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Effects of Air Pollution on Citrus Trees

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ABSTRACT

Agricultural crops can be injured when exposed to concentrations of various air pollutants. Injury ranges from visible markings on the foliage, to reduced growth and yield, to premature death of the plant. The development and severity of the injury depends not only on the concentration of the particular pollutant, but also on a number of other factors. These include the length of exposure to the pollutant, the plant species as well as the environmental factors conducive to a build-up of the pollutant. Effects on vegetation vary and can appear just after short periods of exposure to high concentrations of air pollutants resulting in acute effects, or chronic effects after long exposure periods to low concentrations. This review describes documented effects of air pollutants, such as ozone, fluoride, sulfur dioxide, acidic precipitation and other pollutants on citrus trees, describes the mechanisms of injury and damage of the air pollutants to the plant, discusses the suitability of using dose vs. exposure to define the air pollutant uptake and analyses the past trends and future research needs in the field of effects of air pollutants on citrus trees.

Keywords: acid rain, fluoride, nitrogen dioxide, ozone, sulphur dioxide, suspended particulate matter

Abbreviations: PAN, peroxyacetil nitrate; **SPM**, Suspended Particulate Matter; **TFWT**, Total Fruit Weight per Tree; **TSS**, Total Soluble Solids; **VOC**, Volatile Organic Compounds

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INTRODUCTION

Air pollution is a problem which now affects every part of our planet. While the effects of air pollution on human health are the most important concern, the impacts on crop production, forest vitality and biodiversity may also have considerable implications for human welfare (Emberson *et al.* 2003), with consequences for economic and aesthetic losses (Marx 1975).

Injuries produced on vegetation were one of the first symptoms of pollution effects to be identified, alerting the investigators of a developing problem in urban areas (Marx 1975). Plants may be affected by relatively low concentrations of pollutants and can serve as a kind of early warning system for the build-up of noxious chemicals in the air.

Early air pollution impacts in vegetation characteristically caused severe, but localised, effects close to emission sources and in urban and industrialised areas. Those effects were mainly identified around factories that emitted large amounts of sulphur dioxide (SO_2) and heavy metals, such as power plants and melting factories. Subsequently, studies of damage produced on crops in the surrounding big cities, e.g. Los Angeles in the 1940s, identified oxidant pollutants, such as ozone and peroxyacetil nitrate (PAN), formed in the photochemical smog produced in the city, as the main contributors to the damage of crops and vegetation (Griffiths 2003). The effects of air pollutants have become regional as well as local in extent (Emberson *et al.* 2003).

Today, according to most estimates, ozone accounts for as much as 90% of pollution injury to vegetation. Nevertheless, more localised pollutants, such as SO_2 and hydrogen fluoride have injured vegetation and crops in some localised areas (Marx 1975). Other pollutants recognised as harmful for vegetation are oxidants, including PAN, NO_2, Cl_2 and HCl, heavy metals and particulate matter.

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Invited Review

EFFECTS OF AIR POLLUTANTS ON VEGETATION

Air pollutants are mainly produced by incomplete burning of fossil fuels. The principal source is usually the automobile. The pollutants consist of hydrocarbons, carbon monoxide, nitric oxide, lead derivatives, and many other minor compounds. Some of these emissions react rapidly in sunlight to form photochemical smog which contains ozone as the major component and much lower levels of PAN and NO_2 (Thompson *et al.* 1972).

Major losses to agricultural and forest plants are caused by this mixture of pollutants, especially photochemical smog and fluorides (Thompson 1968). Injury ranges from visible markings on the foliage, to reduced growth and yield, to premature death of the plant. The development and severity of the injury depends not only on the concentration of the particular pollutant, but also on a number of other factors. These include the length and time of day of exposure to the pollutant, the plant species and its stage of development, the interaction between pollutants, soil moisture as well as environmental factors, such as temperature, humidity and the intensity and duration of sunlight previous to the pollutant exposure (i.e. plant preconditioning) as well as the intensity and length of sunlight during the pollutant exposure conductive to a build-up of the pollutant, which make it either susceptible or resistant to injury (Marx 1975; Griffiths 2003).

The effects of pollution in plants are observed in the cell constituents such as membranes, particularly in the cytoplasm and the vacuoles. Pollution also modifies the intracellular pH affecting the metabolic controls. The accumulation of these effects in the membranes and the cellular processes has a repercussion in other processes as photosynthesis, photorespiration and respiration, osmosis, pH and nutrition. At an extracellular level, processes related to transport and allocation are also affected. In vegetation lightly affected by pollutants, homeostatic adjustments in the metabolism may be observed (e.g. adjustment of photosynthesis rate (Johnson and Ball 1990)). Even though, the biochemical processes and physiological controls may affect the plant growth (Granados-Sanchez *et al.* 2001).

A frequently observed consequence of environmental adversity in vegetation is the phenomenon of oxidative stress. By perturbing cellular metabolism, such as photosynthetic processes, many stress factors (e.g. ozone) induce the production of reactive oxygen species, which cause oxidative injury within the plant cell. Protection against oxidative stress is complex and includes both enzymatic and non-enzymatic components. Antioxidant enzymes are key to the defence against the potentially lethal effects of reactive species like superoxide radicals (Mutters *et al.* 1995).

Recent experimental evidence has indicated that physiological and biochemical traits determine the susceptibility of plants to air pollution injury. These processes include changes in stomatal behaviour, which influence plant/water relations, and alterations in carbon and nitrogen metabolism and partitioning. The degree to which crop productivity is impacted depends on the growth stage of the plant, the rate and amount of pollutant entry, and the plant's physiological capacity to detoxify, repair damage, and metabolically compensate for pollutants and its oxidative derivatives. Disturbances in photosynthesis and carbon assimilation may influence plant development, and pollutants can affect the ability of both stomata and other parts of the epidermal layer to regulate gas exchange (Mutters *et al.* 1993).

The leaves are usually the site of injury by gaseous pollutants. Air pollutants enter the leaves through the stomata (Marx 1975), causing different types of injuries. Flecking, bronzing and necrosis are the results of localised death of preciously living tissue. Flecking and bronzing are recognisable generally as small patches of brown or tan discolouration, which turn into necrotic areas after a few days. Silvering and glazing are usually the result of an abnormal increase in sub-epidermal air space. In general, exposure to high enough concentrations of any air pollutant results in such necrotic lesions. Most often leaf veins are the last affected. At low concentrations, PAN, HCl and Cl_2 bronze the abaxial surface of leaves, while ozone and NO_2 injury, appearing as silvering, and affect the adaxial surfaces of the intracostal regions. Damage caused by SO_2 and HF is often restricted to leaf margins or tips. Water logging appears somewhat rapidly following exposure to most pollutants. Except for the water-logging effect, no macroscopic signs of injury occur immediately after exposure to air pollutants. Most appear about 24 hours later (Heath 1980).

The air pollutants currently considered to be most important in causing direct damage to vegetation are SO_2, NO_x, O_3, fluoride and SPM. Direct effects of air pollution can be further classified into visible and nonvisible injury. Visible injury is associated with cell death, a decrease in total leaf photosynthetic activity, and hence reduced leaf productivity. Visible injury normally takes the form of discolorations of the leaf surface caused by internal cellular damage. Such injury can reduce the market value of agricultural crops for which visual appearance is important (e.g. tobacco and spinach). It can also lead to yield reductions, while the damaged parts of the leaf surface can provide points of entry for plant pathogens.

Nonvisible injury results from pollutant impacts on plant physiological or biochemical processes and can lead to significant loss of growth or yield and changes in nutritional quality without visible injury (e.g. protein content) (Ashmore *et al.* 1999). This type of damage can be assessed by actual comparative measurements of productivity (Heath 1980). The nonvisible injury is a consequence of the energy deviation from growth to pollutant damage repair or compensation.

Whilst visible injury tends to be associated with short-term exposures to high pollutant levels, nonvisible injury is generally a consequence of longer-term exposures to moderately elevated pollution concentrations. While visible injury can be identified in the field, loss of yield can only be identified with suitable control plants, and so can go undetected especially if there is little awareness of air pollution issues. Nonvisible effects on crop yields may have very serious consequences, both for the national economy and for the livehoods of individual farmers (Emberson *et al.* 2003).

On the other hand, plants may also offer resistance to air pollution and maintain its capacity to grow and remain free of any visible or invisible injury. This phenomenon of resistance can be explained as a) the plant may resist by exclusion of pollutants closing the stomata in order to avoid stress and b) the plant may be resistant to air pollution if it has a detoxifying mechanism to balance the effects of the air pollutants. In those cases of tolerance to air pollution and stress, the natural selection will imply the adaptation to a wide range of pollutant agents in different species (Granados-Sanchez and Lopez-Rios 2001).

The sensibility and adaptation of each plant to air pollution depends on the balance between the injury repairing processes and all the physiological processes that integrate the metabolism of the plant (Granados-Sanchez and Lopez-Rios 2001).

Effects of oxidants

Sources

Tropospheric ozone (O_3) is the predominant pollutant of a group of chemicals called photochemical oxidants, commonly referred to as photochemical smog. Also present in photochemical smog are formaldehyde, other aldehydes, and peroxyacetyl nitrate. They are all secondary pollutants formed in the atmosphere under conditions of bright sunlight and warm temperature as a result of photochemical reactions involving nitrogen oxides, carbon monoxide and volatile organic compounds as primary pollutant precursors. Sources of these primary pollutants rise mainly from motor-vehicle emissions, stationary combustion sources, and industrial and domestic use of solvents and coatings (Denison *et al.* 2000). However, urban traffic is the principal source (Delgado-Saborit *et al.* 2008).

Effects on vegetation

Ozone is the main pollutant in the oxidant smog complex and is extremely toxic to vegetation at high levels, with chronic effects, the intensity of which depends on meteorological factors, exposition time, age of the plant, leaf position and plant species (Pardos 2006). Its effect on plants was first observed in the Los Angeles area in 1944. Since then, ozone injury to vegetation has been reported and documented in many areas worldwide (Griffiths 2003).

Unlike SO_2 and NO_x, consideration of the toxicity of ozone is not complicated by its role as a source of an essential nutrient. Ozone transfer via the leaf cuticle is negligible and ozone uptake is almost entirely through the stomata. Oxidants are extremely reactive to a variety of substances found within living systems. Such reactions generally involve the formation of free radicals (Heath 1980). On entry to the sub-stomatal cavity, ozone reacts with constituents of the aqueous matrix associated with the cell wall to form other derivatives which result in the oxidation of the sensitive components of the plasmalemma, and subsequently the cytosol. The inability to repair or compensate for altered membrane permeability can manifest itself as symptoms of visible injury, which are generally associated with short-term exposures to high ozone concentrations. Symptoms of acute injury from short exposures characteristically occur on the upper surface of affected leaves and appear as chlorosis, bleaching, bronzing, flecking, stippling and uni- and bifacial necrosis (Emberson *et al.* 2003).

Chronic exposures may or may not result in visible foliar symptoms, usually characterised by chlorosis, premature senescence and leaf abscission. However, reductions in growth from chronic exposures are well documented and can result in crop yield losses, reduction in the quality of the crop (Marx 1975), reductions in annual biomass increments for forest trees and shifts in species composition of semi-natural vegetation (Emberson *et al.* 2003), changes in carbohydrate allocation as well as susceptibility to plagues and illnesses (Pardos 2006) without any visible sign of pollutant stress (Marx 1975). Conversely, some crops can sustain visible foliar injury without any adverse effect on yield. (Griffiths 2003). Chronic exposures to ozone may age plants prematurely and cause premature senescence (Marx 1975).

Susceptibility to ozone injury is influenced by many environmental and plant growth factors. High relative humidity, optimum soil-nitrogen levels and water availability increase susceptibility and severity of injury by ozone (Marx 1975). Injury development on broad leaves is also influenced by the number of stomata, stomata opening and the stage of maturity of the leaf. The youngest leaves, which have less stomata, are resistant. As the leaves begin to expand, they become ozone sensitive at their tips. With continuing expansion, they become successively susceptible at middle and basal portions. The leaves become resistant again at complete maturation, with many of the older leaves susceptible only at their base (Rich 1964; Griffiths 2003).

An indirect effect of oxidant exposure on vegetation is that some leaf diseases caused by fungi are more severe if oxidant injury is also present. On the other hand, pollution may be as harmful to other fungal pathogens as it is to the plant (Marx 1975).

Effects on citrus trees

Plant damage from ozone and other oxidizing materials in polluted air was extensive in 1960s in the Los Angeles basin. Although such damage was readily evaluated on leafy vegetable crops, the effects were more subtle in the case of citrus. Spotting of leaves and fruit were noted at certain times in oranges and grapefruit, but the extent of damage was not determined (Erickson 1968).

During the late 1950s and 1960s, Thompson and Taylor studied the chronic (long-term, low levels) effects of the photochemical oxidants which occur in the Los Angeles basin on navel oranges and lemons. These studies showed reduced water use, reduced photosynthesis, increased leaf drop, reduction in fruit quality and very substantial reductions in yields, associated primarily with reduced numbers of fruit in both crops due to photochemical oxidants. Losses in production occurred even though there were few easily observed leaf injury symptoms on the trees (Thompson 1967, 1968, 1969, 1972; Olszyk *et al.* 1988). According to experiments performed by Thompson at the Air Pollution Research Centre of the University of California at Riverside, oxidants may decrease the yields of citrus crops by as much as 50 to 60%. This may be a due to a decrease in photosynthesis as a consequence of the pollutant probably causing the stomata to close and preventing the uptake of carbon dioxide needed for photosynthesis (Marx 1975). Fruit drop in lemons seemed to be of little importance, but in navel oranges represented a serious problem that occurred in areas of low air pollution and was accentuated by heavy photochemical smog. Apparently when the trees were impoverished by reduced CO_2 absorption and water use plus increased leaf and fruit drop, yields were reduced in some cases to one half (Thompson and Taylor 1969).

Dugger *et al.* (1966) studied the effects of ozone in lemons and reported that visisble symptoms were not observed when lemon seedlings were exposed to 0.25 ppm ozone. They did find that the ozone increased permeability and the rate of respiration of the cells in the leaves. Starch and total carbohydrates decreased in the leaves, although the reducing sugar level was higher (Dugger *et al.* 1966; Erickson 1968). A similar result was reported by Einig *et al.* (1997) in birch cuttings (*Betula pendula* Roth) exposed to different ozone concentrations. They described that chronic ozone exposure leaded to an inhibition of sucrose synthesis and favoured sucrose degradation. They attributed these results to the effect of ozone reducing the photosynthate export (Einig *et al.* 1997).

Olszyk reported in 1989 that ambient oxidants dramatically reduced orange fruit yields. The reduction in fruit weight that comes with exposure to oxidants was associated primarily with a reduced number of fruits per tree and not reduced fruit weight (Olszyk 1988), as previously reported by Thompson *et al.* (1967) and Thompson and Taylor (1969). Oxidants had little effect on fruit quality except for a slightly less orange colour. Ambient oxidants had no effect on overall tree growth, leaf production, immature fruit loss or flower drop. Individual leaves weighed less, with higher oxidant concentrations. Oxidants resulted in stomatal closure and more negative leaf water potentials, indicating increased moisture stress in leaves. The net photosynthetic rate was not affected by oxidants. Leaf starch prior to flowering was higher with increasing oxidant concentrations, thus indicating an effect on carbon allocation which may affect flowering or fruit set. No other biochemical indicators were affected by oxidants (Olszyk 1989; Olszyk *et al.* 1990).

Later, Eissenstat *et al.* (1991) studied the effect of oxidants on freeze resitance of citrus trees. They found that grapefruit (*Citrus paradisi*) exposed to 120 ppb ozone (12-h mean) for eight months exhibited a significant reduction in freeze resistance. The negative effects of ozone were ameliorated by environmental conditions conducive to slow vegetative growth. It is noteworthy that hard freezes, such as the one experimentally reproduced in that study, seldom occur in the citrus growing regions (Eissenstat *et al.* 1991a, 1991b).

Recently, Calatayud *et al.* (2006) studied the effects in photosynthesis and gas exchange of long-term ozone exposure on citrus. Three-year-old Satsuma mandarins (*Citrus reticulata*) were exposed, during one-year in open top chambers, to filtered ($AOT40_{12} = 0$ $mm^3/m^3.h$), ambient air ($AOT40_{12} = 7000$ $mm^3/m^3.h$) and enriched ozone air ($AOT40_{12} = 36000$ $mm^3/m^3.h$). As a result of the higher ozone concentration treatment, net photosynthetic rate and stomatal conductance decreased and intercellular CO_2 concentration increased. Ozone also reduced the development of non-photochemical quenching preventing the dissipation of excess excitation energy and, therefore, generated several alterations in photosynthetic apparatus. The long-term effects in ambient ozone concentration treatment were minor (Calatayud *et al.* 2006).

Iglesias *et al.* (2006) reported the biochemical responses of citrus plants to ozone. Three-year-old 'Clementine' mandarins (*Citrus clementina*) were exposed to ambient (10 nL/L) and high (30 and 65 nL/L) concentrations in open top chambers for a year. The data showed that ozone reduced total chlorophylls, carotenoid and carbohydrate concentration, and increased 1-aminocyclopropane-1-carboxylic acid (ACC) content and ethylene production in leaves. In treated plants, the ascorbate leaf pool was decreased, while lipid peroxidation and solute leakage were significantly higher than in ozone-free controls. The data indicated that ozone triggered protective mechanisms against oxidative stress in citrus (Iglesias *et al.* 2006).

Effects of sulphur dioxide

Sources

Major sources of anthropogenic SO_2 are processes associated with the combustion of fossil fuels containing sulphur, such as coal-burning operations, especially those providing electric power and space heating – domestic and commercial. Sulphur dioxide emissions can also result from the burning of petroleum and the smelting of sulphur-containing ores (Griffiths 2003). Sulphur dioxide is a primary pollutant and therefore concentrations tend to be directly related to the extent of local emissions and the height of emissions (Emberson *et al.* 2003).

Effects on vegetation

Sulphur dioxide enters the leaves mainly through the stomata, but it is also deposited at significant rates on wet surfaces, where it might dissociate to form sulphite or bisulphite and react with cuticular waxes. This can affect the cuticle to such an extent that a certain amount of SO_2 can enter via the damaged cuticle (Wellburn 1994). Critical to the impact of the internal SO_2 dose are the buffering capacities of the internal fluids (Emberson *et al.* 2003). SO_2 act as a poison at specific metabolic sites (such as chloroplast and mitochondria) (unlike oxidants' reactions which are more general) and this initial effect is subsequently translated into further metabolic imbalances (Heath 1980).

Sulphur dioxide causes visible injury characterised by chlorosis of leaf tissue. The visible effects can be classified as either acute or chronic effects. Acute injury is caused by absorption of high concentrations of SO_2 in a relatively short time. The symptoms appear as 2-sided (bifacial) lesions that usually occur between the veins and occasionally along the margins of the leaves. The colour of the necrotic area can vary from a light tan or near white to an orange-red or brown, depending on the time of year, the plant species affected and weather conditions. Recently, expanded leaves are usually the most sensitive to acute SO_2 injury, the very youngest and oldest being somewhat more resistant.

High concentrations of sulphur dioxide can produce acute injury in the form of foliar necrosis, even after relatively short duration exposure. However, such effects are far less important in the field than chronic injury, which results from long-term exposure to much lower concentrations of the gas and is essentially cumulative in nature, taking the form of reduced growth and yield and increased senescence, often with no clear visible symptoms or with some degree of leaf damage, which appears as a yellowing or chlorosis of the leaf, and occasionally as a bronzing on the under surface of the leaves (Griffiths 2003). The effects of a given dose of SO_2 can be modified by prevailing environmental conditions (WHO 2000).

Even when no visible injury is apparent, SO_2 can cause a reduction in growth and yield. However, in sulphur deficient areas, low levels of SO_2 may actually be beneficial to crop growth. SO_2 can also indirectly affect crop yields through effects on the prevalence of plant pathogens and insect pests (Thomas 1961; Bell *et al.* 1993), as can NO_x. Sulphur dioxide can also contribute to acidification of sensitive soils, which may be accompanied by a depletion of base cations, affecting the local vegetation over relatively long timescales (Emberson *et al.* 2003).

Conversely, SO_2 can also modify the response of plants to other environmental stresses, both biotic and abiotic, often exacerbating their adverse impacts (WHO 2000). On the other hand, different plant species and varieties and even individuals of the same species may vary considerably in their sensitivity to SO_2. These variations occur because of the differences in geographical location, climate, stage of growth and maturation (Griffiths 2003).

Effects on citrus trees

Thomas (1961) cited results of O'Gara who reported citrus as being very resistant to acute foliar injury by SO_2 compared to a hundred other crops, ornamental or forest species tested (Thomas 1961). Matsushima and Harada (1964) found that exposures of three species (e.g. *Citrus Unshiu* and *Citrus Hassaku* of 1-year-old citrus to 1 and 5 ppm SO_2 for 2 hr/day for 40 days in closed greenhouses caused no foliar injury (Matsushima and Harada 1964). Later work showed Satsuma orange (*Citrus unshiu*) to have accelerated leaf drop after exposure with 5 ppm SO_2 for 2 hr/day for 34 days. After spraying with Bordeux mixture (i.e. a combination of copper sulphate and hydrated lime), leaf drop was accelerated in 13 days of exposure with SO_2 (Matsushima and Harada 1965, 1966; Olszyk 1988).

Olszyk reported, in 1988 and 1989, on the chronic physiological, growth and productivity effects of SO_2 (0.10 ppm) on 'Valencia' orange trees (*Citrus sinensis*). Sulphur dioxide reduced yields by 23-35% compared to filtered air, mainly as a consequence of both reduced fruit number and reduced fruit weight. Fruit quality effects due to SO_2 were reduced orange colour, increased fruit circumference showing a more elliptical fruit and increased rind thickness. Sulphur dioxide resulted in reduced leaf drop on occasional monthly as well as seasonal and yearly basis compared with filtered air. SO_2 did not have any effect on fruit drop (Olszyk 1988, 1989). Individual leaves weighed less with SO_2 exposure. Sulphur dioxide resulted in a higher leaf transpiration rate than that for under filtered air, but no overall effect were observed on stomatal conductance, net photosynthetic rate, or leaf water potential. Total leaf sulphur concentration was increased with SO_2 exposure, but no other biochemical changes were observed (Olszyk 1989).

Olszyk *et al.* reported in 1990 the effects of combined ambient oxidants (0.009–0.015 ppm O_3) and high concentrations of SO_2 (0.081–0.090 ppm) exposures on 'Valencia' orange fruit yield grown in exposure chambers. Yields for the SO_2-treated were 35% lower compared to trees in filtered air. The yield loss was associated with either reduced fruit size in one studied year and with reduced fruit number in the following studied year. Sulphur dioxide-exposed trees tended to have larger fruit than trees in filtered air. The response occurred for fruit circumference, which might have had been due to the greater availability of reserves to the fewer fruit with the SO_2 exposure. No other external fruit quality characteristics were affected by SO_2. Neither juice Total Soluble Solids (TSS) nor titratable acid concentrations were affected by SO_2 in any year. The TSS and acids (by weight) were 12% and 1%, respectively for the SO_2-exposed trees. Sulphur dioxide had no effect on overall tree growth as the average canopy volume and total weight of leaves dropped from SO_2-exposed trees was the same as for all trees in the chambers. Sulphur dioxide exposure resulted in significantly lower ($p>0.05$) individual leaf weight, and was associated with smaller leaf size, as specific area was actually greater for SO_2-treated leaves (Olszyk *et al.* 1990).

Effects of nitrogen dioxide

Sources

Both nitric oxide (NO) and nitrogen dioxide (NO_2) are known to have impacts on vegetation. NO_2 is predominantly a secondary pollutant formed mainly by reaction between emissions of the primary pollutant nitric oxide and ozone. The rapid conversion of NO to NO_2 results in the atmospheric burden of NO_x being predominantly NO_2 at locations away from sources. These pollutants are produced due to the combination of nitrogen and oxygen at high temperatures during combustion processes, although there is also a small contribution from the combustion of nitrogen contained in the fuel. Combustion of fossil fuels from motor vehicles and stationary sources, such as heating and power generation, are the main sources of NOx (Emberson *et al.* 2003).

Effects on vegetation

Nitrogen dioxide presents low toxicity to plants compared with other pollutants, such as ozone. The predominant pathway of NO_x entry into plant leaves is through the stomata, although cuticular resistance to NO_2 entry are lower than for both SO_2 and O_3. The biochemical effects of NO and NO_2 are quite different and there is some uncertainty over which oxide is more toxic. NO_x can reduce plant growth at high concentrations, although growth stimulation can be caused by low NO_x concentrations, generally under situations of low soil nitrogen (Granados-Sanchez and Lopez-Rios 2001). However, even if growth is stimulated, exposure to NO_x can have adverse effects, such as heightened sensitivity to drought, pest and in some cases, to frost (CLAG 1996).

Rare instances of visible injury caused by exposure to very high concentrations of NO_x (similar to peak levels in polluted cities) are characterised by chlorotic areas on leaves associated with necrotic patches and defoliation (Fenger *et al.* 1999). Prolonged exposure to NO_x has been shown to suppress plant growth via inhibition of photosynthesis (WHO 2000). The combination of NO_x with other pollutants has been found to cause synergistic effects on plants. This is particularly true of NO_2 and SO_2 (Ashden *et al.* 1978), but synergistic effects have also been observed between NO_x and O_3 (CLAG 1996; Emberson *et al.* 2003).

Nitrogen stimulates apical growth in summer, predisposing the tree to injuries in winter. On the other hand, an excess of nitrogen produces an indirect decrease in the roots, making the plant more susceptible to drought and can modify the demand of other elements, such as magnesium, potassium, phosphate, tungsten and boron. These deficiencies may alter the assimilation of nitrogen and may affect the synthesis of the proteins. The same excess may lead to the formation of cells with thin walls, being therefore more susceptible to ambient stress, to fungi and to insects (Granados-Sanchez and Lopez-Rios 2001).

Nitrogen dioxide, as well as SO_2, can also contribute to acidification of sensitive soils. NO_x emissions can also cause long-term eutrophication of nutrient-poor terrestrial ecosystems, although the additional nitrogen deposition may also lead to short-term stimulation of growth.

Effects on citrus trees

Thompson *et al.* (1967, 1969) studied the effect of NO_2 levels on citrus and observed no apparent change in photosynthesis, water use, leaf drop or yield of fruit (Thompson *et al.* 1967; Thompson and Taylor 1969). In a subsequent direct fumigation study, continuous exposure of 'Navel' orange trees to 0.5 and 1.0 ppm of NO_2 caused severe defoliation and chlorosis in 35 days. Exposure to 0.25 ppm, and possibly less, caused increased leaf drop and reduced fruit yield (Thompson *et al.* 1970).

A later study that examined the effect of ambient levels of NO_2 on 'Navel' oranges showed the same effects of photochemical smog in ambient air on leaf drop and yield of 'Navel' oranges as observed previously (Thompson and Taylor 1969). Total leaf drop in ambient air was twice that in filtered air (free of NO_2) and the drop, from selected branches were young leaves, was four times greater. The difference in yield of fruit in trees exposed to ambient NO_2 and to filtered air was more than twofold. The effect of NO_2 on the trees receiving filtered clean air indicated only a trend toward greater leaf drop. Despite the leaf drop and reduced fruit yield, the researchers concluded that measured ambient NO_2 levels (around 7 pphm) were not the cause of significant injury to citrus (Thompson *et al.* 1971).

Effects of fluorine

Sources

The most important fluoride emitting industrial sources are aluminium smelters and fertiliser phosphate factories (Emberson *et al.* 2003). Fluorides are also discharged into the atmosphere from the combustion of coal; the production of brick, tile, enamel frit, ceramics, and glass; and the production of steel, hydrofluoric acid, phosphate chemicals (Griffiths 2003; Marx 1975) plastics manufacture; copper and nickel production; and adhesive production. Some releases may also come from the oil industries (EPAQS 2006).

Effects on vegetation

Plants exposed to elevated levels of gaseous fluorides often build up leaf concentrations of fluoride many thousand times higher than the fluoride concentrations in the air surrounding them. Levels in the air usually are reported in parts per billion, while leaf concentrations are normally expressed as parts per million (Leonard *et al.* 1972).

Both gaseous and particulate fluorides are deposited on plant surfaces and some penetrate directly into the leaf, if the cuticle is old or weathered. Gaseous fluoride is absorbed via the stomata and transported by transpirational flow in the apoplast, and can accumulate at toxic levels in the tips and margins of the leaves (Jacobsen *et al.* 1966). Little injury takes place at the site of absorption, whereas the margins or the tips of the leaves build up injurious concentrations (Griffiths 2003). Fluoride dissolved in water on the leaf surface can also be absorbed by diffusion through the cuticle (Brewer *et al.* 1969) and attack the same areas of the leaf.

Leaf injury in the form of chlorosis and necrosis of leaf tips and margins has been described for a number of species in relation to emissions from aluminium smelters. The injury starts as a gray or light-green water-soaked lesion, which turns tan to reddish-brown. With continued exposure the necrotic areas increase in size, spreading inward to the midrib on broad leaves and downward on monocotyledonous leaves. Studies have also shown reductions in photosynthesis, respiration and metabolism of amino acids and proteins (Emberson *et al.* 2003; Griffiths 2003).

Fluoride has been reported to inhibit the activity of certain enzymes (e.g. UPD-glucose-fructose transglycosylase, phosphoglucomutase, polyphenol oxidase (Weinstein and Davidson 2004)) that occur in plants. Development of fluorine chlorosis on previously green leaves indicates some destruction of chlorophyll. In addition, fluorides may inhibit the synthesis of chlorophyll. In either case, the amount of photosynthesis per unit of leaf area is reduced since chlorophyll is required for photosynthesis. Airborne fluorides also reduce total photosynthesis by decreasing the average size of the leaves. This directly reduces the area of photosynthetic activity. The resulting decrease in production of food by the trees is followed by decreased growth and lower fruit production (Leonard and Graves 1972).

Effects on citrus trees

The effect of fluoride air pollution on citrus growth and fruit production was of considerable concern both in California and in Florida since World War II because of the development and expansion of fluoride emitting industries adjacent to large acreages devoted to citrus production (Brewer *et al.* 1969).

Citrus trees are relatively susceptible to damage from atmospheric fluoride. The various species and varieties of citrus differ considerably in their tolerance to fluoride. Lemon, tangerine and tangelo are very sensitive. 'Valencia' orange was moderately sensitive, whereas 'Hamlin' and 'Pineapple' orange as well as grapefruit were the most tolerant (Woltz *et al.* 1971).

Kaudy *et al.* observed in 1955 that citrus accumulates fluorine poorly when an atmospheric source is absent. The amount of fluorine found in citrus leaves in areas remote from industry varies between 1 and 2 ppm. However, near sources of fluorine pollution in southern California, Kaudy *et al.* found elevated amounts of fluorine in citrus foliage, with as high as 211 ppm adjacent to a steel mill (Kaudy *et al.* 1955).

Fluorine accumulates continually in leaves, particularly in the summer and fall months. Little increase was found after winter rains began (Kaudy *et al.* 1955). The elevated fluorine concentrations found in citrus leaves were not associated with any visible damage to the trees. In fractionating leaves to determine the site of fluoride accumulation, Chang and Thompson (1966) found that 'Navel' oranges had the following decreasing order of subcellular distribution: cell wall, chloroplast, water soluble protein, and mitochondria (Chang *et al.* 1966). However, when allowance was made for cross contamination from chloroplasts and chloroplast fragments, it was concluded that the chloroplast fraction had the highest accumulation of fluoride (Erickson 1968).

Mature leaves of citrus are relatively resistant to injury from moderate concentration of airborne fluorides. In fact, they may accumulate several hundred parts per million of fluoride without showing chlorosis or other evidence of injury. However, young citrus leaves often show fluorine chlorosis when they contain as little as 20-30 ppm fluorine (Leonard and Graves 1972). Reported fluorine in 'Navel' orange leaf toxicity limits ranged from 75 mg/kg dry matter (Brewer *et al.* 1960a) to 100-125 mg/kg dry matter (Chapman 1968; Aucejo *et al.* 1997).

Further attempts to assess the influence of elevated fluoride concentrations in citrus foliage on growth and crop production involved several experimental techniques. Wander and McBride (1956) sprayed young grapefruit foliage with 0.1 N solutions of HF and H_2SiF_6 to reproduce chlorosis patterns observed close to sources. Brewer *et al* (1969) introduced fluoride into 'Washington navel' orange trees via their roots. Although tree growth and fruit yields were substantially reduced by fluoride treatment, which resulted in foliar fluoride concentrations of approximately 50 ppm on a dry weight basis, abnormal root growth resulting from precipitation of insoluble fluoride compounds might have been the primary cause of poor growth (Wander *et al.* 1956; Brewer *et al.* 1969).

In a study of varietal sensitivity, Brewer *et al.* (1960a) exposed seven varieties of young citrus trees in greenhouses to 10 to 12 parts per billion (ppb) of fluorine as hydrogen. The varieties studied were 'Lisbon' and 'Eureka' lemons, pink grapefruit (*CES Redblush No. 3*) and 'Marsh' grapefruit, and 'Temple', 'Navel' and 'Valencia' oranges. After 4 months of exposure to HF, the fumigated trees were less vigorous; their leaves were noticeably smaller, lighter green in colour and more chlorotic than those of the controls. 'Valencia' orange leaves absorbed most fluoride. All varieties were sensitive to fluorine, but not equally so, nor did they respond in the same manner. The apparent order of decreasing sensitivity to airborne fluoride was: 'Navel' orange, 'Lisbon' lemon, 'Valencia' orange, 'Eureka' lemon, red grapefruit, 'Marsh' grapefruit and 'Temple' orange (Leonard and Graves 1972). Lemons developed the most chlorosis, oranges developed the least, and grapefruit were intermediate. On the other hand, oranges showed the most reduction in leaf size and gross growth (Brewer *et al.* 1960a).

Brewer *et al.* (1960b) reported that the growth and vigour of 'Navel' orange trees in greenhouses were reduced by 2 to 3 ppb of hydrogen fluoride in the air, even though visible symptoms were lacking. After five to seven months of continuous exposure to 3 to 5 ppb of hydrogen fluoride in the air, considerable chlorosis and some necrosis of mature leaf tips were found. The tip necrosis bore a resemblance to that produced by an excess of boron. Greater leaf drop was associated with hydrogen fluoride in the air. When citrus leaves were exposed to hydrogen fluoride in the air there was a gradual accumulation of fluorine in the leaves with hardly any translocation to stems and roots. With exposure to 2 to 3 ppb of hydrogen fluoride over a period of a year, the fluorine content in leaves, on a dry-weight basis, was found to increase to about 200 ppm. Fruit quality and yield were adversely affected by hydrogen fluoride, although the fruit accumulated very little fluorine. The most obvious effect was in a coarsening of the peel (Brewer *et al.* 1960b; Erickson 1968). After 13 months, the fumigated trees were much smaller than the controls grown in clean air. Significant reductions in trunk diameter, height of tree, crown volume and average leaf size in the fumigated trees as compared with trees grown in clean air, as well as a detrimental effect on yield and quality of fruit were recorded. Fluorine chlorosis and necrosis were found in leaves containing >75 ppm fluorine (Leonard and Graves 1972) and the fluorine in 'Navel' orange leaf toxicity limit was set at 75 mg/kg dry matter (Aucejo *et al.* 1997).

In 1967, Brewer *et al.* sprayed bearing 'Navel' orange trees periodically with NaF solutions over a period of six years. The symptoms reported were the characteristic intervenial chlorosis pattern, the premature leaf drop and reduced leaf size. Also significant reductions in fruit yield beginning in the third year of treatment were encountered. No effects of the fluoride treatments on fruit quality criteria (taste, vitamin C, citric acid, soluble solids, size, juice content and rind thickness) were observed in that experiment (Brewer *et al.* 1967; Leonard and Graves 1972).

The work performed by Brewer *et al.* (1960a, 1960b) showed that prolonged exposure of citrus trees to a few parts per billion of HF gas under greenhouse conditions with controlled humidity and temperature suggested the potential effects of fluoride air pollution on tree growth and toxicity symptom expression. However, practical interpretation of results of field experiments where trees were enclosed in glass or plastic chambers was complicated by atypical tree performance under greenhouse conditions (Thompson *et al.* 1967). Some of the reasons to have different microclimatic conditions in the greenhouse were assumed to be as consequence of a trend of higher humidity in the greenhouse and because the greenhouse walls were acting as a shield protecting from the effect of the wing.

Thompson and Taylor (1969) studied the effect of fluoride on growth, leaf drop, fruit drop and yield of orange and lemons trees in greenhouses. They found that fluoride levels in the Los Angeles basin, where all levels remained below 50 ppm, had little effect on the responses measured. 'Navel' oranges accumulated only one-half as much fluoride as lemons, although the ambient air levels were very similar (Thompson and Taylor 1969).

In the same year, Brewer *et al.* (1969) studied the effects of various fluoride sources on citrus growth and fruit production by repeated spraying at frequent intervals with very dilute solutions of fluoride solutions (0.001 N HF or NaF solution). The visible chlorosis patterns produced by the two fluoride sources were identical and initially appeared at approximately the same fluoride concentration range, between 50 and 75 ppm. A reduction in top growth of citrus exposed to soluble fluorides was reported to be closely correlated with reduced photosynthetic area resulting from smaller leaves and increased leaf fall. They also reported that intermittent exposure to fluoride was less toxic than continuous exposure to HF gas, because with intermittent exposure there was a chance for the absorbed fluoride to be chemically fixed or translocated between exposures, whereas there is no such "rest period" with continuous exposure. Fluoride content alone was also reported as not always being a valid indicator of plant damage. Widely differing degrees of plant injury may be associated with the same fluoride concentration in the foliage, depending upon the frequency of exposure, the concentrations of HF or other soluble F source present, and the age of the foliage at the time of exposure (Brewer *et al.* 1969).

Woltz *et al.* (1971) conducted an experiment in a 'Valencia' orange grove where fluoride concentrations were high and reported that chlorophyll and leaf development were severely inhibited in young leaves by relatively low levels of fluoride, in the range of 20–50 ppm. Severe fumigation (64–197 ppm) at the time of flower bloom and early fruit set caused blossom or fruit drop, severely reducing yield. Mature leaves that had developed normally accumulated large amounts of fluoride without much apparent damage. Increased fluoride resulted in smaller leaves, higher respiration rate, reduced photosynthetic capacity based on leaf area, and significantly depressed fruit yield as lead fluoride increased (Woltz *et al.* 1971).

Leonard and Graves (1972) performed a fluorine survey in Florida in groves in the surroundings of fluorine sources. They found that groves closer to the sources showed the highest levels of fluoride in the leaves. Levels of nutrient elements (i.e. Mn, Zn, Fe, B, N, O, K, Ca and Mg) were analysed in old and new leaves exposed to airborne fluorine showed no significant effect. Fruit samples were also collected and no significant effect of elevated fluorine levels on the interior quality of citrus was found. Little absorption of fluorine by the fruit was observed and was mostly found in the peel, with very little in the pulp and the juice.

Experiments were also carried out in field greenhouses. In these experiments, wet citrus leaves apparently absorbed more gaseous fluoride than dry leaves due to the greater affinity for water that acid fluoride (Woltz *et al.* 1971). As reported by other researchers (Brewer *et al.* 1960a; Woltz *et al.* 1971), Leonard and Graves (1972) also observed that young leaves were more susceptible to airborne fluorides than old leaves, and that the most damage occurred at the leaf tip where fluoride could accumulate up to 138 ppm compared with the green parts (63 ppm). A trend toward increased respiration rate associated with increased fluoride content in the spring flush was reported. Net photosynthesis and increased respiration apparently caused by airborne fluorides severely limited the gain in useable photosynthate or food supply produced by the leaves. The highest levels of fluorine in the fruit were found in the peel and there was slightly more fluorine in the juice than in the pulp. The fruit quality data showed a trend toward higher acid content and resultant lower °Brix/acid ratio of the juice with increasing leaf fluorides. However, these results were not statistically significant (Leonard and Graves 1972).

Leonard and Graves also performed experiments involving the spraying of citrus trees with HF at different concentrations. Relatively high levels of HF (160–190 ppm) during the spring bloom period were believed to cause the greatest losses in fruit production due to extensive leaf chlorosis, leaf burn, dropping of young leaves and excessive dropping of bloom and small fruit. These symptoms were, however, not observed in greenhouse citrus trees (Leonard and Graves 1972).

Alleviation of fluorine chlorosis

Efforts to alleviate fluorine chlorosis symptoms by application of various liming materials to the soil and spraying mixtures containing >20 lb of hydrated lime per 100 gal had no effect on re-greened fluorine-chlorotic leaves or reduced fluorine absorption by the leaves. However, a mixture containing 25 lb of hydrated lime plus zinc and manganese sulphates, copper, boron, molybdenum, magnesium and urea nitrogen applied as a spray caused at least 90% greening of the fluorine-chlorotic leaves. Similar treatments with less lime (2.5 lb/gal) produced about 70% greening of similar leaves (Leonard and Graves 1972).

Effects of boron

Sources

Boron is a ubiquitous element in rocks, soil, and water. Borate-mineral concentrates and refined products are produced and sold worldwide. They are used in a myriad ways: in glass and related vitreous applications, in laundry bleaches, in fire retardants, as micronutrients in fertilizers and for many other purposes, as well. Modern uses of borate-mineral concentrates, borax, boric acid, and other refined products include glass, fiberglass, washing products, alloys and metals, fertilizers, wood treatments, insecticides, and microbiocides (Woods 1994).

Effects on vegetation

Boron was proven an essential micronutrient for higher plant. Nevertheless, as most essential micronutrient elements, which have a fairly narrow window for concentrations between optimal and toxic, boron is no exception (Blevins *et al.* 1994). Boron in excess concentrations causes similar injuries in plant as excess fluorine: marginal yellowing, tip and marginal burning, and chlorotic patterns (Aucejo *et al.* 1997). Necrotic spots commonly form on leaves following foliar application of boron; however, small quantities of boron (<1/3 kg boron/ha) can be applied safely (Blevins and Lukaszewski 1994).

Boron injury symptoms replaced typical fluoride injury symptoms on foliage as F emissions were reduced. Foliar concentrations of B in excess of 300 ppm accumulated by plants exposed to atmospheric B emissions produced injury symptoms on sensitive species similar to those produced by toxic concentrations of B absorbed through roots. Temple *et al* (1978) reported that silver maples (*Acer saccharinum*) growing near local sources – a fibreglass plant – had delayed growth of new shoots in the spring and had increasingly higher contents of F and B in trunk sap with proximity to the fibreglass plant. Control of F and then B emissions by the company resulted in reduced concentrations of these elements in foliage and a reduction in the degree and extent of vegetation injury around the plant (Temple *et al.* 1978).

Effects on citrus trees

Boron toxicity levels in leaves have been reported for several citrus species (e.g. *C. sinensis*, *C. clementina* L., *C. aurantium* L, *C. paradise Macf x Poncirus trifoliate* L.) (Chapman 1968; Papadakis *et al.* 2003; Keles *et al.* 2004; Papadakis *et al.* 2004). Boron contents of about 100–130 mg/Kg dried leaves are usually considered the limit at which slight to moderate leaf symptoms begin to appear, while contents in the range of 200–250 mg/Kg indicate pronounced boron excess (Aucejo *et al.* 1997).

Effects of acid rain

Sources

Acid rain was classified as a new environmental stress towards the end of 1960. It consists of the transfer of strong acids (e.g. H_2SO_4, HNO_3) and compounds generating them (NH_3, $(NH_4)_2SO_4$, NH_4NO_3) from the atmosphere to the soil. These compounds are secondary pollutants generated from SO_2, NO_x and NH_3 (Pardos 2006).

Effects on vegetation

Plants are frequently exposed to a variety of anthropogenic acid solutions in the form of acid precipitation. Sulphuric acid (H_2SO_4) and nitric acid (HNO_3) are the most common airborne anthropogenic acids, but hydrochloric acid (HCl) also occurs in some locations (Hauser *et al.* 1993). The major regional acidic pollutants associated (oxidised sulphur and nitrogen compounds) represent a threat to a wide range of ecosystems, including agricultural crops, wetlands and heathland vegetation (Fowler *et al.* 1999).

For the acid to directly affect leaf tissues, it must pass through the leaf cuticle. Nevertheless, the leaf cuticle presents the major barrier to penetration of leaf tissues by these compounds because the surface tension of water typically prevents the entry of aqueous solutions by way of the stomata. Damage may occur indirectly by means of cation exchange, accelerating leaching of cations from the leaves, but in many studies it is clear whether the cations were exchanged through the cuticle or merely dissolved from the leaf surface. Cellular activities may be altered either by protons, at a sufficient pH, or by the anions (Hauser *et al.* 1993).

Acid deposition and sulphur in particular, was shown to be the cause of marked reductions in frost hardiness of some plants, such as red spruce. Acid deposition was also shown to be the major contributor to the acidification of soils and, along with the direct effect of SO_2, was the cause of widespread decline in high elevation conifer stands in some Eastern European countries (Fowler *et al.* 1999). Acid rain affects the forest ecosystems generating alterations in the normal interactions of several soil elements (e.g. N, S,H^+, Ca^{2+}, Mg^{2+} and Al^{3+})(Smith 1990).

The effect of acid rain in crops has also been studied. Several studies concluded that acid rain with a pH 3.8–5.1 does not have important effects in crops. However, the growth of several crops may either increase or decrease depending on the pH level. Recent studies suggest that acid deposition may have a small and indirect effect on crop production. The risk is higher when acid rain has a pH lower than 3.0, causing necrosis. Nevertheless, this situation with low pH is very infrequent (Granados-Sanchez and Lopez-Rios 2001).

On the other hand, a recent global assessment showed that modelled deposition of acidity only exceeded critical loads for soil acidification in small areas of Asia in 1990, although this pattern was predicted to change significantly by 2050. There is also little evidence that productive crop systems are sensitive to acidification, while deposition of atmospheric nitrogen is usually significantly smaller than the inputs from organic and/or inorganic fertilisers in agricultural systems (Emberson *et al.* 2003).

Effects on citrus trees

Hart *et al.* (1986) studied the effect of simulated acid rain on the growth and yield of 'Valencia' oranges (*Citrus sinensis*) in Florida. They reported no visible symptoms of injury attributable to pH treatment on foliage. Treatment (pH 3.5-5.5) had no significant effect on shoot growth. However, fruit fresh and dry mass per plant and from selected branches produced by the plants treated with pH 3.5 were approximately twice the mass of fruits produced by plants treated with pH 4.5 or 5.5. In addition, fruit from the pH 3.5 group tended to be larger than fruit from the other two groups and more numerous than fruit from the pH 5.5 group. Mean blossom number per branch also differed between treatment groups. The pH 3.5 group treatment group had the fewest blossoms per branch. No significant differences in growth-medium pH among pH treatments were demonstrated for any of the species tested (Hart *et al.* 1986). In summary, vegetative features of the citrus species studied (i.e. 'Valencia' oranges) showed no effects due to the different pH treatments. Fruit production was more responsive than vegetative growth to differences in pH treatments. Citrus showed no adverse impacts from low-pH rain under the experimental conditions of the study. Fruit weight per plant was highest for the group receiving the lowest pH treatment and fruit tended to be larger in this group. Foliar levels of elements tested did not differ between the pH treatment groups, suggesting that the higher yield of citrus fruit from the pH 3.5 group does not appear then to be attributable to higher nutrient levels resulting from increased sulphur and nitrogen supply in the more acidic solution. Troiano *et al.* (1983) suggested that H^+ at low concentrations may have a stimulatory effect on plant growth (Troiano *et al.* 1983; Hart *et al.* 1986).

Later Eissenstat *et al.* (1991) studied the interaction of simulated acid rain with ozone on freeze resistance, growth and mineral nutrition in citrus. In general, the effects of acid rain on growth and freeze resistance were low. Rain of high acidity (pH 3.3) offset the negative effects of ozone on growth (total leaf mass) in grapefruit/Volkamer lemon trees. In contrast, rain of high acidity magnified the detrimental effects of ozone on electrolyte leakage of leaf disks at subzero temperatures. Freeze resistance, determined by stem and whole-plant survival following freezing temperatures, was lower in the most rapidly growing trees. Consequently, for trees exposed to a combination of ozone and acidic rain, leaf electrolyte leakage did not correlate significantly with stem survival of freezing temperatures. Eissenstat *et al.* concluded that the danger of acidic rain to citrus was rather slight and would only present a potential problem in the presence of extremely high ozone (Eissenstat *et al.* 1991a, 1991b).

Finally, Hauser *et al.* (1993) studied the patterns of effective permeability of leaf cuticles to acids in grapefruits (*C. paradisi*) and lemons (*Citrus limon*). They reported that the leaf cuticle presents an effective short-term barrier to the penetration of protons. This may help explain the lack of dramatic effects of simulated acid rain applied experimentally to plants and the similar low level of immediate impact on foliage of terrestrial vegetation from "natural" acidic precipitation episodes in the field. However, in time the pH inside the cuticle does change, the cuticle serves only to retard, not prevent, the penetration of acids. The increase of effective permeability with time and repeated exposure suggests that the plants are most likely to be affected by acid precipitation when exposed for long periods of time and in mountain clouds or persistent fog. The researchers supported the idea that the cuticle has an outer layer of hydrophobic wax and polar, but uncharged pores which present a major limitation to the permeability of the cuticle ions. The inner cutin layer, known to contain acid groups, would be dissociated at the initial H of the inner solution, but would be progressively protonated as the acid moved through the cuticle (Hauser *et al.* 1993).

Effects of suspended particulate matter

Sources

The term Suspended Particulate Matter (SPM) includes finely divided solids or liquids that range in size from 0.1 to approximately 25 μm in diameter. However, the most common measure of particles used to quantify pollutant concentrations is now PM_{10} and $PM_{2.5}$, the abbreviation for particulate matter having an aerodynamic diameter less than 10 or 2.5 μm respectively. SPMs can be categorised into two groups. Primary particles are emitted directly from source (e.g. heavy metals from vehicles exhaust, dust re-suspension), while secondary particles are formed by interactions with other compounds (e.g. nitrate formation from the photo-oxidation of NO_x) (Emberson *et al.* 2003). Main primary sources of heavy metals in SPM are particularly combustion processes, power generation, smelting, incineration and the internal combustion engine.

In general, most coarse particles (i.e. those between 2.5 to 10 μm) are made up of both natural and organic particles whilst the fine fraction (i.e. less than 2.5 μm) tend to mostly be of anthropogenic origin. The major components of SPM mass, on average, are organic material, sulphate, ammonium, elemental carbon, nitrate, heavy metals and crustal/soil material. Due to the higher rates of deposition closer to particle emission sources, SPM is classified as a local pollutant (Emberson *et al.* 2003).

Effects on vegetation

SPM can produce a wide variety of effects on the physiology of vegetation that in many cases depend on the chemical composition of the particles. Heavy metals and other toxic particles have been shown to cause damage and death of some plant species as a result of both the phytotoxicity and the abrasive action during turbulent deposition (Emberson *et al.* 2003). Visible symptoms of toxicity for lead in plants are unspecific, however, the plants have smaller leaves and experience stunted growth. Leaves may also become chlorotic and reddish with necrosis. Copper, manganese and zinc are essential elements in all higher plants and develop an important function in several physiological processes like, photosynthesis, respiration, protein metabolism, and are important constituents of many enzymes. Manganese is also an essential metal in plants and is constituent of many enzyme systems which have an important function in the oxidation reduction process. Zinc has been demonstrated to be a biologically active metal. Zn concentrations in plants are influenced by the age and vegetative state of the plant. Nevertheless, excess of the heavy metal micronutrients, may cause visible injury to plant foliage and a disruption of physiological and biochemical processes (Caselles 1998).

Cement dust may cause chlorosis and death of leaf tissue by the combination of a thick crust and alkaline toxicity produced in wet weather (Griffiths 2003). Heavy loads of particles can also result in reduced light transmission to the chloroplasts and the occlusion of stomata, decreasing the efficiency of gaseous exchange (and hence water loss) (Emberson *et al.* 2003) and inhibiting the normal respiration and photosynthesis mechanisms within the leaf (Griffiths 2003).They may also disrupt other physiological processes, such as bud-break, pollination and light absorption/reflectance (Emberson *et al.* 2003).

The dust coating also may affect the normal action of pesticides and other agricultural chemicals applied as sprays to foliage (Griffiths 2003). Hence, indirect effects of particulate deposition, such as predisposition of plants to infection by pathogens and the long-term alteration of genetic structure (e.g. differences in frequencies of some alleles and genotypes (Prus-Glowacki and Godzik 1995)), have also been reported (Emberson *et al.* 2003). The effect of SPM in crop yield is not clear as some researchers have reported that particle deposition produced positive growth responses related to the capture and utilisation of nutrient particles from the atmosphere (Becket *et al.* 1998; Emberson *et al.* 2003). However, other researchers reported that the accumulation of alkaline dusts in the soil difficulted the crop growth due to an increase in soil pH to levels adverse to crop growth (Griffiths 2003).

Effects on citrus trees

Lerman and Kopfstein (1988) studied the effects of fly ash on citrus groves located in the vicinity of an oil-fired power plant, where approximately 40% of the citrus yield was rejected for export as a result of necrotic black-brown lesions. The appearance of injury symptoms was described on fruits ranging from young fruit to mature fruits. Microscopic examination of lesions revealed that typical sponge-type fly ash spheres were associated with the lesions. Their chemical composition contained relatively high levels of vanadium in those citrus fruit exposed to the fly-ash, which is a typical component of the fly ash. Crop losses were consistent with the prevailing wind direction from the fly-ash source (Lerman *et al.* 1988).

On the other hand, some researchers have reported the ranges for heavy metals toxicity limits in citrus leaves. These are as follows: lead from 10 mg/kg dried material (Chapman 1966; Pettygrove *et al.* 1984) to 20 mg/kg (Bradford *et al.* 1957); zinc, from 100 mg/kg (Chapman 1968) to 200 mg/kg (Pettygrove and Asano 1984) and nickel 50 mg/kg (Pettygrove and Asano 1984; Aucejo *et al.* 1997).

Recently, Oliva *et al.* (2008) evaluated the levels of some heavy metals on fruits (epicarp and mesocarp), leaves and its fruit marmalade of bitter orange (*Citrus aurantium*) trees and its implications for human health. Samples of bitter orange fruits (epicarp and mesocarp), leaves and its fruit marmalade from sites with different levels of traffic were analysed for Ba, Cd, Cu, Fe, Ni, Ob and Zn concentrations by ICP/AES. The results revealed that Ba, Fe and Mn accumulated in leaves > epicarp > mesocarp, Cu and Ni in leaves > epicarp > mesocarp and Zn leaves > mesocarp > epicarp. The citrus fruits sprayed with metal solution showed a significant increase in the studied elements compared to untreated fruits. The levels of all elements studied were lower than provisional tolerable daily intake values indicating that bitter orange marmalade consumption was safe for consumption (Oliva *et al.* 2008).

Effects of volatile organic compounds

Sources

The major sources of anthropogenic airborne Volatile Organic Compounds (VOCs) are industrial processes (solvents and manufacturing), oil refining and distribution, and transport (exhaust emissions and unburnt fuel). In addition, there are biogenic sources of many VOCs (Kesselmeier *et al.* 1999), of which the most important up to C8 is isoprene (C5).

Some of the VOCs normally considered as anthropogenic are also produced and emitted by plants. For example, ethylene is a plant hormone (Abeles *et al.* 1992) ethane may be emitted by plants that are under stress (Kimmerer *et al.* 1982; Wolfenden *et al.* 1988), methanol and longer-chain alcohols are emitted by many species (Macdonald *et al.* 1993), formaldehyde production has been observed in acorns (Albert *et al.* 1998), and even toluene has been reported to be produced by some species (Heiden *et al.* 1999). This may mean that plants are either tolerant of external VOCs, because there are in-built mechanisms for metabolising them, or that they may be particularly sensitive to anthropogenic VOCs that are normally used as signalling molecules within the plant (Cape 2003).

Effects of vegetation

A very wide range of tolerance to airborne VOCs among plant species has been demonstrated. Bean (*Phaseolus vulgaris*) plants have been shown to be among the more sensitive, with decreases in the harvested pod weight. Other studies have also identified effects on reproductive stages (seed germination, flowering, fruit ripening), in the absence of visible damage or effects on growth.

There have been no relevant experimental studies of the long-term effects of VOCs on plants that specifically include effects on flowering, seed production and viability, and the accumulation of metabolites. Plants can metabolise many airborne VOCs or transport them from leaves to roots. The metabolites may be further degraded, or may be conjugated and accumulate within the leaf, in vacuoles or cell walls. It is possible that these breakdown products are toxic to herbivores or phytophagous insects.

While it appears unlikely that airborne VOCs pose a direct threat to plant health, there is little information on their effects in conjunction with other gaseous pollutants or environmental stresses, such as drought, nutrition or temperature extremes (Cape 2003).

Effects of citrus trees

Very few studies have reported effects of VOCs on citrus trees. Ligor *et al.* (2003) studied the distribution of VOC (1.25 μg/mL of toluene) in citrus, such as kumquats (*Fortunella japonica*), lemons (*Citrus limon*), oranges (*Citrus*) and mandarins (*Citrus reticulata*). The highest concentrations of VOC were observed in the flavedo (0.0-3.9 μg/g of toluene), where the oil glands are located, although part of the VOC concentration also penetrated the pulp (0.0-1.8 μg/g toluene). The data obtained suggested that the high dissolution of aromatic hydrocarbons results from the presence of essential oils in the oil glands (Ligor *et al.* 2003).

Effects of carbon dioxide

Sources

The background CO_2 atmospheric concentration has been steadily increasing for at least 200 years, from around 280 ppmv (parts per million by volume), around the pre-industrial period, to 367 ppmv in 1999. The CO_2 growth rate has been about 1.5 ppmv (0.4%) per year over the past two decades (Apadula *et al.* 2003).

The largest source of CO_2 emissions globally is the combustion of fossil fuels, such as coal, oil and gas in power plants, automobiles, industrial facilities and other sources. A number of specialized industrial production processes and product uses, including mineral production, metal production and the use of petroleum-based products can also lead to CO_2 emissions (USEPA 2008).

Effects on vegetation

The continuing increase in atmospheric CO_2 concentration and projections of possible future increases in global air temperatures have stimulated interest in the effects of these climate variables on plants, and in particular on agriculturally important food crops. Since both CO_2 and temperature can have a large impact on plant growth and yield, it is important to quantify the effects of these climate variables on plants and especially on food crops (Baker *et al.* 1993).

The effect of ozone with CO_2 studied by Gardner *et al.* (2005) showed that an increase of CO_2 decreases the negative effects of ozone by reducing leaf abscission and premature senescence. The action of ozone is associated with cellular expansion where the pollutant attacks the polysaccharides in the cellular wall, which is diminished by high concentrations of CO_2 (Gardner *et al.* 2005; Pardos 2006).

Several plants treated with different levels of CO_2 showed an increase in the photosynthetic rate, and a decrease in crop water use.

Effects on citrus trees

One of the primary direct effects of elevated CO_2 concentrations on plants with the C_3 carbon fixation pathway is almost always an increase in the photosynthetic rate. Baker and Allen studied the effect of different CO_2 to citrus trees. They reported that although citrus presented a low canopy net photosynthetic rate, it displayed a great percentage increase in response to enrichment of CO_2 concentration. This proportional larger response of citrus trees to enrichment was previously reported by Idso and colleagues (Idso *et al.* 1992a, 1992b, 1994). They reported more than a doubling in citrus leaf photosynthetic rate and a reduction by a third in citrus leaf respiration rate in enrichment CO_2 conditions. Enriched citrus trees sequestered more than 3 times more above ground carbon than ambient controls, which might imply that citrus trees may respond proportionally more strongly to CO_2 enrichment than annual plant species (Baker and Allen 1993).

Koch *et al* (1986) found that acclimation to increased CO_2 concentrations did not decrease the activity of Ribulose bisphosphate carboxylase (RuBP carboxylase) in recently expanded flushes of citrus leaves (Koch *et al.* 1986)

Baker and Allen reported that the CO_2 enrichment greatly increased CO_2 uptake by citrus trees and, in most cases, reduced water loss, to a lesser degree. Water-use efficiency was therefore increased by CO_2 concentration enrichment due mainly to increased CO_2 uptake (Baker and Allen 1993).

Utilizing the outdoor, controlled environment chamber system, Koch *et al.* (1983, 1986, 1987) reported very large CO_2 concentration enrichment responses of citrus seedlings relative to reports on other species. The CO_2 enrichment resulted in 1.3-2.0 times greater shoot elongation, 33-100% increases in dry weights of the leaves, stems and roots. The CO_2 enrichment seedlings contained more leaf chlorophyll per gram fresh weight and the leaves were visibly darker green in colour (Koch *et al.* 1983, 1986, 1987). Koch *et al.* (1987) discussed several possible reasons for the relatively large response of citrus to CO_2 enrichment. First was the possibility that C3 plants, like citrus, with an indeterminate growth habit and potentially unlimited number of nodes, may be more responsive to CO_2. In citrus, the node additions occur in flushes and CO_2 enrichment acts to increase the number of growth flushes per growing season. Another possibility discussed by Koch *et al* (1987) was the fact that the citrus materials used were young seedlings. They speculate that juvenile plants may be more responsive to CO_2 than older plants due to increased growth potential and thus greater sink strength for assimilate. Brakke and Allen speculated that woody plants with indeterminate growth may provide a continuous sink for photo-assimilates, and have the potential for greater photosynthetic and growth responses to CO_2 than annual plants (Brakke *et al.* 1995).

CALCULATION OF CROP YIELD LOSSES

Ozone

Olszyk *et al.* (1990) recorded data of crop losses and ambient ozone concentration for 3 years. Thompson and Taylor (1969) studied the effect of air pollutants on citrus trees recording crop yield losses for lemons in different ozone concentrations for 5 consecutive seasons.

As the amount of data collected with which to predict the response of trees in commercial orchards was very limited, these data should be used with caution to indicate possible trends in crop yield losses from ambient ozone as a surrogate for total oxidants. More precise crop loss estimates for citrus crops would have required much more extensive experiments with many trees over more than 3-5 years.

However, because these are still the best available data with which to estimate general trends in effects on 'Valencia' oranges and lemons from oxidants, they were used to develop equations to calculate at least general estimates of yield losses based on ambient ozone concentrations following the general procedure described by Olszyk *et al.* (1988).

The linear equation which describes the relationships between average ozone concentrations and citrus yields is described as:

$$TFWT = \mathrm{a} - (\mathrm{b}^* X_{average}) \qquad (1)$$

where $TFWT$ is the total fruit weight per tree in kg, $X_{average}$ is the average ozone in ppm for all hourly values between 08:00 and 20:00 hr from April through October of the year two years before the harvest year, a is 53.7 kg for orange trees and 74.64 kg for lemon trees and b is 261.1 kg/ppm and 692.02 kg/ppm for orange and lemon trees respectively (Thompson and Taylor 1969; Olszyk 1988, 1989; Olszyk *et al.* 1990).

To calculate the percentage of crop loss, Equation 1 is transformed into Equation 2 as follows:

$$\%Loss = \left[1 - \frac{(a - b * X_{average})}{(a - b * X_{background})}\right] * 100 \qquad (2)$$

where *% Loss* is the estimated crop loss due to ozone levels as a percentage, *a* and *b* are the coefficients defined in Equation 1, $X_{average}$ is the average ozone as described in Equation 1, and $X_{background}$ is a background ozone concentration which represents the potential yield in clean air. Normally, a 08:00-20:00 growing season average concentration of 0.025ppm is used to represent clean air (Delgado-Saborit and Esteve-Cano 2008; Olszyk 1989, 1990).

Olszyk and Thompson (1989) proposed different values for *a* and *b* variables accordingly to data reported by Thompson and Taylor in 1969. The values were 178.0 for a and 19.1280 for b (Olszyk *et al.* 1989).

Kats *et al.* (1985) and Olszyk *et al.* (1990) proposed a version of Equation 2, which took into account harvest cycles of "on" and "off" years (Kats *et al.* 1985):

$$I = 1 - 0.05 * \left[1 - \frac{(a + b * X_{average})}{(a + b * X_{Background})}\right] \qquad (3)$$

Muters and Soret (1995) proposed a equation to calculate Yield loss Index after the work of Thompson and Taylor (1969) assuming that lemon trees cycled between "on" and "off" years comparable to oranges. Ozone was assumed to have no effect on lemons during "off" years. The ozone data were for two years before the harvest year:

$$I = 1 - 0.5 * \left[1 - \frac{(a + b * X_{average})}{(a + b * X_{Background})}\right] \qquad (4)$$

where *I* is the Yield Loss Index as a fraction of 1. If I=1 then no loss from ozone was caused. Constant *a* is 0.5004 and *b* is 0.6224, X is the 12 hours mean ozone concentration (pphm), and $X_{background}$ is a background ozone concentration which represents the potential yield in clean air in pphm. Normally, a 08:00-20:00 growing season average concentration of 2.50 pphm is used to represent clean air (Mutters and Soret 1995).

Fluorine

Leonard and Graves (1972) studied the effect of fluoride in 'Valencia' orange yields, 'Hamlin' oranges and 'Marsh' grapefruit, the latter two on rough lemon rootstock.

'Valencia' orange trees grown in greenhouses receiving filtered air produced significantly more fruit than the trees receiving unfiltered air. There was a highly significant negative correlation (R=-0.907), between yield of fruit and fluorine content of 10 month old spring flush leaves sampled in January. This linear regression was:

$$TFWT^* = 381.91 - 1.3132*X_F \qquad (5)$$

where X_F is ppm fluoride in the leaves and *TFWT ** is total fruit weight per tree expressed as pounds of fruit per tree.

There was also a highly significant negative correlation (R=-0.869), between yield of fruit and fluorine content of old leaves sampled in July. The linear regression formula was:

$$TFWT^* = 417.25 - 0.879*X_F \qquad (6)$$

There was also a significant correlation (R=-0.685), between yield of fruit and fluorine content of 5.5 moth-old leaves sampled in August. The linear regression formula was:

$$TFWT^* = 345.61\text{-}1.912*X_F \qquad (7)$$

'Marsh' grapefruit was sprayed with hydrofluoric acid (HF) 0.01, 0.02 and 0.03 N. The high HF spray treatment produced a yield significantly lower than the low fluoride spray treatment. The correlation coefficient relating yield of fruit to fluorine content of the leaves was highly significant at R=-0.888. The linear regression curve was:

$$TFWT^* = 7.60 - 0.0185*X_F \qquad (8)$$

No significant difference in internal quality of the fruit was found during the experiment (Leonard and Graves 1972).

'Hamlin' oranges were sprayed in the same way as 'Marsh' grapefruit. There was also a highly significant negative correlation between yield and fluorine content of the leaves (R=-0.850). The linear regression formula was (Leonard and Graves 1972):

$$TFWT^* = 6.04 - 0.01206*X_F \qquad (9).$$

Caveats in the use of yield loss equations

There are several points that should be commented before transferring the response functions derived in California and Florida in the 70s and 80s to any specific area. First of all, the conditions where the equations were derived in the Riverside area (California) for ozone and Florida area for fluorine should be comparable with the conditions recorded at each specific area under study as regards the climatology and edaphology.

Secondly, the intercept coefficients represent the production of citrus trees in the absence of an excess of ozone or fluoride. Therefore, the average production per tree in the area under review should be similar to the proposed intercept coefficients.

In the case of Equations 2-4, a review of background levels of surface ozone reveals that current annual average background levels over mid-latitudes in the Northern hemisphere range between approximately 20 and 45 ppb (Vingarzan 2004). Consequently, the value of background ozone concentration ($X_{background}$) proposed by Olszyk in 1989 is still reasonable nowadays. However a revision and update of background levels of surface ozone is advised when using Equations 2 to 4.

Finally, the equation presented for calculating citrus crop yield losses for ozone do not take pollutant uptake via the leaves into account, since no relationship between the two has so far been established to date. Instead, the method is based on mean average ozone concentrations in the air which offers the advantage of simplicity, but also has a limitation as it considers no concentrations dynamics or their effects. Nevertheless, the current trend is to move from concentrations-based to flux-based response relationships (Grünhage *et al.* 2003; Ashmore 2005).

EVOLUTION OF RESEARCH IN AIR POLLUTION EFFECTS ON CITRUS TREES

Historically, the main interest in the effects of air pollutants on citrus trees has been with fluorine compounds produced by factories located very close to the orchards and by photochemical oxidants, such as ozone, produced as secondary pollutants from combustion sources. The former was deeply studied in the 1960s and the later, even the main core of the studies started in 1960s, and they continue into the 2000s. These two pollutants, which in turn are the most harmful to citrus trees, are the only ones for which researchers have described equations that can assess crop yield losses. These equations are based in concentration-dose response. As the new trend is to assess the effects of air pollutants via their stomatal flux, rather by the ambient pollutant concentration, new research should be performed measuring uptake flux data in order to validate the former equations or to derive new ones that could help the scientist, government and farmers to estimate crop yield losses due to air pollution.

The effects of suspended particulate matter, specially fly ash and airborne heavy metals, were also studied since the late 1950s, giving information about heavy metal toxicity on citrus trees. As regards, sulphur dioxide, the effects on citrus trees started to be studied in 1960s, although some other work was completed in the late 1980s. The effects of nitrogen oxides was not studied until the 1970s when also the acids derived from them in 'acid rain' (SO_2 and NO_x) were given some attention.

Lately, climate change has been a hot topic, largely studied since 1990s and therefore, the effects of an increase of CO_2 levels on citrus trees were also documented and reported in the 1990s. More recently, interest has been expressed in the effects of VOCs. The potential for VOCs to have direct effects on plants at current ambient concentrations has been discounted, largely on the basis of short term exposures of vegetation to high concentrations of VOCs. However, as emissions of 'traditional' inorganic pollutants are reduced, the relative role of VOCs and other organic compounds as primary pollutants becomes more important (Cape 2003). So far, studies that detail the effects of VOC on citrus trees are very scarce, and the effects reported are mainly penetration of VOC compounds in the fruit. More information should be gathered as regards the physiological and biochemical response of citrus trees to this range of organic pollutant. Questions such as does organic pollutants and which ones affect the tree growth, flowering and bearing, cause damage to the leaves, affect the photosynthesis or penetrate to the fruit are important to answer in order to assure not only the plant health but also the quality and quantity of citrus crops.

CONCLUSIONS

This review describes documented effects of air pollutants such as ozone, fluorine, sulfur dioxide, acidic precipitation and other pollutants on citrus trees, describes the mechanisms of injury and damage of the air pollutants to the plant and analyses the past trends and future research needs in the field of effects of air pollutants on citrus trees.

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