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Foliar Symptoms of Acute Ozone Injury and Exposure Response Characteristics of Select Native Perennials

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FOLIAR SYMPTOMS OF ACUTE OZONE INJURY AND EXPOSURE RESPONSE CHARACTERISTICS OF SELECT NATIVE PERENNIALS

FOLIAR SYMPTOMS OF ACUTE OZONE INJURY AND EXPOSURE RESPONSE CHARACTERISTICS OF SELECT NATIVE PERENNIALS

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Horticulture

By

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ABSTRACT

Twenty seven perennial species native to the Eastern Temperate Forests Level I Ecoregion were exposed to an acute ozone (O_3) treatment consisting of a target peak O_3 concentration of 2.0 ppm for 30 minutes in a closed chamber environment, during the summer of 2010. Plants were evaluated for visible foliar injury symptoms and symptoms were described and photographically documented. Ten of the 27 species developed visible foliar injury in which interspecific and intraspecific response to O_3 was observed. A severity index was used to compare response to acute ozone exposure for the ten species displaying visible foliar injury. Species showing visible foliar injury in descending order of severity index were *Coreopsis tripteris* L. (tall tickseed), *Coreopsis palmata* Nutt. (stiff tickseed), *Penstemon cobaea* Nutt. (cobaea beardtongue), *Solidago nemoralis* Aiton (gray goldenrod), *Monarda fistulosa* L. (wild bergamot), *Silphium integrifolium* Michx (wholeleaf rosinweed), *Oligoneuron rigidum* (L.) Small var. *rigidum* (stiff goldenrod), *Rudbeckia missouriensis* Engelm. ex C.L. Boynt.(Missouri orange coneflower), *Penstemon pallidus* Small (pale beardtongue), and *Solidago speciosa* Nutt. (showy goldenrod). A range of symptoms was observed including red to purple stipple and tan to yellow or brown flecking; bronzing, leaf margin necrosis, and bifacial necrotic lesions.

Subsequently, in June of 2011, four native *Coreopsis* species (*C. lanceolata*, *C. palmata*, *C. tinctoria*, and *C. tripteris*), were exposed to four acute ozone (O_3) treatment levels with target peak O_3 concentrations of 1.2, 1.7, 2.2, and 2.7 ppm, for 30 minutes in a closed chamber environment. Severity index was used as the criterion for comparing ozone susceptibilities among species. All four species showed foliar injury symptoms following the highest target peak exposure level (2.7 ppm). Two of the four species (*Coreopsis palmata* and *C. tripteris*) exhibited foliar injury symptoms at the lowest target peak exposure level (1.2 ppm). Symptoms

varied among species in the study but were generally uniform within each taxon. *Coreopsis lanceolata* showed the highest degree of ozone tolerance relative to the other three species in the study. Both *Coreopsis palmata* and *C. tripteris* developed visible foliar symptoms at the lowest treatment level (1.2 ppm) indicating ozone susceptibility relative to the other species.

This thesis is approved for recommendation
to the Graduate Council

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DEDICATION

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INTRODUCTION

Ozone (O_3) is currently considered to be the most important air pollutant in many parts of Europe, North and Central America and the Far East not only because of its phytotoxicity, but also because tropospheric concentration of O_3 has increased considerably during the past 60 years (Black et al., 2000).

Ozone is present in the upper atmosphere or stratosphere, as a naturally occurring beneficial layer (the ozone layer) and in the lower atmosphere or troposphere. The troposphere consists of layers that extend above the surface of the earth defined as 8000 m at the poles and 18,000 m at the equator, ending at the tropopause. At ground-level, both natural and artificial sources of O_3 exist and, at supra-ambient concentrations, ground-level O_3 can trigger health problems in people and phytotoxicity in plants. The productivity, quality, and competitive ability of important agricultural and horticultural plants may be adversely affected by current and anticipated concentrations of ground level ozone (Booker et al., 2009).

Natural O_3 consists of lightning generated sources during thunderstorms and downward transport of O_3 via tropopause folding from the upper atmosphere (Krupa et al., 2001). Because of natural sources of O_3 at ground-level, there is a worldwide ambient O_3 concentration of 20 to 30 $nl.l^{-1}$ (ppb). Background values will vary with geophysical conditions and levels of industrialization (Krupa et al., 2001).

As an air pollutant, O_3 is one of the most abundant greenhouse gases in the earth's atmosphere largely resulting when vehicle exhaust gases and industrial emissions such as nitrogen oxides, carbon monoxide, and volatile organic compounds react in the presence of sunlight. Production of the gas occurs primarily through chemical reactions in the atmosphere, driven by sunlight or radiation. Because of seasonal changes in the solar radiational flux, high concentrations of O_3 occur during the plant-growing season (Krupa et al., 2001). As urbanization and industrialization continue; increasing numbers of reports have appeared regarding O_3-induced foliar injury on sensitive plants (Krupa, Tonneijck, and Manning, 1998). Temperature has an important influence on the half-life of O_3 (up to 3 days at 20°C); its decomposition rate increases with increasing temperature. The average lifetime of O_3 is about 16 hours, and once produced, it can be transported long distances to rural agricultural and forested areas. Typical summertime daily maximum surface level O_3 concentrations in urban-suburban areas are 100-400 ppb while those in rural areas range from 50-120. Remote tropical forest and remote marine areas range from about 20-40 ppb (Krupa et al., 2001). Thus, O_3 is a major air pollutant in the United States causing foliar injury to many agronomic and horticultural crops, deciduous trees, and conifers (Krupa, Tonneijck, and Manning, 1998).

Soil and plant disturbance is a common occurrence along roads and highway corridors, around building sites, and within urbanized zones. In addition, efforts to restore altered prairie and wetland habitats; and to bring green space into the urban zone are on the rise. With an increased interest in the use of natural habitats and native plant species comes a need for recommendations of appropriate plant materials for these efforts. Roadside rights-of-way account for more than 10 million acres of land in the United States. This land requires management that protects water quality, reduces erosion, increases wildlife habitat, reduces mowing and herbicide use, enhances natural beauty, controls noxious and invasive species, and protects natural heritage – all objectives of vegetation management (U.S. Department of Transportation, 2007). In urban settings, this type of site could experience episodic high levels of O_3 due to heavy traffic patterns, industrial waste, and other nitrogen oxide producing processes present in highly urbanized areas. Rural areas along major highway interchanges may also be affected by reduced air quality including ozone.

There is limited information available on O_3 injury symptoms and exposure response characteristics of perennial native plant species. Valuable information for the land manager, revegetation specialist and landscape professional may be gathered in this study to facilitate appropriate plant selection and accurate diagnosis of O_3 injury in the field. Information relative to O_3 response of native perennials has the potential to improve the success of native plantings based on the susceptibility/tolerance of plant species in both urban and rural areas. There is also a need to facilitate accurate diagnosis of O_3 injury in the field and to identify species appropriate for use as bioindicators.

Objectives

The objectives of the following experiments were as follows:

1) To induce, describe, and photographically document visible foliar injury symptoms resulting from acute O_3 exposure on select native plant species in a controlled chamber environment.

2) To evaluate the relative sensitivity of four native *Coreopsis* species to acute O_3 exposures and to describe the acute O_3 exposure response characteristics in a closed chamber environment.

3) To compile a list of native plant species that have shown O_3 tolerance for use in Arkansas and other regional vegetation projects.

Hypotheses

1) Visible foliar injury symptoms and the severity of symptoms resulting from acute O_3 exposure will show no variation for the plant species studied.

2) Ozone sensitivity and response characteristics to acute O_3 exposures will show no variation for the plant species studied.

LITERATURE REVIEW

Foliar Ozone Injury on Plants

Ozone (O_3) induced foliar damage was first documented in 1958 on grape (*Vitis vinifera* L.) near San Bernardino California (Krupa et al., 1998; Richards et al., 1958). This documentation resulted from observations of a premature yellowing and leaf fall in a number of vineyards east of Los Angeles, California in 1954. Symptomology included numerous small, discrete spots, or stippling on upper leaf surfaces (Richards et al., 1958). The following year, Heggestad and Middleton (1959) reported that O_3 was the cause of extensive foliar injury on tobacco (*Nicotiana tabacum* L.) in the eastern United States (Krupa, et al., 1998; Heggestad and Middleton, 1959). They found a high correlation between appearance of "weather fleck" in tobacco and high O_3 levels. In addition to the 1954 injury to grape, Heggestad and Middleton (1959) reported that fleck of varying degrees of severity has developed on tobacco at Beltsville, Md., since 1954, and perhaps earlier. They reported that one variety of cigar wrapper tobacco from the Connecticut Valley, designated "C" was so susceptible to fleck that it could not be grown for commercial production. Results of fumigation experiments indicate that the tobacco is relatively resistant to the reaction products of ozone and hydrocarbons, the toxicants responsible for "smog damage." Smog injury appears primarily on the lower leaf surface and can be distinguished from injury caused by O_3, which appears on the upper leaf surface (Heggestad and Middleton, 1959).

Ozone exposure causes several general symptoms on broadleaf species in the field. Among these general symptoms, leaf surface stipple has been described as the classic symptom of O_3 injury on broadleaf species (Krupa et al., 1998; Skelly, 2000). Stipple usually does not affect the veins and veinlets; veinlets often border injury causing an angular appearance of the affected tissue (Skelly, 2000). Ozone induces symptoms other than stipple including foliar reddening, chlorosis, premature defoliation, bronzing, flecking, bleaching, leaf-tip burn, and necrosis (Kline et al. 2008). Such symptoms are not reliable tools however when assessing O_3 injury in the field, since they could be caused by factors other than ozone (Kline et al. 2008, 2009; Orendovici et al. 2003). Both acute and chronic injury may be confused with symptoms of other conditions, such as nutritional disorders, other abiotic stressors, biotic pathogens, or insect infestations; thus sensitive plant species displaying a set of general diagnostic features are particularly useful as bioindicators (Kline et al. 2008; Skelly, 2000).

The sensitivity of leaves to O_3 exposure depends on the leaf age. Many studies have confirmed the high sensitivity of middle-aged leaves over leaves that have not reached the maximum enlargement rate. The period of maximum sensitivity correlates with the stomata becoming fully functional and the formation of intercellular spaces so that O_3 can enter the leaves and reach target sites. These observations correspond to a greater importance in physiological age over chronological age. Leaves of dicotyledonous plants are most sensitive between 65% and 95% of their final size (Krupa et al., 1998).

Mechanisms of Ozone Foliar Injury to Plants

Ozone is deposited from the atmosphere onto plant canopies by diffusion and enters the leaf through stomata. Environmental, biological and cultural (e.g., irrigation) factors that promote stomatal opening increase the risk of O_3 injury to plants (Krupa et al., 1998, 2001). Moist surfaces within the leaf such as the extracellular fluid of the mesophyll tissue allow O_3 to dissolve and diffuse along a concentration gradient similar to that of carbon dioxide. Once in the leaf, O_3 immediately forms other derivatives which show varying degrees of reactivity. Plasma and cell membranes undergo changes in permeability and leakiness of cell membranes to important ions such as potassium. Internal membranes are affected to a lesser extent as the toxic oxidants are diluted and absorbed from the outside towards the inside of the cells (Krupa et al., 1998).

Ozone Bioindicators

Ozone has become an air pollution problem in most industrialized nations resulting in an increased interest in using bioindicator plants on a worldwide basis (Krupa et al., 1998). Two types of plant bioindicators, detectors and sentinels, have been recognized as useful in studies to detect phytotoxic levels of gaseous air pollutants. Detectors are native plants, in general determinate perennial shrubs or trees that respond slowly to O_3 and often fairly late in the growing season. Only the sensitive individuals in a population will respond to O_3 when exposures are sufficient to cause foliar injury; the distribution of the O_3-sensitive genotypes is usually not well known resulting in uncertainty in interpreting the results from detectors.

Sentinels are well defined selections of plants known to be sensitive to O_3 that exhibit diagnostically reliable foliar symptoms when exposed to ambient O_3. These sensitive plants are introduced to an area to serve as early warning devices or checks on the efficiency of abatement practices. An example of a sentinel is the tobacco (Nicotiana tabacum L.) cultivar Bel-W3 (Krupa et al. 1998).

Since 1962, the tobacco cultivar Bel-W3 has been used in many countries as an indicator of the presence of phytotoxic concentrations of O_3. It has a high sensitivity to O_3 and may produce easily recognizable symptoms for several weeks on the new, fully expanded leaves (Heggestad, 1991). Indeed, among all indicator plants, tobacco cv. Bel-W3 and cv. Bel-B are the best described and the most commonly used worldwide (Krupa et al., 2001). The cultivars Bel-B and Bel-C, tolerant and sensitive to O_3, respectively, are sometimes used along with Bel-W3 to increase the value of the data when differences in leaf injury with increasing days of exposure to ambient air are noted. The cultivars were the product of research initiated in 1957 to determine the cause of and reduce losses from tobacco weather fleck. Bel-W3 is so sensitive to O_3 that it probably cannot be grown from the seedling to flowering stage out-of-doors anywhere in the 48 contiguous USA states without some injury. That is, Bel-W3 may be injured when concentrations are only slightly above normal background concentrations. Use of Bel-W3 world-wide as an indicator of elevated O_3 concentrations has been a significant factor in increasing the awareness of O_3 as a pollutant (Heggestad, 1991).

Ozone Effects on Plants

Plants are subjected to acute and chronic exposures of ground-level O_3. An acute exposure consists of high O_3 concentrations (e.g., >80 ppb) from a few consecutive hours to days. In comparison, a chronic exposure consists of relatively low O_3 concentration (e.g., <40 ppb) for the entire life of a plant, with periodic intermittent or random episodes of high concentrations. Plant response to O_3 can vary with the genus, species, cultivar or variety, and genotype (Krupa et al., 2001).

Ozone injures plants mainly following uptake through the stomata in the leaf surface and some of the changes in plant metabolism caused by O_3 become manifest in a variety of visible foliar injury symptoms (Booker et al., 2009). The response of a plant to O_3 depends on the exposure characteristics, plant properties, and external growth conditions. Short term exposure to high concentrations (e.g., >150 ppb) generally result in acute visible foliar injury (Krupa et al., 1998). Ozone negatively impacts a number of plant processes, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield. Many of the physiological functions necessary for growth and reproduction are impaired, but specific cellular sites that undergo damage are not completely known (Krupa 1997; Krupa et al., 2001).

Investigators have shown that chronic, whole growth season or whole life cycle exposures to O_3 can result in losses of marketable yield in agronomic crops and reductions in growth and productivity of forest tree species (Krupa et al., 2001). Fewer studies have been conducted on horticultural plants or native herbaceous plants, but current consensus within the scientific community is that O_3 can negatively impact many crops and horticultural plants (Booker et al., 2009).

Ornamental plants such as petunia (*Petunia* x *hybrida* Juss.), small fruits (blackberry (*Rubus cuneifolius* Pursh)), buddleja (*Buddleja davidii* Franch.), and other landscape shrubs can be injured by ambient O_3 (Booker et al., 2009; Cathey, H.M. and H.E. Heggestad 1982; Chappeka, 2002; Findley et al. 1997a; Findley et al. 1997b). Injury can occur as a loss in biomass or yield, foliar necrosis and pigmentation, a decrease in flowers or species fitness, or alteration in fruit quality (Booker et al., 2009).

Findley et al. (1997a) exposed 26 species and/or cultivars commonly used in landscapes of the southeastern United States to three O_3 levels reported as 12 hour treatment means (sub-ambient (20.2 ± 3 ppb), ambient (35.3 ± 5 ppb), and 2.5x ambient (83.8 ± 8 ppb) for 3-week periods during the spring and summer of 1994, in an experiment designed to determine symptoms of chronic O_3 exposure and sensitivity. Of the 26 species studied, none exhibited foliar injury symptoms at the sub-ambient level. Only two cultivars of *Buddleja* developed visible injury symptoms in the ambient O_3 treatment, and visible injury was detected on all nine cultivars of *Buddleja* in the 2.5x O_3 treatment level along with one cultivar of *Zinnia angustifolia* and three cultivars of *Acer rubrum*. The remaining species did not show visible symptoms.

They found a significant ozone x cultivar interaction for percentage of the leaves injured (PLI) and the Horsfall-Barratt rating (Horsfall and Barratt, 1945), a grading system for measuring plant disease (Findley et al., 1997a).

Subsequently, Findley et al. (1997b) investigated five cultivars of *Buddleja davidii* Franch., in open top chambers for foliar injury, growth index, and inflorescence characteristics during and following three O_3 exposure treatments for 8 weeks. Exposure treatments varied over the 8 week period and were reported as weekly 12 hour treatment means [(sub-ambient (16.4 - 35.3 ppm), ambient (23.5 - 54.6 ppb) and 2.0x ambient (51.9 - 118.9 ppm)]. In this study differential sensitivity to O_3 exposure among the cultivars was studied. Visible foliar injury was present in all cultivars after three and eight weeks of exposure in the 2.0x ambient treatment only. The ozone x cultivar interaction was significant ($p = 0.0117$) for percentage of the leaves injured (PLI) after 3 weeks of exposure. By eight weeks of exposure, sampling mean PLI across cultivars more than doubled, however the ozone x cultivar interaction was not significant indicating no difference among cultivars at 8 weeks. One explanation for the large variation in PLI among cultivars after three weeks of exposure offered by Findley et al. (1997b) is the difference in the number of leaves that had developed. The most severely injured cultivars appeared to develop more slowly than the other cultivars and thus had a higher percentage of mature leaves, i.e. more sensitive to O_3 than immature leaves. Differences in meteorological conditions may have contributed to the variability as well. Studies have shown that foliar O_3 uptake and visible injury are reduced under conditions of high temperature and low relative humidity due to increases in stomatal closure, resulting in less O_3 uptake during these periods. Rainfall was below and temperatures above the 30-year average for the treatment period in 1995. In 1994, rainfall was above the 30-year average and temperatures were near normal. In summary, although differences in foliar injury were present in the short-term (3 weeks) exposure, all cultivars were sensitive to prolonged exposure to elevated levels of tropospheric ozone (Findley et al., 1997b).

In 2001, VanderHayden et al. conducted a study in which O_3 injury symptoms on forest species in Switzerland were identified, verified and related to the current European ozone standard. In Europe, the critical O_3 level for forest trees has been defined at an AOT40 (Accumulated exposure Over a Threshold of 40 ppb) of 10 ppm.h O_3 (10 ppm-hours accumulated exposure of O_3 over a threshold of 40 ppb). The AOT40, also accepted by the United States Environmental Protection Agency, is calculated as the sum of the differences between the hourly concentration (in ppb) and 40 ppb for each hour when the concentration exceeds 40 ppb (de Kluizenaar et al., 2001). The objective of the study was to determine the amount of ambient O_3 required to induce visible foliar symptoms on various forest plant species in southern Switzerland. Species were grown in open top chambers and open forest nursery plots. They found that species differed significantly in terms of the ppb.h exposures needed to cause visible symptoms. Species that first showed evidence of foliar injury demonstrated the most sensitivity throughout the growing season, with symptoms rapidly advancing over 25-30% of the total plant leaf surfaces by the end of the observation period. Conversely, those species that developed symptoms later in the season had far less total injury to plant foliage by the end of the observation period (1.5 to <5% total leaf area injured) (VanderHeyden et al., 2001).

Orendovici et al. (2003) cite recent field surveys in the northeastern USA and in southeastern Spain that revealed many additional plant species that exhibit symptoms typical of ozone-induced injuries. The objective of their study was to confirm O_3 as the cause of the observed foliar symptoms, determine O_3 induced exposure/response relationships, and identify possible bio-indicator species. Thirteen native species of northeastern USA were included in their study. Results confirmed that with few exceptions, symptoms observed in the field were induced by exposures to ambient ozone. Species differed significantly in terms of the exposures required for the initiation of visible symptoms and subsequent injury progression (Orendovici et al., 2003).

Studies conducted by Kline et al. (2008) evaluate the relative O_3 sensitivity of different plant selections and describe ozone-induced foliar symptoms under controlled conditions in continuously stirred tank reactor (CSTR) chambers. Results of the study identify several sensitive plants; American sycamore (*Platanus occidentalis* L.), aromatic sumac (*Rhus aromatica* Aiton), basket willow (*Salix purpurea* L.), Bankers dwarf willow (*Salix* x *cottetii* Kern), bee-balm (*Monarda didyma* L.), black willow (*Salix nigra* Marsh.), buttonbush (*Cephalanthus occidentalis* L.), common milkweed (*Asclepias syriaca* L.), European dwarf elderberry (*Sambucus ebulus* L.), New England aster (*Symphyotrichum novae-angliae* (L.) G.L. Nesom), sandbar willow (*Salix exigua* Nutt.), shining willow (*Salix lucida* Muhl.), silky willow (*Salix sericea* Marsh.), snowberry (*Symphoricarpos* Duham) and swamp milkweed (*Asclepias incarnata* L.) as potential bioindicators. Furthermore, the study confirmed earlier reports that swamp milkweed, European dwarf elderberry, common milkweed, American sycamore and snowberry were sensitive to ozone. They concluded that across much of the US, phytotoxic levels of O_3 occur during each growing season and that O_3-induced injury to native vegetation in these areas will likely continue in future years (Kline et al., 2008).

In a subsequent report, Kline et al. (2009) studied 16 selections of Indian hemp (*Apocynum cannabinum* L.) and nine selections of common milkweed (*Asclepias syriaca* L.) from seed collected from various locations within the Midwest. The selections were exposed to

40 or 80 ppb O_3 under controlled conditions for 7 hr/day, 5 days/week from 14 June to 28 July, 2005 to evaluate their relative O_3 sensitivity. In this study, both species exhibited considerable intraspecific variation in O_3 sensitivity. They suggest that these significant intraspecific differences in response to O_3 may represent genetic differences in O_3 sensitivity among provenances arising randomly or due to selection pressure from spatially different levels of ozone. Variability was too great to assign definitive O_3-sensitivity ratings within geographic regions from which seed was selected; however two locations were identified as possible collection sites for O_3-sensitive selections of both species. They concluded that plants derived from seed from these locations may serve as O_3-sensitive bioindicators (Kline et al., 2009).

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Chapter 1

VISIBLE FOLIAR INJURY SYMPTOMS OF SELECT NATIVE PERENNIALS RESULTING FROM ACUTE OZONE EXPOSURE

Abstract

Twenty seven species of perennials native to the Eastern Temperate Forests Level I Ecoregion were exposed to an acute ozone (O_3) treatment consisting of a target peak O_3 concentration of 2.0 ppm for 30 minutes in a closed chamber environment, during the summer of 2010. Plants were evaluated for visible foliar injury symptoms and symptoms were described and photographically documented. Ten of the 27 species studied developed visible foliar injury in which interspecific and intraspecific response to O_3 was observed. A severity index was calculated for each species and used to compare response to an acute ozone exposure treatment for the ten species displaying visible foliar injury. Species showing visible foliar injury after exposure in descending order of severity index were *Coreopsis tripteris* L. (tall tickseed), *Coreopsis palmata* Nutt. (stiff tickseed), *Penstemon cobaea* Nutt. (cobaea beardtongue), *Solidago nemoralis* Aiton (gray goldenrod), *Monarda fistulosa* L. (wild bergamot), *Silphium integrifolium* Michx (wholeleaf rosinweed), *Oligoneuron rigidum* (L.) Small var. *rigidum* (stiff goldenrod), *Rudbeckia missouriensis* Engelm. ex C.L. Boynt.(Missouri orange coneflower), *Penstemon pallidus* Small (pale beardtongue), and *Solidago speciosa* Nutt. (showy goldenrod). A range of symptoms was documented including red to purple upper leaf surface stipple and tan to yellow or brown flecking; bronzing, leaf margin necrosis, and bifacial necrotic lesions.

Introduction

As an air pollutant, ozone (O_3) is one of the most abundant greenhouse gases in the earth's atmosphere largely resulting when vehicle exhaust gases and industrial emissions such as nitrogen oxides, carbon monoxide, and volatile organic compounds react in the presence of sunlight. Production of the gas occurs primarily through chemical reactions in the atmosphere, driven by sunlight or radiation. Because of seasonal changes in the solar radiational flux, high concentrations of O_3 occur during the plant-growing season (Krupa et al., 2001).

Plants are subjected to acute and chronic exposures of ground-level O_3. An acute exposure consists of high O_3 concentrations (e.g., >80 ppb) from a few consecutive hours to days. In comparison, a chronic exposure consists of relatively low O_3 concentration (e.g., <40 ppb) for the entire life of a plant, with periodic intermittent or random episodes of high concentrations. Plant response to O_3 exposure can vary with the genus, species, cultivar or variety, and genotype (Krupa et al., 2001).

At ground-level, both natural and artificial sources of ozone exist and, at supra-ambient concentrations, ground-level ozone can trigger health problems in people and phytotoxicity in plants (Booker et al., 2009). The productivity, quality, and competitive ability of important agricultural and horticultural plants may be adversely affected by current and anticipated concentrations of ground level ozone (Booker et al., 2009).

Natural ozone consists of lightning generated sources during thunderstorms and downward transport of O_3 via tropopause folding from the upper atmosphere (Krupa et al., 2001; Lefohn, 1992). Because of natural sources of O_3 at ground-level, there is a worldwide ambient O_3 concentration of 20 to 30 $nl \cdot l^{-1}$ (ppb). Background values will vary with geophysical conditions and levels of industrialization (Krupa et al., 2001).

Typical summertime daily maximum surface level O_3 concentrations in urban-suburban areas are 100-400 ppb while those in rural areas range from 50-120 ppb. Remote tropical forest and remote marine areas range from about 20-40 ppb (Krupa et al., 2001). Thus, ozone is a major air pollutant in the United States causing foliar injury to many agronomic and horticultural crops, deciduous trees, and conifers (Krupa, Tonneijck, and Manning, 1998). As urban centers and industries have continued to grow, increasing numbers of reports have appeared in the literature regarding O_3-induced foliar injury on sensitive plants in countries such as Australia, United Kingdom, Canada, Germany, Greece, India, Israel, Italy, Japan, Mexico, the Netherlands, Poland, Spain, and the Ukraine (Krupa, Tonneijck, and Manning, 1998).

The objective of this study was to induce, describe and photographically document the foliar injury symptoms resulting from acute O_3 exposure for select native perennial plant species in a closed chamber environment. This research is intended to provide information on the relative ozone sensitivity of select native perennials and to facilitate accurate diagnosis of O_3 injury in the field by providing descriptions and photographs of ozone injury for perennial species not previously reported in the literature.

Materials and Methods

Plant Culture

This study was conducted at the University of Arkansas, Division of Agriculture Research Farm in Fayetteville, AR (36°06'N, 94°10'W). Twenty seven perennial plant species native to the Eastern Temperate Forests Level I Ecoregion (U.S. Environmental Protection Agency, 2011) were investigated in this study (Table 1.1). Naming conventions in The PLANTS Database (USDA, NRCS, 2011) are followed throughout this document. All species selected have multiple attributes appropriate for revegetation of disturbed sites such as the ability to tolerate drought, heat, humidity, and poor soil conditions; the ability to self-seed and reproduce true-to-form; the ability to provide habitat for insects, birds and other wildlife; and the ability to provide aesthetic qualities in the urban landscape including flowering, form, structure, and color.

Uniform liners of the selected species were obtained in March, 2010 from Missouri Wildflowers Nursery, LLC (Jefferson City, MO 65109). Plants were up-potted to round standard 15 cm (1666 cm^3) pots with Sta-Green Nursery Blend (Spectrum Group, Division of United Industries Corporation, St. Louis, MO) with 0.09N-0.06P-0.05K previously incorporated. Each pot was top-dressed with a one-time application of 15 grams Osmocote PLUS 15N-9P-12K (Scotts-Sierra Horticultural Products Co., Marysville, OH) and 7.5 grams Micromax Micronutrients (Scotts-Sierra Horticultural Products Co., Marysville, OH). Potted plants were maintained on an outdoor gravel nursery pad prior to treatment. Treatments and post-treatment evaluations were done in a greenhouse under 48% shadecloth and ambient air temperature ranging from 24 °C to 35 °C. Plants were watered as needed using the municipal water supply. Light meter readings were obtained the morning of 8 July 2010; a cloudy day, using a LI-COR line quantum sensor, 1 m in length (LI-COR Biosciences, Lincoln, NE). Photosynthetically active radiation (*PAR*) levels in the greenhouse averaged 74.5 $\mu mol \cdot meter^{-2} \cdot sec^{-1}$, and 69.4 $\mu mol \cdot meter^{-2} \cdot sec^{-1}$ inside an exposure chamber in the greenhouse; readings obtained outside the greenhouse on the same day were 186.0 $\mu mol \cdot meter^{-2} \cdot sec^{-1}$.

Ozone Exposure Chambers

Three air-tight 1.83 x .91 x .91 m experimental exposure chambers were constructed from 3 mm thick OPTIX® acrylic sheet (Plaskolite Inc. Columbus, OH) over a pine wood frame using 100% silicon sealant (General Electric Company,' Fairfield, CT) around all seams and hardware attachments. Access ports were built into the chambers using copper compression fittings to allow for injection of ozone and other probes for monitoring chamber conditions. The junction of the top portion of the chamber (chamber cover) and the chamber floor was made air-tight using foam weather stripping. To facilitate handling, the chamber cover can be lifted via a pulley system off of the acrylic chamber floor to move plants in and out of the chamber. Once plants are in place, the cover can then be set onto the acrylic sheet floor creating an air-tight chamber to prevent O_3 loss during treatment (Fig. 1.1). The chamber floor was sectioned into a 2 x 5 grid with all grid sections of equal area, about 1672 cm^2. Each of nine plants (3 plants x 3 species) was placed in the center of a randomly selected grid cell for treatment with one grid cell reserved for a small battery operated portable fan (O2 COOL® Chicago, IL) for air circulation during exposure treatments.

Ozone Generator and Ozone Analyzer

Ozone was generated using a corona-discharge G-6 – V, Variable Ozone Generation System with a rated capacity of 250 $mg \cdot hour^{-1}$ and adjustable output flow rate from 0% to 100% of the rated capacity, custom designed and built for this research (LYNNTECH, Inc., College Station, TX). Ozone gas was transferred from the generator to the chamber using 6.35 mm outside diameter Teflon® coated tubing which does not react with ozone. The gas was allowed to enter via three ports at the top front, middle and back of the chamber. During treatments, the ozone concentration in the chamber was monitored every 10 seconds using a UV absorption analyzer designed specifically for ozone, Ozone Analyzer Model UV-100 (ECO SENSORS, Inc., Santa Fe, NM). Calibration of the Ozone Analyzer Model UV-100 was performed by Eco Sensors, Inc. and is traceable to the U.S. National Institute of Standards and Technology (NIST).

Ozone Treatments

Two treatment levels were administered, the control treatment in which the plants were placed in the chamber under ambient O_3 levels (no O_3 injected into the chambers); and an acute ozone exposure treatment with a peak O_3 concentration target of 2.0 ppm. For both the control and the acute exposure, treatment duration was 30 minutes. Ozone concentration was monitored every 10 seconds and recorded every minute over the 30 minute treatment.

The acute ozone exposure regime was determined by exposing test plants of petunias, marigolds and impatiens; all readily available, to increasing levels of O_3 until foliar symptoms developed. An O_3 level in the range producing moderately severe symptoms in the three test species was used for the perennial species in this study.

Plants were moved from the outdoor gravel nursery pad to the greenhouse the day prior to treatment. The following morning treatments were conducted. Treatments were conducted during the morning hours, beginning after sunrise to avoid excessive heat build-up in the greenhouse and exposure chambers. For each treatment block, three single plant replicates of three plant species (9 plants total per block) were randomly assigned a cell on the chamber floor grid and placed in the center of the cell. A small battery operated fan provided air circulation to mix O_3 throughout the chamber. Plants were removed from the chamber immediately after treatment. Each block was replicated three times (3 plants/species/block x 3 blocks = 9 plants per species). Treatments were conducted during the period from 19 July to 31 August 2010.

Control Treatment: The control treatment was conducted in three chambers simultaneously (all three blocks simultaneously), with no ozone injected into the chambers. Background ambient O_3 levels were monitored throughout the 30 minute treatment duration and recorded every minute (data not shown). During control treatments O_3 levels were typically below 50 ppb and never exceeded 100 ppb.

Acute Ozone Exposure Treatment: Due to the availability of only one ozone generator, the acute exposure treatment was replicated consecutively, all three replications being done on the same day and in the same chamber rather than in three chambers simultaneously as with the ambient ozone control treatment. Once closed, ozone was injected into the chamber until the target peak concentration was reached at which time the ozone generator was turned off and the ozone concentration began to drop.

To illustrate the characteristics of the ozone exposure regime applied for the 27 acute ozone exposure blocks in this study and to verify that target exposure levels were obtained consistently, mean ozone concentration is plotted against the 30 minute treatment duration (Fig. 1.2).

For both the control and the acute ozone exposure treatments, temperature and relative humidity within the chamber was monitored and recorded every 5 minutes during treatments using a battery operated Extech Model RH520 Humidity –Temperature Chart Recorder (Extech Instruments Corporation) (data not shown). After 30 minutes, the plants were removed from the chamber and placed on the greenhouse bench for seven or eight days to allow symptoms to develop. Plants were watered as needed.

Visible Foliar Injury Evaluation

Seven or eight days after treatment, symptoms on each plant were evaluated and digital images were taken of visible leaf injury if present. The injury evaluation method followed the method used by Davis and Coppolino (1974) with modifications. Each plant was assigned a severity factor from 0 (no symptoms) to 5 (very severe symptoms) based on the overall appearance of the plant (Table 1.2).

The percentage of leaves injured was estimated for each plant and the percentage of plants affected evidenced by visible symptoms was determined. A severity index was calculated for each plant by multiplying (severity factor) x (percentage of leaves injured) x (percentage of plants affected). The mean severity index was used as the criterion for comparing ozone susceptibilities of the different species treated (Davis and Coppolino, 1974).

The experimental design consisted of three single plant replications per block and three blocks per treatment level (n = 9) arranged in a randomized design. Severity index and percentage of leaves injured were each analyzed using oneway analysis of variance (ANOVA) by species. To determine whether a mean can be considered the maximum among the means (highest level of injury), with significant separation from all other means, a means comparison of severity index and of percentage of leaves injured was conducted using Hsu's MCB (Multiple Comparison with the Best). All data were analyzed with JMP 9 (SAS Institute, Inc., Cary, NC).

Results

Ten of the 27 species treated showed foliar injury symptoms when evaluated seven or eight days after treatment. Mean severity factor, mean percentage of leaves injured, percentage of plants affected, and mean severity index are given in Table 1.3 for the ten symptomatic species. Descriptions of the foliar injury symptoms are provided in Table 1.4 and photographically documented (Appendix A). Symptoms observed differed by species and, in some cases, within a single taxon. While symptoms may have been present, results from two of the 27 species (*Asclepias syriaca* and *Monarda bradburiana*) were inconclusive due to the presence of spider mite and mildew foliar damage confounding evaluation of the symptoms. Of the 27 species treated, 15 did not show symptoms.

The percent of plants affected varies from 44% - 100%. *Coreopsis tripteris* and *Penstemon cobaea* had the highest number of plants affected within a species (100%) and *Solidago speciosa* showed the lowest number of plants affected (44%) (Table 1.3). The percentage of leaves injured ranged from 10% - 71%. *Coreopsis tripteris* was highest in percentage of leaves injured (71%) but *Penstemon cobaea* ranked closer to the middle (30%) indicating that species with the greatest number of plants affected did not always show the highest percent leaves injured. *Solidago speciosa* had the lowest percent leaves injured (10%). These wide ranges between species indicate a large amount of variability in ozone sensitivity.

Oneway analysis of variance (ANOVA) for both severity index and for percentage of leaves injured showed significant differences among the means for the species tested ($P < 0.0001$). To determine whether a mean is the maximum with a significant separation from the rest of the means and therefore indicative of significantly higher level of injury over the other means, a means comparison of percentage of leaves injured and of severity index was conducted using Hsu's MCB (Multiple Comparison with the Best).

A means comparison of percentage of leaves injured with the Best using Hsu's MCB, $\alpha = 0.05$, showed two species (*Coreopsis tripteris* and *Coreopsis palmata*) with the mean significantly greater than the minimum mean, and all species except *Coreopsis tripteris* and *Coreopsis palmata* with the mean significantly less than the maximum mean (Table 1.3). This comparison shows that the percentage of leaves injured for *Coreopsis tripteris* and *Coreopsis palmata* is significantly higher than the means for all other species showing injury.

Means comparison of severity index with the Best using Hsu's MCB, $\alpha = 0.05$, showed two species (*Coreopsis tripteris* and *Coreopsis palmata*) with the mean significantly greater than the minimum mean and all species with the exception of *Coreopsis tripteris* with the mean significantly less than the maximum mean (Table 1.3). This comparison indicates that the severity index for *Coreopsis tripteris* is significantly higher than the mean for all other species showing injury. Thus *Coreopsis tripteris* showed the greatest injury levels of all species studied and *Coreopsis palmata* showed the second highest level of injury symptoms.

Discussion

Ten of the 27 species treated in this study showed visible foliar injury symptoms from acute ozone exposures over a 30 minute exposure period and a peak concentration target of 2.0 ppm (Table 1.5). Thus, the exposure regime applied under the experimental conditions of this study represents a useful ozone dose at which slightly more than one third of the species studied developed visible foliar injury symptoms. Susceptible species showed a wide range of symptoms; the most common being upper leaf surface stipple and flecking of various colors. Symptoms of greater severity observed included leaf edge and tip necrosis and relatively small interveinal bifacial necrotic areas with the most severe symptoms being bifacial necrotic coalesced lesions on *Coreopsis tripteris* and *Penstemon cobaea*. Other less frequently observed symptoms included bronzing and chlorosis (Table 1.4). Symptoms observed in this study correspond to foliar ozone injury symptoms described and documented for numerous other plant species and cultivars (Davis et al., 1981; Davis and Coppolino, 1974; Findley et al., 1997a; Kline et al., 2009; Orendovici et al., 2003; Richards et al., 1958).

Percent of plants affected within a susceptible species ranged from 44-100% with only two of the ten susceptible species showing 100% of plants affected (Table 1.3). These results indicate interspecific and intraspecific differences in susceptibility for the species tested. Interspecific and intraspecific differences in response to ozone are well known and have been documented for numerous plant species (Davis and Coppolino, 1974; Davis and Wood, 1972; Findley et al., 1997b; Kline et al., 2009).

Plant species unaffected by the acute O_3 exposure regime applied in this study show tolerance and may represent wise choices for use in areas susceptible to high levels of ozone (Table 1.5). A decision to avoid the ten species that developed foliar injury after treatment

(Table 1.3), particularly species with the highest level of injury (*Coreopsis tripteris* and *C. palmata*), may be appropriate in areas prone to high levels of ozone.

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Table 1.1. Plants evaluated for visible foliar injury symptoms following an acute ozone exposure regime.

Scientific name	Common name	Plant symbol	Link to NRCS PLANTS Database profile
Asclepias syriaca L.	common milkweed	ASSY	http://plants.usda.gov/java/profile?symbol=ASSY
Coreopsis lanceolata L.	lanceleaf tickseed	COLA5	http://plants.usda.gov/java/profile?symbol=COLA5
Coreopsis palmata Nutt.	stiff tickseed	COPA10	http://plants.usda.gov/java/profile?symbol=COPA10
Coreopsis tripteris L.	tall tickseed	COTR4	http://plants.usda.gov/java/profile?symbol=COTR4
Echinacea pallida (Nutt.) Nutt.	pale purple coneflower	ECPA	http://plants.usda.gov/java/profile?symbol=ECPA
Echinacea paradoxa (J.B.S. Norton) Britton	Bush's purple coneflower	ECPA2	http://plants.usda.gov/java/profile?symbol=ECPA2
Echinacea simulata R.L. McGregor	wavyleaf purple coneflower	ECSI	http://plants.usda.gov/java/profile?symbol=ECSI
Liatris aspera Michx.	tall blazing star	LIAS	http://plants.usda.gov/java/profile?symbol=LIAS
Liatris mucronata DC.	cusp blazing star	LIMU	http://plants.usda.gov/java/profile?symbol=LIMU
Liatris pycnostachya Michx.	prairie blazing star	LIPY	http://plants.usda.gov/java/profile?symbol=LIPY
Monarda bradburiana Beck	eastern beebalm	MOBR2	http://plants.usda.gov/java/profile?symbol=MOBR2
Monarda fistulosa L.	wild bergamot	MOFI	http://plants.usda.gov/java/profile?symbol=MOFI
Oligoneuron rigidum (L.) Small var. *rigidum*	stiff goldenrod	OLRIR	http://plants.usda.gov/java/profile?symbol=OLRIR

Penstemon cobaea Nutt.	cobaea beardtongue	PECO4	http://plants.usda.gov/java/profile?symbol=PECO4
Penstemon digitalis Nutt. ex Sims	talus slope penstemon	PEDI	http://plants.usda.gov/java/profile?symbol=PEDI
Penstemon pallidus Small	pale beardtongue	PEPA7	http://plants.usda.gov/java/profile?symbol=PEPA7
Rudbeckia fulgida Aiton var. *speciosa* (Wender.) Perdue	orange coneflower	RUFUS3	http://plants.usda.gov/java/profile?symbol=RUFUS3
Rudbeckia missouriensis Engelm. ex C.L. Boynt. & Beadle	Missouri orange coneflower	RUMI	http://plants.usda.gov/java/profile?symbol=RUMI
Rudbeckia subtomentosa Pursh	sweet coneflower	RUSU	http://plants.usda.gov/java/profile?symbol=RUSU
Silphium integrifolium Michx	wholeleaf rosinweed	SIIN2	http://plants.usda.gov/java/profile?symbol=SIIN2
Solidago rugosa Mill. ssp. *aspera* (Aiton) Cronquist	wrinkleleaf goldenrod	SORUA	http://plants.usda.gov/java/profile?symbol=SORUA
Solidago nemoralis Aiton	gray goldenrod	SONE	http://plants.usda.gov/java/profile?symbol=SONE
Solidago speciosa Nutt.	showy goldenrod	SOSP2	http://plants.usda.gov/java/profile?symbol=SOSP2
Tradescantia ernestiana E.S. Anderson & Woodson	Ernest's spiderwort	TRER4	http://plants.usda.gov/java/profile?symbol=TRER4
Tradescantia ohiensis Raf.	bluejacket	TROH	http://plants.usda.gov/java/profile?symbol=TROH
Tradescantia subaspera Ker	zigzag spiderwort	TRSU2	http://plants.usda.gov/java/profile?symbol=TRSU2

Gawl.

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Table 1.2. Severity factor ratings and descriptions

Rating	Description
0	no visible symptoms
1	symptoms only detected upon close inspection
2	symptoms appear minor
3	symptoms are an obvious concern
4	symptoms are a serious concern
5	symptoms causing severe damage or death

Table 1.3. Mean severity factor (SF), mean percentage of leaves injured (PLI), percentage of plants affected (PPA), and mean severity index (SI) for the ten species with visible foliar injury symptoms, following ozone treatment.

Scientific name	SF	PLI (%)	PPA (%)	SI[z]
Coreopsis tripteris L.	3.8	71 a[yx]	100	29522 a[yx]
Coreopsis palmata Nutt.	1.8	59 a	89	11966 ab
Penstemon cobaea Nutt.	2.1	30 b	100	8300 b
Solidago nemoralis Aiton	1.7	27 b	67	6030 b
Monarda fistulosa L.	1.3	19 b	78	4203 b
Silphium integrifolium Michx	1.3	20 b	56	2837 b
Oligoneuron rigidum (L.) Small var. *rigidum*	1.2	19 b	89	2818 b
Rudbeckia missouriensis Engelm. ex C.L. Boynt. & Beadle	1.1	18 b	89	2710 b
Penstemon pallidus Small	0.9	14b	67	2271b
Solidago speciosa Nutt.	0.6	10b	44	621b

[z] Mean severity index (SI) was calculated by summing severity indexes for nine individual treatment plants and dividing by nine.

[y] Values within columns followed by the letter “a” indicate mean significantly greater than the minimum using Hsu’s MCB ($\alpha = 0.05$).

[x] Values within columns followed by the letter “b” indicate mean significantly less than the maximum based on Hsu’s MCB ($\alpha = 0.05$).

Mean SF standard error = 0.38
Mean PLI standard error = 9.4
Mean SI standard error = 2721

Table 1.4. Visible foliar injury symptoms from acute ozone exposures after a 30 minute exposure period and a peak ozone concentration target of 2.0 ppm

Scientific name	Symptoms
Coreopsis palmata Nutt.	Bronzing, red-purple stippling with larger veins unaffected, leaf edge and tip bifacial necrosis
Coreopsis tripteris L.	Flecking and tan interveinal necrosis, bifacial necrotic coalesced lesions
Monarda fistulosa L.	Upper surface tan and red stipple, chlorosis
Oligoneuron rigidum (L.) Small var. *rigidum*	Light tan to yellow flecking of upper leaf surface
Penstemon cobaea Nutt.	Tan to brown upper surface stipple and flecking, leaf margin necrosis, interveinal bifacial coalesced necrotic lesions
Penstemon pallidus Small	Tan upper surface flecking, leaf margin necrosis, large light brown bifacial necrotic areas
Rudbeckia missouriensis Engelm. ex C.L. Boynt. & Beadle	Yellow to tan flecking, Reddish stipple, bifacial necrosis of leaf tip and margin
Silphium integrifolium Michx	White flecking, red stippling
Solidago nemoralis Aiton	Upper surface bleaching, white to yellow flecking
Solidago speciosa Nutt.	Yellow stipple

Table 1.5. Plants evaluated for visible foliar injury symptoms and symptom development following an acute ozone exposure regime.

Scientific name	Symptoms
Asclepias syriaca L.	I
Coreopsis lanceolata L.	N
Coreopsis palmata Nutt.	Y
Coreopsis tripteris L.	Y
Echinacea pallida (Nutt.) Nutt.	N
Echinacea paradoxa (J.B.S. Norton) Britton	N
Echinacea purpurea (L.) Moench	N
Echinacea simulata R.L. McGregor	N
Liatris aspera Michx.	N
Liatris mucronata DC.	N
Liatris pycnostachya Michx.	N
Monarda bradburiana Beck	I
Monarda fistulosa L.	Y
Oligoneuron rigidum (L.) Small var. *rigidum*	Y
Penstemon cobaea Nutt.	Y
Penstemon digitalis Nutt. ex Sims	N
Penstemon pallidus Small	Y
Rudbeckia fulgida Aiton var. *speciosa* (Wender.) Perdue	N
Rudbeckia missouriensis Engelm. ex C.L. Boynt. & Beadle	Y
Rudbeckia subtomentosa Pursh	N
Silphium integrifolium Michx	Y
Solidago rugosa Mill. ssp. *aspera* (Aiton) Cronquist	N

Solidago nemoralis Aiton	Y
Solidago speciosa Nutt.	Y
Tradescantia ernestiana E.S. Anderson & Woodson	N
Tradescantia ohiensis Raf.	N
Tradescantia subaspera Ker Gawl.	N

Y indicates visible foliar injury symptoms developed after an acute ozone exposure.
N indicates visible foliar injury symptoms did not develop after an acute ozone exposure.
I indicates symptoms were inconclusive and therefore, not analyzed due to confounding factors.

Fig 1.1. Experimental exposure chambers with chamber covers raised by a pulley system and treatment plants in place in the center of individual cells on the chamber floor grid.

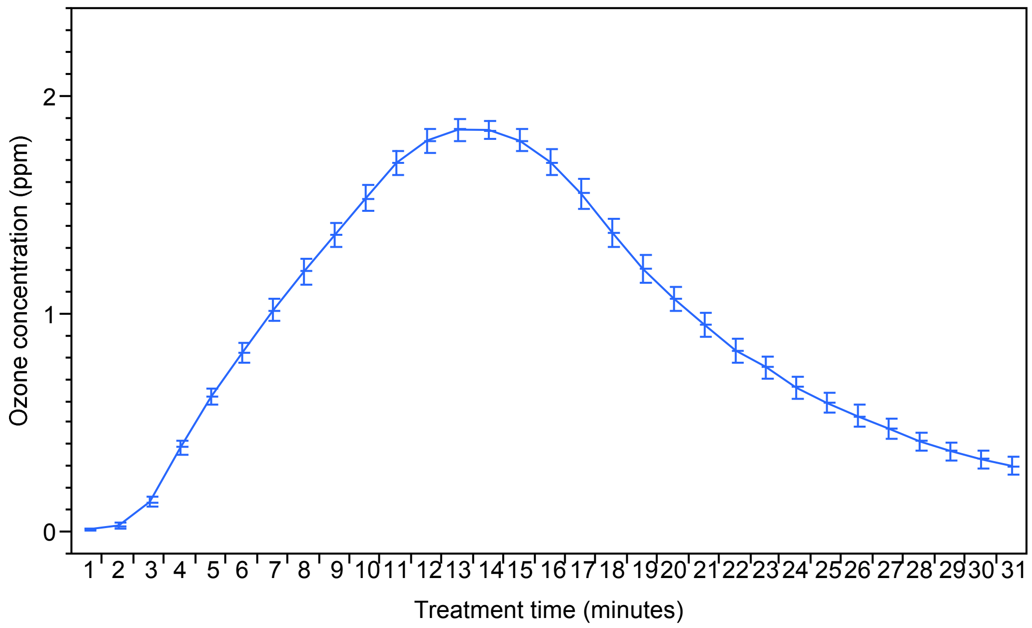
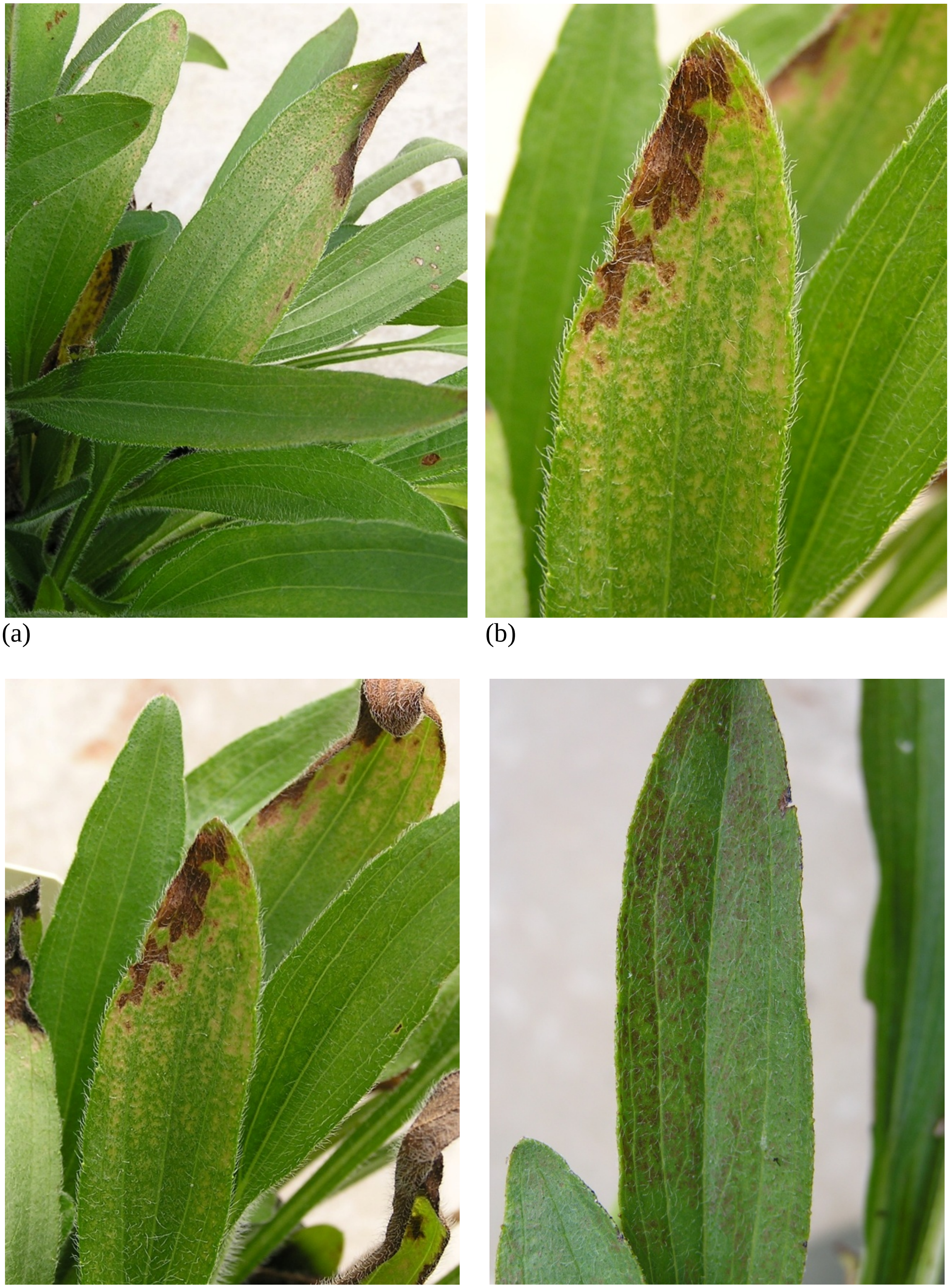


Fig. 1.2. Mean ozone concentration (ppm) for the 27 blocks of the study plotted against the 30 minute treatment duration (minutes). Ozone concentration means are connected by a line and mean standard error bars are displayed.

APPENDIX 1.A

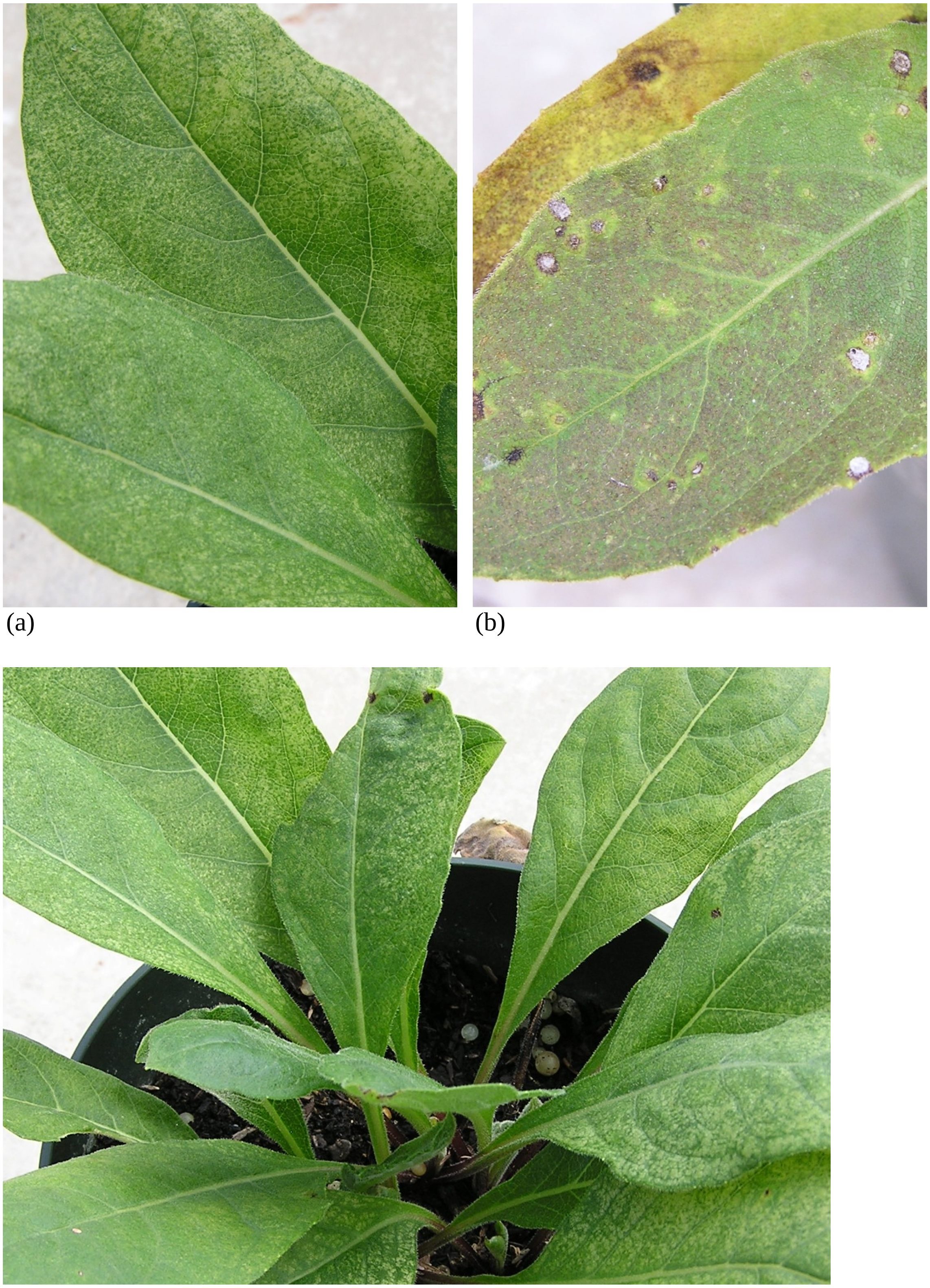
A PHOTOGRAPHIC ATLAS OF VISIBLE FOLIAR INJURY SYMPTOMS FOR TEN PLANT SPECIES DISPLAYING ACUTE OZONE EXPOSURE INJURY



(a) (b)

(c) (d)

Figure 1.A.1. Ozone-induced flecking (a,b,c); reddish stipple (d); and bifacial necrosis (a,b,c) of leaf tip and margin of *Rudbeckia missouriensis* (Missouri orange coneflower).



(a)

(b)

(c)

Figure 1.A.2. Ozone-induced white flecking (a,c) and reddish stipple (b) of *Silphium integrifolium* (wholeleaf rosinweed).

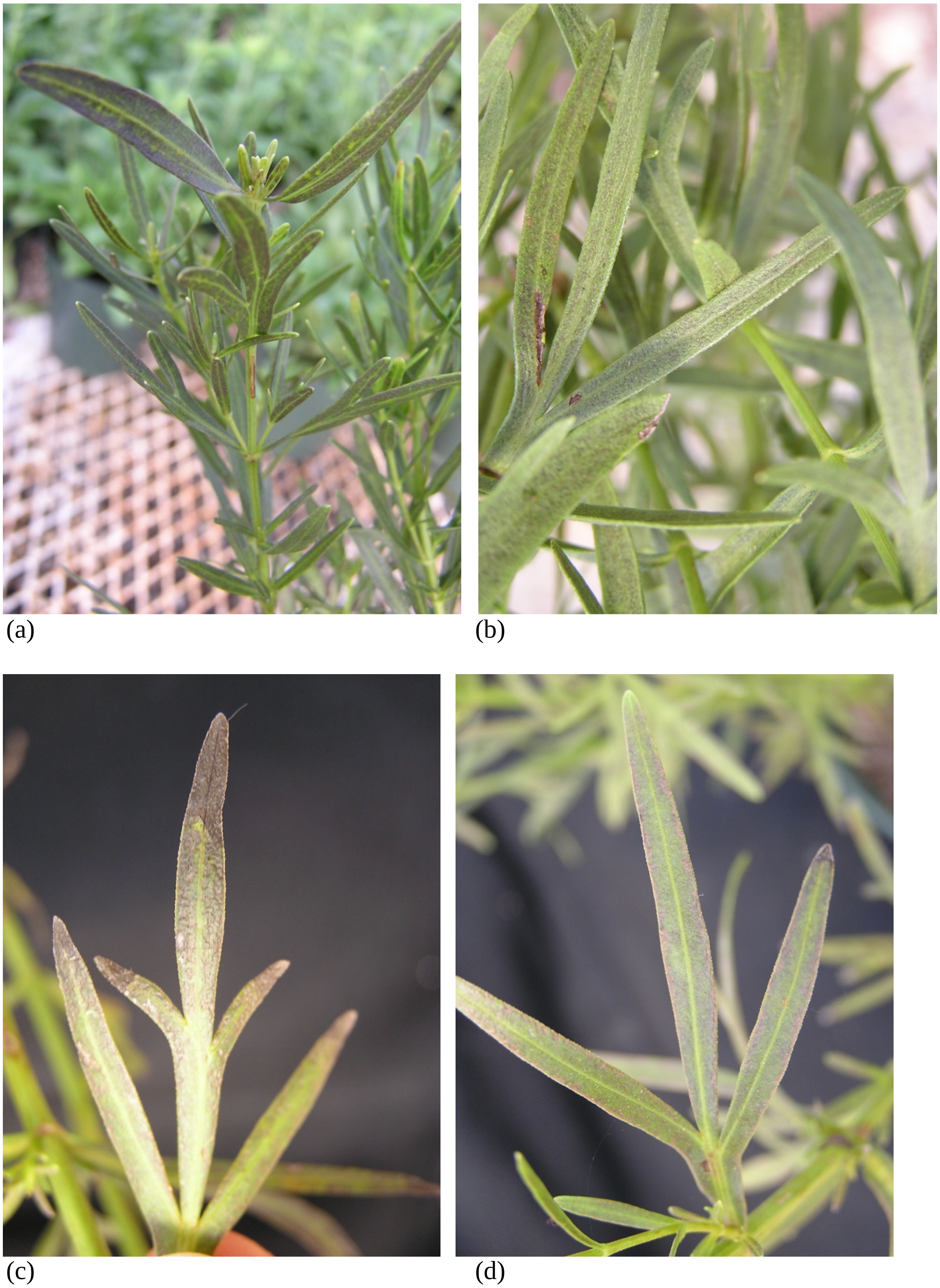

(a) (b) (c) (d)

Figure 1.A.3. Ozone-induced red-purple stippling and bronzing with larger veins unaffected (a,b); and leaf edge and tip bifacial necrosis(c,d) of *Coreopsis palmata* (stiff tickseed).

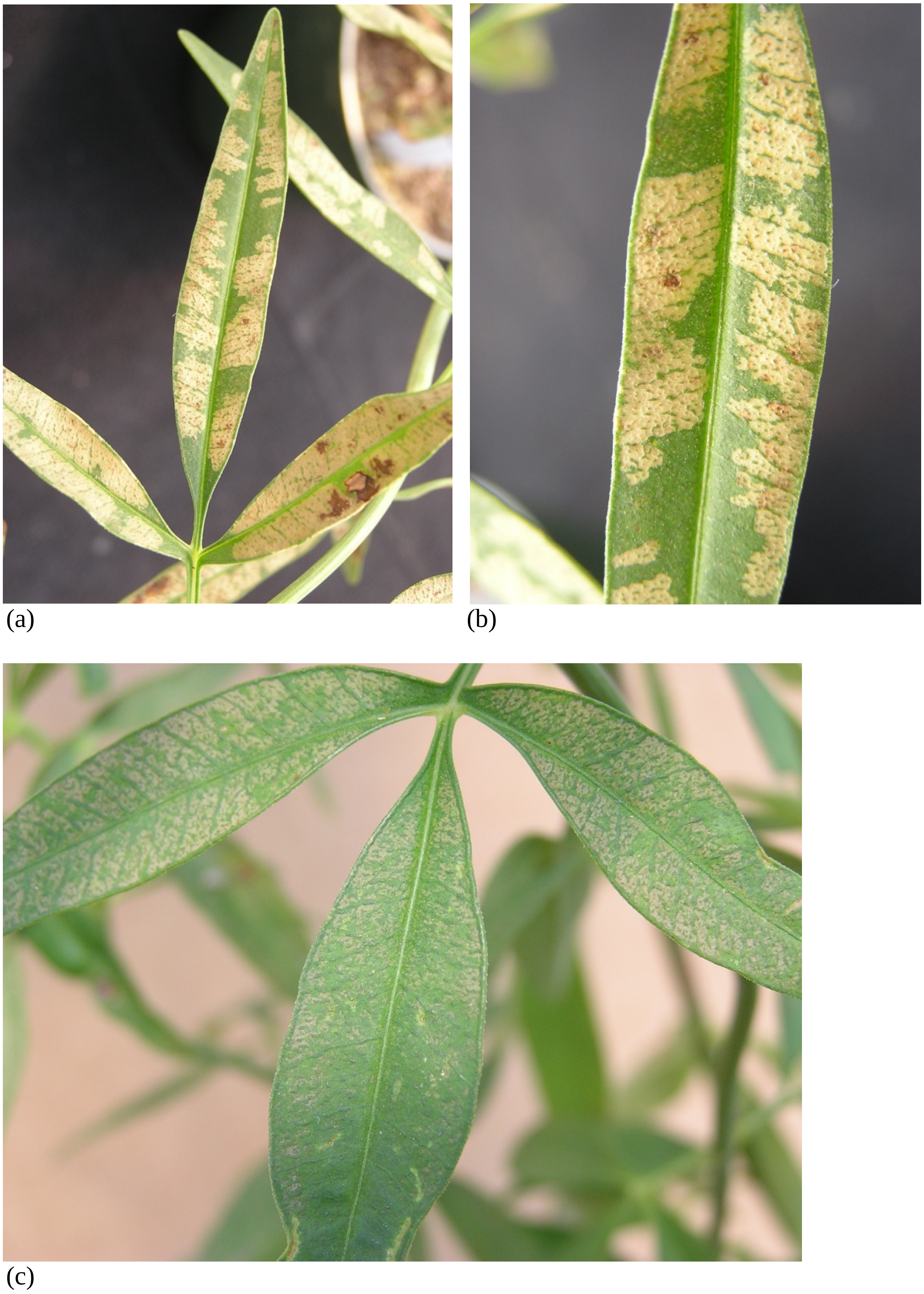


Figure 1.A.4. Ozone-induced flecking (c), tan interveinal necrosis (a,b,c), and bifacial coalesced necrotic lesions (a,b) of *Coreopsis tripteris* (tall tickseed).

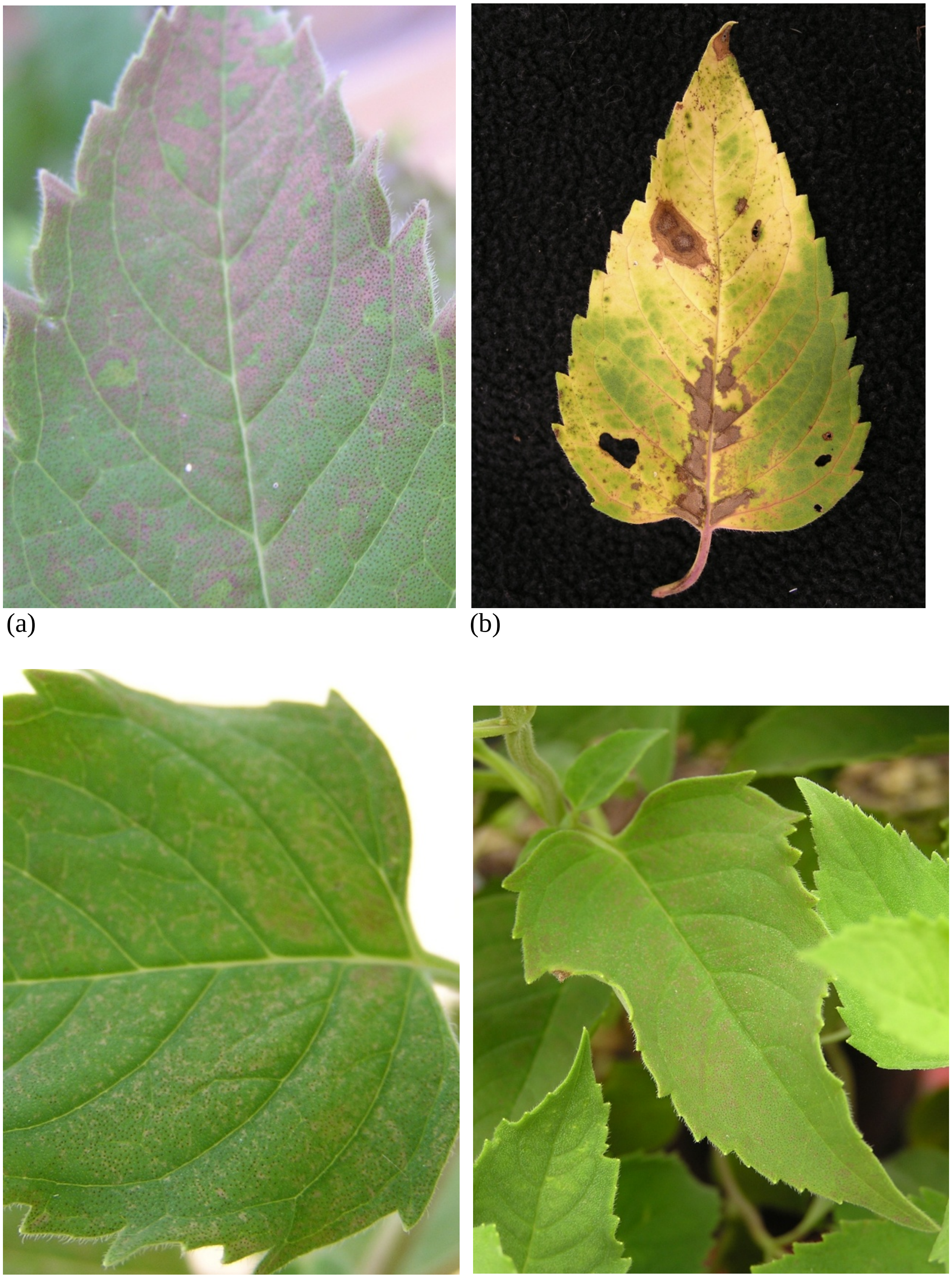
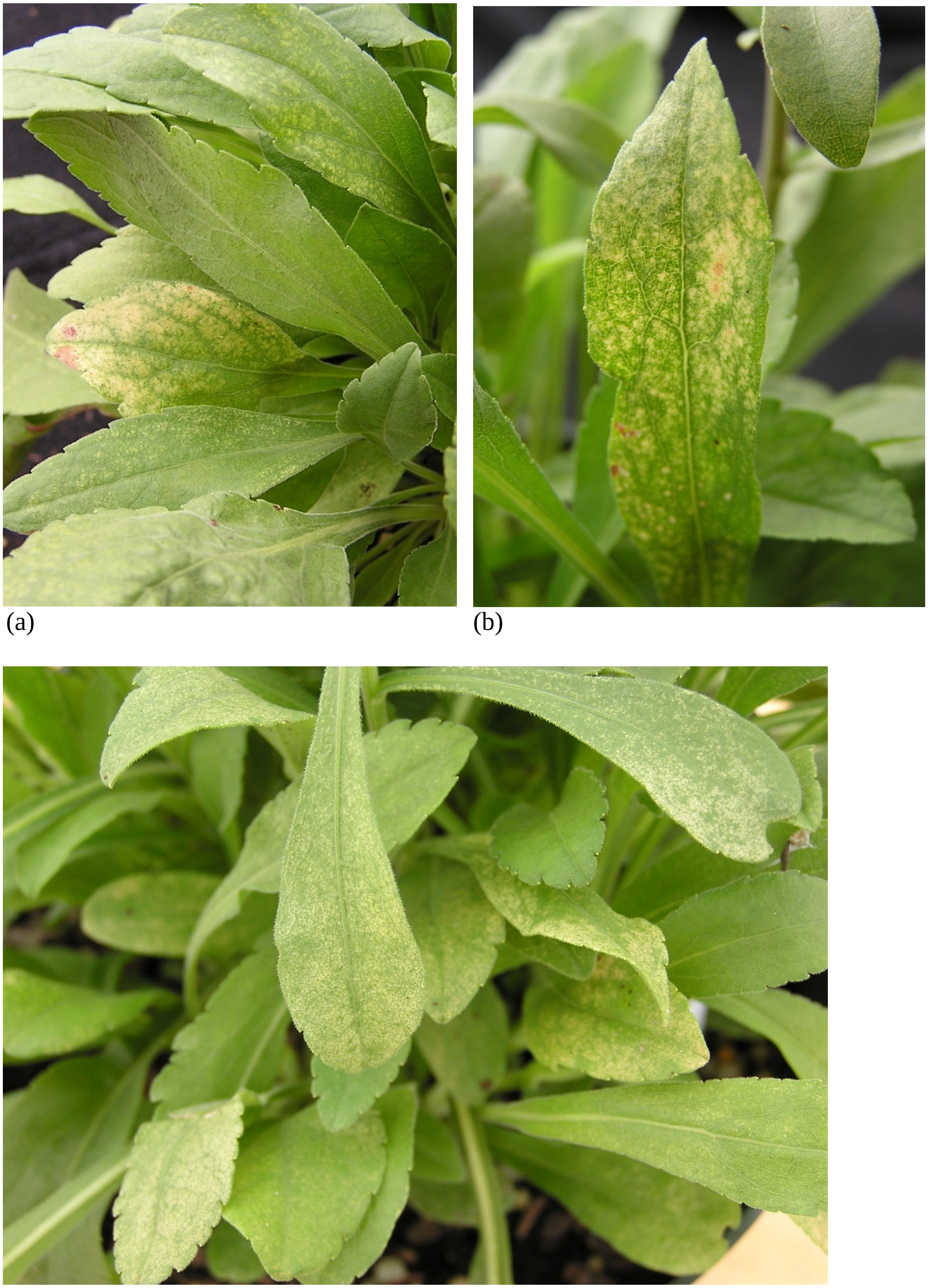


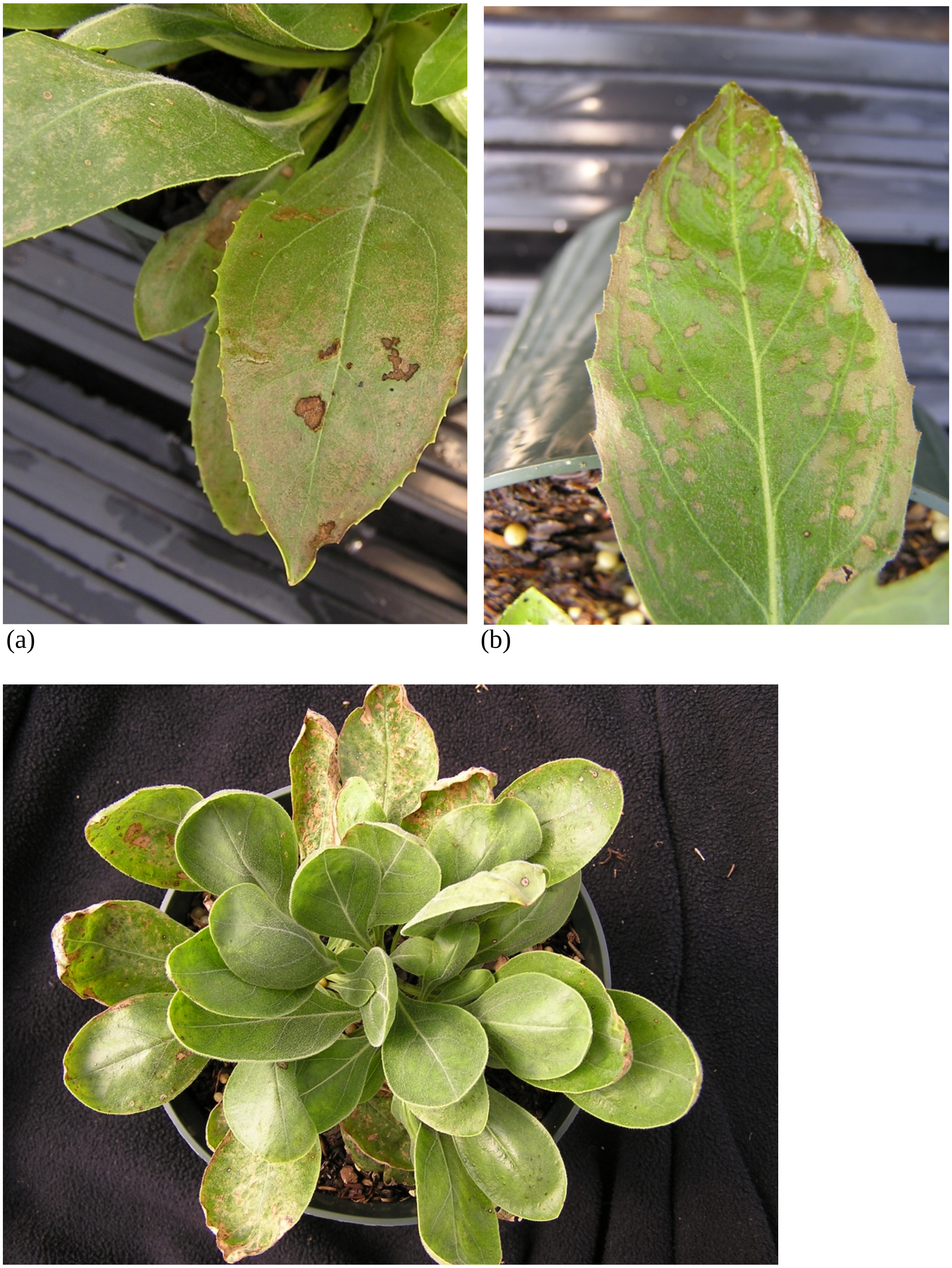
Figure 1.A.5. Ozone-induced upper surface tan and red stipple (a,c,d) and chlorosis (b) of *Monarda fistulosa* (wild bergamot).



(a) (b)

(c)

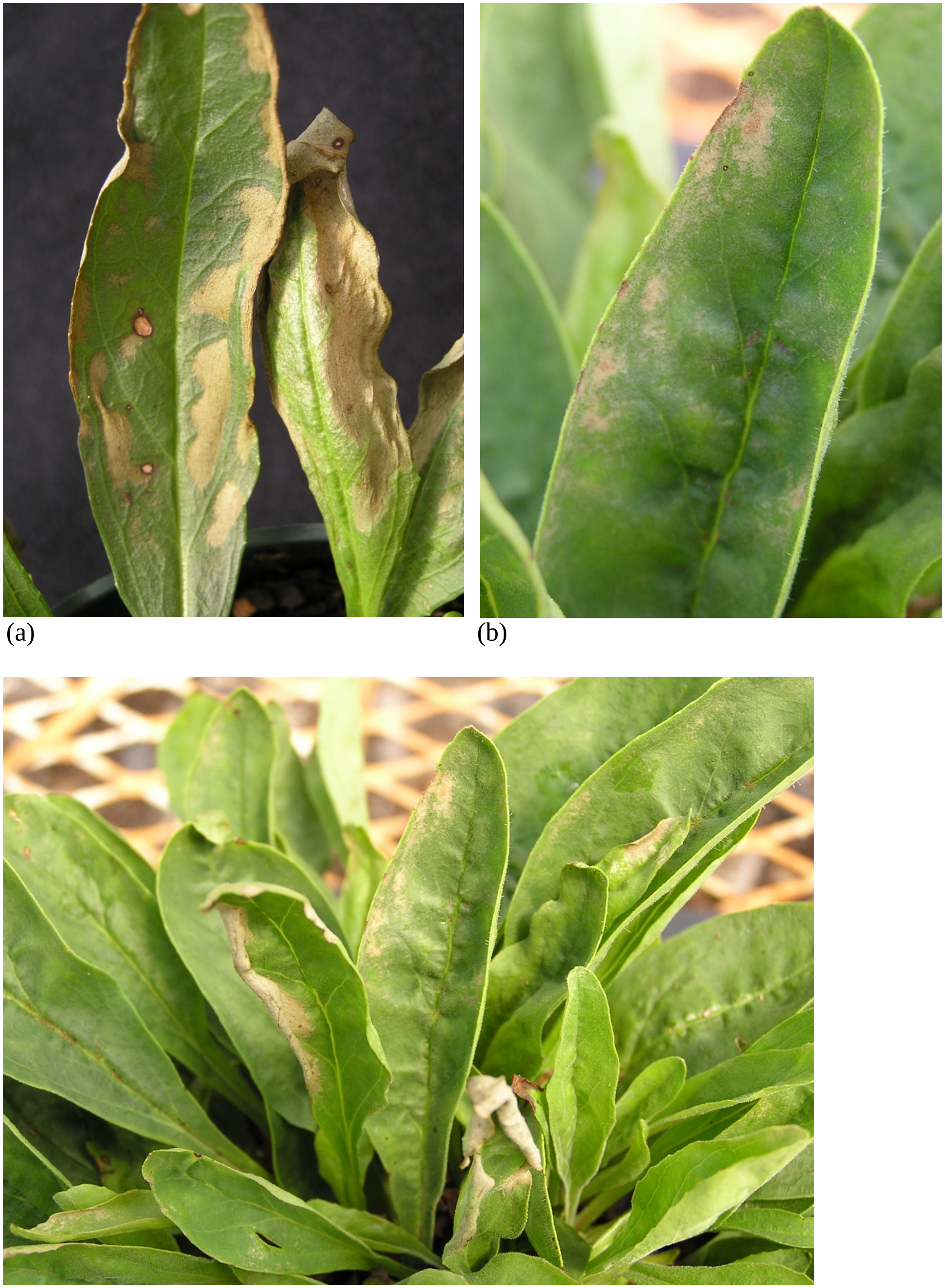
Figure 1.A.6. Ozone-induced upper surface bleaching (a,b) and white to yellow flecking (a,b,c) of *Solidago nemoralis* (gray goldenrod).



(a) (b)

(c)

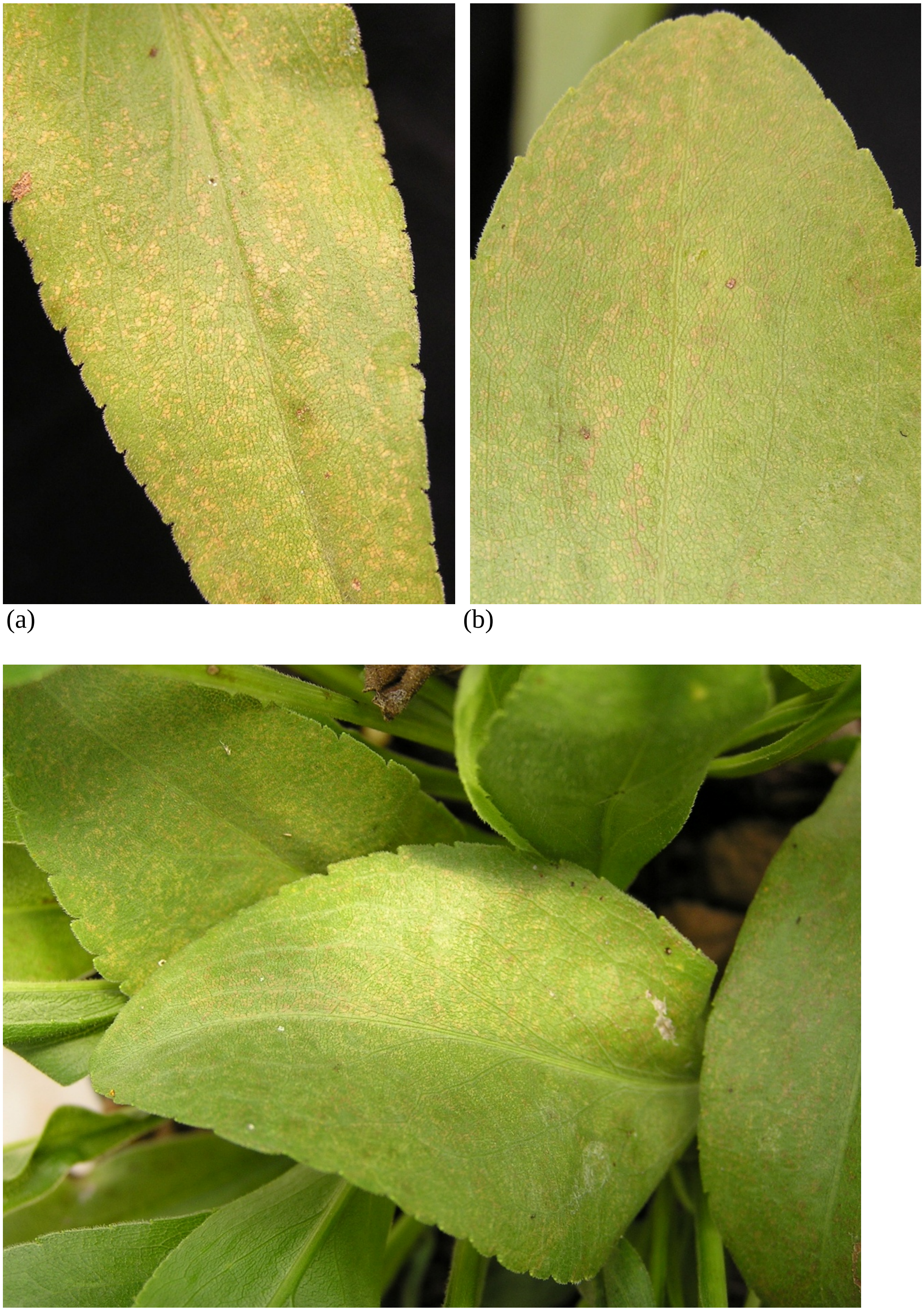
Figure 1.A.7. Ozone-induced upper surface stipple and flecking (a,c), leaf margin necrosis (b,c), interveinal bifacial coalesced necrotic lesions (b,c) of *Penstemon cobaea* (cobaea beardtongue).



(a) (b)

(c)

Figure 1.A.8. Ozone-induced upper surface flecking (b,c), leaf margin necrosis (a,c), large light brown bifacial necrotic areas (a,c) of *Penstemon pallidus* (pale beardtongue).



(a) (b)

(c)

Figure 1.A.9. Ozone-induced upper leaf surface flecking (a,b,c) of *Oligoneuron rigidum* var. *rigidum* (stiff goldenrod).

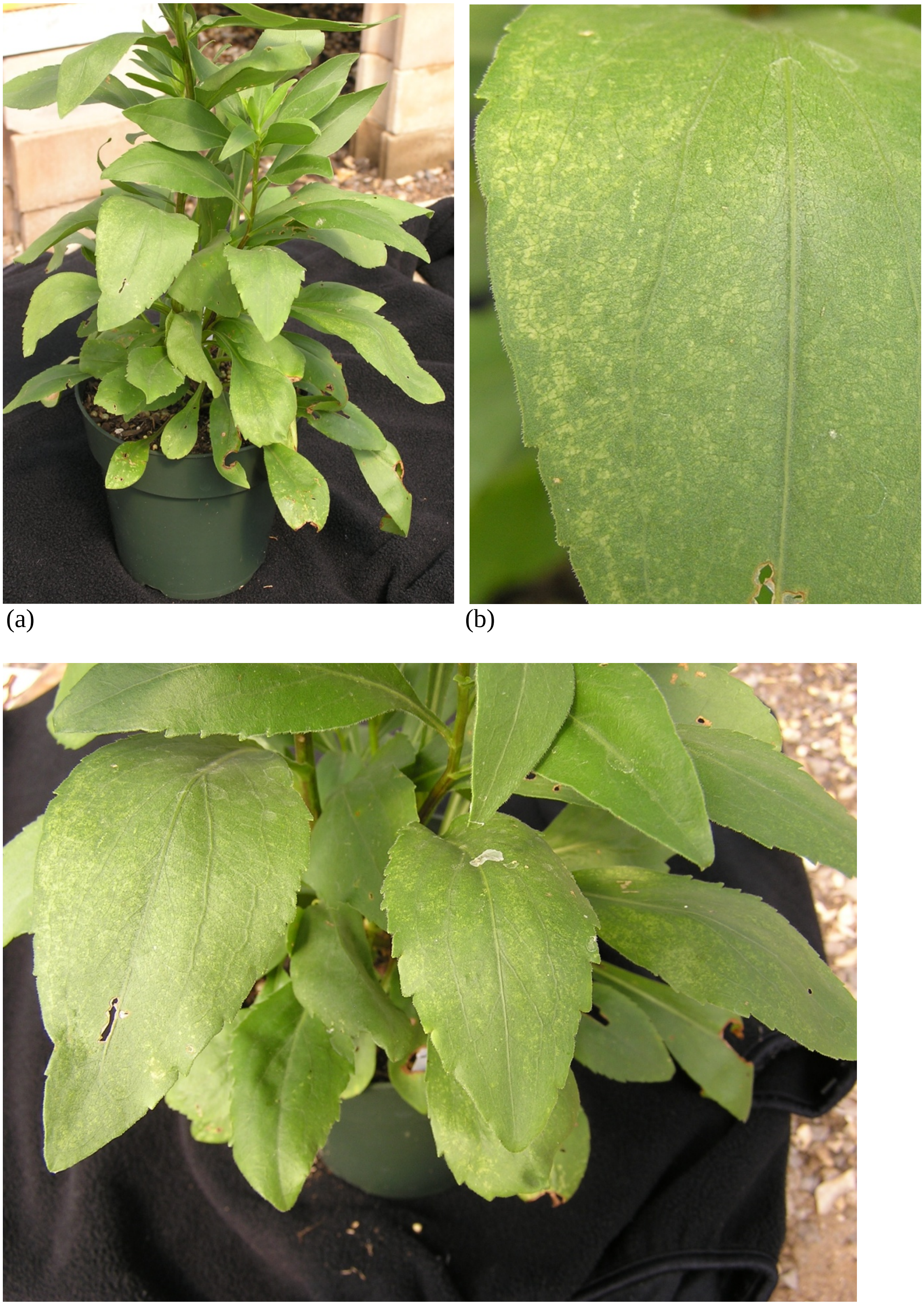

(a) (b) (c)

Figure 1.A.10. Ozone-induced yellow stipple of *Solidago speciosa* (showy goldenrod).

Chapter 2

EVALUATION OF THE RELATIVE SENSITIVITY OF SELECT NATIVE COREOPSIS SPECIES AND THEIR ACUTE OZONE EXPOSURE RESPONSE CHARACTERISTICS

Abstract

Four *Coreopsis* species (*C. lanceolata*, *C. palmata*, *C. tinctoria*, and *C. tripteris*), all native to the Eastern Temperate Forests Level I Ecoregion, were exposed to four acute ozone (O_3) treatment levels with target peak O_3 concentrations of 1.2, 1.7, 2.2, and 2.7 ppm for 30 minutes in a closed chamber environment, during June of 2011. Plants were evaluated for visible foliar injury symptoms and symptoms were described and photographically documented. Severity index was calculated for each species and used as the criterion for comparing ozone susceptibilities among species. All four species in this study showed foliar injury symptoms following the highest target peak exposure level (2.7 ppm). Three of the species tested (*Coreopsis palmata*, *C. tinctoria*, and *C. tripteris*) exhibited symptoms following the target peak 2.2 and 1.7 ppm exposure levels. Two of the four species (*Coreopsis palmata* and *C. tripteris*) exhibited foliar injury symptoms at the lowest target peak exposure level (1.2 ppm). Visible foliar injury symptoms varied among species in the study but were generally uniform within each taxon. *Coreopsis lanceolata* showed the highest degree of ozone tolerance relative to the other three species in the study. Both *Coreopsis palmata* and *C. tripteris* developed visible foliar symptoms at the lowest treatment level (1.2 ppm) indicating ozone susceptibility relative to the other species.

Introduction

As an air pollutant, ozone (O_3) is one of the most abundant greenhouse gases in the earth's atmosphere largely resulting when vehicle exhaust gases and industrial emissions such as nitrogen oxides, carbon monoxide, and volatile organic compounds react in the presence of sunlight. Production of the gas occurs primarily through chemical reactions in the atmosphere, driven by sunlight or radiation. Because of seasonal changes in the solar radiational flux, high concentrations of O_3 occur during the plant-growing season (Krupa et al., 2001).

Plants are subjected to acute and chronic exposures of ground-level O_3. An acute exposure consists of high O_3 concentrations (e.g., >80 ppb) from a few consecutive hours to days. In comparison, a chronic exposure consists of relatively low O_3 concentration (e.g., <40 ppb) for the entire life of a plant, with periodic intermittent or random episodes of high concentrations. Plant response to O_3 can vary with the genus, species, cultivar or variety, and genotype (Krupa et al., 2001).

Ozone injures plants mainly following uptake through the stomata in the leaf surface, and some of the changes in plant metabolism caused by O_3 become manifest in a variety of visible foliar injury symptoms (Booker et al., 2009). The response of a plant to O_3 depends on the exposure characteristics, plant properties, and external growth conditions. Short term exposure to high concentrations (e.g., >150 ppb) generally result in acute visible foliar injury (Krupa et al., 1998). Ozone negatively impacts a number of plant processes, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield. Mechanisms of action are not completely known (Krupa, 1997; Krupa et al., 2001). Investigators have shown that chronic, whole growth season or whole life cycle exposures to O_3 can result in losses of marketable yield in agronomic crops and reductions in growth and productivity of forest tree species (Krupa et al., 2001). Fewer studies have been conducted on horticultural plants or native herbaceous plants. Current consensus within the scientific community is that current and anticipated concentrations of ground level O_3 can negatively impact the productivity, quality, and competitive ability of important agricultural and horticultural plants (Booker et al., 2009).

At ground-level, both natural and artificial sources of ozone exist. Natural ozone consists of lightning generated sources during thunderstorms and downward transport of O_3 via tropopause folding from the upper atmosphere (Lefohn, 1992 and Krupa et al., 2001). Because of natural sources of O_3 at ground-level, there is a worldwide ambient O_3 concentration of 20 to 30 $nl \bullet l^{-1}$ (ppb). Background values will vary with geophysical conditions and levels of industrialization (Krupa et al., 2001). As urbanization and industrialization continue; increasing numbers of reports have appeared regarding O_3-induced foliar injury on sensitive plants (Krupa et al., 1998).

Typical summertime daily maximum surface level O_3 concentrations in urban-suburban areas are 100-400 ppb while those in rural areas range from 50-120. Remote tropical forest and remote marine areas range from about 20-40 ppb (Krupa et al., 2001). Thus, ozone is a major air pollutant in the United States causing foliar injury to many agronomic and horticultural crops, deciduous trees, and conifers (Krupa et al., 1998).

Ozone induced foliar damage was first documented in 1958 on grape (*Vitis vinifera* L.) (Richards, 1958). Since then, studies have documented several general symptoms on broadleaf species in the field. Among these general symptoms, leaf surface stipple has been described as the classic symptom of ozone injury on broadleaf species (Skelly, 2000; Krupa et al., 1998).

Stipple usually does not affect the veins and veinlets. Veinlets often border injury causing an angular appearance of the affected tissue (Skelly, 2000).

Ozone induces symptoms other than stipple including foliar reddening, chlorosis, premature defoliation, bronzing, flecking, bleaching, leaf-tip burn, and necrosis (Kline et al. 2008). Such symptoms are not reliable tools however when assessing ozone injury in the field, since they could be caused by factors other than ozone (Kline et al. 2008, 2009; Orendovici et al. 2003). Both acute and chronic injury may be confused with symptoms of other conditions, such as nutritional disorders, other abiotic stressors, biotic pathogens, or insect infestations; thus sensitive plant species displaying a set of general diagnostic features are particularly useful as bioindicators (Skelly, 2000; Kline et al. 2008).

An example of a bioindicator is the tobacco (Nicotiana tabacum L.) cultivar Bel-W3 (Krupa et al. 1998). Since 1962 cv. Bel-W3 has been used in many countries as an indicator of the presence of phytotoxic concentrations of O_3. It has a high sensitivity to O_3 and may produce easily recognizable symptoms for several weeks on the new, fully expanded leaves (Heggestad, 1991). Among all indicator plants, tobacco cv. Bel-W3 and cv. Bel-B are the best described and the most commonly used worldwide (Krupa et al., 2001). The cultivars were the product of research initiated in 1957 to determine the cause of and reduce losses from tobacco weather fleck. Bel-W3 is so sensitive to O_3 that it probably cannot be grown from the seedling to flowering stage out-of-doors anywhere in the 48 contiguous USA states without some injury. That is, Bel-W3 may be injured when concentrations are only slightly above normal background concentrations. Use of Bel-W3 world-wide as an indicator of elevated O_3 concentrations has been a significant factor in increasing the awareness of O_3 as a pollutant (Heggestad, 1991).

There is limited information available on ozone injury symptoms and exposure response characteristics of perennial native plant species. Valuable information for the land manager, revegetation specialist and landscape professional can be gathered from this study to facilitate appropriate plant selection and accurate diagnosis of ozone injury in the field. Information relative to ozone response of native perennials has the potential to improve the success of native plantings based on the susceptibility/tolerance of plant species in both urban and rural areas. There is also a need to facilitate accurate diagnosis of ozone injury in the field and to identify species appropriate for use as bioindicators.

The objective of this study was to evaluate the relative sensitivity of four *Coreopsis* species native to the Eastern Temperate Forests Level I Ecoregion (U.S. Environmental Protection Agency, 2011) to acute ozone exposures and to describe their acute exposure response characteristics in a closed chamber environment.

Materials and Methods

Plant Culture

This study was conducted at the University of Arkansas, Division of Agriculture Research Farm in Fayetteville, AR. Species selected for this study were determined from the results of Study 1 (see Chapter 1). Selection was based on the development of interesting or unique foliar symptoms and differences in ozone response between species, particularly those species displaying differences in ozone response within a single taxon.

Three perennial *Coreopsis* species from Study 1 and one annual *Coreopsis* species, all native to the Eastern Temperate Forests Level I Ecoregion were investigated in this study (Table 2.1). Naming conventions in The PLANTS Database (USDA, NRCS, 2011) are followed throughout this document. The four species selected have multiple attributes appropriate for revegetation of disturbed sites such as the ability to tolerate drought, heat, humidity, and poor soil conditions; the ability to self-seed and reproduce true-to-form; the ability to provide habitat for insects, birds and other wildlife; and the ability to provide aesthetic qualities in the urban landscape including flowering, form, structure, and color. Plants of the three perennial species (*Coreopsis lanceolata*, *C. palmata*, and *C. tripteris*) were purchased in 10 cm (900 cm^{3}) pots and the annual *Coreopsis tinctoria* was purchased as seed, all from Missouri Wildflowers Nursery, LLC, (Jefferson City, MO). Plants and seed were received by March 9th, 2011. *Coreopsis tinctoria* seeds were germinated in a 128 cell plug tray in Fafard germinating mix (Hummert International, St. Louis, MO) on March 10th, 2011. The plugs were transplanted at the 2-4 leaf stage to round standard (315 cm^{3}) pots using Sta-Green Nursery Blend with 0.09N-0.06P-0.05K previously incorporated (Spectrum Group, Division of United Industries Corporation, St. Louis, MO). Potted plants were maintained on an outdoor gravel nursery pad before exposure treatment and in a greenhouse during and after treatment. Plants were watered as needed in both locations from the municipal water supply.

Treatments and post-treatment evaluations were done in a greenhouse under 48% shadecloth and ambient air temperature ranging from 24 °C to 35 °C. Plants were watered as needed. Light meter readings were obtained the morning of 8 July 2010; a cloudy day, using a LI-COR line quantum sensor, 1 m in length (LI-COR Biosciences, Lincoln, NE). Photosynthetically active radiation (*PAR*) levels in the greenhouse averaged 74.5 $\mu\text{mol}^{\cdot}\text{meter}^{2\cdot}\text{sec}^{-1}$, and 69.4 $\mu\text{mol}^{\cdot}\text{meter}^{-2\cdot}\text{sec}^{-1}$ inside an exposure chamber in the greenhouse; readings obtained outside the greenhouse on the same day were 186.0 $\mu\text{mol}^{\cdot}\text{meter}^{-2\cdot}\text{sec}^{-1}$.

Ozone Exposure Chambers

Three air-tight 1.83 x .91 x .91 m experimental exposure chambers were constructed from 3 mm thick OPTIX® acrylic sheet (Plaskolite Inc. Columbus, OH) over a pine wood frame using 100% silicon sealant (General Electric Company, Fairfield, CT) around all seams and hardware attachments. Access ports were built into the chambers using copper compression fittings to allow for injection of ozone and other probes for monitoring chamber conditions. The junction of the top portion of the chamber (chamber cover) and the chamber floor was made air-tight using foam weather stripping. To facilitate handling, the chamber cover can be lifted via a pulley system off of the acrylic chamber floor to move plants in and out of the chamber. Once plants are in place, the cover can then be set onto the acrylic sheet floor creating an air-tight chamber to prevent O_3 loss during treatment (Fig. 2.1). The chamber floor was sectioned into a 2 x 5 grid with all grid sections of equal area, about 1672 cm^2. No more than nine plants were treated at one time in a single chamber. Each treatment plant was placed in the center of a randomly selected grid cell with one grid cell reserved for a small battery operated, portable fan (O2 COOL® Chicago, IL) for air circulation during exposure treatments.

Ozone Generator and Ozone Analyzer

Ozone was generated using a corona-discharge G-6 – V, Variable Ozone Generation System rated at 250 $mg \cdot hour^{-1}$ with adjustable output flow rate from 0% to 100% of the rated capacity, costom designed and built for this research (LYNNTECH, Inc., College Station, TX). Ozone gas was transferred from the generator to the chamber using 6.35 mm outside diameter Teflon® coated tubing which does not react with ozone. The gas was allowed to enter via three ports at the top front, middle and rear of the chamber. During treatments, the ozone concentration in the chamber was monitored every 10 seconds using a UV absorption analyzer designed specifically for ozone, Ozone Analyzer Model UV-100 (ECO SENSORS, Inc., Santa

Fe, NM). Calibration of the Ozone Analyzer Model UV-100 was performed by Eco Sensors, Inc. and is traceable to the U.S. National Institute of Standards and Technology (NIST).

Ozone Treatments

Five treatment levels were administered; the control treatment in which plants were placed in the chambers under ambient O_3 levels for the 30 minute treatment duration (no O_3 injected into the chambers), and four acute ozone exposure treatments with peak ozone concentration targets of 1.2, 1.7, 2.2, and 2.7 ppm . Ozone concentration within the chamber was recorded every minute over the 30 minute treatment duration.

Target peak ozone concentrations for the four acute ozone exposure regimes for this study (1.2, 1.7, 2.2, and 2.7 ppm) were determined as follows. A target peak ozone concentration of 2.2 ppm was used for the initial treatment for all plant species. This level is slightly higher than the target peak ozone concentration in Study 1 and was selected based on the response of *Coreopsis palmata*, *C. tripteris*, and *C. lanceolata* in which both *Coreopsis palmata* and *C. tripteris* developed a high degree of foliar injury symptoms and *C. lanceolata* did not develop visible foliar injury symptoms. Subsequent target peak ozone concentrations for Study 2 were based on the response of the treatment species.

Plants were moved from the outdoor gravel nursery pad to the greenhouse prior to treatment. All treatments were conducted during the morning hours, beginning after sunrise to avoid excessive heat build-up in the greenhouse and exposure chambers. For the three perennial species in this study (*Coreopsis lanceolata*, *C. Palmata*, and *C. tripteris*), each treatment block consisted of three single plant replicates of each of the three species (9 plants in the chamber) randomly assigned a cell on the chamber floor grid and placed in the center of the cell. For the single annual species (*Coreopsis tinctoria*) each treatment block consisted of three single plant replicates of *Coreopsis tinctoria* only (3 plants in the chamber) randomly assigned a cell on the chamber floor grid and placed in the center of the cell. Each block was replicated three times (total 9 plants per species). A small battery operated fan provided air circulation to mix O_3 throughout the chamber. Plants were removed from the chamber immediately after treatment. Treatments were conducted during the period from 6 June to 27 June 2011.

Control Treatment: The control treatment was conducted in three chambers simultaneously (three blocks simultaneously), with no ozone injected into the chambers. Background ambient O_3 levels were monitored throughout the 30 minute treatment duration and recorded every minute (data not shown). During control treatments O_3 levels were typically below 40 ppb and never exceeded 70 ppb.

Acute Ozone Exposure Treatments: Due to the availability of only one ozone generator and the need to monitor ozone concentration in the chamber continuously throughout the treatment duration, the acute exposure treatments were replicated consecutively in one chamber, rather than simultaneously in three chambers as with the ambient ozone control treatment. Once closed, ozone was injected into the chamber until the target peak concentration was reached at which time the ozone generator was turned off and the ozone concentration began to drop.

For both the control and the acute ozone exposure treatments, temperature and relative humidity within the chamber were monitored and recorded every 5 minutes during treatments using a battery operated Extech Model RH520 Humidity –Temperature Chart Recorder (Extech Instruments Corporation). After 30 minutes, the plants were removed from the chamber and placed on the greenhouse bench for six days to allow symptoms to develop. Plants were watered as needed.

To illustrate the characteristics of the ozone exposure regimes applied for each acute ozone exposure level (6 blocks per level) and to verify that target exposure levels were obtained consistently; ozone concentration for each block within a treatment level was plotted against the 30 minute treatment duration for each treatment level (Fig. 2.2).

Visible Foliar Injury Evaluation

Six days after treatment, symptoms on each plant were evaluated and digital images were taken of visible leaf injury if present. The injury evaluation method followed the method used by Davis and Coppolino (1974) with modifications. Each plant was assigned a severity factor from 0 (no symptoms) to 5 (very severe symptoms) based on the overall appearance of the plant (Table 2.2).

Percentage of leaves injured was estimated for each plant and percentage of plants affected was determined. Severity index was calculated for each plant by multiplying (severity factor) x (percentage of leaves injured) x (percentage of plants affected). Mean severity index was used as the criterion for comparing ozone susceptibilities among species and treatment levels (Table 2.3) (Davis and Coppolino, 1974).

The experimental design consisted of three blocks per ozone treatment level replicated over time with three single plant replicates per block (n = 9). To test if there is a difference in the mean response for the four *Coreopsis* species and four acute ozone exposure regimes in this study, analysis of variance (ANOVA) was conducted. Severity index data were log 10 (severity index + 1) transformed prior to conducting ANOVA. A bivariate fit of severity index by treatment was performed. In addition a contrast test was conducted to determine significant differences in the rates of response for each species (slope of the line) to O_3. Data were analyzed with JMP 9 (SAS Institute, Inc., Cary, NC).

Results

Typical symptoms of ozone injury in broad-leaved plants include pigmentation or bronzing in which leaves turn red-brown to brown as phenolic pigments accumulate; chlorosis; flecking or small necrotic areas due to death of palisade cells; stippling or tiny white, black, red, or red-purple spots; bifacial necrosis ranging in color from white to dark orange-red; and premature senescence of leaves, flowers, or fruit (Krupa et al., 1998).

All four species in this study showed foliar injury symptoms following the highest target peak exposure level (2.7 ppm). Three of the species tested (*Coreopsis palmata*, *C. tinctoria*, and *C. tripteris*) exhibited symptoms following the target peak 2.2 ppm and the target peak 1.7 ppm exposure levels. Two of the four species (*Coreopsis palmata* and *C. tripteris*) exhibited foliar injury symptoms at the lowest target peak exposure level (1.2 ppm) (Table 2.4). Visible foliar injury symptoms varied among species in the study but were generally uniform within each taxon (Table 2.4).

The two species with cauline growth habit (*Coreopsis palmata* and *C. tripteris*) exhibited more injury symptoms on fully expanded leaves near the center of the stem while the newest leaves near the apical meristem appeared more tolerant to ozone exposure. This pattern of injury corresponds to earlier studies confirming the high sensitivity of middle-aged leaves over leaves that have not reached the maximum enlargement rate. The period of maximum sensitivity correlates with the stomata becoming fully functional and the formation of intercellular spaces so that O_3 can enter the leaves and reach target sites. Leaves of dichotomous plants are most sensitive between 65% and 95% of their final size (Krupa et al., 1998). A similar pattern of symptoms was not observed on the basal rosette growth of *C. lanceolata*. The faster growing annual, *C. tinctoria* showed tolerance to O_3 exposure in the center of the developing rosette where new foliar growth originated.

Severity index data were log 10 (severity index + 1) transformed prior to performing analysis of variance (ANOVA). Least squares means are presented (Table 2.5). A significant ($P < 0.0001$) treatment x species interaction was observed.

Bivariate fit of severity index by treatment was performed. Significant positive linear relationships in response to increasing O_3 concentration were exhibited for *Coreopsis palmata*, *C. tinctoria*, and *C. tripteris* (Table 2.5). In addition, differences in the rate of response to increasing O_3 concentration (slope) were analyzed using a contrast test (Table 2.5).

Discussion

The main objectives of this study were to evaluate the relative susceptibility of four *Coreopsis* species and to describe their acute ozone exposure response characteristics in a controlled chamber experiment. This study provides needed information on sensitivity and symptoms of O_3 injury for four *Coreopsis* species native to the Eastern Temperate Forests Level I Ecoregion.

The mean percent of plants affected within a species at the lowest treatment dosage (1.2 ppm) ranged from 0-56% with two of the four species resulting in 0% plants affected. The mean percent of plants affected within a species at the highest treatment dosage (2.7 ppm) ranged from 11-100% with three of the four species exhibiting 100% plants affected (Table 2.3). These results indicate interspecific and intraspecific differences in susceptibility for the species tested. Interspecific and intraspecific differences in response to ozone are well known and have been documented for numerous plant species (Davis et al., 1974; Davis et al., 1972; Findley et al., 1997b; Kline et al., 2009).

All four Coreopsis species exhibited symptoms in at least one treatment level. The highest degree of tolerance to the acute ozone dosage in this study was exhibited by *C. lanceolata* in which only one of the plants tested developed visible foliar injury symptoms at the highest ozone dosage (2.7 ppm); no symptoms were exhibited by *C. lanceolata* at the three lower ozone dosages (1.2, 1.7, 2.2 ppm). Linear response to O_3 treatment levels was non-significant for *C. lanceolata*.

Both *Coreopsis palmata* and *C. tripteris* exhibited symptoms at the lowest acute ozone dosage (1.2 ppm) indicating the highest degree of susceptibility of the four species studied. Symptoms exhibited by *C. tripteris* include purplish stipple on the upper leaf surface which in addition to being considered one of the most common general symptoms on broadleaf species in the field, is described as the classic symptom of ozone injury on broadleaf species (Skelly, 2000; Krupa et al. 1998). Since sensitive broadleaf species that produce classic stipple may serve as useful bioindicators when conducting field surveys to evaluate ozone injury (Kline et al. 2009), *C. tripteris* represents the greatest potential for use as a bioindicator of the four species in this study. Its use as a bioindicator could eliminate confusion with symptoms of other conditions, such as nutritional disorders, other abiotic stressors, biotic pathogens, or insect infestations. Further research to study the response of *C. tripteris* to ozone at lower dosages; those closer to field conditions, would be needed to determine if classic stipple symptoms would develop under realistic field conditions of lower ozone concentrations and longer exposure duration.

Increased use of natural habitats and native plant species along roads and highway corridors, around building sites, and within urbanized zones; and increased efforts to restore altered prairie and wetland habitats, creates a need for recommendations of appropriate plant materials for these efforts. The high degree of tolerance exhibited by *C. lanceolata* makes it a good option for this type of planting in sites prone to high ozone levels. Use of native plants with a high degree of ozone tolerance has the potential to increase revegetation success.

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Table 2.1. Plants evaluated for relative ozone sensitivity and exposure response characteristics following an acute ozone exposure.

Scientific name	Common name	Plant symbol	Link to NRCS PLANTS Database profile
Coreopsis lanceolata L.	lanceleaf tickseed	COLA5	http://plants.usda.gov/java/profile?symbol=COLA5
Coreopsis palmata Nutt.	stiff tickseed	COPA10	http://plants.usda.gov/java/profile?symbol=COPA10
Coreopsis tinctoria Nutt.	golden tickseed	COTI3	http://plants.usda.gov/java/profile?symbol=COTI3
Coreopsis tripteris L.	tall tickseed	COTR4	http://plants.usda.gov/java/profile?symbol=COTR4

USDA, NRCS. 2011. The PLANTS Database National Plant Data Team, Greensboro, NC. 3 August 2011. <http://plants.usda.gov>.

Table 2.2. Severity factor ratings and descriptions

Rating	Description
0	no visible symptoms
1	symptoms only detected upon close inspection
2	symptoms appear minor
3	symptoms are an obvious concern
4	symptoms are a serious concern
5	symptoms causing severe damage or death

Table 2.3. Mean severity factor (SF), mean percentage of leaves injured (PLI), percentage of plants affected (PPA), and mean severity index (SI) for the four study species at each ozone treatment level.

	Coreopsis lanceolata			
Treatment (ppm)	SF	PLI (%)	PPA (%)	SI
1.2	0	0	0	0
1.7	0	0	0	0
2.2	0	0	0	0
2.7	0.3	8	11	257
	Coreopsis palmata			
Treatment (ppm)	SF	PLI (%)	PPA (%)	SI
1.2	0.4	6	33	257
1.7	0.9	29	56	3173
2.2	1.3	46	78	7644
2.7	3.2	88	100	28944
	Coreopsis tinctoria			
Treatment (ppm)	SF	PLI (%)	PPA (%)	SI
1.2	0	0	0	0
1.7	0.1	0	11	1
2.2	1.7	25	89	6378
2.7	2.1	37	100	12133
	Coreopsis tripteris			
Treatment (ppm)	SF	PLI (%)	PPA (%)	SI
1.2	0.7	11	56	933
1.7	1.6	29	78	5130
2.2	2.8	70	100	23400
2.7	2.9	57	100	20689

Table 2.4. Symptomatic exposure levels and visible foliar injury symptoms from acute ozone exposures after a 30 minute exposure period and peak ozone concentration targets of 1.2, 1.7, 2.2, and 2.7 ppm.

Scientific name	Symptomatic exposure levels (ppm)	Symptoms
Coreopsis lanceolata	2.7	Light colored flecking
Coreopsis palmata	1.2, 1.7, 2.2, 2.7	Bronzing, red-purple flecking with larger veins unaffected, leaf edge and tip bifacial necrosis
Coreopsis tinctoria	1.7, 2.2, 2.7	purplish flecking and coalesced necrotic lesions
Coreopsis tripteris	1.2, 1.7, 2.2, 2.7	Upper leaf surface purple stipple and tan interveinal necrosis with larger veins unaffected

Table 2.5. Least squares means of log 10 transformed severity index for four *Coreopsis* species subjected to four acute ozone exposures[z]

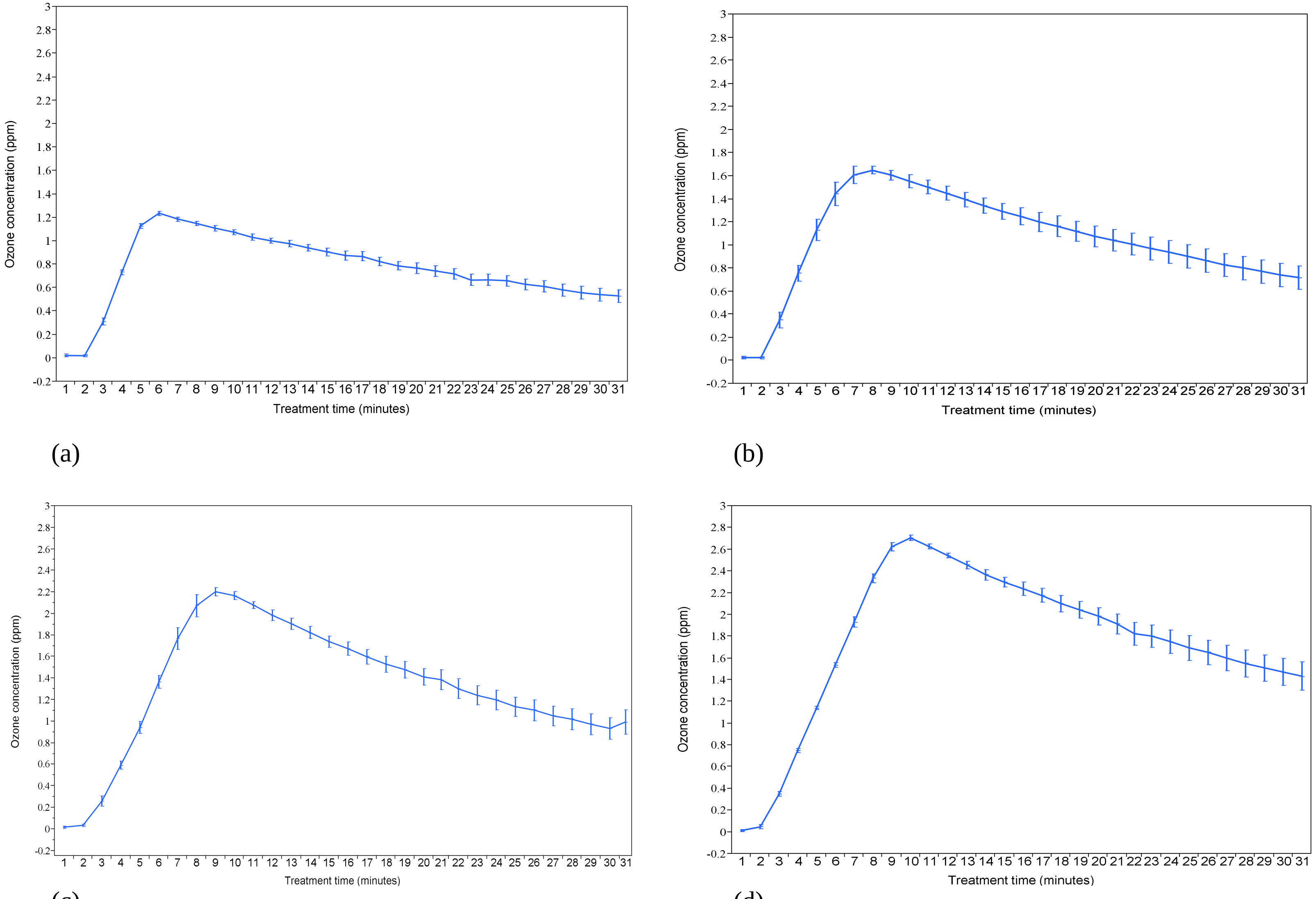
Species	Treatment (target peak concentration, ppm)				Effect[y]	Slope[x]
	1.2	1.7	2.2	2.7		
Coreopsis lanceolata	2.66e-15	4.44e-16	-3.33e-16	0.37	NS	0.224a
Coreopsis palmata	0.94	1.95	2.78	4.43	L	2.257bc
Coreopsis tinctoria	2.11e-15	0.12	3.0	3.49	L	2.672c
Coreopsis tripteris	1.65	2.72	4.06	3.94	L	1.642b

[z] Treatment x species interaction significant at $P < 0.0001$. Data collected from 9 plants per species per treatment were Log 10 (severity index + 1) transformed before analysis and represented as least squares means.

[y] NS, L: Nonsignificant or linear response significant at $P \leq 0.001$.

[x] Rates of response to O_3 exposure (Slope) followed by the same letter are not significantly different at $P \leq 0.05$; separated by slope contrast.

Fig 2.1. Experimental exposure chambers with chamber covers raised by a pulley system and treatment plants in place in the center of individual cells on the chamber floor grid.



(a)

(b)

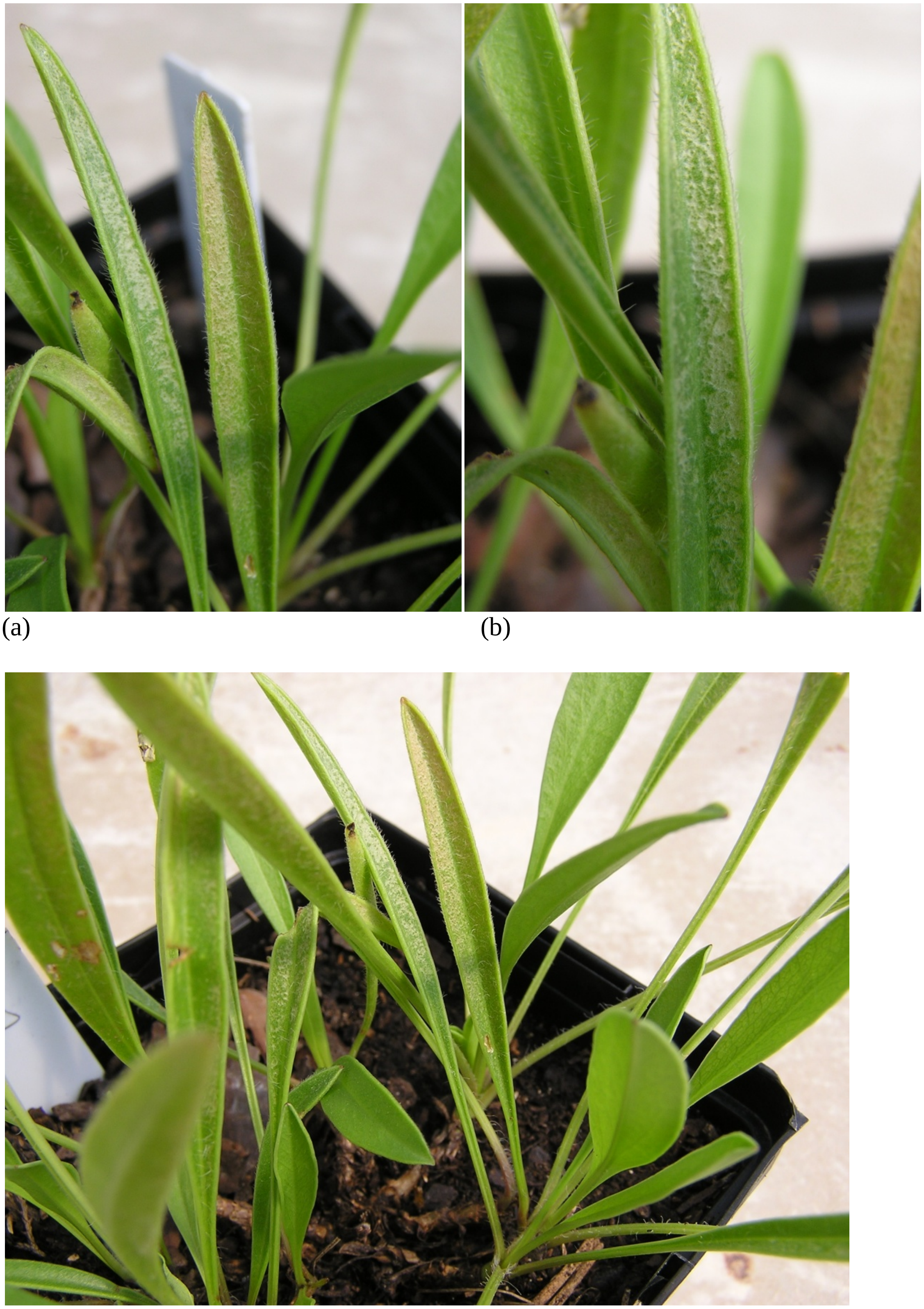
(c)

(d)

Fig. 2.2. Ozone concentration (ppm) plotted against the 30 minute treatment time (minutes) for each of four exposure regimes: (a) 1.2 ppm target peak concentration, (b) 1.7 ppm target peak concentration, (c) 2.2 ppm target peak concentration, (d) 2.7 ppm target peak concentration. Ozone concentration means are connected by a line and mean standard error bars are displayed.

APPENDIX 2.A

A PHOTOGRAPHIC ATLAS OF VISIBLE FOLIAR INJURY SYMPTOMS FOR FOUR COREOPSIS SPECIES DISPLAYING ACUTE OZONE EXPOSURE INJURY

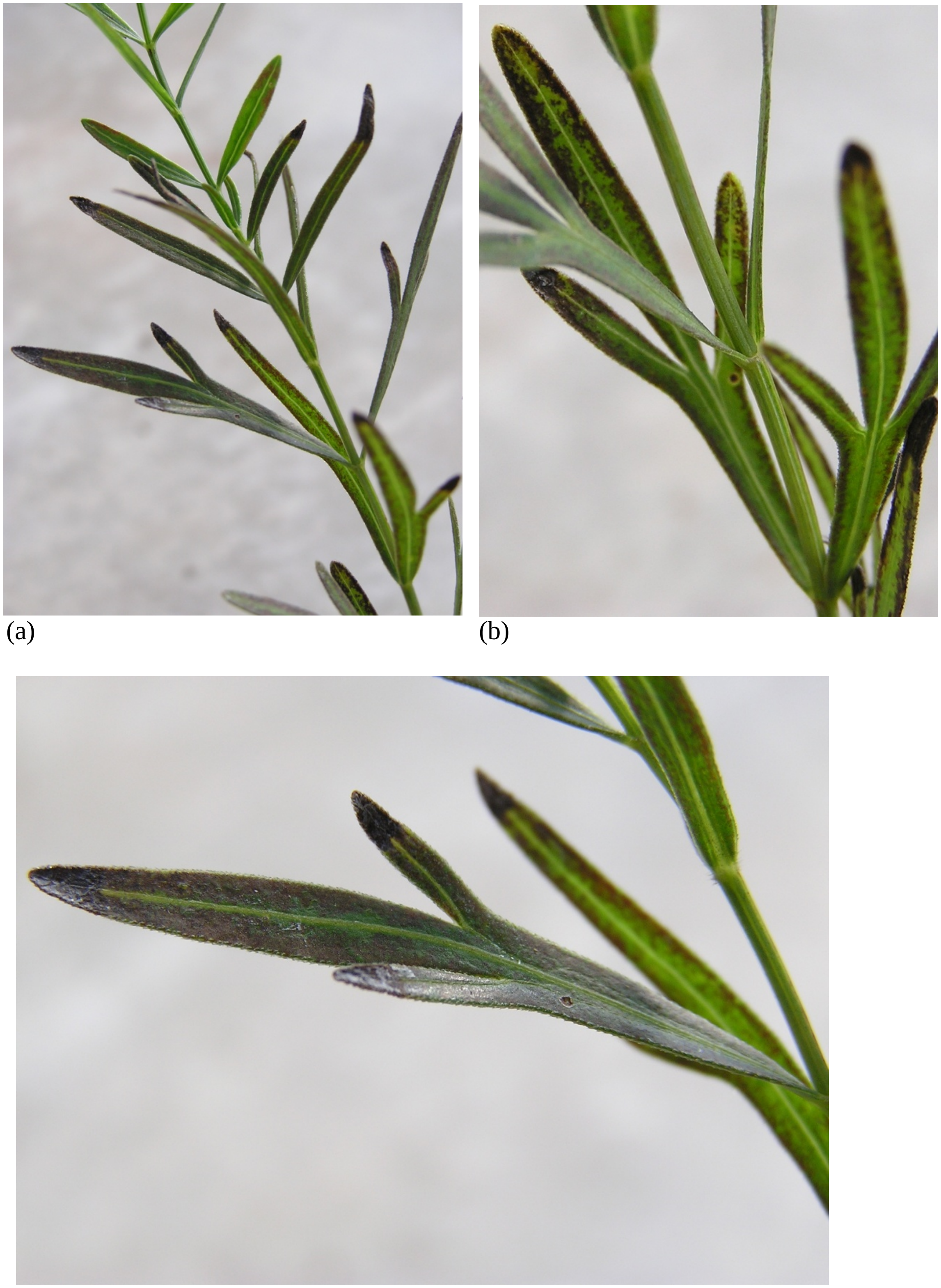


(a)

(b)

(c)

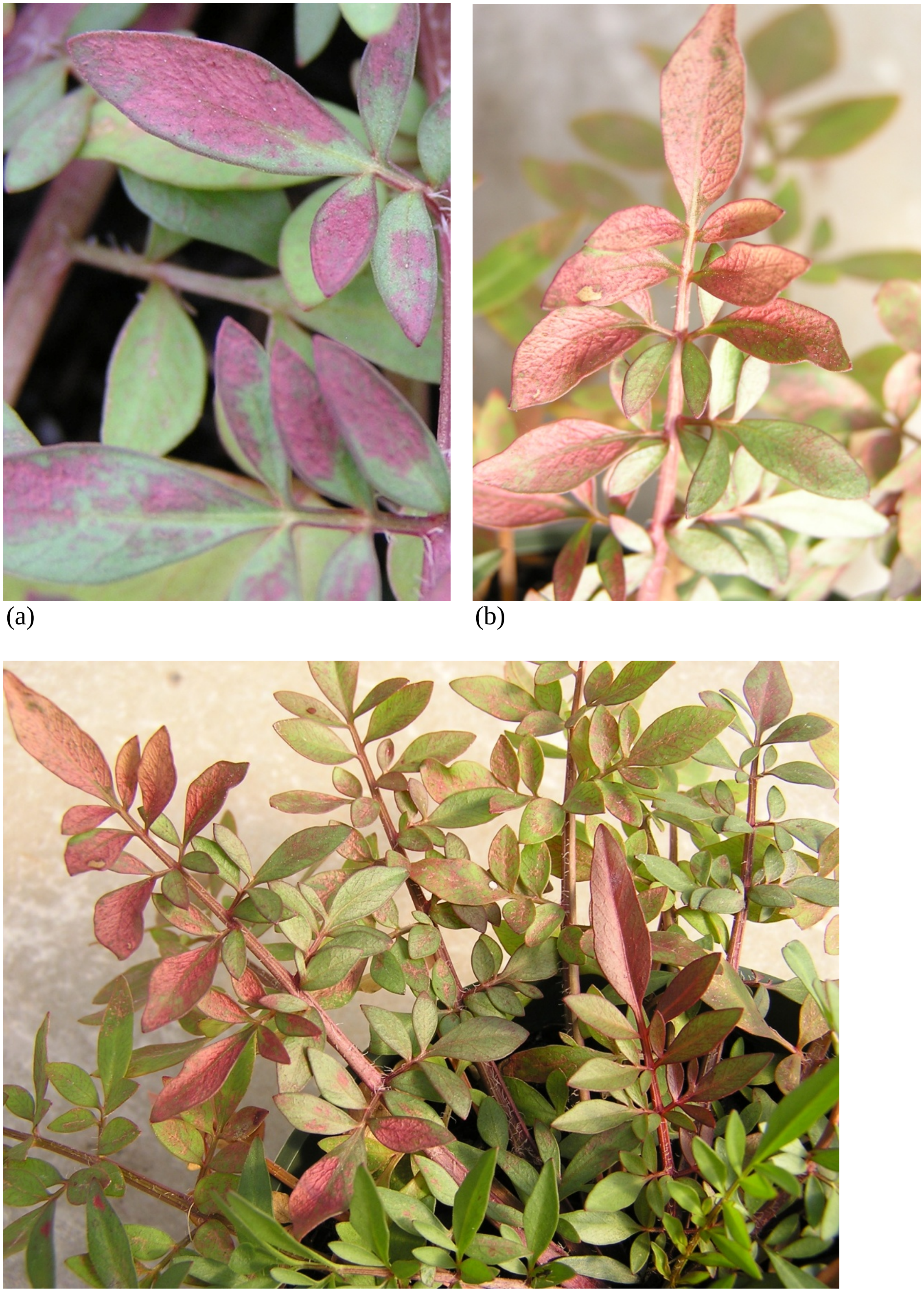
Fig. 2.A.1. Ozone-induced flecking (a,b,c) of *Coreopsis lanceolata* (lanceleaf tickseed).



(a) (b)

(c)

Figure 2.A.2. Ozone-induced red-purple stippling and flecking with larger veins unaffected; leaf edge and tip bifacial necrosis (a,b,c) of *Coreopsis palmata* (stiff tickseed).

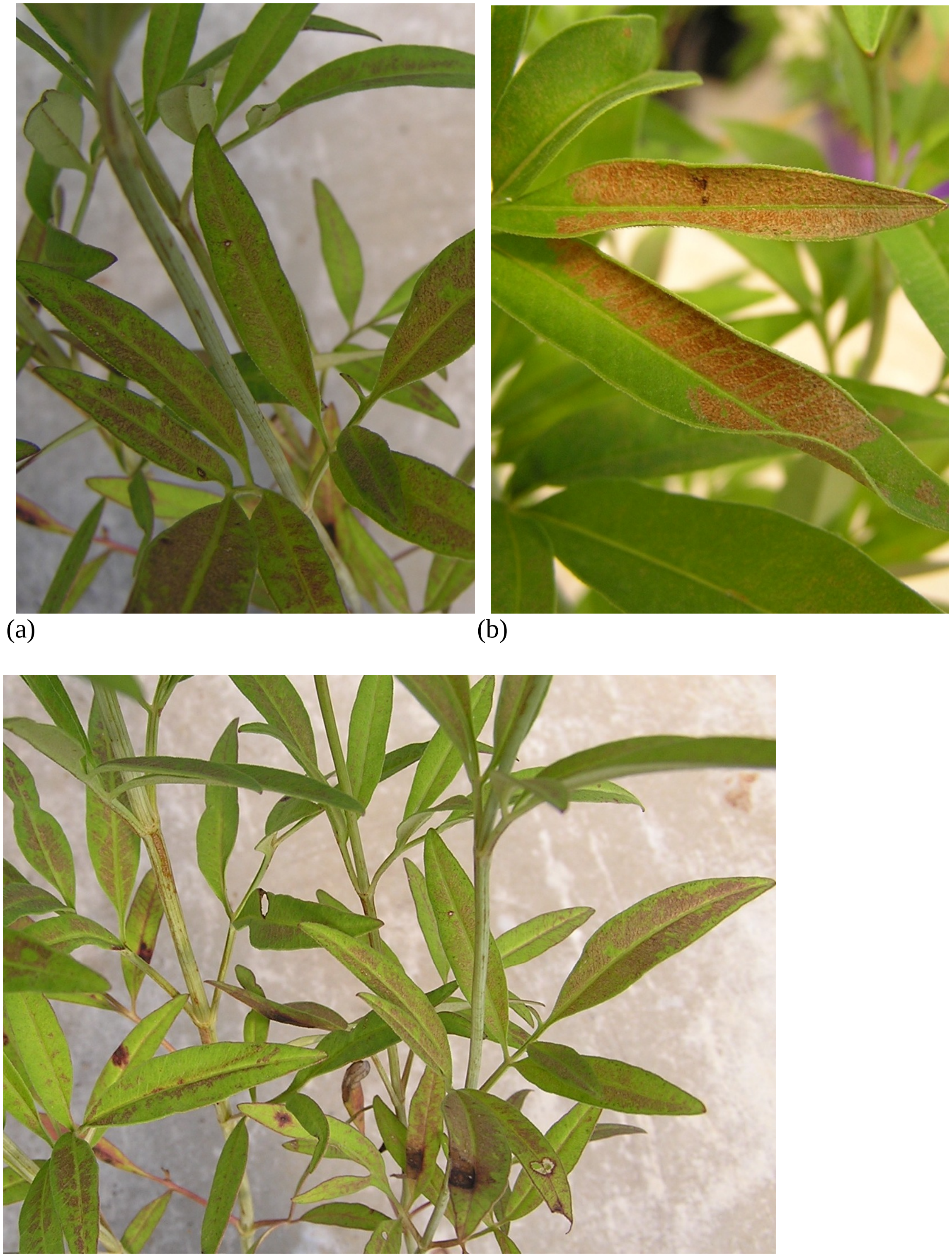


(a)

(b)

(c)

Figure 2.A.3. Ozone-induced purplish flecking and coalesced necrotic lesions (a,b,c) of *Coreopsis tinctoria* (golden tickseed).


(a) (b)

(c)

Figure 2.A.4. Ozone-induced purple stipple with larger veins unaffected (a, c), tan necrosis with larger veins unaffected (b) of *Coreopsis tripteris* (tall tickseed).

CONCLUSIONS

The objective of Study 1 was to induce, describe and photographically document the foliar injury symptoms resulting from acute O_3 exposure for select native perennial plant species in a closed chamber environment.

Ten of the 27 species treated in Study 1 showed foliar injury symptoms after treatment. Descriptions and digital images of the foliar injury symptoms not previously reported in the literature are now documented. Symptoms observed differed by species and, in some cases, within a single taxon. Wide ranges in percentage of leaves injured and percentage of plants affected indicate a high degree of interspecific and intraspecific variation for the species studied. *Coreopsis tripteris* showed the greatest injury levels and *Coreopsis palmata* showed the second highest level of injury symptoms. Of the 27 species treated, 15 did not develop visible injury symptoms after treatment. Plant species unaffected by the acute O_3 exposure applied in Study 1 show tolerance and may represent wise choices for use in areas susceptible to high levels of ozone. Avoidance of the ten species that developed foliar injury after treatment, particularly species with the highest level of injury (*Coreopsis tripteris* and *C. palmata*), is recommended in areas prone to high levels of ozone.

The exposure regime applied under the experimental conditions of Study 1 represents a useful ozone dose at which slightly more than one third of the species studied, developed visible foliar injury symptoms. Susceptible species showed a wide range of symptoms; the most common being upper leaf surface stipple and flecking of various colors. Symptoms of greater severity observed included leaf edge and tip necrosis and relatively small interveinal bifacial necrotic areas with the most severe symptoms being bifacial necrotic coalesced lesions. Other less frequently observed symptoms included bronzing and chlorosis. Symptoms observed in this study are consistent with foliar ozone injury symptoms previously documented for other plant species.

Study 2 provides descriptions and digital images of the visible foliar injury symptoms as well as an evaluation of the relative sensitivity of four *Coreopsis* species to acute O_3 exposures. Study 2 also provides an analysis of the acute O_3 exposure response characteristics for the four study species in a controlled chamber experiment.

All four *Coreopsis* species exhibited symptoms at the highest treatment level (2.7 ppm). As with Study 1, interspecific and intraspecific differences in sensitivity for the four *Coreopsis* species tested were observed.

Both *Coreopsis palmata* and *C. tripteris* exhibited symptoms at the lowest acute ozone dosage (1.2 ppm) indicating the highest degree of sensitivity of all four species in Study 1 and as well as the species in Study 2. Symptoms exhibited by *C. tripteris* include purplish stipple on the upper leaf surface which in addition to being considered one of the most common general symptoms on broadleaf species in the field, is described as the classic symptom of ozone injury on broadleaf species. Thus *C. tripteris* represents the greatest potential for use as a bioindicator of all the species studied. Further research to investigate the response of *C. tripteris* to ozone at lower dosages; those closer to field conditions, would be needed to determine if classic stipple symptoms would develop under more realistic field conditions of lower ozone concentrations and longer exposure duration.

Overall, this research provides valuable information on O_3 injury symptoms and exposure response characteristics of native perennial species. The findings herein are intended to facilitate appropriate plant selection for sites prone to high O_3 levels, and to facilitate accurate diagnosis of O_3 injury in the field. The findings also identify one species that may be appropriate for use as a bioindicator. Information gathered from this research relative to O_3 response of native perennials has the potential to improve the success of native plantings based on the sensitivity/tolerance of plant species in both urban and rural areas.

Appendix A.

NATIVE PERENNIALS WITH OZONE TOLERANCE:

A Partial List for Consideration in

Midwest and Eastern Regional Landscaping and Revegetation Projects

Ozone (O_3) is considered one of the most important air pollutants at ground level in the United States and elsewhere causing injury to agronomic and horticultural crops, deciduous trees, and conifers. Near ground level, ozone is formed when pollutants such as nitrogen oxides, carbon monoxide, and volatile organic compounds (collectively called ozone precursors) are emitted from vehicle exhaust, industrial processes and other sources. Ozone precursors react in the presence of sunlight to form ozone. High concentrations of O_3 are most likely to occur during the summer months and consequently during the plant-growing season when high temperatures and sunlight make conditions favorable for its formation. Ozone precursors can be moved by wind currents hundreds of miles to rural areas where reaction in the presence of sunlight can occur. As a result, rural areas can also experience high ozone levels and its harmful effects. Peak ozone levels occur during calm, hot, summer days.

As urbanization and industrialization continue; reports of O_3-induced foliar injury on sensitive plants have increased. Roadside rights of way and highly urbanized areas could experience episodic high levels of ozone due to heavy traffic patterns, industrial waste, and other ozone precursor producing processes. Soil and plant disturbance is a common occurrence along roads and highway corridors, around building sites, and within urbanized zones. In addition, efforts to restore altered prairie and wetland habitats; and to bring green space into the urban zone are on the rise. With an increased interest in the use of natural habitats and native plant species comes a need for recommendations of appropriate plant materials for these efforts.

There is limited information available on native perennials that tolerate ozone pollution. This list provides information for the land manager, revegetation specialist and landscape professional to facilitate appropriate plant selection for sites that are prone to high ozone levels. Choosing landscape plant materials based on their tolerance to ozone has the potential to improve the success of native plantings in both urban and rural areas.

Of 27 native perennial species tested during the 2010 and 2011 field season at the University of Arkansas, Fayetteville, 15 were found to be tolerant of ozone in an acute (short term, high concentration) dosage. The 15 species are listed.

Table 3.1. Plant species found to be tolerant of ozone in an acute dosage: A partial list for consideration in Midwest and eastern regional vegetation projects

Common name	Scientific name
lanceleaf tickseed	*Coreopsis lanceolata*
pale purple coneflower	*Echinacea pallida*
Bush's purple coneflower	*Echinacea paradoxa*
eastern purple coneflower	*Echinacea purpurea*
wavyleaf purple coneflower	*Echinacea simulata*
tall blazing star	*Liatris aspera*
cusp blazing star	*Liatris mucronata*
prairie blazing star	*Liatris pycnostachya*
talus slope penstemon	*Penstemon digitalis*
orange coneflower	*Rudbeckia fulgida* var. *speciosa*
sweet coneflower	*Rudbeckia subtomentosa*
wrinkleleaf goldenrod	*Solidago rugosa* ssp. *aspera*
Ernest's spiderwort	*Tradescantia ernestiana*
bluejacket	*Tradescantia ohiensis*
zigzag spiderwort	*Tradescantia subaspera*

Appendix B. Meteorological Data

Daily maximum and minimum temperatures (°F), barometric pressure (inches), precipitation (inches), and cloud cover recorded at the University of Arkansas, Division of Agriculture Research Farm in Fayetteville, AR, during data collection.

Mar-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	51	34	36	29.82		CLOUDY
2	43	22	30	29.90		CLOUDY
3	35	20	23	29.86		CLEAR
4	48	22	27	29.92		CLEAR
5	56	32	39	30.00		CLEAR
6	66	40	48	30.13		CLEAR
7	64	51	53	30.12		CLEAR
8	67	43	49	29.90		CLOUDY
9	62	48	53	29.40	0.62	CLEAR
10	65	49	54	29.39		CLEAR
11	72	40	40	29.40	0.3	CLOUDY
12	66	37	39	29.50		CLOUDY
13	43	37	37	29.74		CLOUDY
14	48	43	43	29.34		CLOUDY
15	50	43	43	29.92		CLOUDY
16	58	40	41	30.01		CLOUDY
17	52	40	40	30.00		CLOUDY
18	50	39	40	29.92		CLEAR
19	61	39	44	29.78		CLEAR
20	67	30	37	29.86	0.75	CLEAR
21	55	29	30	29.76	0.38	CLOUDY
22	35	29	33	29.80	0.75	CLEAR
23	49	30	40	29.72		CLEAR
24	69	40	50	29.70		CLOUDY
25	63	50	51	29.52	0.55	RAIN
26	55	30	35	29.60	0.51	CLEAR
27	62	46	50	29.61		CLOUDY
28	64	39	42	29.86		CLEAR
29	53	32	34	29.84	0.18	CLEAR
30	63	40	43	29.74		CLEAR
31	71	60	61	29.60		CLEAR

Apr-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	81	60	62	29.70		CLEAR
2	80	62	62	29.60		CLOUDY
3	68	45	47	29.91	0.84	CLEAR
4	69	58	63	29.87	0.04	CLEAR

5	76	66	67	29.80		CLEAR
6	74	61	62	29.60		CLEAR
7	74	56	56	29.50	0.04	CLOUDY
8	74	36	39	29.80		CLEAR
9	59	38	40	29.90		CLEAR
10	70	48	56	30.13		CLEAR
11	74	46	53	30.19		CLEAR
12	78	51	54	30.01		CLEAR
13	78	44	49	30.00		CLEAR
14	79	49	58	30.01		CLEAR
15	78	60	62	30.10		CLEAR
16	79	56	56	30.00		CLEAR
17	76	51	54	30.10	0.03	CLOUDY
18	57	50	51	30.10	0.09	CLOUDY
19	61	48	49	29.90	0.03	CLOUDY
20	63	40	41	29.80		CLEAR
21	68	41	52	29.79		CLOUDY
22	67	52	58	29.60		CLEAR
23	77	58	65	29.40	0.26	CLOUDY
24	73	53	55	29.43	1.5	CLOUDY
25	66	51	53	29.52	0.01	CLOUDY
26	63	49	49	29.60		CLEAR
27	60	40	42	29.68		CLOUDY
28	58	38	42	29.72		CLEAR
29	69	42	62	29.50		CLEAR
30	79	62	68	29.20		CLOUDY

May-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	75	55	59	29.51	0.01	CLOUDY
2	69	52	57	29.60	0.1	CLOUDY
3	70	58	61	29.62		CLEAR
4	79	55	57	29.80		CLEAR
5	79	57	69	29.53		CLEAR
6	81	58	61	29.60		CLEAR
7	84	61	71	29.42		CLEAR
8	75	44	49	30.28		CLEAR
9	65	50	52	30.23		CLEAR
10	65	49	49	29.90	1.17	CLOUDY
11	66	49	60	29.64	0.5	CLOUDY
12	74	66	70	29.60		CLEAR
13	81	60	60	29.80	0.4	CLOUDY
14	71	58	58	29.92	0.64	CLOUDY
15	71	62	62	29.91	1.63	CLOUDY
16	74	60	62	29.95	0.44	CLOUDY
17	74	59	59	29.80	0.36	CLOUDY

18	72	53	55	29.80		CLEAR
19	72	52	59	29.72		CLEAR
20	70	57	59	29.60	1.52	CLOUDY
21	73	56	59	29.72		CLEAR
22	78	61	72	29.82		CLEAR
23	85	72	74	29.82		CLEAR
24	85	72	72	29.74		PC
25	86	69	71	29.80		CLEAR
26	82	62	64	29.82	1.2	CLOUDY
27	84	62	67	29.85	0.24	CLEAR
28	85	62	66	29.80		CLEAR
29	84	65	68	29.80		CLEAR
30	84	65	70	29.76		CLEAR
31	86	68	70	29.85		CLEAR

Jun-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	85	65	68	29.72		CLEAR
2	89	67	70	29.61		CLEAR
3	87	64	64	29.62	0.3	RAIN
4	83	64	70	29.60		CLEAR
5	89	70	77	29.30		CLEAR
6	89	72	74	29.86		CLEAR
7	84	65	67	29.80		CLEAR
8	77	64	76	29.72	0.77	PC
9	84	64	72	29.80		CLOUDY
10	83	69	71	29.72		CLOUDY
11	83	69	74	29.80	0.02	CLOUDY
12	87	76	77	29.89		CLEAR
13	89	76	80	29.91		CLEAR
14	90	68	66	29.80	0.11	CLOUDY
15	88	66	67	29.72		CLOUDY
16	88	66	75	29.90		CLEAR
17	87	68	75	29.80	0.17	CLEAR
18	92	75	80	29.94		CLEAR
19	94	75	81	29.96		CLEAR
20	90	74	78	29.80		CLEAR
21	93	78	78	29.82		CLEAR
22	93	72	76	29.82		CLEAR
23	93	75	78	29.90		CLEAR
24	94	74	77	29.89		CLEAR
25	93	70	73	29.84		CLEAR
26	93	75	80	29.85		CLEAR
27	91	70	81	29.90		CLEAR
28	93	69	70	29.70	0.30	CLOUDY
29	86	60	68	29.80		CLEAR

30	92	65	65	29.81		CLEAR

Jul-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	84	61	66	30.00		
2	87	65	68	29.72		CLEAR
3	80	74	76	29.91	0.21	CLEAR
4	86	75	76	29.87		CLEAR
5	90	75	76	29.80		CLOUDY
6	89	76	77	29.78		CLOUDY
7	88	68	72	29.90	0.08	CLOUDY
8	82	70	73	29.80	0.52	CLOUDY
9	78	71	71	29.90	0.58	CLOUDY
10	82	70	71	29.95	2.65	CLOUDY
11	89	73	76	29.77		CLOUDY
12	90	75	75	29.60	0.02	CLOUDY
13	82	67	69	29.62	4.95	CLOUDY
14	87	67	77	29.80		CLEAR
15	89	76	79	29.80		PC
16	91	61	72	29.82	3.34	CLEAR
17	93	73	79	29.86	1.1	CLOUDY
18	97	75	79	29.80		CLOUDY
19	94	77	79	29.80		CLEAR
20	91	76	76	29.72		CLEAR
21	90	76	77	29.82		CLEAR
22	90	75	78	29.60		CLEAR
23	91	75	78	29.80		CLEAR
24	91	78	79	29.92		CLEAR
25	91	72	78	29.92	0.04	CLEAR
26	92	71	73	29.90	0.01	CLEAR
27	90	71	73	29.82	0.4	PC
28	91	74	75	29.90		CLEAR
29	89	74	75	29.92		CLEAR
30	91	76	80	29.78	0.01	CLEAR
31	93	74	82	29.78		CLEAR

Aug-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	96	80	80	29.77		CLEAR
2	98	80	82	29.70		CLEAR
3	98	77	79	29.80		CLEAR
4	98	76	76	29.80		CLEAR
5	97	76	79	29.60		PC
6	89	72	77	29.82		CLEAR

7	89	77	80	29.77		CLEAR
8	98	78	80	29.70		CLEAR
9	97	79	80	29.72		CLEAR
10	97	71	76	29.72		CLEAR
11	98	76	79	29.70		CLEAR
12	98	75	79	29.60		CLEAR
13	97	79	80	29.50		CLEAR
14	99	78	79	29.75		CLEAR
15	99	78	77	29.85		CLEAR
16	98	70	71	29.90		CLEAR
17	92	64	68	29.80		CLEAR
18	85	68	73	29.73		CLEAR
19	91	69	71	29.72		CLEAR
20	97	70	79	29.60		CLEAR
21	97	82	82	29.71		CLEAR
22	98	74	76	29.86		CLEAR
23	96	72	75	29.80		CLEAR
24	96	67	68	29.80		CLEAR
25	95	64	67	29.92		CLEAR
26	86	56	58	30.00		CLEAR
27	86	55	51	29.90		CLEAR
28	91	60	65	29.93		CLEAR
29	93	73	76	29.89		CLEAR
30	87	73	75	29.90		CLEAR
31	90	76	78	29.90		CLEAR

Sept-2010		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	92	76	78	29.72		CLEAR
2	86	70	72	29.72	1.84	CLOUDY
3	86	60	60	29.90	0.33	PC
4	78	50	55	30.09		CLEAR
5	80	55	61	29.91		CLEAR
6	85	69	71	29.58		CLEAR
7	89	73	74	29.82		PC
8	88	60	68	29.90	0.21	CLOUDY
9	85	68	69	29.68	2.71	RAIN
10	81	69	75	26.60	0.82	CLEAR
11	90	68	71	29.77		CLEAR
12	83	61	64	30.09		CLEAR
13	84	61	62	29.90		PC
14	89	59	66	29.90	0.05	RAIN
15	88	62	66	29.82	1.03	CLOUDY
16	85	65	71	29.80	0.04	PC
17	81	63	63	29.82		CLEAR
18	89	67	69	29.99		CLEAR
19	92	68	71	30.01		CLEAR

20	92	66	66	29.90		CLEAR
21	89	66	70	29.72		CLEAR
22	84	67	69	29.80		PC
23	84	69	73	29.82		CLEAR
24	84	67	69	29.82	1.46	CLOUDY
25	82	55	57	30.09		CLEAR
26	77	50	57	29.99		CLOUDY
27	62	45	45	29.82		CLEAR
28	67	47	55	29.78		CLEAR
29	74	50	55	29.70		CLEAR
30	75	50	53	29.80		CLEAR

Mar 2011		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	48	27	37	30.10	0.06	CLEAR
2	65	34	35	30.00		CLEAR
3	67	35	48	29.92		PC
4	67	48	58	29.70		PC
5	67	28	31	30.12	0.67	CLOUDY
6	42	26	27	30.23		CLOUDY
7	55	36	38	29.80		CLOUDY
8	53	42	46	29.62		CLOUDY
9	53	35	35	29.80	0.01	CLOUDY
10	40	27	28	30.10		CLEAR
11	50	28	45	30.00		CLEAR
12	68	47	52	29.97		CLEAR
13	73	36	51	30.06		CLEAR
14	66	40	40	29.90	1.16	CLOUDY
15	36	33	33	30.00	0.02	PC
16	54	33	40	30.00		PC
17	68	40	60	29.82		CLEAR
18	73	60	62	29.82		CLEAR
19	72	52	52	30.19		CLOUDY
20	71	59	60	30.02		CLOUDY
21	78	59	63	29.90		CLOUDY
22	78	51	64	29.68		PC
23	71	57	58	29.50		CLEAR
24	67	35	36	29.90		CLEAR
25	57	46	46	30.09		PC
26	58	36	42	29.87		CLOUDY
27	47	33	34	29.63	0.21	CLOUDY
28	40	35	36	29.90		PC
29	54	42	43	29.60	0.02	CLOUDY
30	46	36	36	29.82	0.12	CLOUDY
31	40	33	36	29.70		CLEAR

April 2011		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	40	33	33	29.60	0.55	CLOUDY
2	64	38	42	29.97		CLEAR
3	75	65	67	29.61		CLEAR
4	84	45	45	29.50	0.19	CLOUDY
5	50	30	33	29.80	0.1	CLEAR
6	66	33	57	29.64		CLOUDY
7	72	56	61	29.60		PC
8	67	61	66	29.60		CLEAR
9	89	70	72	29.77		CLEAR
10	86	62	72	29.67		CLEAR
11	82	56	56	29.68	1.48	CLOUDY
12	63	41	45	29.90		CLEAR
13	72	45	48	29.80		CLEAR
14	77	48	62	29.70		PC
15	76	45	45	29.40	0.75	CLOUDY
16	58	38	38	29.90	0.16	CLEAR
17	61	46	55	29.86	0.01	CLEAR
18	74	61	62	29.60		CLEAR
19	78	62	70	29.40		PC
20	83	43	43	29.90	0.25	CLOUDY
21	64	44	50	29.90		PC
22	71	50	65	29.58	1.76	PC
23	79	52	56	29.72	"	RAIN
24	66	54	56	29.86	"	CLOUDY
25	60	57	58	29.50	8.3	CLOUDY
26	69	48	49	29.40	4.9	CLOUDY
27	62	49	51	29.20		CLOUDY
28	53	41	45	29.88	0.55	CLEAR
29	69	46	56	29.82		CLEAR
30	76	62	63	29.58	0.01	CLEAR

May 2011		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	71	42	56	29.93	1.29	CLOUDY
2	44	43	43	30.02	0.11	CLOUDY
3	44	33	36	30.05	0.48	FOG
4	64	36	45	30.10	0.01	CLEAR
5	71	45	52	30.01		CLEAR
6	69	42	45	29.90	0.11	CLEAR
7	69	43	68	29.60		CLEAR
8	74	62	68	29.71		CLEAR
9	81	62	62	29.71		CLEAR
10	86	82	62	29.50		CLOUDY

11	82	71	72	29.60		CLEAR
12	82	59	62	29.70	0.17	CLEAR
13	78	54	55	29.60	0.37	CLOUDY
14	61	47	47	29.96	0.06	CLEAR
15	55	42	46	29.95		CLEAR
16	58	45	45	29.90		CLEAR
17	66	40	45	29.80		CLEAR
18	68	45	53	26.62		PC
19	71	53	66	29.60	0.13	CLOUDY
20	76	66	71	29.60		CLOUDY
21	72	62	66	29.74	2.01	CLOUDY
22	80	63	73	29.68		CLOUDY
23	82	68	68	29.52	0.89	CLOUDY
24	73	58	62	29.60	2.57	CLOUDY
25	78	62	62	29.40	0.48	PC
26	76	53	54	29.70	0.21	CLOUDY
27	66	52	55	29.72		CLEAR
28	78	66	72	29.56		CLEAR
29	86	73	75	29.62		CLEAR
30	87	73	75	29.81		CLEAR
31	84	75	75	29.90		PC

June 2011		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	86	71	71	30.00		PC
2	86	69	72	29.90		PC
3	88	72	76	29.82		CLEAR
4	90	69	78			CLEAR
5	91	69	78			CLEAR
6	91	76	76	29.80		CLEAR
7	90	70	73	29.74		CLEAR
8	90	73	75	29.70		CLEAR
9	88	71	74	29.70		CLEAR
10	88	71	75	29.70		PC
11	89	66	77	29.79		CLEAR
12	87	66	67	29.87	0.4	CLEAR
13	88	67	78	29.70		CLEAR
14	90	78	80	29.60		CLEAR
15	89	63	67	29.60	0.25	CLEAR
16	89	65	71	29.68		CLOUDY
17	83	68	73	29.60	0.11	PC
18	89	81	82	29.68		CLEAR
19	91	78	78	29.64		CLEAR
20	91	77	77	29.50		CLEAR
21	89	68	75	29.50		PC
22	87	65	67	29.60		CLEAR

23	86	63	69	29.70		CLEAR
24	90	68	72	29.70	0.07	CLEAR
25	93	74	79	29.69		CLEAR
26	94	77	81	29.74		CLEAR
27	94	81	81	29.70		CLOUDY
28	93	72	76	29.80		PC
29	81	67	72	29.90	0.22	CLEAR
30	91	72	76	29.90		CLEAR

July 2011		AIR TEMP °F				
DATE	MAX	MIN	OBS	BAR	PRECIP	CLOUD
1	94	72	76	29.80		CLEAR
2	98	72	82	29.93	0.06	CLEAR
3	99	70	78	29.88		CLEAR
4	98	72	75	29.81	0.07	CLEAR
5	92	72	72	29.80	0.19	PC
6	95	72	74	29.80		CLEAR
7	93	74	78	29.74		PC
8	101.2	72	73	29.70		CLOUDY
9	91	72	79	29.76		CLEAR
10	100.7	80	85	29.76		CLEAR
11	102.8	81	84	29.80		CLEAR
12	100.1	81	81	29.80		CLEAR
13	100	70	71	29.80	0.11	PC
14	84	72	74	29.70	0.25	PC
15	95	74	77	29.70		CLEAR
16	98	75	84	29.80		CLEAR
17	98	77	83	29.95		CLEAR
18	96	76	76	29.94		CLEAR
19	95	72	77	29.90		CLEAR
20	96	74	77	29.80		CLEAR
21	97	76	77	29.70		CLEAR
22	99	79	82	29.72		CLEAR
23	99.6	81	83	29.85	0.04	CLEAR
24	101.5	79	84	29.87		PC
25	101.2	76	77	29.82		PC
26	94	73	77	29.72		CLEAR
27	100	76	76	29.70		CLEAR
28	100	73	73	29.80		CLEAR
29	99	73	76	29.90		CLEAR
30	98	77	83	29.93	0.02	CLEAR
31	101	75	86	29.96		CLEAR