximity to the city of Nelspruit.

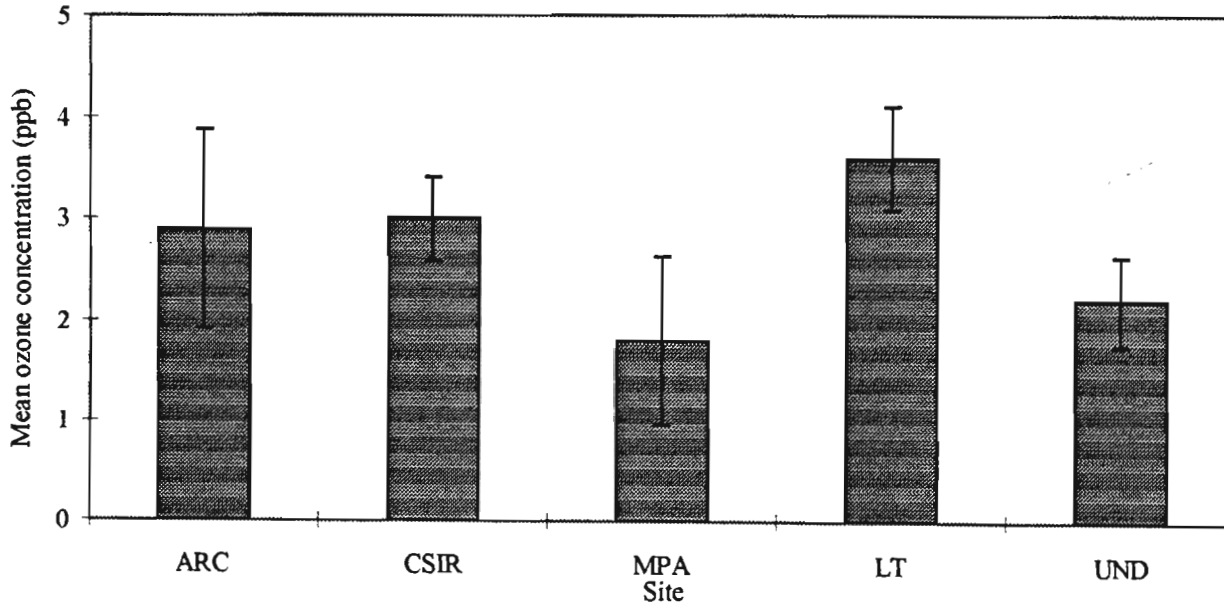


Figure 3.11: Mean ozone concentrations (ppb) measured at the four Mpumalanga sites, the CSIR, the Agricultural Research Council (ARC), Mpumalanga Provincial administration (MPA), Long Tom Pass (LT), and one KwaZulu-Natal site, the University of Natal (UND), using passive diffusion tubes (n=4). Standard errors shown as vertical bars.

The coefficients of variation were calculated for the data obtained from the O_3 diffusion tubes shown in Table 3.10. The coefficients of variation were relatively high in this study, ranging from 20.3% at the CSIR site to 49.6% at the ARC site. These coefficient of variation values were similar to those of the O_3 diffusion tubes used in the Greater Durban study (Table 3.5), which ranged from 7.8% to 42%. Thus, precision was also low in the O_3 diffusion tube measurements made during the Mpumalanga study.

Table 3.10: Coefficients of variation (%) for the mean O_3 concentrations calculated for each site during the study period (n=4).

Site	V(%)
ARC	49.6
CSIR	20.3
MPA	41.9
LT	25.6
UND	22.2

3.2.2.2 NO_2

The mean NO_2 concentrations as well as the calculated standard errors are displayed in Figure 3.12. A multiple range test showed that there were significant differences ($p<0.01$) in the NO_2 concentrations between the CSIR site and the LT and MPA site in Mpumalanga, the NO_2 concentrations being higher at the CSIR site, but there was no significant ($p>0.05$) difference in the NO_2 concentration between the ARC site and the other Mpumalanga sites. The NO_2

concentrations of the UND site was significantly higher ($p<0.01$) than the NO_2 concentrations measured at all four of the sites in Mpumalanga.

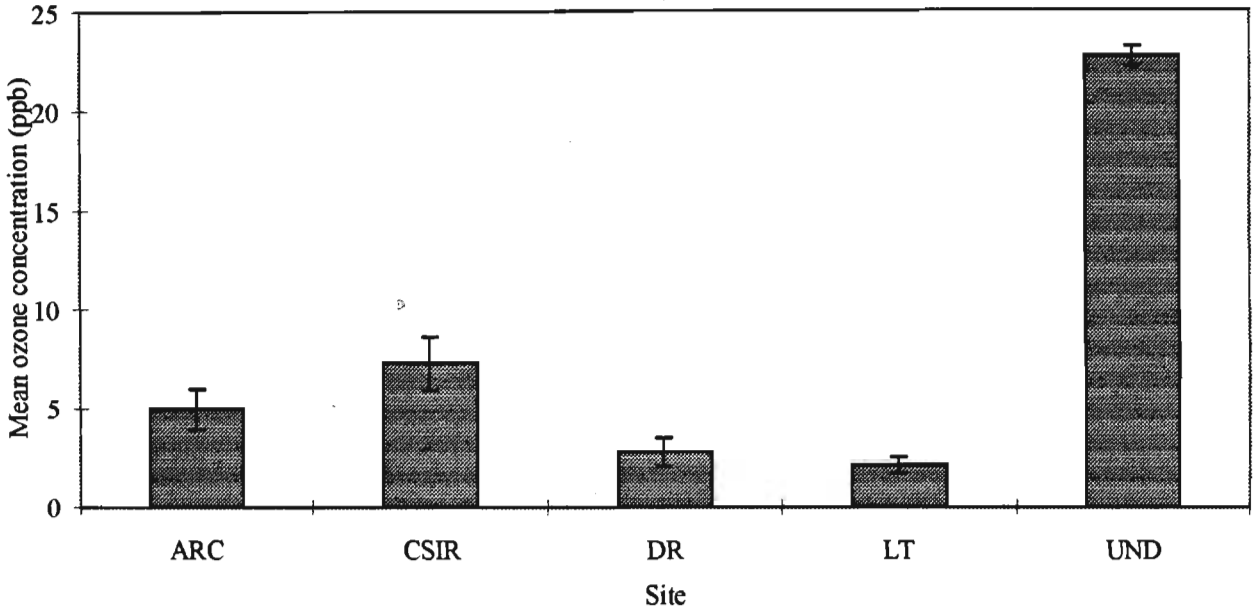


Figure 3.12: Mean NO_2 concentrations measured at the four Mpumalanga sites, the CSIR, the Agricultural Research Council (ARC), Mpumalanga Provincial Administration (MPA), Long Tom Pass (LT), and one KwaZulu-Natal site, the University of Natal (UND), using the passive diffusion tubes (n=4). Standard errors shown as vertical bars.

Thus, generally the concentration of NO_2 measured at the Mpumalanga sites was much lower than that measured at the one site in Durban.

Coefficients of variation were calculated for the NO_2 concentrations measured with the diffusion tubes (Table 3.11). It was found that these coefficients of variation were high, ranging from 19.6% at the Long Tom Pass site to 66.7% at the CSIR site. The coefficient of variation values for the NO_2 diffusion tubes measured for Durban ranged from 8.5% to 261.5%.

Therefore, the high values obtained in the Mpumalanga study could once again imply a lack of precision in the use of this technique.

Table 3.11: Coefficients of variation for the mean NO_2 concentrations calculated for each site during the study period.

Site	V(%)
ARC	53.0
CSIR	66.7
MPA	36.6
LT	19.6
UND	26.4

3.2.3 Bioindicators

3.2.3.2 Ozone phytotoxicity

None of the seeds placed at each site germinated, so a comparison between the two week old plants and the plants germinated on site was not possible. The mean damage index values and standard errors for the two week old plants placed at three Mpumalanga sites and the UND site in Durban are displayed in Figure 3.13. A Kruskal-Wallis test revealed that there were no significant differences ($p>0.05$) in the observed leaf injury between the various sites. This might be expected since there were no significant differences in the diffusion tube measurements of the O_3 concentration between these sites.

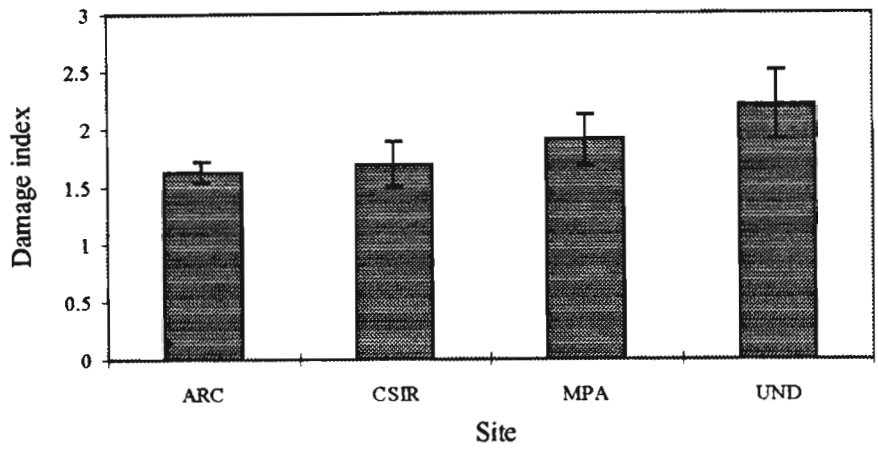


Figure 3.13: Mean calculated damage index values and standard error bars for the two week old plants placed at three of the Mpumalanga sites, the CSIR, the Agricultural Research Council (ARC), and the Mpumalanga Provincial Administration (MPA), as well as the University of Natal (UND) site in KwaZulu-Natal (n=5). Plants at the Long Tom Pass site in Mpumalanga died during the sampling period.

3.2.3.3 Chlorophyll analysis

The mean chlorophyll a:b ratios, carotenoid:chlorophyll a ratios and dry weights and the calculated standard errors are shown in Table 3.12. Analysis of variance showed that there were no significant differences ($p>0.05$) in the chlorophyll a:b ratios or the carotenoid:chlorophyll a ratio for each of the five sites. This would be expected since there was no significant difference recorded in the visible damage to the leaves. A multiple range test showed, however, that the dry weights of the plants at the UND (11.7 ± 1.9 mg) and ARC (11.8 ± 0.8 mg) sites were significantly higher ($p<0.001$) than at the CSIR (3.5 ± 0.6 mg) and MPA (3.9 ± 0.9 mg) sites.

Table 3.12: Mean dry weight, chlorophyll a:carotenoid ratio and chlorophyll a:b ratio and the respective standard errors, measured for plants exposed at three sites in Mpumalanga and one site in KwaZulu-Natal from 22 September to 6 October 1997 (n=5). Measurements for the fourth site (Long Tom Pass) as missing as all the plants at this site died during the study.

Site	Dry weight(mg)	Chla/carotenoid ratio	Chla:chlb ratio
ARC	11.8±0.8	1.6±0.01	3.6±0.1
CSIR	3.5±0.6	1.8±0.04	3.7±0.1
MPA	3.9±0.9	1.7±0.01	3.2±0.3
UND	11.7±1.9	1.7±0.04	4.1±0.3

4. DISCUSSION

4.1 Evaluation of methods

Of the three methods used in this study (the passive diffusion tubes, the bioindicators, and the Dasibi), the Dasibi appeared to provide the most accurate results. The Dasibi is well maintained and regularly serviced, and is calibrated by running another Dasibi next to it simultaneously. Thus, it is most likely that the data from the Dasibi are reliable.

The precision and accuracy of the results obtained using both the O_3 diffusion tubes and the NO_2 diffusion tubes, however, is questionable. The coefficients of variation calculated for both these sets of data were high. Also, the coefficients of variation increased as the mean concentration values increased. This would imply then that these techniques may have intrinsic errors in the way they were designed and used. However, when the passive diffusion technique was first developed, Levaggi *et al.* (1973) concluded that their field data indicated that the method was accurate and precise, since it provided results that agreed with automated instruments. Similarly, other studies have led to the conclusion that this is an effective technique to use (Grosjean *et al.*, 1992; Bytnerowicz *et al.*, 1993; Dhammapala *et al.*, 1995).

The main problem associated with the use of diffusion tubes seems to be the effects of wind turbulence (Koutrakis *et al.*, 1993; Gair & Penkett, 1995; Grosjean *et al.*, 1995). In the present study, since the passive samplers were not protected in any way, they were exposed directly to varying wind velocities and turbulence typically encountered outdoors. This could account for their lack of accuracy and precision. Koutrakis *et al.* (1993) found that, barring the influence

from other unknown interferences, the large differences that can occur between sampling tube collection rates is primarily due to wind velocity effects. Gair and Penkett (1995) have found that air turbulence resulted in NO_2 concentrations being over-estimated by up to 40% by the diffusion tubes. This was attributed to a shortening of the effective diffusion path along the tube caused by wind-induced turbulence at the opening of the sampler. The influence of turbulence on the sampler efficiency is likely to be site dependent. Providing a relationship exists between wind speed and the reduction in diffusion length, data from diffusion tube samplers could be corrected for the effects of turbulence by measuring the wind speed at the exposure site, and using a correction factor (Gair & Penkett, 1995). According to Gair and Penkett (1995), the relationship between the reduction in molecular diffusion length and wind speed, however, is highly variable. Thus, corrected measurements can only be used at sites recording wind speed continually, thus reducing the simplicity of the technique. So Gair and Penkett (1995) concluded that further research is necessary to determine the type and significance of the relationship between wind speeds and turbulence and the reduction in diffusion length.

An effective way of reducing the effect of air turbulence would be the use of protective screens. In those studies that found good agreement between the data from the passive samplers and continuous monitors, the diffusion tubes were fitted with either wire mesh filters (Dhammapala *et al.*, 1995) or Teflon filters (Grosjean *et al.*, 1992; Bytnerowicz *et al.*, 1993) to reduce the effects of wind turbulence. These filters provided a constant diffusion-limited flow rate. Thus, to ensure precision and accuracy from the data obtained from diffusion samplers, the most feasible solution for further studies would be the use of filters, or alternatively to place the samplers in protective devices to reduce extremes in wind velocities.

During this study, errors could also have resulted during the preparation and analysis of the O_3 and NO_2 tubes. It is preferable that all preparation and analysis be performed in O_3- and NO_2-free chambers. Since these were not available for use in this study, errors may have occurred during the preparation and analysis of the tubes. During the analysis, it was also observed that dust had collected on the filters. Therefore, before analysis, the solutions were filtered, thus increasing exposure period of the solutions. Also, any sand dissolved in the solutions, or dust particles not removed, could have increased errors in the spectrophotometry readings. Therefore, in future studies, the protective device, mentioned above, would be essential to prevent moisture and dust accumulating in the tubes, as well as reducing extreme wind velocities, and the possible effects of rain and sunlight. This protective device would also prevent insects and spiders from nesting inside the tubes. This was another problem encountered in the present study. Other improvements could include establishing the effects of ambient humidity on diffusion rate.

The tobacco bioindicators were the third means of monitoring O_3 in this study. The results of the bioindication study yielded contradictory results, in that the results obtained for the two week old plants were the opposite to those obtained for the plants germinated on site. There were no differences in the damage index values between the four sites for the two week old plants, whereas differences were noted in damage index values of the plants germinated on site. For the two week old plants, the highest damage index values were observed in winter and the lowest in summer, but for the plants germinated on site the highest damage index values occurred in summer, and there were no significant differences in the damage index values between autumn and winter. Those plants germinated on site would be exposed to different

climatic conditions at the different sites, thus the degree of damage exhibited on the plants could be influenced by physiological response of the plants to these different climatic conditions, as well as the concentration of O_3. As Markert (1994) has stated, seasonal changes may be occurring in the pollutants, but similarly the seasons will affect the physiology of the bioindicator and thus their response to the pollutant. This method of germinating the plants on site probably does not provide an accurate reflection of the true effects and amounts of O_3 concentration.

With those plants put out when two weeks old, although not grown in O_3-free conditions initially, they were all exposed to the same conditions during initial growth. Any new developments (with more damage occurring or no change) when the plants were placed at the various sites, should be comparable and should indicate whether there are differences in O_3 concentration between these sites, even though they would not provide an indication of the actual concentration being experienced. Thus, this second method of plant bioindication is probably the more effective indicator and these results more indicative of the true situation.

The damage index values for the two week old plants correlated positively with the Dasibi data, but these damage index values did not correlate with the concentrations measured with the O_3 diffusion tubes placed at each of the sites. This is further evidence that accuracy and precision were lacking in these O_3 diffusion tubes.

Lorenzini (1994) has stated that the threshold for visible injury in these tobacco plants is around 40-50 ppb for some four hours of exposure. In this study, however, O_3-induced injury was

occurring on the leaves of the tobacco plants at concentrations of below 30 ppb. Thus, either this threshold is incorrect, or the plants became more susceptible to injury due to other biotic or environmental factors. Another possibility is that the effects of O_3 are accumulative, or alternatively there may be a stochastic response as opposed to a threshold response. As mentioned previously (Chapter 1), it is thought that earlier exposure to O_3 predisposes tobacco plants to greater foliar injury during subsequent exposures (Heagle & Heck, 1974). Heck *et al.* (1986) have observed that yield loss is caused by the accumulative negative effects of daily exposures over the entire growth period.

There are limitations to indicator systems that depend solely on visual estimates of foliar injury. Ozone can cause a variety of symptom types and intensities of injury depending on numerous modifying factors. This leads to a degree of subjectivity is required, which usually results in different estimates by different individuals for a given sample. Also, other stresses can cause symptoms that mimic those caused by O_3, making it difficult or impossible to separate them from O_3 effects. As mentioned above, another major limitation to this system is that climatic factors and previous O_3 exposure can affect the degree of plant response to O_3 (Heagle & Heck, 1974). Thus, a given amount of O_3 does not necessarily cause a given degree of response (Heagle *et al.*, 1994). Knudson *et al.* (1977) have concluded that the chlorophyll determination procedure is a practical, precise alternative method of evaluating O_3 injury to plants. They found that this method estimated the same aspect of injury as visual observations with more precision than the latter and without human bias. They also found this technique to be useful in the evaluation of tobacco plants.

In this study, since O_3 concentrations were relatively low, no definite conclusions about this method (chlorophyll analysis) of O_3 detection and monitoring in this study can be made. More work with tobacco plants grown in widely varying O_3 concentrations would be necessary before these conclusions could be made.

4.2 Ozone concentrations in the Greater Durban area and Mpumalanga

The concentrations of O_3 measured with the continuous monitor at the UND site were relatively low, with the mean hourly values ranging from 0 ppb to 11.7 ppb, and the highest mean hourly maximum O_3 values recorded over all the sampling periods being 40 ppb. Diab and Helas (1998), monitoring surface O_3 from September 1994 to October 1997 at the same location as this study, also concluded that, in general, the values were low, with most hourly values lying between 5 ppb and 10 ppb, and the highest hourly mean maximum recorded during their study being 39 ppb. The extreme O_3 episodes (values between 100-400 ppb) that are characteristic of some urban-industrial areas, are absent in this area. Thus, the O_3 concentrations being experienced in the Durban area are lower than the South African standard for human health of 120 ppb (one-hour average) and lower than the exposure limit of 60 ppb (24-hour average) as set by the WHO. The values were also lower than the critical level of 40 ppb set in Europe for the effects on plants.

Ozone monitoring has also been carried out at Cape Point (South Africa) ($34^0$21'S $18^0$29'E) 210m above sea level since 1979.The monitoring station at Cape Point is regarded as an O_3 background monitoring station. It is ideally suited as a background monitoring station as it is removed from direct pollution impacts and because air masses which reach the station are

mainly of oceanic origin (Combrink *et al.*, 1995). Here the annual O_3 means for 1982 and 1983 were 20 ppb and 21 ppb respectively. Up until 1985, the highest O_3 concentration recorded at Cape Point with a Dasibi analyser was 78 ppb (April 1984) (Brunke & Allen, 1985). These values approximated measurements made at other baseline stations in the southern hemisphere, such as at Cape Grim (Tasmania) and the South Pole (Brunke & Allen, 1985), and are probably higher than would have been expected from the UND site during the same period. The O_3 concentrations measured at the UND site, therefore, appeared to be below those observed at the Cape Point station. Thus, it would appear that O_3 levels in the greater Durban area are low enough so as not to cause concern with regards to damage to human health, crops and natural vegetation damage.

Seasonal differences in O_3 concentrations measured with the Dasibi were noted. Mean concentrations of the highest one-hour means were higher during the spring, however, significant differences were only observed between the summer and spring concentrations for highest one-hour means, and summer and autumn concentrations, as well as the summer and spring concentrations for the 24 hour means. The seasonality thus observed indicated a general spring maximum and a summer minimum. The study performed by Diab and Helas (1998), where observations of the O_3 concentrations from September 1994 to October 1997 were observed, indicated winter maxima and summer minima at this same location. Since winter and spring conditions in the Durban area vary relatively little, it can be deduced that a general late winter/early spring maximum is the rule for this area.

Ozone measurements, taken from a global network of sites, primarily in the Atlantic and Pacific Ocean areas (Barrow, AK; Mauna Loa, Hawaii; American Samoa; and South Pole) (Oltmans, 1994) indicated that summer and early autumn minima were the rule in the northern hemisphere, as well as the southern hemisphere. The seasonal cycle at the surface in the southern hemisphere indicates that O_3 destruction is occurring even in a relatively low NO_X regime (Oltmans & Levey, 1994). According to Olson *et al.* (1996), the cause of this observed seasonality, which also occurs at various background monitoring stations, is not completely understood. They have, however, suggested that these winter/spring maxima may be due to annual minima in both water vapour and the ultraviolet radiation required to initiate photochemical O_3 destruction (in the absence of high NO_X concentrations). Combrink *et al.* (1995) also believe that the summer minima are due to low NO_X values which provide a photochemical sink for O_3, and in contrast highly polluted sites show spring/summer maxima. Oltmans and Levey (1994) concluded that at the South Pole, low O_3 concentrations are probably associated with transport of O_3-poor air from lower latitudes. Also, in the northern hemisphere, winter maxima observed in Barbados and the spring maxima observed in Bermuda are linked to the transport from higher latitudes and altitudes of air with higher O_3 concentrations.

Many clean air sites, for example Cape Point (South Africa), remote from the effects of industrial activity, typically depict a winter O_3 maximum and a summer minimum. This pattern of seasonal change is probably due to the injection of stratospheric O_3-rich air into the troposphere, which appears to be most effective in the late winter and spring months (Viezee *et al.*, 1983; Levey *et al.*, 1985; Combrink *et al.*, 1995). Olson *et al.* (1996) also pointed out that

because biomass burning is quite prevalent during the dry winter period, especially in the southern tropics of Africa, an influence from transport of emissions from tropical biomass burning in the southern hemisphere regions may occur. Galbally *et al.* (1986) have observed that the winter maximum is related to seasonal variation in the mixing within the surface to 2km layer. They have suggested that surface destruction is more efficient in summer and downward mixing of O_3 from higher altitudes is more likely in winter.

Following on from this, Diab and Helas (1998) have suggested that it is likely that the winter/spring maximum observed at the UND site can be partly explained by increased subsidence in the prevailing anticyclonic circulation in winter. It could also be due to reduced water vapour and lower rainfall in the winter. In this present study, there was only a significant negative correlation between the amount of rainfall during the summer sampling period and the mean highest one-hour O_3 concentrations. Thus, rainfall appeared to have little influence on the measured O_3 concentrations. Of the three meteorological variables that were measured, the maximum daily temperature appeared to have the only influence. It was interesting to note, however, that this influence of temperature was greatest during spring, when O_3 concentrations were generally the highest, and during summer, when O_3 concentrations were the lowest. Also, even though temperatures were greatest in summer, O_3 concentrations were the lowest. During winter, when the O_3 concentrations were intermediate between summer and spring, meteorological conditions seemed to have no effect on the daily O_3 concentration. It is expected that there should be a direct relationship between solar radiation and O_3 formation, however the study performed by Diab and Helas (1998) revealed that there was no correlation between daily solar input and daily maximum O_3. Similarly, they observed no dependence on

daily maximum temperature. Khemani *et al.* (1995) also observed that while the diurnal maximum temperature occurred during the afternoon period, when solar radiation was maximal, the highest O_3 concentrations were observed in winter and not in summer. As Vukovich (1995) has concluded, a strong correlation between O_3 and any meteorological variable (for example temperature) does not necessarily mean that there is a functional relationship between that variable and O_3. Vukovich (1995) did observe that high O_3 is found behind high-pressure systems, but the relationship between O_3 and surface pressure was a weak one. Thus, it would appear that the seasonal variations in O_3 concentrations measured at the UND site were strongly influenced by meteorological variables.

These observations of seasonality (summer minima and winter/spring maxima in the Greater Durban area) are in contrast to sites in Europe, for example Athens in Greece (Gusten *et al.*, 1988), parts of the UK, and the USA, for example the eastern USA (Vukovich, 1995), where O_3 concentrations are highest in summer. Heavily populated and industrialized regions of the USA are characterised by broad summer maxima in surface O_3 which persists from spring into summer (March to August) (Logan, 1985). This is characteristic of highly polluted urban-industrial environments in the northern hemisphere. In a study performed at Patras (a medium-sized city in the Mediterranean), Greece, it was observed that the O_3 concentrations reached their maximum values during the summer months with peak values occurring in August and minimum monthly values occurring in the winter months (Danalatos & Glavas, 1996). Danalatos and Glavas (1996) have also found that there appears to be a tendency towards late spring monthly O_3 maxima at high latitudes, in the northern hemisphere, compared with late summer at lower latitudes, probably reflecting the monthly solar intensity variation.

In addition to differences in the phasing of the seasonal O_3 cycle between the northern hemisphere and the southern hemisphere, there is an observable difference in the amount of O_3, with greater concentrations at a comparable latitude in the northern hemisphere than in the southern hemisphere (Oltmans & Levy, 1994). The reason for this hemispheric difference is not clear (Oltmans *et al.*, 1994). It could be that the northern hemisphere has more sources of NO_2 emissions due to a greater degree of industrialization, than the southern hemisphere, thus producing more precursors to O_3.

The diurnal characteristics of the O_3 concentrations measured at the UND site were typical of that found in most polluted urban environments, the magnitude, however, being substantially lower. The minima were in the early morning (approximately 08h00 in autumn, winter and spring, and between 04h00 and 08h00 in summer), followed by an increase during the morning to reach a maximum between 12h00 and 14h00. Danalatos and Glavas (1996) have suggested that the minimum value which occurred at around 08h00 could be due to the morning traffic emissions of NO which rapidly destroys O_3. The distribution of the late-evening and night minima is probably due to the cessation of O_3 production after sunset in conjunction with its continuing destruction by NO and other loss processes. This diurnal cycle is also seasonally consistent.

In contrast to the Durban data, the clean air site of Cape Point exhibits a weak diurnal cycle (Combrink *et al.*, 1995). Here the amplitude of the mean monthly diurnal wave has been found to be no more than 2 ppb (Combrink *et al.*, 1995). In a global study of O_3, Oltmans and Levey (1994) found that there is evidence that over almost all of the ocean in the southern hemisphere

and over much of the northern hemisphere oceans, conditions with low NO_2 concentrations prevail, and hence O_3 production is weak. For high latitude sites such as Barrow and South Pole and Westman Islands in Iceland, there was no real sign of a diurnal variation, reflecting the lack of daily varying solar radiation for all or much of the year, combined with low NO_2 concentrations (Oltmans & Levey, 1994).

It was interesting to note in this study, that in summer and spring the peaks in mean O_3 concentration occurred at midday (12h00), whereas the peaks in concentration for autumn and winter occurred approximately two hours later. The opposite was observed by Danalatos and Glavas (1996) in their study on O_3 concentration in Patras, Greece. They observed that the winter O_3 maximum occurred at 14h00, approximately two hours after the maximum daily solar intensity occurred at midday. In the summer months, however, the maximum O_3 occurred between 16h00 and 17h00 because the solar intensity was maintained very close to the maximum for a longer time since Greece experiences longer daylight hours than South Africa, and thus O_3 also continued to be produced at almost the maximum rate for a longer period of time.

Another interesting and quite unusual feature noted in this study was the presence of a night-time secondary O_3 maximum in concentration. This double peak was observed to be most prevalent in the autumn and winter sampling periods. This second peak occurred during the early morning (01h00 to 06h00). This feature was also observed during the spring sampling period (August to October), but it was most predominant in autumn/winter (April to August). In the study performed by Danalatos and Glavas (1996), where night maximum values were

also observed, these night maxima also occurred frequently in the winter, when the maximum O_3 produced during the day was not high, and often became the maximum value for a particular 24 hour period.

A number of explanations have been proposed that can account for this unusual nocturnal O_3 secondary peak. The most common explanation is the breakdown in the nocturnal surface inversion (Diab & Helas, 1998). In the normal situation (without a secondary peak), the low night-time surface readings would be due to the absence of sunlight and the trapping of NO and other O_3 reducers near the surface in a relatively shallow (400m) inversion layer created by radiational cooling. Above the surface inversion, O_3 concentrations are thought to remain relatively high throughout the night (Samson, 1978). After the morning surface heating erodes the nocturnal surface inversion (three to five hours after sunrise), mixing proceeds vertically until heat flux into the mixing layer from the surface and from warmer air above is insufficient to maintain further growth. This mixing is important in distributing the surface emissions through the boundary layer in the early afternoon. This process leads to the expected simple diurnal oscillation as mentioned above. It has been suggested that the secondary O_3 maximum is either a result of vertical mixing of stratospheric O_3, the vertical mixing of the daytime O_3 found above the night inversion (Samson, 1978; Danalatos & Glavas, 1996), or that it is due to transport from long distances, or possibly all three (Danalatos & Glavas, 1996). At low elevations the source of O_3 is above the surface inversion in the remnant daytime boundary layer. This nocturnal mixing process could lead to the nocturnal rise in O_3. Samson (1978) deduced that the lack of a nocturnal maximum at certain sites, or during certain periods (as noted in this study), is probably due to the existence of more O_3 precursors in the surface

inversion in that area, or during that time period, than at other sites or periods. Therefore, it could be that the nocturnal rise will be most pronounced at locations removed from local NO or hydrocarbon sources downwind of large urban or industrial areas.

Another mechanism proposed for this secondary peak is that of horizontal transport of O_3 from other locations. Diab and Helas (1998) have hypothesised that the observed night-time peaks occurring in Durban is due to the long range transport of relatively O_3-rich air from a non-urban, high altitude environment, which is not characterised by the O_3 depletion that occurs in more polluted urban areas, and which has higher O_3 mixing ratios because of its greater altitude. The mechanism that has been proposed for Durban is the mountain-plain wind circulation that exists in KwaZulu-Natal (Tyson & Preston-Whyte, 1972). Diab and Helas (1998) have also observed that these secondary night-time peaks frequently occurred in association with low wind speeds. This advection of relatively O_3-rich air from inland high altitude areas towards the coast occurs predominantly in winter and autumn. It has also been suggested that a close proximity to the sea can result in this secondary night-time peak (Baird, 1995). As a result of peculiar atmospheric conditions above the sea, such as a greater atmospheric stability, limited vertical exchanges and lower destruction processes, O_3 can be carried for distances of several tens of kilometres without undergoing noticeable dilution and transformation. This implies that under a sea-land breeze wind regime pollutant recirculation may take place, with a possible return towards land of O_3-rich air masses from the open sea during the evening, resulting in night-time O_3 peaks.

The results obtained from the passive diffusion tubes indicated that there were no differences in O_3 concentrations between the various sites the Greater Durban area study. There also appeared to be no seasonal differences in O_3 concentration. No differences were found in the O_3 concentrations between the sites in the Mpumalanga study. Similarly, no differences were found between the Mpumalanga sites and the UND site. The highest O_3 concentration observed was at the Long Tom site, the site furthest from the city of Nelspruit and at the highest elevation. However, since the diffusion tubes in the Greater Durban study and the Mpumalanga study lacked accuracy and thus the results were questionable, the use of these results for comparison of sites is not taken further here.

The damage index values for the two week old tobacco plants were highest in winter. This was associated with the Dasibi findings, where O_3 concentrations were highest in winter and spring. However, the only significant correlation observed was that between the damage index values of the plants at the UND site and the highest one-hour daily means of O_3 above 15 ppb, measured with the Dasibi, for all the seasons combined.

The winter O_3 maxima may have significant effects on vegetation. In species that overwinter, survival of frost and desiccation depends on optimum morphological development and accumulation of adequate reserves during the summer and on hardening in the autumn (Barnes *et al.*, 1988). A pollutant that impedes, or alters, the development of the cuticle or increase weathering of epicuticular waxes may reduce resistance to winter desiccation. Barnes *et al.* (1988) have demonstrated that O_3 can cause latent injury, which may then decrease the resistance of herbaceous species and trees to freezing. When O_3 maxima occur in winter, these

elevated concentrations are occurring outside of the growing season, so perennials and over-wintering annuals may be subjected to the combined stresses of pollution, plus chilling, freezing, and winter desiccation (Barnes *et al.*, 1988). Future studies on vegetation in parts of South Africa where harsh conditions occur, would indicate if the effects of O_3 on this vegetation is more pronounced in winter.

Concern for crop decline in several parts of the world is based on lower than expected yields. Breeders of crops make the mistake of only considering the commonly accepted stresses as contributing factors in crop decline but do not consider the possible effects O_3 or other air pollutants (Heck *et al.*, 1986). Awareness of air pollutant effects is necessary before breeding programmes, effective for development of resistant cultivars, can be initiated (Heck *et al.*, 1986). This is an essential aspect that must be considered in crop production in South Africa. Although O_3 concentrations appear to be relatively low in comparison to other countries, the effects, if any, on the crops grown in South Africa are not known. This is certainly an area where more studies could be carried out. In the United States, of all the pollutants, O_3 is the one of most concern, having the greatest effect on agriculture and forestry production, as it is found at damaging concentrations in most parts of the country (Heck *et al.*, 1986). Estimates in the USA of annual crop losses due to O_3 are from $1 billion to $5 billion (Heck *et al.*, 1986). These estimates must be considered preliminary for the following reasons: 1) only one or several cultivars of the most important species had been studied; 2) effects of soil moisture on dose-response relationships are poorly understood; and 3) the effects of other factors (e.g. climate, exposure dynamics) on plant response to O_3 in the dose-response relationship were unquantified (Heck *et al.*, 1986). These factors need to be taken into account when establishing

whether or not O_3 is a potential threat not only to crops and forestry, but also to the natural ecosystems, in South Africa.

Since there were no observed significant differences in the amount of injury occurring on the two week old plants at the various sites, it was not surprising that there were also no significant differences between the measured chlorophyll contents. In the study performed with the two week old plants it was interesting to note that the chlorophyll a:b ratios were highest in the winter sampling period, when the damage index values were also the highest observed. This observation is contrary to what might be expected. It has been stated that the chlorophyll a:b ratios decrease with increasing amounts of injury (Knudson *et al.*, 1977). Alternatively, the chlorophyll a:b ratios decrease as the total concentration of chlorophyll decreases. There are two possible reasons for this decrease in chlorophyll a:b ratios: firstly chlorophyll a may be more readily degraded by O_3 than chlorophyll b; and secondly, O_3 may affect the synthesis of new chlorophyll so that the synthesis of chlorophyll a is reduced or the synthesis of chlorophyll b is increased relative to uninjured leaves (Knudson *et al.*, 1977).The dry weights of the plants were highest during the summer. This could be due to reduced O_3 concentrations in summer, but it is most probably due to the fact that summer is the growth season. However, as mentioned previously, it is difficult to make conclusions about the usefulness of chlorophyll analysis in tobacco bioindication, since the O_3 concentrations measured in this study were low.

While it would appear from this and other preliminary studies, that the O_3 concentrations being experienced in South Africa are well below those concentrations where effects on plants are

significant, measures should be taken to ensure that O_3 concentrations remain low, thus preventing detrimental effects to plants in the future.

4.3 NO_2 concentrations in the Greater Durban area and Mpumalanga

The highest measured NO_2 concentrations, in the Greater Durban area study, were at the UND and Botanic Gardens (BG) sites, and lowest at the other two sites (Kloof and Mooi River). These observations are what may be expected since the UND and BG sites are closer to the city centre, whereas the Kloof and Mooi River sites could be classed as suburban and rural respectively. NO_2 concentrations are usually higher closer to the city centre as a result of the combustion of fuels, for example in road transport, power stations, commercial and domestic heating, railways, and refineries (Bell & Ashenden, 1997). Urban concentrations of NO_2 are dominated by motor vehicle emissions (UK Department of Environment, 1993). Road transport accounts for about half of the United Kingdom NO_2 emissions and a greater proportion of ground-level concentrations in urban areas. In the UK emissions from road transport have increased by a factor of 1.4 between 1986 and 1990. Campbell *et al.* (1994) observed that mean NO_2 urban concentrations ranged from less than 10 ppb in northern Scotland to around 50 ppb at near-road sites in London (July to December 1986 and 1991).

In this study, the highest NO_2 concentration measured in the city centre was approximately 31 ppb during the late autumn season. This figure is double the critical level of NO_2 for vegetation of 16 ppb as set by the UN-ECE, but much lower than the standards set by the USA (50 ppb), the WHO (75 ppb), and South Africa (50 ppb) for human health.

There were no recorded significant differences in NO_2 concentration between the four seasons at the UND, Kloof and Mooi River sites. Seasonal differences did occur, however, at the BG site, with the highest concentrations occurring in autumn and winter. Although statistical analysis indicated no seasonal difference in the NO_2 concentrations at the UND site, the concentrations appeared to be somewhat higher during the late autumn and winter seasons. Surveys of NO_2 in rural areas, remote from sources such as roads, have shown NO_2 to be present throughout the year, with winter levels approximately twice those found in summer (Campbell, 1988; Ashenden & Bell, 1989). Several factors are thought to cause this seasonal variation (Bell & Ashenden, 1997). Fossil fuel use for power generation and heating may be increased during the winter months. Seasonal variation may also be enhanced by changes in NO_2 sinks from summer to winter. Winter episodes of elevated NO_2 concentration can occur during cold stagnant anticyclones when dispersion is poor (UK Department of Health, 1993). Concentrations of total NO_2 are high (often greater than 1000 ppb) and NO_2/NO_X ratios are typically only 0.1-0.3. The precise method of formation of the NO_2 in such situations is not completely clear, although the reaction between NO and O_2 probably makes a significant contribution. At normal ambient levels of NO this reaction is very slow and makes no contribution to NO_2 formation. However, in the high concentrations found during winter stagnation episodes this is not the case. The rate of the reaction between NO and O_2 is also faster at lower temperatures (UK Department of Health, 1993). The diurnal pattern of NO_2 in urban areas tends to follow quite closely that of traffic activity. In the UK, future trends in urban concentrations of NO_2 will almost exclusively be dictated by motor vehicle activity (UK Department of Health, 1993).

The short survey undertaken in this study of NO_2 concentrations in parts of Mpumalanga, indicated that NO_2 concentrations were higher in the city of Nelspruit than in the surrounding areas. Once again this could be attributed to emissions from vehicles. The concentrations measured simultaneously at the UND site were shown to be considerably higher than those in and around Nelspruit, being more than double the mean concentrations measured within the city centre of Nelspruit. This would be expected since Durban has more industry and a greater population and, therefore, has more vehicular emissions than Nelspruit.

Although a study of seasonal variation in NO_2 concentrations in Mpumalanga was not carried out in this study, other investigations have indicated that seasonal variations in NO_2 concentrations do occur in this area. In a study carried out by Held *et al.* (1993) in Mpumalanga, it was observed that nitrate concentrations followed a very distinct seasonal variation with minimum values recorded during January/February (summer) and maximum values during August/September (winter). The mean values calculated for the study varied from a minimum of $0.28ug.m^{-3}$ (0.15 ppb) in summer to a maximum high of $1.2ug.m^{-3}$ (0.62 ppb) during winter. A steady increase in pollutant concentration seems to occur over a period of six months from about February to August, while the decline is quicker, extending only over a four month period from September to January. Peak values occurred during a period varying from a week to a month while minimum concentrations were only observed for a few days. Annegarn *et al.* (1996b) have similarly observed that higher monthly means of NO_2 occurred during winter than in summer. They found that the highest NO_2 values were from coal burning and exhaust emissions during rush hour traffic. Thus seasonal variations of NO_2 do exist in the Mpumalanga region.

The effects of NO_2 on crops and ecosystems in South Africa are not known. As with O_3 pollution, high concentrations of NO_2 may result in changes in competitive relationships between various plant species, thus resulting in changes and/or loss of biodiversity. Injury to plants, both visible and invisible, only occurs at very high concentrations of NO_2. When NO_2 occurs alone in ambient air, it is not considered an important phytotoxicant except near point sources at which it exceeds 1000 ppb (Heck *et al.*, 1986). The concentrations that were experienced in the Greater Durban area during this study were much lower than 100 ppb. It would appear, therefore, that NO_2 would not have a direct effect on crops plants and ecosystems in this area. However, NO_2 can affect vegetation in indirect ways: a) as an important ingredient in photochemical reactions (forming O_3); and b) when present in association with SO_2 or O_3 (Heck *et al.*, 1986). It is, therefore, interesting to note that the UN-ECE set the critical level for NO_2 as low as 16 ppb. This may be to compensate for the effects that can occur when NO_2 is present in association with other pollutants. Thus, even though the concentrations of NO_2 in South Africa may not be high enough to cause concern with regard to vegetation, future studies should look at the effects of NO_2 in association with other air pollutant gases. It would also be essential to maintain these low levels of NO_2 (through control measures) in order to maintain low levels of O_3.

4.4 Conclusions

Although this study showed that O_3 and NO_2 levels in the Greater Durban area are generally low, it can be concluded that a wider monitoring network is necessary throughout South Africa before considering the current O_3 and NO_2 levels as of having little effect on agriculture and forestry. It would be necessary to use cheap and simple equipment for such a widescale

monitoring network. This means that the problems encountered with the diffusion tubes need to be overcome so that this technique can be used effectively. Similarly, if tobacco bioindicators were to be used again, it would be necessary to establish whether or not these plants do exhibit injury over a threshold value, or whether their sensitivity is being increased at exposures to low O_3 concentrations. That is, it would be necessary to establish whether these plants are exhibiting a stochastic response to O_3 or if there is a threshold response as previously reported (Lorenzini, 1994). The threshold of 40-50 ppb was obviously not applicable for this study. Therefore, either, this threshold is incorrect, or, there are other factors influencing the sensitivity of these tobacco plants. These other factors would have to be identified if these plants were to be used as bioindicators again. For future studies the white clover biomonitor should be investigated together with chlorophyll analysis, as this species has been found to be very effective as a biomonitor (e.g. Karlsson, 1995).

The O_3 levels measured in this study were below the threshold levels set in Europe, implying that the South African crops and natural vegetation should be unaffected by the O_3 concentrations being experienced. However, since the bioindicators were exhibiting injury at levels much lower than this threshold, it is important that future studies establish whether or not other plant species in South Africa are being similarly affected by these lower O_3 levels. Similarly, the extent of the effects of NO_2 alone and in combination with other pollutants, like O_3 and SO_2, on the South African vegetation, needs to be established.

5. REFERENCES

AEA Technology (1996). http://www.slb.mf.stockholm.se/e/chem.htm.

Annegarn, H. J., Rorich, R. P., Burns, Y. & Engelbrecht, W. G. (1996a). Standards, equipment and monitoring. In *Air pollution and its impacts on the South African Highveld* (ed. G. Held, B. J. Gore, A. D. Surridge, G. R. Tosen, C. R. Turner and R. D. Walmsley), Environmental Scientific Association and National Association for Clean Air, Cleveland and Parklands. pp 20-24.

Annegarn, H. J., Turner, C. R., Helas, G., Tosen, G. R. & Rorich, R. P. (1996b). Gaseous pollutants. In *Air pollution and its impacts on the South African Highveld* (ed. G. Held, B. J. Gore, A. D. Surridge, G. R. Tosen, C. R. Turner and R. D. Walmsley), Environmental Scientific Association and National Association for Clean Air, Cleveland and Parklands. pp 25-34.

Ashenden, T. W. & Bell, S. A. (1989). Rural concentrations of nitrogen dioxide pollution throughout Wales. *Environmental Pollution* **58,** 179.

Ashmore, M. R. (1994). Critical levels and agriculture in Europe. In *Critical levels for ozone: A UN-ECE workshop report* (ed. J. Fuhrer and B. Achermann), Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bern. pp 22-41.

Badiani, M., Fuhrer, J., Paolacci, A. R. & Sermanni, G. G. (1996). Deriving critical levels for ozone on peach trees (Prunus persica (L.) Batsch) grown in open-top chambers in central Italy. *Fresenius Environmental Bulletin* **5,** 592-597.

Baird, C. (1995). *Environmental Chemistry*. W.H. Freeman, New York.

Barnes, J. D., Reiling, K., Davison, A. W. & Renner, C. J. (1988). Interaction between ozone and winter stress. *Environmental Pollution* **53,** 235-254.

Bell, J. N. B. (1992).Biotic and abiotic interactions with air pollutants. Commission of the European Communities.

Bell, S. & Ashenden, T. W. (1997). Spatial and temporal variation in nitrogen dioxide pollution adjacent to rural roads. *Water, Air & Soil Pollution* **95,** 87-98.

Bobbink, R., Boxman, D., Fremstad, E., Heil, G., Houdijk, A. & Roelofs, J. (1992). Critical loads for nitrogen eutrophication of terrestrial and wetland ecosystems based upon changes in vegetation and fauna. In *Critical loads for nitrogen: Report from a workshop held at Lokeberg, Sweden* (ed. P. Grennfelt and E. Thornelof), Nordic Council of Ministers, Lokeborg. pp 111-160.

Bonte, J. & Mathy, P. (1989).The European Communities research project on open-top chambers results on agricultural crops 1987-1988. Commission of the European Communities.

Braun, S. & Fluckiger, W. (1994). Critical levels of ambient ozone for growth of tree seedlings. In *Critical levels for ozone: A UN-ECE workshop report* (ed. J. Fuhrer and B. Achermann), Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bern. pp 88-97.

Bronnimann, S. & Neu, U. (1997). Weekend-weekday differences of near-surface ozone concentrations in Switzerland for different meteorological conditions. *Atmospheric Environment* **31,** 1127-1135.

Brunke, E.-G. & Allen, R. J. (1985). Measurement of atmospheric ozone and other oxidants at three localities in the Cape Peninsula, South Africa. *South African Journal of Science* **81,** 678-681.

Bull, K. R. (1992). An introduction to critical loads. *Environmental Pollution* **77,** 173-176.

Bytnerowicz, A., Manning, W. J., Grosjean, D., Chmiellewski, W., Dmuchowski, W., Grodzinska, K. & Godzik, B. (1993). Detecting ozone and demonstrating its phytotoxicity in forested areas of Poland: A pilot study. *Environmental Pollution* **80,** 301-305.

Campbell, G. W. (1988). Measurements of nitrogen dioxide concentrations at rural sites in the United Kingdom using diffusion tubes. *Environmental Pollution* **55,** 251.

Campbell, G. W., Steadman, J. R. & Stevenson, K. (1994). A survey of nitrogen dioxide concentrations in the United Kingdom using diffusion tubes, July-December 1991. *Atmospheric Environment* **28,** 477-486.

Combrink, J., Diab, R. D., Sokolic, F. & Brunke, E. G. (1995). Relationship between surface, free tropospheric and total column ozone in two contrasting areas in South Africa. *Atmospheric Environment* **29,** 685-691.

Corporation, D. E. (1989). *U.V. photometric ozone analyzer: Operating and service manual.* Dasibi Environmental Corporation, California.

Cox, R. M. (1992). Air pollution effects on plant reproductive processes and possible consequences to their population biology. In *Air pollution effects on biodiversity* (ed. J. R. Barker and D. T. Tingey), Van Nostrand Reinhold, New York. pp 131-158.

Dammgen, U., Grunhage, L., Jager, H.-J. & Haenel, H.-D. (1996). Model considerations for the use of passive sampling techniques to determine trace gas concentrations and flux densities. *Water, Air & Soil Pollution* **88,** 21-37.

Danalatos, D. & Glavas, S. (1996). Diurnal and seasonal variation of surface ozone in a Mediterranean coastal site, Patras, Greece. *The Science of the Total Environment* **177,** 291-301.

Darley, E. F. (1960). Use of plants for air pollution monitoring. *Journal of Air Pollution Control Association* **10,** 198-199.

Davison, A. W. & Barnes, J. D. (1998). Effects of ozone on wild plants. *New Phytologist* **139,** 135-152.

Department of National Health and Population Development (1986). *Air quality guidelines.* DNHPD, Pretoria.

Dhammapala, R. S., Pienaar, J. J., Lachmann, G., Ayers, G. P. & Gillette, R. W. (1995). Monitoring regional air quality in Southern Africa using diffusive samplers. In *Proceedings of the National Association of Clean Air Conference 1995* (Durban, South Africa).

Diab, R. D. & Helas, G. (unpub.). Characteristics of surface ozone mixing ratios at Durban.

Diaz, G., Barrantes, O., Honrubia, M. & Gracia, C. (1996). Effect of ozone and sulphur dioxide on mycorrhizae of *Pinus halepensis* Miller. *Ann. DSci. For.* **53,** 849-856.

Dodd, J. (1996). NO_X air pollution in the Durban Metropolitan area. B.Sc. Honours thesis, University of Natal, Durban.

Dunning, J. A. & Heck, W. W. (1977). Response of bean and tobacco to ozone effect and light intensity, temperature and relative humidity. *Journal of the Air Pollution Control Association* **27,** 882-886.

Durham, J. L. & Kenneth, L. D. (1985). Atmospheric acidification chemistry: A review. In *Acid deposition: Environmental, economic, and policy issues* (ed. D. D. Adams and W. P. Page). Plenum Press, New York. pp 17-30.

Evans, L. S. (1989). Effects of acidic precipitation on crops. In *Acidic precipitation. Volume 2: Biological and ecological effects. Advances in Environmental Science Series* (ed. D. C. Adriano and A. H. Johnson), Springer-Verlag, New York. pp 29-60.

Fernandez, I. J. (1985). Acid deposition and forest soils: Potential impacts and sensitivity. In *Acid deposition: Environmental, economic, and policy issues* (ed. D. D. Adams and W. P. Page), Plenum Press, New York. pp 223-240.

Fernandez, I. J. (1989). Effects of acidic precipitation on soil productivity. In *Acidic precipitation. Volume 2: Biological and ecological effects. Advances in Environmental Science Series* (ed. D. C. Adriano and A. H. Johnson), Springer-Verlag, New York. pp 61-83.

Ferris, B. G. (1978). Health effects of exposure to low levels of regulated air pollutants: A critical review. *Journal of the Air Pollution Control Association* **28,** 482-497.

Folinsbee, L. J., McDonnell, W. F. & Hortsman, D. H. (1988). Pulmonary function and symptom responses after 6.6 hour exposure to 0.12 ppm ozone with moderate exercise. *Journal of the Air Pollution Control Association* **38,** 28-35.

Fuhrer, J. & Achermann, B. (1994). *Critical levels for ozone: A UN-ECE workshop report.*

Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bern.

Gair, A. J. & Penkett, S. A. (1995). The effects of wind speed and turbulence on the performance of diffusion tube samplers. *Atmospheric Environment* **29,** 2529-2533.

Gair, A. J., Penkett, S. A. & Oyola, P. (1991). Development of a simple passive technique for the determination of nitrogen dioxide in remote continental locations. *Atmospheric Environment* **25A,** 1927-1939.

Gair, A. J. & Penkett, S. A. (1995). The effects of wind speed and turbulence on the performance of diffusion tube samplers. *Atmospheric Environment* **29,** 2529-2533.

Galbally, I. E., Miller, A. J., Hoy, R. D., Ahmet, S., Joynt, R. C. & Attwood, D. (1985). Surface ozone at rural sites in the Latrobe Valley and Cape Grim, Australia. *Atmospheric Environment* **20,** 2403-2422.

Goren, A. & Donagi, A. (1979). Use of Bel-W3 tobacco as indicator plant for atmospheric ozone during different seasons in the coastal zone of Israel. *International Journal of Biometeorology* , 331-335.

Gradko International Limited (1995). Nitrogen dioxide: Preparation of diffusion tubes.

Grennfelt, P. & Thornelof, E. E. (1992). *Critical loads for nitrogen.* Nordic Council of Ministers, Copenhagen.

Grosjean, D. a. H., M.W.M. (1992). A passive sampler for atmospheric ozone. *Journal of Air Waste and Management.* **42,** 169-173.

Grosjean, D., Williams, E. L. & Grosjean, E. (1995). Monitoring ambient ozone with a network of passive samplers: A feasibility study. *Environmental Pollution* **88,** 267-273.

Gusten, H., Heinrich, G., Cvitas, T., Klasinc, L., Ruscic, B., Lalas, D.P. and Petrakis, M. (1988). Photochemical formation and transport of ozone in Athens, Greece. *Atmospheric Environment.* **22,** 1855-1861.

Haines, B. L. & Caelson, C. L. (1989). Effects of acidic precipitation on trees. In *Acidic precipitation. Volume 2: Biological and ecological effects. Advances in Environmental Science Series* (ed. D. C. Adriano and A. H. Johnson), Springer-Verlag, New York. pp 1-27.

Heagle, A. S. (1989). Ozone and crop yield. *Annual Review of Phytopathology* **27,** 397-423.

Heagle, A. S. & Heck, W. W. (1974). Predisposition of tobacco to oxidant air pollution injury by previous exposure to oxidants. *Environment Pollution* 7, 247-252.

Heagle, A. S., Miller, J. E. & Sherrill, D. E. (1994). Atmospheric pollutants and trace gases. *Journal of Environmental Quality* **23,** 613-621.

Heagle, A. S., Miller, J. E., Chevone, B. I., Dreschel, T. W., Manning, W. J., McCool, P. M., Morrison, C. L., Neeley, G. E. & Rebbeck, J. (1995). Response of a white clover indicator system to tropospheric ozone at eight locations in the United States. *Water, Air & Soil Pollution* **85,** 1373-1378.

Heagle, A. S., Reinert, R. A. & Miller, J. E. (1996). Response of white clover to ozone in different environments. *Journal of Environmental Quality* **25,** 173-278.

Heck, W. W., Dunning, J. A. & Hindawi, I. J. (1965). Interactions of environmental factors on the sensitivity of plants to air pollution. *Journal of the Air Pollution Control Association* **15,** 511-515.

Heck, W. W., Heagle, A. S. & Shriner, D. S. (1986). Chapter 5: Effects on vegetation: Native, Crops, Forests. In *Air pollution,* Academic Press. pp 247-333.

Heck, W. W., Taylor, O. C. & Tingey, D. T. (1987). *Assessment of crop loss from air pollutants*. Elsevier Applied Science.

Heggestad, H. E. (1991). Origin of Bel-W3, Bel-C and Bel-B tobacco varieties and their use as indicators of ozone. *Environmental Pollution* **74,** 264-291.

Held, G., Snyman, G. M. & Scheifinger, H. (1993). Seasonal variation and trends of atmospheric particulate on the South African Highveld: 1982-1990. *The Clean Air Journal* **8,** 4-11.

Hendrey, G. R. (1985). Acid deposition: A national problem. In *Acid deposition: Environmental, economic, and policy issues* (ed. D. D. Adamas and W. P. Page), Plenum Press, New York. pp 1-16.

Hill, A. C., Heggestad, H. E. & Linzon, S. N. (1970). Ozone. B1-B22. Air Pollution Control Association. Pittsburgh.

Hogsett, W. E., Weber, J. E., Tingey, D., Herstrom, A., Lee, E. H. & Laurence, J. A. (1997). Environmental auditing: An approach for characterising tropospheric ozone risk to forests. *Environmental Management* **21,** 105-120.

Hortsman, D. H., Folinsbee, L. J., Ives, P. J., Abdul-Salaam, S. & McDonnell, W. F. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12ppm. *American Review Respiratory Diseases* **142,** 1158-1163.

Horvath, S. M. & McKee, D. J. (1994). Acute and chronic health effects of ozone. In *Tropospheric ozone: Human health and agricultural effects* (ed. D. McKee, J,), Lewis Publishers, Boca Raton. pp 39-84.

Jacobson, J. S. & Hill, A. C. (1970). Introduction. In *Recognition of air pollution injury to vegetation: a pictorial atlas*, Pittsburgh.

Karlsson, G. P., Sellden, G., Skarby, L. & Pleijel, H. (1995). Clover as an indicator plant for phytotoxic ozone concentrations: Visible injury in relation to species, leaf age and exposure dynamics. *New Phytologist* **129,** 355-365.

Khemani, L. T., Momin, G. A., Rao, P. S. P., Vijayakumar, R. & Safai, P. D. (1995). Study of surface ozone behaviour at urban and forested sites in India. *Atmospheric Environment* **29,** 2021-2024.

Knudson, L. L., Tibbits, T. W. & Edwards, G. E. (1977). Measurement of ozone injury by determination of leaf chlorophyll concentration. *Plant Physiology* **60,** 606-608.

Koutrakis, P., Wolfson, J. M., Bunyaviroch, A., Froelich, S. E., Hirano, K. & Mulik, J. D. (1993). Measurement of ambient ozone using a nitrite-coated filter. *Analytical Chemistry* **65,** 209-214.

Krupa, S. V. & Manning, W. J. (1988). Atmospheric ozone: Formation and effects on vegetation. *Environmental Pollution* **50,** 101-137.

Krupa, S. V., Manning, W. J. & Nosal, M. (1993). Use of tobacco cultivars as biological indicators of ambient ozone pollution: An analysis of exposure-response relationships. *Environmental Pollution* **81,** 137-146.

Lefohn, A. S. & Foley, J. K. (1992). NCLAN results and their application to the standard-setting process: Protecting vegetation from surface ozone exposures. *Journal of the Air and Waste Management Association* **42,** 1046-1052.

Lefohn, A. S. (1997). A new ozone standard in the United States. *Atmospheric Environmental* **31,** 3851-3852.

Levaggi, D. A., Siu, W. & Feldstein, M. (1973). A new method for measuring average 24-hour nitrogen dioxide concentrations in the atmosphere. *Journal of the Air Pollution Control Association* **23,** 30-33.

Levey, M., Mahlman, J. D., Moxim, W. J. & Lin, S. C. (1985). Tropospheric ozone: The role of transport. *Journal of Geophysical Research* **90,** 3753-3772.

Lioy, P. J., Vollmuth, T. A. & Lippman, M. (1985). Persistence of peak flow decrement in children following ozone exposures exceeding the national ambient air quality standard. *Journal of the Air pollution Control Association* **35,** 1068-71.

Liu, L.-J. S., Delfino, R. & Koutrakis, P. (1997). Ozone exposure assessment in a Southern California community. *Environmental Health Perspectives* **105.**

Ljungstrom, E. & Hallquist, M. (1996). Nitrate radical formation rates in Scandinavia. *Atmospheric Environment* **30,** 2925-2923.

Logan, J. A. (1985). Tropospheric ozone seasonal behaviour, trends and anthropogenic influence. *Journal of Geophysical Research* **20,** 10463-10482.

Lorenzini, G. (1994). A miniaturised kit for ozone biomonitoring. *Applied Biochemistry and Biotechnology* **48,** 1-4.

Markert, B. (1991). Inorganic chemical investigations in the Forest Biosphere Reserve near Kalinin, USSR. I. Mosses and peat profiles as bioindicators for different chemical elements. *Vegetatio* **95,** 127-135.

Markert, B. (1993). *Instrumentelle multielementanalyse an pflanzenproben*. VCH-Verlagsgesellschaftmbh, Weinbein, New York, Tokyo.

Markert, B. (1994). Plants as Biomonitors: Potential advantages and problems. In *Biogeochemistry of trace elements* (ed. D. C. Adriano, Z. S. Chen and S. S. Yang), Northwood. pp 601-613.

McCurdy, T. R. (1994). Concentrations of ozone in the lower troposphere (ambient air). In *Tropospheric ozone: Human health and agricultural impacts* (ed. D. J. McKee), Lewis Publishers, Boca Raton. pp 19-38.

McKee, D. J. (1994a). Introduction. In *Tropospheric ozone: Human health and agricultural impacts* (ed. D. J. McKee), Lewis Publishers, Boca Raton. pp 3-8.

McKee, D. J. (1994b). Legislative requirements and ozone NAAQS review. In *Tropospheric ozone: Human health and agricultural impacts* (ed. D. J. McKee), Lewis Publishers, Boca Raton. pp 9-18.

McLaughlin, S. B. & Taylor, G. E. (1981). Relative humidity: Important modifier of pollutant uptake by plants. *Science* **211,** 167-169.

Mehlhorn, H., Seufert, G., Schmidt, A. & Kunert, K.J. (1986). Effect of SO_2 and O_3 on production of antioxidants in conifers. *Plant Physiology* **82**, 336-338.

Merienne, M. F., Jenouvrier, A. & Coquart, B. (1995). The NO_2 absorption spectrum. I: Absorption cross-sections at ambient temperature in the 300-500 nm region. *Journal of Atmospheric Chemistry* **20,** 281-297.

Miller, D. P. (1988). Low-level determination of nitrogen dioxide in ambient air using the Palmes tube. *Atmospheric Environment* **22,** 945-947.

Nikki, H., Daby, E. E. & Weinstock, B. (1972). Mechanisms of smog reactions. In *Photochemical smog and ozone reactions: Advances in chemistry 113* (ed. R. F. Gould), American Chemical Society, Washington D.C. pp 16-57.

Olbrich, K. (1994). Air pollution impacts on South African commercial forests: An overview

1988-1993. CSIR, Division of Forest Science and Technology. Nelspruit. 14 January 1994. p 1-12.

Olson, J. R., Fishman, J., Kirchhoff, V. W. J. H., Nganga, D. & Cros, B. (1996). Analysis of the distribution of ozone over the southern Atlantic region. *Journal of Geophysical Research* **101** (D19), 24083-24093.

Oltmans, S. J. (1994). Surface ozone measurements from a global network. *Atmospheric Environment* **28,** 9-24.

Ormrod, D. P. (1982). Air pollutant interactions in mixtures. In *Effects of gaseous air pollution in agriculture and horticulture* (ed. M. H. Unsworth and D. P. Ormrod), Butterworths, London. pp 307-332.

Palmes, E. D. & Gunnison, A. F. (1973). Personal monitoring device for gaseous contaminants. *American Industrial Hygiene Association Journal* **34,** 78.

Palmes, E. D., Gunnison, A. F., DiMattio, J. & Tomczyk, C. (1976). Personal sampler for nitrogen dioxide. *American Industrial Hygene Association Journal* **37,** 570-577.

Pardo, L. H. & Driscoll, C. T. (1993). A critical review of mass balance methods for calculating critical loads of nitrogen for forested ecosystems. *Environmental review* **1,** 145-156.

Pardo, L. H. & Driscoll, C. T. (1996). Critical loads for nitrogen deposition: Case studies at two northern hardwood forests. *Water, Air, & Soil pollution* **89,** 105-128.

Penuelas, J., Fillele, I., Elvira, S. & Inclan, R. (1995). Reflectance assessment of summer ozone fumigated Mediterranean white pine seedlings. *Environmental and Experimental Botany* **35,** 299-307.

Preston-Whyte, R. A. & Tyson, P. D. (1988). *The atmosphere and weather of Southern Africa.* Oxford University Press, Cape Town.

Rich, S., Taylor, G. S. & Tomlinson, H. (1969). Crop damaging periods of ambient ozone in Connecticut. *Plant Disease Reptr.* **53,** 960-973.

Richards, B. L., Middleton, J. T. & Hewitt, W. B. (1958). Air pollution with relation to agronomic crops. V. Oxidant stipple of grape. *Agronomy Journal* **50,** 559-5561.

Rorich, R. P. (1991).Ambient monitoring network annual data report for 1990. Eskom TRI. Johannesburg. TRR/S91/116.

Rorich, R. P. (1992).Ambient monitoring network annual data report for 1991. Eskom TRI. Johannesburg. TRR/S92/059.

Rorich, R. P. (1993).Ambient monitoring network annual data report for 1992. Eskom TRI.

Johannesburg. TRR/S93/043.

Samson, P. J. (1978). Nocturnal ozone maxima. *Atmospheric Environment* **12,** 951-955.

Schenkel, A. a. B., B. (1982). Interference of some trace gases with ozone measurements by the KI method. *Atmospheric Environment* **16,** 2187-2190.

Scholes, M. C., Olbrich, K. A. & Van Rensburg, E. (1996). The environmental impact of atmospheric pollution in the industrial Highveld and adjacent regions: Crops, indigenous vegetation and commercial forests. In *Air pollution and its impacts on the South African Highveld* (ed. G. Held, B. J. Gore, A. D. Surridge, D. R. Tosen and R. D. Walmsley), Environmental Scientific Association and National Association for Clean Air, Cleveland and Parklands. pp 89-101.

Schuurman, G. & Klein, W. (1988). Advances in bioconcentration. *Chemosphere* **17,** 1551-1574.

Shooter, D., Watta, S. F. & Hayes, A. J. (1995). A passive sampler for hydrogen sulphide. *Environmental Monitoring and Assessment* **38,** 11-23.

Shriner, D. S. & Johnston, J. W. J. (1985). Acid rain interactions with leaf surfaces: A review. In *Acid deposition: Environmental, economic, and policy issues* (ed. D. D. Adams and W. P. Page), Plenum Press, New York. pp 241-254.

Shriner, D. S., Heck, W. W., McLaughlin, S. B., Johnson, D. W., Irving, P. M., Joslin, J. D. & Peterson, C. E. (1990).Response of vegetation to atmospheric deposition and air pollution. Acidic Deposition: State of Science and Technology, Vol III. National Acid Precipitation Assessment Program. Washington DC. NAPAP SOS/T Report 18.

Skarby, L. (1994). Critical levels for ozone to protect forest trees. In *Critical levels for ozone: A UN-ECE workshop report* (ed. J. Fuhrer and B. Achermann), Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bern. pp 74-87.

Smith, R. I., Anderson, C. W. & Castro Rivadeneyra, I. (1995). Estimation of the uncertainty in crop loss predictions through statistical modelling of the AOT40s for ozone. *Water, Air, & Soil Pollution* **85,** 1491-1496.

Stadelmann, F. X. & Roch, P. (1994). Preface. In *Critical levels for ozone: A UN-ECE workshop report* (ed. J. Fuhrer and B. Achermann), Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bien.

Tanner, R. L. (1990). Sources of acids, bases, and their precursors in the atmosphere. In *Acidic precipitation: Volume 3. Sources, deposition, and canopy interactions* (ed. S. E. Lindberg, A. L. Page and S. A. Norton), Springer-Verlag, New York. pp 1-19.

Taylor, G. J. (1970). *Recognition of Air Pollution injury to vegetation: A pictorial Atlas,* Pittsburgh.

Taylor, G. J. (1989). Aluminium toxicity and tolerance in plants. In *Acidic precipitation: Volume 2. Biological and ecological effects. Advances in Environmental Science* (ed. D. C. Adriano and A. H. Johnson), Springer-Verlag, New York. pp 327-361.

Terblanche, A. P. S. & Sithole, J. S. (1996). The impacts on human health. In *Air pollution and its impacts on the South African Highveld* (ed. G. Held, B. J. Gore, A. D. Surridge, G. R. Tosen, C. R. Turner and R. D. Walmsley), Environmental Scientific association and National Association for Clean Air, Cleveland and Parklands. pp 108-118.

Tingey, D. T., Heck, W. W. & Reinert, R. A. (1971). Effect of low concentrations of ozone and sulfur dioxide on floiage, growth and yield of radish. *Journal of the American Society of Horticultural Science* **96,** 369-371.

Tingey, D. T., Olszyk, D. M., Herstrom, A. A. & Lee, H. E. (1994). Effects of ozone on crops. In *Tropospheric ozone: Human health and agricultural effects* (ed. D. J. McKee), Lewis Publishers, Boca Raton. pp 175-206.

Department of Environmental Affairs and Tourism, & Department of Water Affairs and Forestry (1997).Discussion document towards a white paper on integrated pollution control and waste management. May, 1997.

Treshow, M. & Anderson, F. K. (1989). *Plant stress from air pollution.* Wiley, New York. p 131-142.

Tyson, P. D. & Preston-Whyte, R. A. (1972). Observations of regional topograhically-induced wind systems in Natal. *Journal of Applied Meteorology* **11,** 643-650.

Tyson, P. D., Kruger, F. J. & Louw, C. W. (1988).Atmospheric pollution and its implications in the Eastern Transvaal Highveld. South African National Scientific Programmes. CSIR. Pretoria. 150. p 144.

United Kingdom Department of Health (1993).Oxides of nitrogen. Advisory Group on the Medical aspects of Air Pollution Episodes. London. Third Report. p 137.

Utell, M. J. (1989). Health effects of ozone: Critical review discussion papers. *Air and Waste Management Association* **39,** 1188-1189.

Viezee, W., Johnson, W. B. & Singh, H. B. (1983). Stratospheric ozone in the lower troposphere. II. Assessment of downward flux and ground-level impact. *Atmospheric Environment* **17,** 1979-1993.

Vukovich, F. M. (1995). Regional-scale boundary layer ozone variations in the eastern united states and their association with meteorological variations. *Atmospheric Environment* **29,** 2259-2273.

Wellburn, A. R. & Higginson, C. (1981). Biochemical explanations of more than additive

inhibitory low atmospheric levels of SO_2 and NO_2 upon. *New Phytologist* **88,** 223-237.

Wellburn, A. R. (1982). Effects of SO_2 and NO_2 on metabolic function. In *Effects of gaseous air pollution in agriculture and horticulture* (ed. M. H. Unsworth and D. P. Ormrod), Butterworths, London. pp 169-187.

Wellburn, A. (1988). *Air pollution and acid rain: The biological impact*. Longman Scientific & Technical, Harlow, Essex. p 274.

Wellburn, A. (1994). *Air pollution and climate change: The biological impact*, Second edition. Longman Scientific and Technical, Essex.

Wolfenden, J., Fox, D. G., Shaw III, C. G. & Moir, W. H. (1992). Action of pollutants individually and in combination. In *Air pollution effects on biodiversity* (ed. J. R. Barker and D. T. Tingey), Van Nostrand Reinhold, New York. pp 72-92.

World Health Organisation (1987). *Air quality guidelines for Europe*. WHO, Geneva.