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Alberta
ENVIRONMENT

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# THE POTENTIAL USE OF CHEMICAL PROTECTANTS TO MEASURE ATMOSPHERIC OXIDANT DAMAGE IN ALBERTA CROPS

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## FOREWORD

Ozone in air at ground-level originates from two sources. Stratospheric ozone (the protective ozone that helps screen the Earth's surface from UV light) may be brought to ground-level as a result of atmospheric turbulence. Ozone at ground-level is also formed as a consequence of reactions among substances naturally present in the atmosphere, and among substances that are produced by human activity. A powerful oxidant, ozone has the potential to harm natural, agricultural, and horticultural plant species. Research into the effects of ozone has been extensive, but despite this effort, there is still a great deal of uncertainty regarding the levels of ozone that are harmful to plants.

Much of the scientific research effort to date has concentrated on examining the effects of ozone that has been added to the air, either in growth chambers, field chambers, or through field systems that do not use enclosures. While these efforts have provided important information on the concentration of ozone, and the duration of ozone exposure that may affect plant growth, the artificial nature of these experimental conditions has limited the applicability of much of the information.

Adding ozone to air and evaluating the effects on plants is one way to approach this problem, and this has been the approach most commonly used. A lesser used method is to remove ozone from the air and observe changes in plant growth. A third way to approach this problem is to treat plants with a substance that "protects" the plant from ozone exposure. Several chemical substances have been found to have the properties of a "protectant" – they cause the plant to become insensitive to ozone, or they react with ozone before the ozone can react with plant tissues. Use of these substances has recently expanded the ability of researchers to investigate the mechanisms by which ozone in air may affect plant growth.

The Air Research Users Group of Alberta Environment has commissioned this report in order to provide a review and interpretation of the scientific literature that describes the use and effects of chemical ozone protectants. The authors of this report were asked to determine if there was sufficient information available on these substances to warrant a field evaluation of their use. This was done as part of Alberta Environment's efforts to determine if ozone has the potential to cause negative (or positive) effects on plants in the province, with a focus on agricultural crops. The results of this review and interpretation will assist Alberta Environment in making a decision on whether or not to conduct a field investigation into the potential use of chemical ozone protectants as part of an evaluation of the possible effects of ozone on Alberta crops.

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# EXECUTIVE SUMMARY

Ozone is an important phytotoxic gaseous pollutant in Canada, the USA and many other industrialized countries. Crop injury and loss induced by elevated ground level ozone has been reported in these countries leading to a widespread effort at the protection of plants using various means. Protection may be achieved by coating the leaf surface and providing physical and/or chemical protection, through the alteration of gas exchange or through the alteration of plant metabolism.

Although several reviews assessed the usefulness of protectants against ozone damage a more comprehensive review of plant protectants against ozone is needed in order to develop a tool to quantify the effects of elevated ground level ozone on Alberta crops. Our intent was to conduct a literature search and to provide a comprehensive review of this subject, and an interpretation of the literature, including detailed recommendations regarding the use of protectants for the evaluation of the potential for ozone effects on Alberta crops.

The earliest report of plant protection from ozone showed that pinto bean leaves could be protected from ozone injury by spraying them with aqueous suspensions of manganese (maneb) or zinc ethylenebis dithiocarbamate (zineb) prior to fumigation. The use of chemicals that cause stomatal closure such as phenylmercuric acetate and monoethyl esters of decenylsuccinic acid can protect plants from entry of ozone into leaves. Freebairn (1960) and Freebairn and Taylor (1960) were the first to modify plant metabolism to protect plants from ozone by applying vitamin C as a spray. Since then, a large number of chemicals used singly and in combination have been evaluated for their abilities to protect plants from ozone injury.

Ozone protectants can be grouped as pesticides, including fungicides, insecticides, and herbicides, plant growth regulators, dusts and mechanical barriers, and antioxidants, such as ethylene diurea (EDU). Several studies suggest that the application of these chemical protectants against ozone might be a reliable means by which to assess ozone effects on crops under field conditions. While many chemicals have been shown to convey partial or total protection against ozone injury, many are ineffective and have unacceptable side-effects rendering them of little value for the purpose of assessment of crop effects in the field. This is true of some of the most promising antiozonants. For example, even though the fungicide benomyl has been found to effectively control ozone injury in a number of plants, it would be impossible to separate the fungicidal benefits from its antiozonant properties in the field.

In the late 1980's and throughout the 1990's the focus has been on the evaluation and understanding of EDU as a protectant from ozone injury. Researchers reported that EDU reduced and/or delayed the appearance of ozone damage to developing foliage and delayed plant senescence and leaf abscission. These findings showed promise for the use of EDU as a general protectant against ozone damage but in order to be useful as a tool for the determination of crop losses due to ozone exposure, it was also necessary to verify whether EDU caused side-effects in the absence of ozone.

Since then, studies have been conducted to establish optimized protocols for the use of EDU in programs aiming at the quantification of the effects of ozone on vegetation and to understand the

process by which EDU conveys resistance to ozone. From the extensive literature on the subject, it is apparent that the effects of EDU are species- and sometimes cultivar-specific, that the dose, frequency and mode of application are critical and that one must take into consideration the length and frequency of ozone exposure as well as environmental conditions in developing a protocol for EDU use.

Dose response experiments will not only allow for the determination of the optimal dosage of EDU to convey resistance to ozone but will also allow for the determination of possible side-effects of EDU by application of EDU to plants grown in the absence of ozone in parallel to application to plants being exposed to ozone. In the first studies by Carnahan *et al.* (1978) in which plants were exposed to acute ozone treatments, various dosages of EDU were tested. Since then, while proper dose response protocols have been followed in many experiments, several studies have been conducted using EDU dosages that were extrapolated from previous experiments. Some of these extrapolations were done from acute to chronic ozone studies and have led to under- or over-dosages of EDU. The misuse of the EDU method has led to ineffective protection by EDU and EDU-induced toxicity resulting in reduced yields.

Applications of EDU as a soil drench and as a foliar spray have been successful in conveying resistance to ozone injury in plants but the possibility of soil accumulation of EDU and the subsequent possibility of toxicity argue for the use of foliar applications. Perhaps the greatest factors determining the appropriate mode of application are practical issues. On the large scale and in the field, it is perhaps not feasible to apply soil drenches at all stages of plant development, especially in crops that are not grown in rows such as hay and broadcasted cereal crops where it would not be possible to apply EDU to the soil without simultaneous application onto above-ground organs. The application of EDU as a soil drench at field scale would also require large volumes of solution while surface applications would depend on precipitation to carry the chemical to the plant roots. Foliar application appears to be the most practical especially for large-scale field studies.

We conclude that EDU is specific in the suppression of ozone injury in a wide variety of plant species. Studies conducted to date have shown that EDU has few side-effects and is effective on almost all plants studied. If appropriate exposure/response and toxicological studies are conducted with EDU and ozone, it should be possible to use EDU for assessing crop effects in the field. Therefore we recommend that the EDU method be adopted for studies aimed at the assessment of crop effects under field conditions in Alberta. EDU at a concentration of 250 – 500 ppm should be applied to the foliage to runoff every 7 to 10 days throughout the vegetation period. This should allow for the partial or total mitigation of ozone effects in chronic exposures at concentrations of up to 80 ppb. For the preliminary determination of the potential use of EDU in assessing effects of ozone on crops of Alberta, we recommend that studies be conducted at sites where ozone levels are greatest, perhaps Fort Saskatchewan or east Edmonton, with an ozone control site established near Vegreville where ozone levels are very low. Information on the relative sensitivity of common Alberta crops is lacking, making it difficult to determine which species should be used in these studies. We recommend that at least two species be studied. Based on their relative importance, we recommend that barley (*Hordeum vulgare*) and canola (*Brassica napus* – the most common species of canola) be used.

# ACKNOWLEDGEMENTS

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# 1. INTRODUCTION

Deleterious effects of atmospheric oxidants have been recognized since the first observations of damage to ornamental plants and crops in California in the early 1940s. While a number of oxidants have been found to be toxic to plants, few are found in sufficient quantities in the Canadian environment to cause noticeable effects on vegetation. Ozone is recognized as the most prevalent photochemical oxidant and its effects on vegetation have been extensively studied.

Studies of the potential for protection of vegetation from ozone injury using chemical applications have been conducted over the last four decades. Rich (1964) reviewed some aspects of protection with emphasis on early attempts using fungicides and inert surface-active materials. In 1974, Ormrod and Adedipe published a paper on "Protecting Horticultural Plants from Atmospheric Pollutants: A Review" in HortScience. The article contained a short historically-oriented section on chemical protectants. Ormrod and Adedipe divided chemical protectants into fungicides, antioxidants and growth regulators. In 1977, the Committee on Medical and Biologic Effects of Environmental Pollutants published a list of chemicals used in protecting plants against oxidant injury in a book chapter "Plants and Microorganisms" in "Ozone and Other Photochemical Oxidants". The list contains chemicals used against ozone and general oxidant injury and suggests a research focus on tobacco, bean and ornamentals such as poinsettia and petunia.

Manning and Krupa (1992) presented a list of examples of chemicals used to protect plants from ozone injury with references spanning from 1960 to 1991. The most recent work has been on EDU (ethylene diurea N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea) and EDU as a protectant against ozone damage has shown the most promise. The review by Pandey and Agrawal (1993) echoed this opinion and recent discussions on the use of ozone protectants have made little reference to chemicals other than EDU (Musselman and Hale 1997). These authors suggest that "there appears to be no current research being conducted on the usefulness of fungicides and other antioxidants for $O_3$ injury to vegetation". A second observation that can be made from the present review is that many researchers have focused their attention on the use of a number of chemicals in protecting tobacco from injury in southern Ontario, Canada (Gayed 1983; Bisessar and Palmer 1984; Walker 1961,1966,1967), in the northeastern United States (Bertinuson *et al.* 1961; Silber 1964; Taylor and Rich 1961,1962,1974; Taylor 1970; Miller and Taylor 1970; Miller *et al.* 1976; Reinhart and Spurr 1972; Sung and Moore 1979; Reilly and Moore 1982; Moyer and Smith 1995; Godzik and Manning 1998), Japan (Fukuda *et al.* 1975a,b; Toshikiyo *et al.* 1976; Koiwai 1977; Koiwai and Hiroshi 1975; Koiwai and Kisaki 1976) and Italy (Lorenzini *et al.* 1987).

The purpose of this report is to develop recommendations for the use of ozone protectants in Alberta in order to evaluate the effects of ozone on crops. The recommendations will be based upon a review of the literature.

# 2. PLANT PROTECTION AGAINST OZONE INJURY

The literature on the effects of ozone on agricultural crops of Canada has been recently reviewed (Pearson and Percy, 1997) and hence, the effects of ozone on crops will not be reviewed in detail in this document. Briefly, ozone diffuses through stomatal pores at the leaf surface, dissolves and decomposes rapidly to produce toxic oxygen species. Visual symptoms of ozone injury are primarily observed on leaves causing bronzing, necrosis and desiccation and in advanced stages results in rapid and premature senescence and leaf drop. Retardation of growth and severe (up to 45%) loss of yields in crop plants may occur (Pearson and Percy, 1997).

Research into the potential protection of plants from oxidant damage has included a number of approaches (Tables 1 - 5). Protection may be achieved by coating the leaf surface and providing physical and/or chemical protection, through the alteration of gas exchange or through the alteration of plant metabolism.

The earliest report of plant protection from ozone is by Middleton *et al.* (1953) who showed that pinto bean leaves could be protected from ozone injury by spraying them with aqueous suspensions of manganese (maneb) or zinc ethylenebis dithiocarbamate (zineb) prior to fumigation. Rich (1964) and Seidman *et al.* (1965) reported on the use of chemicals that cause stomatal closure such as phenylmercuric acetate and monoethyl esters of decenylsuccinic acid to protect plants from entry of ozone into leaves. Freebairn (1960) and Freebairn and Taylor (1960) were the first to use metabolic effectors to protect plants from ozone by applying vitamin C as a spray (Table 4). Since then, a large number of chemicals used singly and in combination have been evaluated for their abilities to protect plants from ozone injury. In the late 1980's and throughout the 1990's the focus has been on the evaluation and understanding of EDU as a protectant from ozone injury. While caveats on the use of EDU for this purpose have arisen, EDU is currently widely used (Table 5) and is part of the research conducted within the framework of the International Cooperative Program on the effects of air pollutants on crops and non-woody plants (ICP-Crops) established as part of the United Nations/Economic Commission for Europe (UN-ECE) working group (for example: Schenone *et al.* 1995; Tonneijck and Van Dijk 1996).

## *2.1. The use of pesticides as ozone protectants*

A number of studies have made use of pesticides such as fungicides, herbicides and insecticides to protect plants against ozone injury and a summary of those studies is provided in Tables 1 and 2.

### *2.1.1. Fungicides*

Because of the extensive use of benzimidazole, carboxin and their derivatives we have elected to treat these in a separate section below. A report on the use of fungicides other than those mentioned above is given in the section entitled 'Various fungicides'.

Table 1. Pesticides used to protect plants from ozone injury; plant species studied and references.

| **Chemicals** | **Formulas** | **Type of Protectant** | **Plant Species** | **References** |
|---|---|---|---|---|
| maneb, zineb<br>ferbam, ziram, thiram | ethylene bis dithiocarbamates (maneb -Mn and zineb - Zn) | fungicides | bean, tobacco | Middleton *et al.* (1953), Kendrick *et al.* (1954), Bertinuson *et al.* (1961), Kendrick *et al.* (1962), Reinert and Spurr (1972) |
| a large number of fungicides, too many to list – many were dithiocarbamate derivatives and compounds closely related to thiuram derivatives | too many to list | fungicides | bean | Kendrick *et al.* (1962) |
| BAS 3191 F<br>Calixin | 2-5-dimethyl-3-furylanilde<br>N-tridecyl-2,6-dimethyl | fungicides | bean | Seem (1972) |
| triarimol and its monochlorphenyl cyclohexyl analogue | α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine-methanol and α-(2-dichlorophenyl) - α-cyclohexyl-5-pyrimidinemetha-nol | fungicides | bean, marigold | Seem *et al.* (1972), Klingaman and Link (1975) |

| Chemicals | Formulas | Type of Protectant | Plant Species | References |
|---|---|---|---|---|
| thiophanate methyl analogue | 1,2-bis (3- ethyl and its ethoxycarbonyl) benzene and 1,2-bis (3-methoxycarbonyl-2-thioureido) | fungicides | bean, marigold | Seem *et al.* (1973), Klingaman and Link (1975) |
| DPA | diphenylamine | fungicides | apple, bean, melon, tobacco, petunia | Walker and Barlow (1974), Gilbert *et al.* (1975, 1977), Lisk (1975), Elfving *et al.* (1976), Koiwai *et al.* (1977) |
| Santoflex 13 | N-(1,3-dimethyl butyl)-N'-phenyl-p-phenylene-diamine | | | |
| Santoflex 77 | N,N'-bis(1,4-dimethylpentyl)-p'-phenylenediamine | | | |
| dodine | dodecylguanadine acetate | fungicide | tobacco | Reinert and Spurr (1972) |
| triadimefon | 1-(4-chlorophenoxy)-3,3-dimethyl-1-([H-],2,4-triazol-1-yl)-2-butanone | fungicide | bean | Fletcher and Hofstra (1985) |
| diphenamid | N,N-dimethyl-2,2-diphenyl acetamide | herbicide | tobacco | Sung and Moore (1979), Reilly and Moore (1982) |
| isopropalin | 2,6-dinitro-N,N-dipropyl cumidine | herbicide | tobacco | Sung and Moore (1979), Reilly and Moore (1982) |
| pebulate | S-propyl butylethylthio-carbamate | herbicide | crop plants, tobacco | Carney *et al.* (1973), Sung and Moore (1979), Reilly and Moore (1982) |

| Chemicals | Formulas | Type of Protectant | Plant Species | References |
|---|---|---|---|---|
| Atrazine | 2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine | herbicide | bean | Seem (1972) |
| spectracide 25EC<br>lannate 90SP | diazinon<br>methomyl | insecticides | bean | Teso *et al.* (1979) |
| aldicarb | 2-methyl-2-(methylthio)propion-aldehyde 0-(methylcarbomyl)oxime | insecticides | bean | Seem (1972) |
| carbofuran | 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate | | | |
| disulfoton | 0,0-diethyl s-(2-[ethylthio]ethyl) phosphorodithioate | | | |
| Butox, piperonyl butoxide, PB | α-[2-(2-butoxyethoxy) ethoxy]-4,5-methylenedioxy-2-propyltoluene | insecticide - antioxidant | tobacco, bean | Koiwai *et al.* (1974, 1976, 1977), Kitano *et al.* (1975), Rubin *et al.* (1980) |
| methylenedioxy-phenyl and derivatives – all functionally or structurally related to piperonyl butoxide | too many to list, more than 100 chemicals | insecticides and insecticide synergists | tobacco | Koiwai *et al.* (1974), Koiwai (1977), Fukuda *et al.* (1975a) |

Table 2. Benzimidazole, carboxin and their derivatives used to protect plants from ozone injury; plant species studied and references.

| Chemicals | Formulas | Type of Protectant | Plant Species | References |
|---|---|---|---|---|
| Benzimidazole (BZI, Bd) | | non-fungitoxic | azalea, bean, cucumber, tobacco | Pellissier (1971), Pellissier *et al.* (1971b, 1972), Tomlinson and Rich (1973b), Fukuda *et al.* (1975), Kitano *et al.* (1975), Lee *et al.* (1990) |
| benomyl | methyl-1-butyl-carbamyl-2-benzimidazole-carbamate | fungicide | bean, cucumber, grapevines, potato, poinsettia, soybean, tobacco, turfgrass | Miller and Taylor (1970), Taylor (1970), Pellissier (1971), Pellissier *et al.* (1971a,b; 1972a), Pellissier and Lacasse (1972), Manning and Papia (1972), Manning *et al.* (1972), Reinert and Spurr (1972), Kender *et al.* (1973), Manning and Vardaro (1973a,b), Manning *et al.* (1973a,b,c; 1974), Curtis *et al.* (1974; 1975), Moyer *et al.* (1974a), Taylor and Rich (1974), Fukuda *et al.* (1975), Rufner *et al.* (1975), Miller *et al.* (1976), Pell (1976), Littlejohns *et al.* (1976), Papple and Ormrod (1977), Clarke *et al.* (1978), Hofstra *et al.* (1978), Walker and Melin (1978), Musselman (1985), Lee *et al.* (1990) |
| thiabendazole | 2-(4-thiazol)-benzimidazole | fungicide | bean | Pellissier *et al.* (1971b), Pellissier *et al.* (1972a) |
| NC 2983 | 5,6-dichloro-2-trifluoromethyl-benzimidazole | fungicide | bean | Seem (1972) |
| carboxin (Vitavax) | 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide | fungicide | azalea, bean, cotton, soybean, tobacco, tomato, turfgrass | Curtis *et al.* (1973; 1974; 1975), Moyer *et al.* (1974b), Rich *et al.* (1974), Taylor and Rich (1974), Miller *et al.* (1976), Papple and Ormrod (1977), Hofstra *et al.* (1978) |
| carboxin derivatives | 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamides | fungicides | azalea, bean, tobacco | Curtis (1973), Curtis *et al.* (1973; 1974; 1975), Moyer *et al.* (1974b), Rich *et al.* (1974), Fukuda *et al.* (1975a) |

### *Various fungicides*

Middleton *et al.* (1953) and Kendrick *et al.* (1954) were among the first to report on the protective qualities of specific sprays and dusts. They showed that plant injury caused by ozonated gasoline or hexene-1 could be prevented using sprays or dusts of zinc ethylene bis dithiocarbamate (zineb), manganese ethylene bis dithiocarbamate (maneb), tetramethylthiuram disulfide (thiram), or ferric dimethyl dithiocarbamate (ferbam). In these studies, they found that fungicides such as 2,3-dichloro-1,4-naphthoqinone (dichlone) or tetrachloro-p-benzoquinone (chloranil) did not protect bean plants. They later showed that the degree of protection was directly related to the concentration of the chemicals. In studies conducted in Connecticut between 1955 and 1959, Bertinuson *et al.* (1961) also found that zineb could offer some degree of protection to shade-grown tobacco plants. Kendrick *et al.* (1962) later published results of extensive studies on the use of fungicides as well as antioxidants from the rubber industry as ozone protectants. Because the action of the protectants was localized and not systemic they suggested that the effect was that of deactivation of the oxidants upon application of the chemical protectant. They also found that the degree of protection was related to accumulated periods of exposure to toxicants. Seem (1972) reported on the potential of the herbicide atrazine, the fungicides BAS 3191 F, calixin and NC 2983 and the insecticides aldicarb, carbofuran and disulfoton in protecting bean plants against ozone damage. They found that the systemic fungicide α-2,4-dichlorophenyl-αphenyl-5-pyrimidinemethanol (triarimol) reduced ozone injury. Seem *et al.* (1973) also showed that thiophanate ethyl and its methyl analogue were highly effective in suppressing ozone injury in bean plants. Reinert and Spurr (1972) showed that while dodine and maneb did reduce ozone injury in tobacco leaves, they were not as effective as benomyl.

Walker (1966) found that the application of diphenylamine (DPA) as a foliar spray was highly effective in protecting flue-cured tobacco from weather fleck. Dust and liquid applications of DPA to apple and dust applications of DPA to bean, muskmelon and petunia were shown to provide protection against ozone (Gilbert *et al.* 1975). This fungicide was also used to quantify the effects of ambient oxidants on plants during monitoring of air quality in Georgia, U.S.A. (Walker and Barlow 1974). Lisk (1975) found that foliar application of DPA at 1000ppm (for apple) and 1% (for bean, melon, petunia and tobacco) reduced ozone damage by 50% or more. A combination of DPA and the antitranspirant Wilt Pruf proved to be even more effective in protecting apple foliage from ozone damage than either used singly (Elfving *et al.* 1976). The DPA derivative Santoflex 13, an ozone protectant used to protect rubber products, has been shown to offer significant protection to tobacco, muskmelon and bean from ozone injury while another similar derivative Santoflex 77 did not (Gilbert *et al.* 1977).

### *Benzimidazole, carboxin and their derivatives as ozone protectants*

Benzimidazole, carboxin and their derivatives, all of which are fungicides with the exception of benzimidazole, have been tested extensively for their abilities in reducing ozone effects in plants including bean, soybean, cucumber, potato, tobacco, grapevines, turfgrass, poinsettia, azalea and cotton. A summary of a number of such studies is given in Table 2. Like research on other pesticides described above, most studies on the potential benefits of benzimidazole, carboxin and their derivatives date from the 1970's and later studies are uncommon.

Based on the finding that benzimidazole and benomyl supplied at benzimidazole-equivalent dosages equally protected pinto beans from ozone injury, Pellissier *et al.* (1972a) concluded that it was the benzimidazole moiety that was responsible for the antiozonant effect. In 1973, Tomlinson and Rich (1973a) reported on the protection of chlorophyll and free sterols in membranes of bean plants exposed to ozone. Following the evaluation of an extensive number of chemicals as protectants of tobacco against ozone injury, Fukuda *et al.* (1975a,b) and Kitano *et al.* (1975) found that benzimidazole was among the most effective chemicals used with benzimidazole and some of its derivatives being surpassed only by piperonyl butoxide. Like Tomlinson and Rich (1973), Fukuda *et al.* found that benzimidazole protected chlorophyll from damage by ozone. At that time and in subsequent years, a number of the benzimidazole derivatives were studied.

The fungicide benomyl is by far the most studied benzimidazole derivative as evidenced by the list shown in Table 2. The vast majority of studies using benomyl have focused on its application to tobacco and bean. From 1972 to 1974, Manning and co-workers published a series of papers on the use of benomyl as an ozone protectant in beans and poinsettia. Manning *et al.* (1972) published results of experiments in which they looked at the effects of benomyl application as a soil amendment on growth and nodulation in pinto beans. They found that while benomyl could protect plants temporarily from ozone, plants exposed to benomyl showed either equal or decreased growth and nodulation compared to controls. Manning *et al.* (1973a) also used soil amendments of benomyl to study the response of pinto bean to repeated exposures to low levels of ozone. They found that benomyl amendments were not effective in overcoming the long-term deleterious effects of ozone and that benomyl caused dose-dependent toxicity symptoms. In 1973, Manning *et al.* (1973b) applied benomyl as a foliar spray to bean plants grown in field plots. They found that benomyl provided 70-80% suppression of oxidant injury. In the same year, Manning and Vardaro (1973) showed that benomyl supplied as a soil drench could significantly reduce the incidence of chronic ozone injury in two cultivars of poinsettia. Manning *et al.* (1974) found that benomyl applied as a foliar spray suppressed ozone injury by up to 80% in two sensitive cultivars of bean while no beneficial effects were found when benomyl was applied to an ozone resistant cultivar that suffered only minor visible injury. They also found that benomyl only caused yield recoveries in the most ozone-sensitive cultivars.

While in the early 1970's, the discovery that benomyl could protect plants against ozone injury opened a new area of investigation, the discovery that carboxin had similar beneficial effects rapidly led to side-by-side comparisons of the two chemicals. Manning and Vardaro (1973b) showed that while benomyl sprays protected bean plants from ozone injury, carboxin applied over seed at planting provided complete suppression of oxidant injury for up to 40 days. However, Taylor and Rich (1974) found that while both benomyl and carboxin could reduce the amount of visible injury, applications of carboxin to the soil supporting tobacco plants could lead to toxicity while benomyl, applied at greater dosages, had mostly beneficial effects. Rich *et al.* (1974) had also found that when carboxin was applied to soil, the dosage necessary to protect plants from ozone injury was close to the phytotoxic dose. They observed that soil treatments that were sufficient to protect plants from ozone caused the yellowing of leaf margins and stunting. Curtis *et al.* (1974) reported that foliar sprays of carboxin and its sulfoxide analogue (F-831) were more effective in preventing yield loss in white beans than was benomyl. After Miller and Taylor (1970) reported beneficial effects of combining benomyl with nematicides that increase ozone sensitivity in preventing weather fleck of tobacco, Miller *et al.* (1976) combined either benomyl or carboxin with contact nematicides to attempt to reduce the severity of ozone damage to tobacco and bean plants. They found that bean plants grown in soil amended with benomyl or carboxin either alone or with nematicides were ozone resistant. They also found that the combination of benomyl with the nematicide, fensulfothion, induced ozone resistance in less time than did benomyl applied singly. Papple and Ormrod (1977) compared the efficacy of benomyl and carboxin in reducing ozone injury in turfgrasses. They found that benomyl effectively reduced ozone-induced injury but that carboxin did not perform well and had direct toxic effects on leaves of the three species studied. In 1978, Hofstra *et al.* found that benomyl and carboxin were equally effective in causing yield recovery in navy beans exposed to ozone although neither was as effective as EDU.

Other studies have looked at the possible benefits of benomyl with other chemicals thought to have similar effects. For example, Pellissier *et al.* (1972b) compared the effectiveness of benomyl and benomyl-folicote (an antitranspirant) treatments in reducing ozone injury in beans. They found that both benomyl and folicote used singly afforded the same degree of protection (~99%). They also found that a greater concentration of benomyl was needed when it was used as a soil drench rather than a foliar spray, although this was improved when a surfactant was added to benomyl in a soil drench.

Other derivatives of benzimidazole have been used to protect plants from ozone. Pellissier *et al.* (1971b) and Pellissier *et al.* (1972a) tested the possibility that thiabendazole applied to soil could impart protection to bean plants. They found that this chemical offered no protection from ozone and hypothesized that the failure of thiabendazole in protecting plants against ozone might have been due to low uptake. Seem (1972) found that the experimental fungicide NC 2983 conveyed a high degree of ozone resistance to bean plants with the complete elimination of leaf injury when applied at higher dosages. Curtis (1973) tested the efficiency of a number of oxathiin (carboxin) analogues apply as foliar sprays in protecting white bean from ozone injury. Curtis reported that carbathiin and the sulfoxide analogue were highly effective in reducing injury in plants grown both in controlled environments and in the field while other related oxathiin and thiazole analogues were ineffective. Curtis *et al.* (1973) showed that while treatment with protectants prior to ozone exposure was necessary to convey maximal protection, the effectiveness of antiozonant carboxin analogues was lost 5-10 days after application as foliar sprays in field-grown white bean plants. Curtis *et al.* (1973, 1974) and Rich *et al.* (1974) hypothesized that because carboxin is rapidly oxidized in soil and leaves that it was likely that the sulfoxide form was responsible for protection against ozone.

### *2.1.2. Insecticides*

Koiwai *et al.* (1974) and Fukuda (1975a,b) published reports of studies testing numerous insecticides for the protection of tobacco in Japan. Koiwai *et al.* (1974) reported that while five out of ninety chemicals tested, namely 3,4-methylenedioxyphthaldehyde, benzimidazole, safroxane, xanthone and piperonal showed high protective capacities, they were all less effective than peperonyl butoxide. Fukuda *et al.* (1975a,b) reported that while many of the benzimidazole, oxathiin and methylenedioxyphenyl derivatives were effective in controlling ozone injury to tobacco leaves, piperonyl butoxide was most effective, followed by benzimidazole and safroxane. Teso *et al.* (1979) studied the interactions of spectracide 25EC (diazinon) and lannate 90SP (methomyl) and there effects on bean plants. While they found that diazinon alleviated ozone injury, the combination of methomyl and ozone was more injurious than ozone alone. The use of antiozonant insecticides for the purpose of crop effects surveys would only be useful in situations where no crop loss due to insects occurs, otherwise it would be impossible to separate the benefits of insect control from those of ozone protection.

### *2.1.3. Herbicides*

In the late 1970's and early 1980's Sung and Moore (1979) and Reilly and Moore (1982) published work on the effects of herbicides diphenamid, isopropalin and pebulate on ozone injury in tobacco. Sung and Moore (1979) found that sensitivity was either decreased or unaffected by herbicide application. Reilly and Moore (1982) found no consistent effect of pebulate but found that isopropalin and diphenamid reduced ozone injury for two to four weeks in field grown tobacco. Similarly, Carney *et al.* (1973) found that the intensity of ozone injury to tobacco was either increased, decreased or unaffected by the herbicides pebulate, benefin and chloramben, respectively.

While the many studies on the use of pesticides have demonstrated that a number of chemicals were promising for the protection of plants to ozone, most of these were abandoned shortly thereafter. Teso *et al.* (1979) nevertheless underscored the importance of research on air pollution-pesticide interactions as they may have a profound effect on integrated pest management. The possible dual purpose of some chemical agents in controlling pests and mitigating ozone effects remains interesting.

## ***2.2. The use of growth regulators, dusts and mechanical barriers and other chemicals as ozone protectants***

A summary of studies that have made use of growth regulators, dusts and mechanical barriers and various other chemicals is given in Table 3. Various growth regulators have been used in attempts at preventing ozone damage to plants. Seem (1972) used a whole host of growth regulators to protect bean plants from ozone injury. Seem found that while foliar application of SADH and Chloro IPC reduced ozone injury to leaves by approximately 50%, chlormequat applied as a soil drench provided near complete protection. Fletcher *et al.* (1972) found that it was possible to considerably reduce ozone injury in bean plants by causing stomatal closure using ABA. Adedipe and Ormrod (1972) observed protective effects of N-6-benzyladenine (BA), gibberellic acid (GA) and indole acetic acid (IAA) from ozone in radish plants where BA was found to be the most effective protectant. Runeckles and Resh (1975) found that the cytokinins BA and kinetin both reduced the loss of chlorophyll caused by ozone and stimulated leaf growth but did not prevent ozone-induced decreases in stem and root growth. Cathey and

Table 3. Various chemicals used to protect plants from ozone injury; plant species studied and references.

| Chemicals | Formulas | Type of Protectant | Plant Species | References |
|---|---|---|---|---|
| a large number of growth regulators including plant hormones | too many to list | growth regulators | bean | Seem (1972) |
| GA | gibberellic acid | hormone, growth regulator | radish, bean | Adedipe and Ormrod (1972), Seem (1972) |
| IAA | indole-3-acetic acid | hormone, growth regulator | radish, bean | Adedipe and Ormrod (1972), Seem (1972) |
| cytokinins | kinetin (K), N-6-benzyladenine (BA) | hormones, antisenescence agents, growth regulators | bean, radish, marigold | Tomlinson and Rich (1973a), Adedipe and Ormrod (1972), Runeckles and Resh (1975) |
| ABA | abscisic acid | hormone, growth regulator | bean | Fletcher *et al.* (1972) |
| SADH | succinic acid 2,2-dimethyl hydrazide | growth retardant | petunia, bean, marigold | Cathey and Heggestad (1972), Seem (1972), Klingaman and Link (1975), Lee *et al.* (1990) |
| CBBP | 2,4-dichloro-benzyl tributyl phosphonium chloride | growth retardant | petunia | Cathey and Heggestad (1972) |
| ancymidol | a-cyclopropyl-a-(4-methoxyphenyl) –5-pyrimidine-methanol | growth retardant | poinsettia, marigold and soybean | Seem (1972), Cathey and Heggestad (1973), Klingaman and Link (1975), Ross *et al.* (1976), Lee *et al.* (1990) |

| Chemicals | Formulas | Type of Protectant | Plant Species | References |
|---|---|---|---|---|
| chlormequat | | growth retardant | poinsettia, bean | Seem (1972), Cathey and Heggestad (1973) |
| polyamines | putrescine, spermidine, spermine | antisenescence agents | tomato, tobacco | Ormrod and Beckerson (1986), Bors *et al.* (1989) |
| peroxidase | | enzyme | bean, tobacco | Larkin (1973) |
| folicote | paraffinic hydrocarbon waxes | antitranspirants | bean, solanaceous crops, marigold | Knapp and Fieldhouse (1970), Pellissier *et al.* (1972), Klingaman and Link (1975) |
| Wilt Pruf | | antitranspirants | apple | Elfving *et al.* (1976) |
| charcoal, diatomaceous earth, clay, ferric oxide, kaolin, zinc ethylene bisdithiocarba-mate, road dust | | dusts | tobacco, bean | Bialobok (1984), Jones (1963), Burtinuson *et al.* (1961), Vasiloff and Drummond (1974) |

Heggestad (1972) found that a combination of SADH, L-ascorbic acid and the antitranspirant folicote was very effective in protecting petunia from ozone injury. In the same study they found that while soil drenches of the growth retardant CBBP reduced ozone effects by approximately 50%, ancymidol and chlormequat offered less protection. In a later study, Cathey and Heggestad (1973) used the growth retardants, ancymidol and chlormequat, to protect poinsettias from ozone damage. Klingaman and Link (1975) found that while ancymidol offered protection against ozone injury to marigold leaves, it delayed anthesis and reduced flower count. Ross *et al.* (1976) also found significant protective effects of ancymidol against ozone in marigolds but did not report negative effects on flower production. More recently, Lee *et al.* (1990) found that ancymidol offered some protection against ozone in soybeans and reduced ozone-induced senescence. Other antisenescence compounds such as the polyamines putrescine, spermidine and spermine have been shown to offer some protection from ozone injury to tomato and tobacco (Ormrod and Beckerson 1986; Bors *et al.* 1989). Growth regulators affect a wide range of plant growth and development processes and therefore, are not specific to ozone protection and are not useful as protectants to establish effects of ozone on plants.

Dusts, waxes and antitranspirants have also been used to protect plants against ozone injury by reducing gas exchange. Substances such as charcoal, diatomaceous earth, clay, ferric oxide, kaolin and zinc ethylene bisdithiocarbamate have been used as physical barriers (Bertinuson *et al.* 1961; Jones 1963; Bialobok 1984). Knapp and Fieldhouse (1970) and Pellissier *et al.* (1972b) used the antitranspirant folicote to protect bean and solenaceous crops against ozone injury, while Elfving *et al.* (1976) used the antitranspirant Wilt Pruf to protect the foliage of apple trees. In 1974, Vasiloff and Drummond published a report of studies conducted to test the potential of road dust as an ozone protectant. They found that dusted pinto bean plants exposed to ozone for 6 hours suffered significantly fewer ozone lesions than did undusted plants. The effects of dusts waxes and antitranspirants are not specific and therefore, they are not useful as protectants to establish effects of ozone on plants.

While growth regulators, dusts, waxes and antitranspirants used singly or in combination offered some protection from ozone injury, the effects were generally inconsistent and confounding effects were observed.

### *2.3. The use of antioxidants as ozone protectants*

A rather heterogeneous group of antioxidants have been found to prevent ozone damage with varied success primarily through the inhibition of oxidative processes (Table 4). While a number of chemicals described in other sections of this report have antioxidant properties, only those that appeared to be used primarily as antioxidants were placed into this section.

As an antioxidant, ascorbic acid and its salts have been used with success in reducing plant injury due to ozone in bean, celery, lettuce, barley, citrus and petunia (Freebairn 1960; Freebairn and Taylor 1960; Dass and Weaver 1968; Lee *et al.* 1990; Macher and Wasescha 1995). Soil application of potassium and calcium salts of ascorbic acid has been shown to protect bean plants from ozone injury (Freebairn 1963). In contrast, Siegel (1962) reported that ascorbic acid failed to provide appreciable protection from ozone to cucumber plants. Ozoban, an isomer of ascorbic

Table 4. Antioxidants used to protect plants from ozone injury; plant species studied and references.

| **Chemicals** | **Formulas** | **Type of Protectant** | **Plant Species** | **References** |
|---|---|---|---|---|
| ascorbic acid | K-ascorbate, N-ascorbate, sodium erythorbate (Ozoban) | antioxidants | bean, celery, citrus, lettuce, petunia, mandarin, tangelo, barley, shortleaf pine | Freebairn (1960), Freebairn and Taylor (1960), Dass and Weaver (1968), Lee *et al.* (1990), Flagler and Toups (1992), Flagler *et al.* (1994), Macher and Wasescha (1995) |
| metal-quinolinol | manganous 1,2-napthoqinone-2-oxime; Co and Mn chelates of 8-quinalinol | antioxidants | tomato | Rich and Taylor (1960) |
| NBC | nickel-N-dibutyl dithiocarbomate | antioxidant | bean | Dass and Weaver (1968) |
| Phenylurea | | antioxidant | bean | Tomlinson and Rich (1974) |
| Glutathione | | antioxidant | soybean | Lee *et al.* (1990) |
| BHT | butylhydroxytoluene | antioxidant | soybean | Lee *et al.* (1990) |
| TBHQ | tertiary butylhydroquinone | antioxidant | soybean | Lee *et al.* (1990) |
| 6-BAP | 6-benzylaminopurine | antioxidant | soybean | Lee *et al.* (1990) |

acid that is marketed by Pfizer Chemical Company as an antioxidant spray to reduce yield loss by ozone damage, was developed to protect Thompson seedless grapes from ozone damage in California. Field tests with Ozoban on grapes in Riverside, California, yielded mixed results with no consistent protective effects on yield of fruit (PM McCool in Flagler *et al.* 1994). Ozoban has also been used by Flagler and Toups (1992), Flagler and Lock (1994) and Flagler *et al.* (1994) to protect shortleaf pines from ozone injury in east Texas. In a short-term (1.5 years) study, Ozoban was found to provide some protection from ambient ozone. Recent studies by Kuehler and Flagler (1999) on loblolly pine showed that Ozoban can reduce photosynthetic rates in low-ozone environments and appeared to be harmful to chloroplast pigments in plants exposed to elevated ozone levels. Conflicting results as to the effectiveness of Ozoban in protecting pines from ozone injury are found in the literature and little information exists on its effects on annual crops. Extensive research would be required to establish the potential of Ozoban as a chemical protectant against ozone in crops of Alberta.

Field-grown tomato plants treated with manganous and cobaltous chelates of 8-quinolinol showed protection against visible ozone injury (Rich and Taylor 1960). Nickel-N-dibutyl dithiocarbamate was also found to be protective to bean plants and more protective than ascorbic acid (Dass and Weaver 1968). In 1974, Tomlinson and Rich reported that bean plants treated with the antioxidant phenylurea became highly resistant to ozone injury within 24 hours of application. The effects were shown to last for approximately 7 days. Based on experiments using leaf discs of bean, Tomlinson and Rich showed that phenylurea protected the chlorophyll pigment. Recently, Lee *et al.* (1990) tested the efficacy of a number of antioxidants in protecting soybean leaves from ozone injury. They found that while glutathione and BHT did not convey ozone protection, TBHQ and 6-BAP reduced ozone injury and chlorophyll damage to soybean leaves by more than 50%. While Lee *et al.* (1990) showed that a number of antioxidants afforded protection from ozone, none were nearly as effective as EDU, which offered total protection against 2 to 4 hour exposures to 350ppb ozone.

### *2.4. The use of ethylene diurea (EDU) as an ozone protectant*

Ethylene diurea (EDU – chemical name: N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea) is a systemic antioxidant that protects plant tissues from oxidant stipple and from early senescence caused by ozone. It was first developed by the duPont Chemical company in the 1970's specifically for this purpose. Although it contains urea, it apparently does not act as a plant nutrient, nor does it show pesticide or plant regulatory effects (Manning 1992). EDU does not affect photosynthesis, dark respiration and transpiration even when applied at dosages (1000 ppm soil drench) causing decreased growth of new tissues (Roberts 1987, Cannon *et al.* 1993). It appears to be specific for the suppression of ozone injury, having no effects on peroxyacetylnitrate (PAN) or $SO_2$ injury (Cathey and Heggestad 1982a, Lee *et al.* 1992). While EDU is systemic, it apparently is not redistributed to new tissues and repeated applications are required to protect newly-emerging leaves. The precise nature of the protective effects of EDU remains unclear.

The uptake and partitioning of EDU has recently been studied using HPLC (Regner-Joosten *et al.* 1994). Autoradiographic studies conducted by Roberts *et al.* (1987) on woody plants showed that EDU injected into stems accumulated in the leaves and persisted for approximately 10 days, a time line congruent with many reports on the length of protection afforded by an EDU application. EDU has been used to modify $O_3$ sensitivity in many plant species (Manning 1988; Manning and Krupa 1992). In studies conducted in the ozone-polluted regions of eastern Canada in the late 1970's and early 1980's, researchers reported that EDU reduced and/or delayed the appearance of ozone damage to developing foliage and delayed plant senescence and leaf abscission. These findings showed promise for the use of EDU as a general protectant against ozone damage.

Since these early reports, a multitude of studies have been conducted using EDU as an ozone protectant. Researchers have sought to establish optimized protocols for the use of EDU in programs aiming at the quantification of the effects of ozone on vegetation and to understand the process by which EDU conveys resistance to ozone. From the extensive literature on the subject, it is apparent that the effects of EDU are species- and sometimes cultivar-specific, that the dose, frequency and mode of application are critical and that one must take into consideration the length and frequency of ozone exposure as well as environmental conditions in standardizing the EDU method. Table 5 provides a summary of experiments conducted since the development of EDU in the late 1970s.

### *Methods of Application*

In 1974, Tomlinson and Rich reported on the use of phenylurea to protect bean leaves from ozone injury and to inhibit senescence. In 1978, Carnahan *et al.* described the beneficial effects of a new chemical that contained phenylurea, EDU, in increasing resistance to ozone in pinto beans by 30-fold. They performed full dose response experiments using both soil drenches and foliar applications. They suggested that EDU would become a useful survey tool in the identification and quantification of ozone damage in vegetation. EDU was soon put to the test and in the same year Musselman *et al.* (1978) and Cathey and Heggestad (1978) described beneficial effects of EDU in grapevines and on a number of florist and nursery crops, respectively. While Musselman *et al.* found that soil drenches were largely ineffective, Cathey and Heggestad found drenches to be as effective as foliar applications. Also in 1978, Clarke *et al.* published results of tests performed to verify the potential benefits of EDU application to potato plants exposed to ozone. They found that soil application of EDU was highly effective in preventing foliar injury but tuber yield, size and specific gravity were similar whether plants had been treated or not.

Applications of EDU as a soil drench and as a foliar spray have been successful in conveying resistance to ozone injury in plants but the possibility of soil accumulation of EDU and the subsequent possibility of toxicity argue for the use of foliar applications. Perhaps the greatest factors determining the appropriate mode of application are practical issues. On the large scale and in the field, it is perhaps not feasible to apply soil drenches at all stages of plant development, especially in crops that are not grown in rows such as hay and broadcasted cereal crops. The application of EDU as a soil drench at field scale would require large volumes of solution while surface applications would depend on precipitation to carry the chemical to the

Table 5. Dosage, mode and number of applications, ozone treatments, plant species and references for studies that have made use of the antioxidant EDU to protect plants from ozone injury. References are arranged in chronological order from the earliest to the most recent. CE = Controlled environment. OTC = open-top chamber. CSTR = continuous-stirred tank reactor. N/A = data not available.

| **Dosage** | **Mode of application** | **Number of applications** | **Ozone Treatment** | **Plant species** | **References** |
|---|---|---|---|---|---|
| 20-500 μg/ml – 6 mL/plant<br>0.2 mg/mL – 20 mL/plant | foliar to run off<br>soil surface application | 1 | acute CE<br>800 ppb | pinto bean | Carnahan *et al.* (1978) |
| 0.56 Kg/ha | foliar | weekly, biweekly or triweekly | chronic (field)<br>conc. not given | grapevines | Musselman *et al.* (1978) |
| N/A | foliar or soil drench | N/A | chronic (ambient)<br>conc. not given | a number of florist and nursery crops | Cathey and Heggestad (1978) |
| 6.7 kg a.i./ha | soil application | triweekly | chronic (ambient)<br>accumulated dosage AOT40 0.20 – 3.87 ppm | potato | Clarke *et al.* (1978) |
| 1.12 kg/ha | foliar | Every 7 to 10 days starting at flowering | chronic (ambient)<br>conc. not given | navy bean | Hofstra *et al.* (1978) |
| 5-100 mg/pot | soil application | 1 | chronic (ambient) CE<br>conc. not given | bush bean | Bennett *et al.* (1978) |
| N/A | foliar and soil combination | N/A | chronic (ambient)<br>conc. not given | watermelon | Fieldhouse (1978) |
| N/A | N/A | N/A | chronic CE<br>conc. not given | cucumber – 9 cultivars | Proctor (1978) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 1000 ppm | foliar | 3 days prior to each fumigation | 9 weekly 4 h fumigations<br>0 – 400 ppb | tree seedlings – white ash, black cherry | McClenahen (1979) |
| 0-5000 µg/g | Foliar | 1 at various growth stages | acute CE<br>0 – 750 ppb | pinto bean | Weidensaul (1980) |
| 0-12.5 g/L<br>0-0.15 g/L | foliar and soil drench | 1 | acute CE (on 2 subsequent days) | tomato | Legassicke and Ormrod (1981) |
| 1-2.5 g/L – to run off<br>0.15 and 0.07 g/plant | foliar and soil drench | June to August (foliar) and 3 times for soil drench | chronic (ambient) field<br>250 ppb | | |
| 9 kg/ha<br>747 L/ha | soil drench<br>foliar - run off | 2<br>6 | chronic (ambient) field<br>conc. not given | navy bean | Saettler (1981) |
| 1.1 kg a.i./ha | foliar spray to run off | 5 times at 10 day intervals | chronic (ambient) field<br>27 – 80 ppb | potato | Bisessar (1982) |
| 250 – 5000 ppm<br>soil – 100 mL/pot | foliar spray and soil drench | 1 | acute CE<br>350 – 950 ppb | petunia | Cathey and Heggestad (1982a) |
| 500 ppm | foliar spray and soil drench | 1 | acute CE<br>0 – 600 ppb | 40 + herbaceous species | Cathey and Heggestad (1982b) |
| 500 ppm - 250 mL<br>500 ppm – run off | soil drench foliar | 1 | acute CE<br>0 – 950 ppb | 13 woody species | Cathey and Heggestad (1982c) |
| 1000 mg/L<br>1.25 kg/ha | foliar spray to run off | 7 and 5 times at weekly | chronic (ambient) field<br>220 – 1400 ppb | bush bean | Hucl and Beversdorf (1982) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 2000 ppm solution | foliar spray to run off | 3-4 times at 10 day intervals | chronic (ambient) field<br>conc. not given | white bean | Toivonen *et al* (1982) |
| 6.7 kg a.i./ha | soil drench | triweekly from June to Sept. | chronic (ambient) field<br>61 – 107 ppm • hr | potato | Clarke *et al.* (1983) |
| 1.1 kg/ha | 1 soil drench followed by 5 foliar sprays | foliar sprays biweekly | chronic CE<br>250 ppb | potato | Foster *et al.* (1983) |
| 5.6 kg/ha and 0.56 kg/ha, respectively | soil drench + foliar sprays | 1 soil + 1, 2 or 3 foliar | chronic field<br>conc. not given | tobacco | Gayed (1983) |
| 1500 ppm a.i.<br>1.68 kg a.i./ha | foliar spray | 4-5 times at 10 day intervals | chronic field<br>59 – 73 ppm · hr and<br>chronic CE<br>150 ppb | potato | Hofstra *et al.* (1983) |
| 1 kg a.i./ha | foliar spray to run off | 7 times at 8 day intervals | chronic field<br>0 – 126 ppb | tobacco | Bisessar and Palmer (1984) |
| 0.4 and 0.6 % w/v | Soil | 1 | acute CE<br>800 ppb | bush bean | Chanway and Runeckles (1984) |
| N/A | N/A | N/A | chronic field<br>50 – 110 ppm • hr | potato | Clarke *et al.* (1984) |
| 500 ppm | soil drench | weekly | OTC chronic<br>up to 210 ppb | green ash, white ash | Elliot *et al.* (1985) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 2.0 g (50% WP)/L | foliar spray to run off | 1<br>6-8, at 7 or 14 d intervals | chronic CE<br>220 ppb<br>chronic (ambient) field<br>up to >220 ppb | peanut | Ensing *et al.* (1985) |
| 9 kg/ha<br>2.24 - 6.16 kg/ha | soil applications<br>foliar sprays | 2<br>4-11 times every 1-3 weeks | chronic field and natural field 'episodes'<br>conc. not given | grapevines | Musselman (1985) |
| 500 ppm - 250 mL<br>500 ppm - 5 mL | soil drench<br>stem injection | 1<br>1 | CSTRS acute (CE)<br>0 – 950 ppb | 2 year old woody seedlings (4 spp) | Roberts and Jensen. (1985) |
| 500 ppm | foliar spray | 4 – at 12 day intervals | chronic field<br>conc. not given | potato | Bambawale (1986) |
| 150 ppm<br>500 ppm<br>4L/row | soil drench<br>soil drench | 1<br>biweekly from June to Aug. | acute CE (on 2 consecutive days)<br>200 ppb<br>chronic (ambient) field<br>5 ppm • hr | soybean | Brennan *et al.* (1987) |
| 6.7 kg a.i./ha | soil drench | every 3 weeks, June to Sept. | chronic (ambient) field<br>50 – 110 ppm • hr | potato | Brennan *et al.* (1987) |
| N/A | N/A | N/A | acute treatment 2 consecutive days, CE and OTC's<br>200 ppb | soybean | Greenhalgh *et al.* (1987) |
| 500 µg/mL | foliar | 3 | OTC and field (ambient)<br>conc. not given | bean | Laguette *et al.* (1987) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 1000 ppm<br>1 mL | stem injection | 1 | none, the paper focused on EDU distribution within the plant<br>conc. not given | 4 woody species | Roberts *et al.* (1987) |
| 500 ppm<br>4 L/row | soil drench | biweekly | chronic (ambient) field<br>54 – 62 ppb | soybean | Smith *et al.* (1987) |
| 500 ppm | Foliar | weekly or biweekly | chronic field<br>113 – 116 ppm • hr | white pine | Eberhardt *et al.* (1988) |
| 500 ppm | soil drench | biweekly | chronic field<br>conc. not given | soybean – but also references work on several other crops under field and CE conditions | Heggestad (1988) |
| 500 ppm, 4 L/row<br>and<br>dose response using 0, 125, 250, 500 and 1000 ppm | soil drench | biweekly | chronic (ambient) field<br>54 – 65 ppb | soybean | Brennan *et al.* (1990) |
| 500 ppm<br>6.7 kg a.i./ha | soil drench | triweekly | chronic (ambient) field<br>conc. not given | potato | Clarke *et al.* (1990) |
| 0.5 mg/mL<br>100 mL each | soil application | 1 | acute CE<br>400 ppb | snapbean | Whitaker *et al.* (1990) |
| 150 ppm<br>25 mL/cell | soil drench | 1 | acute CE<br>200 – 280 ppb | field pea | Zilinskas *et al.* (1990) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 40-140 mL/tree increasing based on increased leaf area per year | foliar spray to run off | 7 per growing season at 10 day intervals for 3 growing seasons | chronic (ambient) field<br>0 to >40 ppb | black cherry | Long and Davis (1991) |
| 0.5 mL of 500 mg/L and 0.25 mL of 1000 mg/L | stem injection | 3 in total, once before each exposure to $O_3$ | semi-OTC chronic field<br>30 – 80 ppb | beech | Ainsworth and Ashmore (1992) |
| 500 mg/L | soil drench | 1 | acute CE<br>0 – 900 ppb | snapbean and soybean | Lee *et al.* (1992) |
| 0.3 mg/mL | soil drench | 1 | acute CE<br>300 ppb | snapbean | Pitcher *et al.* (1992) |
| 100 mg/L - 2 L/m row and 200 mg/L – 1 L/m row | soil drench | 1 | chronic (ambient) field<br>40 – 54 ppb | radish | Kostka-Rick and Manning (1992a) |
| 150 mg/L<br>60 mL/plant | soil drench | 1 | chronic CE<br>70 – 120 ppb | radish | Kostka-Rick and Manning (1992b) |
| 100 mg/L - 2 L/m row (1.67 g/$m^2$ or 10 mg/plant) and 200 mg/L - 1 L/m row | soil drench | 1 | chronic (ambient) field<br>42 – 52 ppb | radish | Kostka-Rick et al. (1993) |
| 0 to 800 mg/L and 0 to 400 mg/L<br>100 mL/pot | soil drench | 2 | chronic CE and chronic CE with an ‘episode’ occurring once per week<br>60 – 140 ppb | radish | Kostka-Rick and Manning (1993a) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 0 to 800 mg/L and 0 to 400 mg/L<br>200 mL/pot | soil drench | 4<br>2 | chronic (ambient) CE, and chronic and acute CE combined (ie. chronic with 2 'episodes')<br>60 – 140 ppb | bush bean | Kostka-Rick and Manning (1993b) |
| 100 mg/L 200 mL/plant | soil drench | 2 | chronic (ambient)<br>25 – 88 ppb | bush bean | Kostka-Rick and Manning (1993c) |
| 0, 150, 300 and 450 ppm | foliar | monthly | chronic CE<br>0, 50, 100, 200 ppb | shortleaf pine | Flagler *et al.* (1994) |
| 300 ppm | foliar | monthly | chronic field<br>up to 120 ppb | | |
| 0-120 and 0-32 mg a.i. /L potting medium<br>500 mL/pot | soil drench | 4 | OTC chronic field<br>0 to >60 ppb | snap bean | Miller *et al.* (1994) |
| 200 ppm<br>50 mL per pot | soil drench | 3 (initial and then 2 more at 2 week intervals following first application) | OTC chronic field<br>3.8 – 61.6 ppb | white clover | Ommen *et al.* (1994) |
| 420 µg/mL | foliar spray | 1 | acute CE<br>150 ppb | tobacco | Valenti *et al.* (1994) |
| 0-300 mg/L<br>200 mL/pot | soil drench | 2 or 3 depending on experiment at 10 or 20 day intervals | CE<br>40 – 50ppb | bush bean | Astorino *et a.l* (1995) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 150 ppm 200 mL/pot | soil drench | biweekly | OTC chronic field<br>0 - 110 ppb | bean | Brunschön-Harti *et al.* (1995a, b) |
| 150 ppm 100 mL/pot | soil drench | biweekly | chronic CE and chronic field<br>up to 51.3 ppb | bush bean | Fagnano and Zoina (1995) |
| 500 mg/L 200 mL/pot | soil drench | 3, 10 day intervals | chronic field<br>80 ppb | radish, turnip | Hassan *et al.* (1995) |
| 50 mg/L/pot | soil drench | 1 | acute CE<br>300 ppb | snapbean | Lee (1995) |
| 150 ppm<br>100 mL/pot | soil drench | 2, biweekly | chronic (ambient) field and OTC (chronic-3 day episode)<br>24 – 39 ppb | clover | Pihl *et al.* (1995) |
| 150 ppm | soil drench | biweekly | chronic field<br>AOT40<br>5 – 11 ppm • hr | subterranean clover, lettuce, bean, tomato | Postiglione and Fagnano (1995) |
| 150mg $L^{-1}$<br>200 mL/plant | soil drench | triweekly | chronic field<br>conc. not given | bean | Ranieri and Soldatini (1995) |
| 3 total doses ranging from 0-280 mg/plant | soil application | initial, then biweekly | chronic field<br>10 – 60 ppb | bush bean | Schenone *et al.* (1995) |
| N/A | N/A | N/A | chronic field<br>40 –120 ppb | bean, grapevine, wheat | Tiedemann (1995) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 100-250 ppm<br>200 mL/pot | soil drench | initial, then biweekly | chronic (ambient) field<br>51 – 55 ppb<br>and chronic CE<br>103 – 104 ppb | bush bean | Vandermeiren *et al.* (1995) |
| 0.5 mL of 250 mg/L and 1.0 mL of 1000 mg/L | stem injection | 1 | chronic (10 – 8hr fumigations) CE<br>85 ppb | hybrid poplar | Ainsworth *et al.* (1996) |
| 0-250 ppm a.i. | soil drench | 1 | chronic CE – continuously stirred tank reactor (CSTR)<br>100 ppb | potato | Eckardt and Pell (1996) |
| 150 ppm 100 mL/pot | soil drench | 4, biweekly | OTC chronic<br>24 – 53 ppb<br>and field chronic<br>60 –69 ppb | white clover | Fumagalli *et al.* (1997) |
| 300 ppm | Foliar | 1 | chronic ambient<br>24.7 – 34.8 ppb | tobacco | Godzik (1997) |
| 0.5 mg/mL<br>100 mL/pot | soil application | 1 | acute CE<br>300 ppb | snap bean | Lee *et al.* (1997) |
| N/A | Foliar | biweekly | chronic (field)<br>40 – 60 ppb | red clover | Salam and Soja (1997) |
| 100 mg/L<br>200 mL/pot | soil drench | 5 times at 2 week intervals | chronic field<br>0 – 0.6 ppm • hr | bush bean | Tonneijck and Van Dijk (1997a) |
| 150 mg/L<br>100 mL/pot | soil drench | biweekly until harvest | chronic field<br>21 – 58 ppb | subterranean clover | Tonneijck and Van Dijk (1997b) |

| Dosage | Mode of application | Number of applications | Ozone Treatment | Plant species | References |
|---|---|---|---|---|---|
| 150 ppm, 100 mL | soil drench | biweekly for 3 months | chronic (ambient) field<br>0.3 – 12 ppm • hr | white clover | Ball *et al.* (1998) |
| 250-1000 ppm | foliar spray to run off | 1 | chronic CE<br>(3 to 14 d) 75 ppb | snap bean | Gillespie *et al.* (1998) |
| 300 ppm | foliar spray to run off | 1 | chronic CE<br>(5 to 6 d) 80 ppb | tobacco | Godzik and Manning (1998) |
| 300 ppm | Foliar | weekly | OTC chronic field<br>38 – 82 ppb | spreading dogbane | Bergweiler and Manning (1999) |
| 400 ppm | soil drench | 12 day intervals from March-June | chronic field<br>45 – 46 ppb | tomato | Varshney and Rout (1998) |
| 0-300 ppm | Crown spray to run off | biweekly | OTC chronic field<br>10 – 200 ppm • hr | loblolly pine | Kuehler and Flagler (1999) |
| 0.2g (100% WP)/L<br>100 mL/pot | soil drench | 2 times, 14 days apart | chronic (ambient) field<br>20 – 57 ppb | radish | Pleijel *et al.* (1999) |

plant roots. Foliar application appears to be the most practical especially for large-scale field studies.

### *Application Dose*

As emphasized by Manning (1988, 1992, 1995) and Kostka-Rick and Manning (1993a,b), it is of paramount importance that proper dose response studies be conducted prior to the use of EDU as a survey tool for the measurement of ozone effects. Dose response experiments will not only allow for the determination of the optimal dosage of EDU to convey resistance to ozone but will also allow for the determination of possible side-effects of EDU by application of EDU to plants in the absence of ozone in parallel to application to plants being exposed to ozone. In the first studies by Carnahan *et al.* (1978) in which plants were exposed to acute ozone treatments, various dosages of EDU were tested. Since then, while proper dose response protocols have been followed in many experiments, several studies have been conducted using EDU dosages that were extrapolated from previous experiments. Some of these extrapolations were done from acute to chronic ozone studies and have led to under- or over-dosages of EDU. In these cases, EDU has led to ineffective protection and to EDU-induced toxicity resulting in reduced yields. Manning (1992) provides a review of uses and misuses of EDU.

Ever since Carnahan *et al.* (1978) found that a 500 ppm EDU spray applied to foliage was the optimal rate for protecting bean plants from acute exposure to ozone, many studies have shown this rate of application to be suitable. For example, Cathey and Heggestad (1982a,b) conducted exposure/response screening trials on 4 cultivars of petunia and 44 species of herbaceous plants and found that 500 ppm as a foliar spray or soil drench was optimal. Soil drenches at 500 ppm EDU were also shown to be best for woody species (Cathey and Heggestad 1982c). Based on a number of studies, 500 ppm EDU is the appropriate concentration to protect plants from acute ozone injury (Carnahan *et al.* 1978; Cathey and Heggestad 1982a,b,c; Weidensaul 1980). Based on these findings, later studies making use of EDU to protect plants against chronic exposures to ozone were designed with the assumption that repeated, weekly or biweekly, applications of EDU would not be injurious and that the same concentration (500 ppm) of EDU would be protective. This method was used with varied success (Bambawale 1986; Brennan *et al.* 1990; Clarke *et al.* 1983, 1990; Heggestad 1988; Hofstra *et al.* 1983; Legassicke and Ormrod 1981; Toivonen *et al.* 1982). In several of these studies plants were over-dosed with EDU and negative effects such as browning of leaf edges and reductions in yield were found. In recent studies EDU has been used without proper preliminary dose-response studies. For example, the standard protocol for the UN-ECE ICP-Crops program is to test various species and cultivars for ozone damage in pot studies in which EDU at a concentration of 150 ppm is added as a 100 mL soil drench at biweekly intervals. Postiglione and Fangnano (1995) used EDU to test for effects of ambient ozone on lettuce, subterranean clover, bean and tomato while Fumagalli *et al.* (1997) used both EDU and open-top chambers to study the effects of ambient ozone on white clover plants in the Milan region of Italy. In both studies researchers found no ozone-related effects of EDU.

In 1992, Kostka-Rick and Manning (1992a) examined the dose response to EDU applied as a soil drench in radish exposed to ozone. They showed that the concentration and dose of EDU could be reduced by 3 to 7-fold relative to earlier studies without compromising the effectiveness of the product. They found that at these dosages plant growth at all stages examined (early to late hypocotyl thickening) was completely preserved in the face of ozone stress although EDU did cause slight leaf margin necrosis and hyponastic leaf deformations. These studies point to the importance of conducting dose response experiments prior to the use of the EDU method in assessments of crop effects. Kostka-Rick and Manning further emphasized this caveat in papers published over the following two years from work on EDU protection of radish and bean (Kostka-Rick and Manning 1993a,b).

### *Timing of Application*

Clarke *et al.* (1983) pointed to the importance of the timing of the exposure episode in relation to tuber formation in determining whether EDU could impart beneficial effects. Hofstra *et al.* (1978) compared the efficacy of EDU to carboxin and benomyl in reducing ozone-related yield losses in navy bean. They found that EDU was the most effective and that the timing of application was critical in eliciting optimal effect. In 1979, McClenahen tested the efficacy of EDU in protecting white ash and black cherry from ozone injury. McClenahen (1979) found that weekly application of EDU to seedling foliage provided near complete protection from ozone at concentrations of up to 300 ppb. In 1980, Weidensaul showed that pinto bean plants were best protected from ozone injury when EDU was applied 3 to 7 days prior to ozone exposure but that EDU afforded no protection to foliage that had not yet formed when the chemical was applied.

### *Side-effects*

The possible side-effects of EDU were discussed by Legassicke and Ormrod (1981) and Foster *et al.* (1983) who showed that EDU did not increase yield in the ozone resistant cultivar of tomato 'New Yorker' nor in the 'White Rose' potato, respectively. Similar findings were reported by Clarke *et al.* (1983) in the potato cultivar 'Green Mountain'. Foster *et al.* (1983) also showed that EDU applied every 2 weeks did not cause increased yields in ozone-sensitive cultivars of potato grown in ozone-free air. Bisessar and Palmer (1984) used approximately the same rate of application of EDU for tobacco as did Foster *et al.* but applied it every 7-10 days. Increasing the frequency of application and perhaps over-dosing the plants caused side-effects of EDU on root and shoot biomass. Greenhalgh *et al.* (1987) used open-top chambers to verify whether EDU had side-effects when it was applied to soybean. They found no differences in plant height, pod number, seed yield, chlorophyll content and foliar injury between soybean plants grown in ozone-free air in the presence or absence of EDU.

## 3. CONCLUSIONS AND RECOMMENDATIONS

Several studies suggest that the application of certain chemical protectants against ozone might be a reliable means by which to assess crop effects under field conditions.
While many chemicals have been shown to convey partial or total protection against ozone injury, many are ineffective and have unacceptable side-effects rendering them of little value for the purpose of crop effects assessments in the field. This is true of some of the most promising antiozonants. For example, even though the fungicide benomyl has been found to effectively control ozone injury in a number of plants, it would be impossible to separate the fungicidal benefits from its antiozonant properties in the field.

We conclude that EDU is specific in the suppression of ozone injury in a wide variety of plant species. Studies conducted to date have shown that EDU has few side-effects and is effective on almost all plants studied. If appropriate exposure/response and toxicological studies are conducted with EDU and ozone, it should be possible to use EDU for assessing crop effects in the field. Therefore we recommend that the EDU method be adopted for studies aimed at the assessment of crop effects under field conditions in Alberta. EDU at a concentration of 250 – 500 ppm should be applied to the foliage to runoff every 7 to 10 days throughout the vegetation period. This should allow for the partial or total mitigation of ozone effects in chronic exposures at concentrations of up to 80ppb. For the preliminary determination of the potential use of EDU application in assessing effects of ozone on crops of Alberta, we recommend that studies be conducted at sites where ozone levels are greatest, perhaps Fort Saskatchewan or east Edmonton, with an ozone control site established near Vegreville where ozone levels are very low. Information on the relative sensitivity of common Alberta crops is lacking, making it difficult to determine which species should be used in these studies. We recommend that at least two species be studied. Based on their relative importance, we recommend that barley (*Hordeum vulgare*) and canola (*Brassica napus* – the most common species of canola) be used.

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