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Screening Buddleia Cultivars for Acute Ozone Sensitivity[1]

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Abstract

Eight cultivars of buddleia were exposed to ozone (O_3) concentrations up to 375 ppb for two 4-hour periods on consecutive days in 1995. Visible injury to all cultivars consisted of small, discrete spots, reddish purple in color, on the adaxial leaf surface. Cultivars differed in sensitivity to acute O_3 exposure with 'Empire Blue' and 'Opera' being the most tolerant and 'Black Knight', 'Nanho Blue', 'Pink Delight', and 'Royal Red' the most sensitive. Severe injury occurred on the most sensitive cultivars with O_3 exposures of 250 or 375 ppb. At the highest O_3 concentration, the severity index (SI), an indicator of foliar injury, ranged from 1.3 (less injury) for 'Opera' to 4.6 (more injury) for 'Pink Delight'. An exposure-response study with 'Black Knight' resulted in visible injury increasing as both concentration and number of exposures increased, with the most severe injury to plants given the most exposures (5 days) to the highest concentration (375 ppb, SI of 6.3).

Index words: tropospheric ozone, air pollution.

Species used in this study: Butterfly bush (*Buddleia davidii* Franch. 'Black Knight', 'Charming Summer', 'Empire Blue', 'Opera', 'Pink Delight', and 'Royal Red', *B. fallowiana* Balf. 'Lochinch', and *B. davidii nanhoensis* (Chitt.) Rehd. 'Nanho Blue').

Significance to the Nursery Industry

Buddleia has enjoyed a resurgence in popularity in recent years due to the introduction of numerous cultivars. Foliage of these cultivars varies in sensitivity to acute ozone (O_3) exposure with 'Black Knight', 'Nanho Blue', 'Pink Delight', and 'Royal Red' being more sensitive than 'Empire Blue' or 'Opera'. At concentrations currently found in urban areas of the southeastern United States, injury from acute O_3 exposure would be relatively minor and should not preclude the use of buddleia in the landscape. However, as previously reported (4), foliar injury from chronic O_3 exposure may occur under ambient conditions with certain buddleia cultivars.

Introduction

Ozone (O_3) was identified as a significant phytotoxic air pollutant during the 1950s (17) and has progressively become a major air pollutant across the United States. Normally associated with urban areas with large numbers of automobiles, tropospheric O_3 is readily transported long distances to non-urban or rural areas (23). The major effects of O_3 on terrestrial vegetation include visible injury, and reductions in growth, productivity, and plant quality (16). Ozone concentrations ≥ 120 ppb exceed EPA National Ambient Air Quality Standards (23).

Visible injury from acute O_3 exposures, *i.e.* exposure to high concentrations for short periods of time, has been observed on a number of landscape plants in the northeastern United States including azalea (*Rhododendron* spp. L.), sweet mock-orange (*Philadelphus coronarius* L.), tea viburnum (*Viburnum setigerum* Hance), spreading cotoneaster (*Cotoneaster divaricata* Rehd. & E.H. Wils.), and multiflora rose (*Rosa multiflora* Thunb.) (2, 3). Studies involving agronomic crops such as tobacco (*Nicotiana tabacum* L.) and pinto bean (*Phaseolus vulgaris* L.) have reported that the amount of visible injury is a function of both exposure duration and O_3 concentration with increasing injury as either duration of exposure or concentration increases (9, 14).

Cultivar differences in sensitivity to O_3 have been reported in azalea (7), trembling aspen (*Populus tremuloides* Michx.) (12), and white pine (*Pinus strobus* L.) (6). In a screening of landscape plants common in the southeastern United States to chronic ozone exposure, cultivar differences were observed in buddliea (*Buddleia* spp.) (4). Chronic O_3 effects are caused by exposure to frequent, relatively low hourly concentrations, with periodic random, intermittent peaks of relatively high hourly concentrations on one or more days, as opposed to acute effects caused by exposure to high hourly concentrations for short durations (15). Sensitivity to chronic and acute O_3 exposures are not necessarily correlated (21). Based on these results and the fact that even minor foliar injury can make a plant undesirable or unmarketable, the objectives of this study were to elucidate differences among buddleia cultivars in sensitivity to acute concentrations of O_3 and to determine the relative importance of both O_3 concentration and number of exposures in visual symptom development.

Materials and Methods

Cultivar sensitivity. Rooted cuttings of eight buddleia cultivars (*Buddleia davidii* Franch. 'Black Knight', 'Charming Summer', 'Empire Blue', 'Opera', 'Pink Delight', 'Royal Red', *B. fallowiana* Balf. 'Lochinch', and *B. davidii nanhoensis* (Chitt.) Rehd. 'Nanho Blue') were transplanted into 10.2 cm (4 in) square pots containing a pine bark:sand medium (7:1 by vol) in August 1995. The medium was amended per m^3 (yd^3) with 4.7 kg (8 lb) 22N–2.6P–11.6K (Polyon 22–6–14, Pursell Industries, Sylacauga, AL), 0.9 kg (1.5 lb) Micromax (The Scotts Company, Marysville, OH), and 3.0 kg (5 lb) dolomitic limestone. Plants were grown under 47% light exclusion and irrigated by overhead rotary nozzles twice daily for 30 minutes per application. Mean ambient O_3 concentrations in July and August 1995 were 33 and 30 ppb, respectively.

Plants were selected for uniformity and placed into one of four blocks in September 1995. Within each block two plants per cultivar were assigned to one of four O_3 treatments: 0,

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125, 250, or 375 ppb. Thus for each cultivar, eight plants were exposed to each O_3 concentration. Plants were exposed to the assigned O_3 treatment for 4 hours on two consecutive days in continuously stirred tank reactors (CSTRs) located within a walk-in growth chamber (9).

Air was filtered through activated carbon prior to adding O_3 to help maintain uniform O_3 concentrations within the CSTRs. Ozone was generated by passing pure oxygen through a high intensity electrical discharge source (Ozone Research and Equipment Corporation, Phoenix, AZ), and O_3 concentrations were monitored continuously during exposures with a UV Photometric Ozone Analyzer (Thermo Environ. Instruments, Inc., Hopkinton, MA). Photosynthetic photon flux at canopy level within the CSTRs was 520 ± 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Temperature and relative humidity during the exposures were 27C ± 2C (80.6F ± 3.6F) and 75% ± 3%, respectively. Due to only four CSTRs being available, treatments were replicated over time for a total of four blocks.

Plants were watered before and after each exposure period to avoid drought stress. After exposure, plants were returned to the shade structure and evaluated two and seven days later for percentage of the leaves injured (PLI) and leaf area injured. Leaf area injured was rated for those leaves exhibiting visible injury using the Horsfall-Barratt foliar injury scale (H-B rating) (10) with 1 = 0, 2 = 1–3, 3 = 3–6, 4 = 6–12, 5 = 12–25, 6 = 25–50, 7 = 50–75, 8 = 75–87, 9 = 87–94, 10 = 94–97, 11 = 97–99, and 12 = 100% of the leaf area injured. A severity index (SI), similar to the one described by Davis and Coppolino (1), was developed by multiplying (PLI / 100) × (H-B rating) × (percentage of the population injured / 100). The range for SI is 0–12 with 12 occurring when the entire leaf area of every plant treated is injured (1.00 × 12 × 1.00). The SI was utilized to estimate overall ozone injury.

Exposure response. A second experiment investigated the effects of both O_3 concentration and number of exposures. 'Black Knight' was exposed to O_3 concentrations of 0, 125, 250, or 375 ppb for one to five days for 4 hours daily. Conditions were similar to those in the previous experiment with four-node cuttings taken in August 1995 and O_3 exposures initiated in September 1995 under similar environmental conditions. Plants were selected for uniformity and placed into one of three blocks. Within each block, two plants were assigned to each O_3 concentration and exposure number combination. Thus, six plants were exposed to each O_3 concentration and exposure number combination. Plants were evaluated for PLI and H-B rating two and seven days after final exposure, and a SI was calculated.

Statistical analyses. A randomized complete block design with four blocks (replicated over time) containing two plants/cultivar/ozone treatment was used to test for ozone treatment effects. The second study was a four × five factorial experiment (four concentrations × five exposure numbers) arranged in a randomized complete block design. All data were arc-sin transformed prior to performing analysis of variance (ANOVA), using the SAS General Linear Model procedure, and Duncan's multiple range test to compare cultivars. Linear regression was used to test O_3 concentration and exposure number, and single degree of freedom contrasts were used to compare specific exposures (22).

Results and Discussion

Cultivar sensitivity. The most common O_3 injury symptom was stippling of the upper leaf surface, which consisted of numerous small, discrete spots reddish-purple in color. At O_3 concentrations of 250 and 375 ppb, visible injury was observed on both the oldest and most recently matured leaves. Visible injury was observed under all O_3 concentrations except the control for all cultivars except 'Charming Summer' and 'Lochinch' in which visible injury was only observed under the two higher concentrations (250 and 375 ppb).

An ozone treatment × cultivar interaction was significant for the SI (Table 1). When exposed to 125 ppb O_3, 'Black Knight', 'Opera', and 'Royal Red' had the highest SI, 0.03, 0.02, and 0.02, respectively, while the other five cultivars had SI of 0.005 or less. Although injury at 125 ppb was minor, these concentrations of O_3 have been recorded in urban areas of the southeastern United States during the summer (13), indicating a potential for injury under ambient conditions.

The SI ranged from 0.20 for 'Charming Summer' to 1.23 for 'Royal Red' when plants were exposed to O_3 concentrations of 250 ppb. Visible injury detected at this concentra-

Table 1. Severity index of visible foliar injury for buddleia cultivars exposed to four levels of ozone.[z]

Ozone concn. (ppb)	Cultivar							
	Black Knight	Charming Summer	Empire Blue	Lochinch	Nanho Blue	Opera	Pink Delight	Royal Red
0	0.0a[y]	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
125	0.03a	0.0b	0.002b	0.0b	0.005b	0.02a	0.001b	0.02a
250	1.08a	0.20c	0.44bc	0.80abc	0.75abc	0.27bc	0.83ab	1.23a
375	3.65abc	2.65cd	2.07de	3.27bc	3.62abc	1.28e	4.56a	3.95ab
Significance[x]	Q***	C**	Q**	Q***	Q***	Q***	C***	Q**

[z]Ozone treatment × cultivar interaction significant ($p \leq 0.001$). Severity index (SI) data were arc-sin transformed before analysis; retransformed mean values are presented. SI = (% of leaves injured expressed as a decimal) × (Horsfall-Barratt rating) × (% of population injured expressed as a decimal); higher SI indicates more visible injury.

[y]Mean separation within rows by Duncan's multiple range test, $p \leq 0.05$.

[x]Q, C: Quadratic, or cubic response significant at $p \leq 0.01$ (**) or 0.001 (***).

tion was more severe than at lower concentrations with larger stipples and more leaves affected, resulting in an increased SI for all cultivars. The SI ranged from 1.28 for 'Opera' to 4.56 for 'Pink Delight' when exposed to 375 ppb O_3. Foliar injury was extensive and was visible within 24 hours of the first exposure on the most severely injured cultivars, 'Black Knight', 'Nanho Blue', 'Pink Delight', and 'Royal Red'. One week after the final exposure the most severely injured leaves on all cultivars were senescing.

These results agree with previous studies demonstrating intraspecific differences in visible foliar injury with acute O_3 exposure (2, 4, 6, 7). In addition, 'Black Knight' and 'Royal Red' were sensitive to both chronic (4) and acute O_3 exposures. However, 'Nanho Blue' and 'Pink Delight', which were relatively tolerant to chronic O_3 exposure (4), were sensitive to acute O_3 exposure. Our results support previous observations with other plants that sensitivity to chronic and acute O_3 exposures are not necessarily correlated (21). For example, Musselman et al. (1983) working with bean (*Phaseolus vulgaris* L. 'Red Kidney') reported that plants exposed to higher concentrations (acute exposure) exhibited more visible injury and less growth than plants exposed to a lower concentration at an equivalent dose. Acute O_3 exposures result in direct effects on metabolic processes such as injuring chloroplasts and direct denaturing of proteins. Chronic exposures result in indirect effects to metabolic processes, which can result in cumulative effects. These include membrane leakage, effects to stomatal apparatus, and increases in phenolic compounds for repair and defense (1, 21).

We also observed that plants exposed to the higher O_3 concentrations were more likely to be attacked by spider mites (*Tetranychus urticae* Koch). Although these findings were purely observational, this apparent feeding preference for foliage exposed to ozone is similar to that reported with spider mites on white clover (*Trifolium repens* L.) and peanut (*Arachis hypogeae* L.) (8). This is an area that warrants further research.

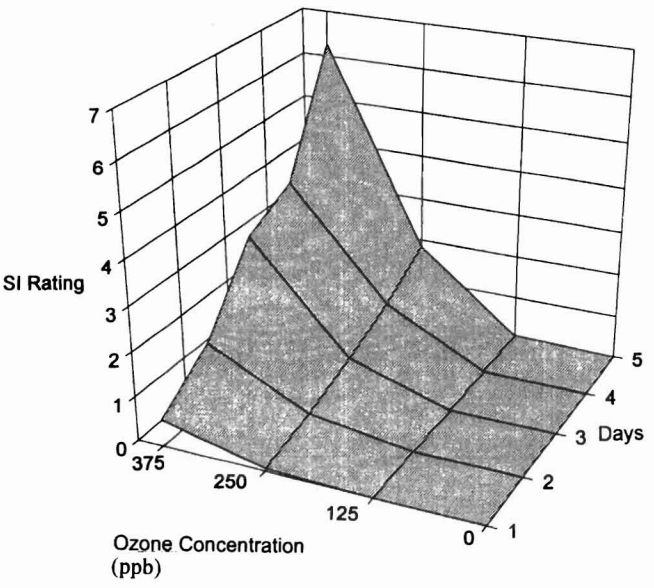

Fig. 1. Severity index rating (SI) of 'Black Knight' buddleia exposed to four ozone concentrations for one to five days, 4 hours daily. SI = (% of leaves injured/100) × (Horsfall-Barratt rating) × (% of population injured/100).

Exposure-response. The effects of a 4-hour exposure to O_3 for one to five days was evaluated using 'Black Knight', an O_3-sensitive cultivar. At the higher O_3 concentrations, 250 and 375 ppb, visible injury was observed on both the oldest and recently matured leaves. At these concentrations, the most severely injured leaves had small, necrotic areas and stippling after four exposures, and the lower pairs of leaves senesced within one week after the last exposure. Early leaf senescence has been reported in other landscape plants exposed to O_3 such as American beech (*Fagus sylvatica* L.) (18) and black cherry (*Prunus serotina* Ehrh.) (5).

The SI increased as both O_3 concentration and number of exposures increased with the highest SI (6.28) observed for plants given the most exposures of the highest O_3 concentration (Fig. 1). No visible injury was observed on plants given the most exposures to 0 ppb O_3 for any number of episodes and only minor visible injury was observed on plants exposed to 125 ppb of O_3 (SI of 0.0006 for one day to 0.06 for five days of exposure). The SI ratings were similar for plants exposed to 250 ppb of O_3 for one or two days, as well as for plants exposed to 0 or 125 ppb of O_3 for one to five days. Plants exposed to 375 ppb of O_3 for one day or 250 ppb of O_3 for two or three days had similar SI ratings (SI of 0.60, 0.34, and 0.73, respectively). The SI also was similar for plants exposed to two days of 375 ppb of O_3 or four days of 250 ppb of O_3 (1.47 and 1.22, respectively).

For a specific ozone exposure, which is the product of O_3 concentration and exposure duration (19), concentration was more important in inducing visible injury than number of exposures. No difference in SI was determined for plants exposed to 125 ppb of O_3 for four days and plants exposed to 250 ppb of O_3 for two days even though the total exposure was the same. However, the SI was higher for plants exposed for one day at 375 ppb of O_3 (SI of 0.6) than for plants exposed to three days of 125 ppb of O_3 (SI of 0.01). The SI was also higher for plants exposed to two days of 375 ppb of O_3 (SI of 1.47) compared to three days of 250 ppb of O_3 (SI of 0.73). This type of response has been reported for pinto beans (14) and tobacco (9), as well as elatior begonia (*Begonia semperflorens-cultorum* Hort.) (20).

These studies demonstrate that acute exposure to O_3 can cause visible injury to buddleia cultivars. Visible foliar injury is particularly detrimental to the use of landscape plants since visual appearance is a primary attribute in selection and use. The most common injury symptom was stippling on the upper leaf surface. As injury increased, stipples coalesced to cover larger areas of the leaf surface. Exposure to O_3 for relatively short periods induced similar visible symptoms among all cultivars tested with various degrees of injury. Cultivars that appear most tolerant to elevated ozone concentrations include 'Empire Blue' and 'Opera', while 'Black Knight', 'Nanho Blue', 'Pink Delight', and 'Royal Red' appear more sensitive. Data collected in the O_3 exposure-response study indicate the importance of both concentration and number of exposures on visible injury. At concentrations currently found in urban areas of the southeastern U. S., injury from acute exposure to O_3 would be relatively minor, but after extended periods of time (chronic exposure) injury could become extensive (3).

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