Nutritive Quality of Highbush Blackberry (*Rubus argutus*) Exposed to Tropospheric Ozone

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Abstract

Numerous studies have examined the impacts of ground level O_3 on plants that are important for human consumption, but native species that are important for wildlife have received less scrutiny. During May–August 2004 we examined the effects of O_3 on biomass production and nutritive quality of highbush blackberry (*Rubus argutus* Link), an important forage for white-tailed deer (*Odocoileus virginianus* Zimmerman) and other herbivorous mammals. Plants were fumigated in open-top chambers with three levels of O_3 in a randomized-block experiment with three replicates of each treatment. Our three experimental treatments were carbon-filtered air, characteristic of clean air quality; nonfiltered air, representative of air quality in Auburn, AL; and air with double (2×) the ambient concentration of O_3. Although biomass production was not influenced by O_3 exposure, nutritive quality of plants was associated negatively with O_3 concentration. Specifically, neutral detergent fiber was greater and relative feed value was less in plants exposed to elevated levels of O_3. Similarly, in vitro dry matter digestibility tended to be less in plants exposed to elevated O_3. Nutritive quality of regrowth vegetation followed a similar pattern, where neutral detergent fiber was greater and relative feed value was less in plants exposed to elevated levels of O_3. These data suggest that elevated levels of ground level O_3 could have implications for diet selection of herbivorous mammals.

Resumen

Numerosos estudios han examinado el impacto del nivel del suelo de O_3 en las plantas que son importantes para el consumo humano; pero las especies nativas que son importantes para la fauna silvestre han recibido menos atención. Durante mayo a agosto del 2004, examinamos los efectos de O_3 sobre la producción de la biomasa y la calidad nutritiva de highbush (*Rubus argutus* Link), una especie forrajera muy importante para los venados de cola blanca (*Odocoileus virginianus* Zimmerman) y de otros mamíferos herbívoros. Las plantas fueron fumigadas en cámaras abiertas con tres niveles de O_3 en un experimento en bloques completamente al azar con tres repeticiones por tratamiento. Nuestros tres tratamientos experimentales fueron aire carbón-filtrado, característico de calidad del aire limpio; aire no-filtrado, que representa la calidad del aire en Auburn, AL; y aire con el doble (2×) de la concentración ambiente de O_3. A pesar de que la producción de la biomasa no fue influenciada por la exposición de O_3, la calidad nutritiva de plantas se asoció negativamente a la concentración O_3. Específicamente, la fibra detergente neutra fue mayor y el valor relativo del alimento fue menor en las plantas expuestas a los niveles elevados de O_3. De la misma manera, la digestibilidad en vitro de la materia seca fue menor en plantas expuestas a niveles elevados de O_3. La calidad nutritiva del rebrote siguió un patrón similar, donde la fibra detergente neutra fue mayor y el valor relativo del alimento fue menor en las plantas expuestas a los niveles elevados de O_3. Estos datos sugieren que los niveles elevados a nivel del suelo de O_3 podrían tener implicaciones para la selección de la dieta de mamíferos herbívoros.

Key Words: ground-level ozone, herbivory, *Odocoileus virginianus*, pollution, white-tailed deer

INTRODUCTION

Every industrialized nation in the world is now exposed to potentially phytotoxic levels of tropospheric (ground-level) ozone (O_3) to a variable extent (Chameides et al. 1994; Ashmore 2005; Ren et al. 2007). High-temperature combustion of fossil fuels creates hydrocarbons and nitrogen oxides, the primary precursors of O_3; in the presence of sunlight, these precursors react to form O_3 (National Center for Environmental Assessment 1996). It has been predicted that O_3 levels will continue to increase at rates of $\sim 0.5–2\% \cdot yr^{-1}$ for the next 50 yr in the midlatitudes of the Northern Hemisphere if current trends continue (Vingarzan 2004). However, metropolitan centers are not the only areas affected by ground-level O_3. Damaging concentrations of O_3 may be transported thousands of miles from industrial areas to rural agricultural and forested lands (Chameides et al. 1994).

Previous research has focused on impacts of O_3 on crops that are important to human food production (Heck et al. 1988). However, recent evidence suggests O_3 can dramatically influence native vegetation (Chappelka et al. 2003; Orendovici et al. 2003; Powell et al. 2003; Lewis et al. 2006). Alterations in biomass production and/or nutrient allocation may directly and/or indirectly influence wildlife communities and species dependent on these plant species to meet life history needs. Specifically, only a few researchers have conducted studies focused on understanding the relationship between ruminant herbivores and forages exposed to elevated O_3 (Krupa et al.

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2004; Lewis et al. 2006). Ozone exposure can cause increases in foliar injury and reductions in biomass yield, as well as alter resource allocation and nutritive quality (Krupa and Manning 1988; Kängasjarvi et al. 1994; Krupa et al. 2004; Szantoi et al. 2007). Although the impact of O_3 on economically important crops has been estimated in the millions of dollars (US Environmental Protection Agency [US EPA] 2006), little has been done to quantify O_3 impacts on native vegetation and subsequent effects on herbivores that utilize those resources. Today, land managers are faced with the challenge of producing crops, wildlife, and forests on the same land base while meeting the demands of the wood products industry and requirements of conservationists (Burger 2006). Compounding this challenge is the fact that rural ecosystems are susceptible to anthropogenic disturbances such as O_3. Because ground-level O_3 has the potential to alter both spatial and absolute availability of nutrients to free-ranging herbivores, it is important that we identify key forage species that may be susceptible to injury from pollutants such as O_3.

Highbush blackberry (*Rubus argutus* Link) is a principal wildlife browse in the southeastern United States and is consumed by a variety of herbivores, including white-tailed deer (*Odocoileus virginianus* Zimmerman) and Eastern cottontails (*Sylvilagus floridanus* Allen). The soft mast is consumed by both mammals and a significant number of song and game birds (Miller and Miller 1999), and Martin et al. (1951) noted the importance of blackberry as escape cover for small mammals. Considering the importance of blackberry to native fauna, it is critical that we link sources of damage and/or disturbance to affected species at multiple levels. Linkages such as these could help explain how negative effects such as reduced biomass yield and nutritive quality could alter utilization of damaged plants by herbivores.

Blackberry as a species is considered to be very sensitive to O_3. Skelly (2000) identified Allegheny blackberry (*Rubus allegheniensis* Porter) as a perennial bioindicator for O_3 in open-top chamber (OTC) investigations in central Pennsylvania. Duchelle et al. (1983) also noted common blackberry (*Rubus* spp.) showed stippling (reddish–purple flecking) on the upper leaf surface when exposed to ambient air with high O_3 concentrations in Shenandoah National Park. Sand blackberry (*Rubus cuneifolius* Pursh) has been shown to have an initial acceleration in flowering when exposed to elevated O_3 levels, but there was no difference in number of fruits among plants exposed to different O_3 concentrations (Chappelka 2002). However, this same study also found that fruits were larger and riper in chambers with ambient air and chambers in which approximately 50% of ambient O_3 had been removed, indicating a potential link between O_3 exposure and reproductive quality. Avoidance of damaged fruits by omnivorous or frugivorous animals that consume blackberries could negatively influence seed dispersal of blackberry, thereby reducing its prevalence in mixed plant communities (Chappelka 2002). This is of primary importance if blackberry species are integral in meeting the nutritional needs of local herbivores. Additionally, litter decomposition of a blackberry (*R. cuneifolius*):bluestem (*Andropogon virginicus* L.) mixture was slower in an ozone environment, primarily due to increases in permangenate lignin in the blackberry (Kim et al. 1998), indicating a shift in resource allocation toward lignin for blackberry. If O_3 effects on reproduction and foliage extend to nutritive quality of exposed blackberry plants, the consequences to plants in a community could be negative in terms of plant abundance and dispersal, as well as to herbivores that consume affected plant species. Herbivores that do not modify their utilization patterns of damaged foliage could encounter greater difficulty in meeting nutritional requirements.

We examined highbush blackberry because of the foliar sensitivity of *Rubus* spp. (Smith et al. 2003; Kohut 2007) to O_3 and its importance as a ruminant forage species in natural plant communities (Miller and Miller 1999). Whereas researchers have documented visible injury and reproductive abnormalities in *Rubus* species exposed to ground-level O_3 (Evans et al. 1996; Barbo et al. 1998; Chappelka 2002; Kohut 2007), effects on nutritive quality and biomass production have yet to be determined. We hypothesized that in order to have an effect on herbivory, previous reports of visible injury should be accompanied by reductions in nutritive quality of this forage. We predicted that declines in biomass yield would occur in tandem with increases in concentrations of refractory cell wall constituents, and decreases in crude protein concentration and *in vitro* dry matter digestibility. Our specific objectives were to determine if exposure to elevated O_3 levels influenced 1) aboveground biomass and 2) nutritive quality of highbush blackberry.

METHODS

Species

Plants were grown from root cuttings collected in March 2004 from a single stand of blackberry at the Louise Kreher Forest Ecology Preserve (lat 32°40′N, long 85°29′W), an Auburn University demonstration forest located approximately 2 km from campus in Lee County, Alabama. Root cuttings of 15–20 cm were placed in 5.68-L pots and filled with a soil mix of peat moss (Pro-Mix®) and sand (1:1 by volume). Plants were maintained at the Plant Sciences Research Center, a controlled-environment facility of the Alabama Agricultural Experiment Station located on the Auburn University campus approximately 1 km from the O_3 fumigation site. Plants sprouted and were allowed to grow unrestricted for 5 wk. Pots were transferred to OTCs on 3 May 2004.

O_3 Exposure System

Nine OTCs, each 4.8 m high and 4.5 m in diameter (Heagle et al. 1989), were used as the exposure system. The system was maintained on a 1.5-ha fenced area on the Auburn University campus (lat 32°36′N, long 85°30′W) in Lee County, Alabama. One month prior to the start of fumigation, the bottom (bare ground) of each chamber was sprayed with glyphosate (Roundup®) and mulched with straw to reduce weed growth. Fifteen potted blackberry plants were placed in each OTC and arranged in triangles of equal surface area that radiated from the center of the chamber. Plants were allowed to acclimate to chamber conditions for 1 wk prior to start of fumigation on 10 May and were fertilized once during this acclimation period with 3 g of controlled-release fertilizer (14–14–14 of $N:P_2O_5:K_2O$). Plants were irrigated twice daily (1100 and 1600 hours) for 10 min per watering episode regardless of ambient rainfall. Ozone was generated by passing pure oxygen

(O_2) through a high-intensity electrical discharge source (Griffin Inc., Lodi, NJ) and applied to the chambers 12 $h \cdot d^{-1}$ (0900–2100 hours), 7 $d \cdot wk^{-1}$. Fans were turned off from 2300 to 0500 hours to permit natural dew formation within the chambers. Ozone concentrations were monitored with the use of a US EPA approved instrument (Thermo Environmental Instruments, Inc., Hopkinton, MA) that was calibrated periodically according to US EPA quality-assurance guidelines. Each monitoring port was read two times per hour on a time-shared basis.

Study Design

The experimental design was a completely randomized block design (three blocks) in which the OTC was the experimental unit. We fumigated blackberry plants with three concentrations of O_3 from 10 May 2004 to 11 August 2004. Carbon-filtered air (CF; $n = 3$), nonfiltered ambient air (NF; $n = 3$), and twice ambient O_3 conditions (2×; $n = 3$) were the treatments utilized during the experiment. The CF treatment removed ~ 50% of the ambient O_3, reducing levels to ~ 15–20 parts per billion (ppb), representing a pristine environment (Vingarzan 2004).

Three primary harvests (P1, 8 June; P2, 8 July; P3, 9 August) and one regrowth harvest (R1, 11 August) were performed at approximately 4 wk, 8 wk, and 12 wk following the initiation of fumigation. Five plants were harvested in each of three periods from all nine chambers, except where mortality limited harvest to four plants. Regrowth forage was harvested 9 wk following the P1 harvest. Plants were cut approximately 3 cm above the soil surface. To simulate forage available to browsing herbivores, all leaf material was separated from the stem by clipping the petioles of leaves approximately 1 cm posterior to the junction of the leaflets. Both leaves and stems were placed in paper bags, and weight of total aboveground biomass was recorded following drying at 50°C.

Dried leaf material from each plant was ground in a Wiley mill to pass a 1-mm screen. Because of limited quantities of leaf material, leaves were then pooled by chamber for laboratory assessment of nutritive quality. We used an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY) to fractionate forage cell-wall constituents into neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin following the procedures of Van Soest et al. (1991). Crude protein (CP) concentration of blackberry leaves was determined with the use of the Kjeldahl procedure (Kjeldahl $N \times 6.25$; Association of Official Analytical Chemists 1990). Association of Official Analytical Chemists (1990) methods were used to determine concentration of dry matter (DM) in harvested material. All samples were analyzed in duplicate. Prediction equations (Linn and Martin 1989) employing NDF and ADF concentrations were used to determine relative feed value (RFV; Rohweder et al. 1978). In vitro dry matter digestibility was determined by the Goering and Van Soest (1970) modification of the Tilley and Terry (1963) procedure. Rumen fluid was obtained 16 November 2004 and 6 January 2005 from two hunter-harvested, female white-tailed deer at the Piedmont Substation, a 5 666-ha tract of land owned by Auburn University approximately 40 km from the main campus. This area was dominated by meadows sown in winter wheat (*Triticum aestivum* L.), bermudagrass (*Cynodon dactylon* L.), and tall fescue (*Festuca arundinacea* Schreber), in a matrix of mixed hardwood-pine forest. Does were aged at 4.5 and 2.5 yr with the use of the tooth replacement and wear method (Severinghaus 1949). To maintain anaerobic conditions, deer rumina were double-tied at both ends and removed immediately after harvest. They were then transferred to a prewarmed insulated chest and transported to the laboratory. Blackberry leaf material was placed in four digestion vessels containing, in duplicate, forages harvested from each of the periods and two blanks (three primary and one regrowth harvest; 20 fiber bags per vessel). Samples were incubated for 48 h at 39°C and then rinsed with distilled water. Fiber bags were frozen until neutral detergent extraction was performed. Filter bags were dried overnight at 100°C in a drying oven, and then were weighed.

Statistical Analysis

For analysis of primary-growth forage data, we used general linear models procedures (PROC GLM; SAS Institute 1990), with OTC within treatments as the error term for treatment main effects. Residual mean square was the error term for harvest period (subplot) and period × treatment interaction. When significant interactions were detected (protected F test), we used multiple comparison tests (PROC GLM, least-squares means, Tukey adjustment) to determine differences among treatments within period and differences among periods within treatment. We tested for differences between regrowth forage and P1 harvested forage with the use of the REPEATED command in PROC MIXED (Littell et al. 1996).

RESULTS

Meteorological Data

Ambient rainfall was less than the 30-yr average (1971–2000; Table 1) for June (− 1.5 cm) and July (− 5.5 cm) and greater than average for August (+ 5.3 cm). Temperatures were similar to 30-yr averages. We found that in this study ambient O_3 concentrations were 10–40 ppb less than in previous years (Muntifering et al. 2000, Powell et al. 2003) at the same location. Mean daily daytime (0900–2100 hours) O_3 concentrations were 21 ppb, 19 ppb, and 25 ppb for Periods 1, 2, and 3, respectively, for the CF treatment (Fig. 1). NF-treated forages were exposed to daytime mean concentrations of 32 ppb, 28 ppb, and 37 ppb of O_3, whereas the 2× treatment registered daily means concentrations of 70 ppb, 69 ppb, and 81 ppb for Periods 1–3.

Table 1. Mean monthly temperature and precipitation for May–August 2004 and 30-yr averages (1971–2000) for Auburn, Alabama (data source: Agricultural Weather Information Service, Inc., Auburn, AL).

	Air temperature (°C)		Precipitation (cm)	
Month	2004	30-yr average	2004	30-yr average
May	23.3	21.4	9.4	9.4
June	25.6	25.1	8.5	10.1
July	27.2	26.6	8.7	14.6
August	25.7	26.3	13.1	7.7

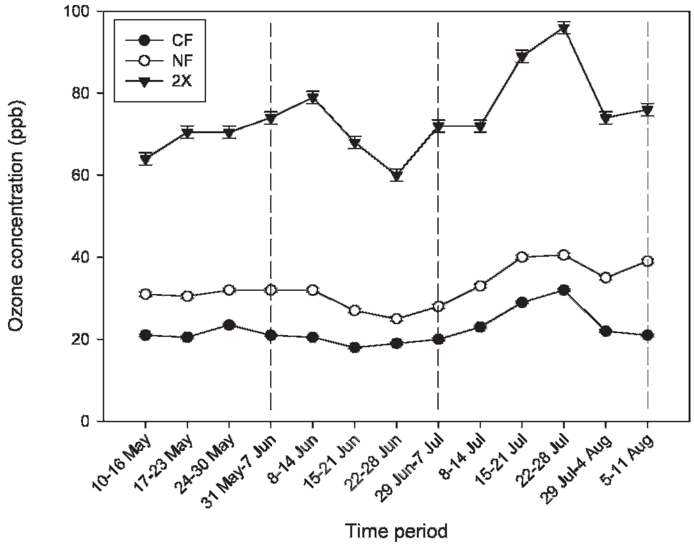

Figure 1. Weekly 12-h (0900–2100) O_3 concentrations (parts per billion [ppb]; CF, carbon-filtered pristine ozone concentrations; NF, nonfiltered ambient air; 2×, twice ambient ozone concentrations) for 10 May–11 August, 2004. Bars represent standard error, and vertical dashed lines represent times of vegetation sampling.

Primary Growth

We detected O_3-treatment effects for NDF ($F_{2,10} = 9.85$; $P = 0.013$) concentration and RFV ($F_{2,10} = 8.83$; $P = 0.016$). However, no O_3-treatment effects ($P \geq 0.073$) were found for biomass yield, in vitro dry matter digestibility (IVDMD), and CP, ADF, and lignin concentrations (Table 2). Pairwise comparisons indicated that concentrations of NDF were 16.6% and 18.9% greater ($P \leq 0.043$) in Period 3 than in Period 1 for 2× and CF plants, respectively (Table 3). Additionally, RFV was decreased ($P \leq 0.050$) by 15.9% and 17.75% from Period 1–3 for 2× and CF-treated plants, respectively. Biomass, and concentrations of NDF, ADF, and lignin increased ($P \leq 0.002$) from Periods 1–3, whereas RFV, IVDMD, and CP concentration declined ($P \leq 0.001$) over the same time period.

Secondary Growth

Growth-stage differences were detected for NDF concentration and RFV between regrowth and primary-growth blackberry forage (Table 3). NDF concentration of 2×-treated regrowth forage increased ($F_{2,6} = 6.47$; $P = 0.032$) by 14.2%, whereas RFV decreased ($F_{2,6} = 5.02$; $P = 0.052$) by 14.1% in relation to primary growth. Across all treatments, NDF, ADF, and lignin concentrations of regrowth were greater ($P \leq 0.003$) than those of primary-growth forage, whereas RFV, IVDMD, and CP concentrations were less ($P \leq 0.001$; Table 3).

DISCUSSION

Period effects were significant for all nutritive quality variables measured, which was expected because cell-wall fractions become more lignified and accumulate in maturing tissues at the expense of nonstructural cell contents (Holechek et al. 2004). We hypothesized a priori that concentration of fiber fractions would increase and that CP concentration, RFV, and IVDMD would decrease over time as a result of natural plant development. Cellular changes in developing tissues increase plant rigidity and structural integrity while simultaneously increasing concentrations of secondary phenolic compounds (i.e., lignin and tannins) that can deter herbivory (Briske 1991). We found that some measures of nutritive quality (NDF concentration and RFV) of highbush blackberry were associated negatively with level of exposure to tropospheric O_3. Van Soest (1994) reported that NDF concentration is associated negatively with voluntary DM intake of forages by ruminants, whereas ADF concentration is inversely related to DM digestibility. RFV integrates intake and digestibility predicted from concentrations of NDF and ADF, respectively, into a single index that can be used to compare nutritive quality among different forages based on their capacity to support intake of digestible DM (Rohweder et al. 1978). Muntifering et al. (2006b) found that alfalfa (*Medicago sativa* L.) exhibits declines in RFV following short-term (1–4 d) exposure to elevated concentrations of O_3. Significant variation in plant response to O_3 fumigation among treatments within an individual period might also reflect qualitative changes in plant physiological and biochemical processes in response to phytotoxic O_3 concentrations. For example, within Period 3, CP concentration and aboveground DM of biomass were associated negatively with increasing O_3 concentration, and NDF, ADF, and lignin concentrations were associated positively with increasing O_3 concentration.

CP concentrations recorded during Periods 1 and 2 (12.9–17.3%) were sufficient for growth and maintenance of white-tailed deer, but CP concentrations during Period 3 (9.1–9.4%) may have been below those required for maximum growth.

Table 2. Table of main effects for highbush blackberry (*Rubus argutus*) primary growth exposed to three ozone concentrations from 10 May to 11 August 2004 and harvested during three periods at 30-d intervals.

	Period		Treatment		Period × treatment	
Main effect	F[1]	*P*	F	*P*	F	*P*
Biomass (g dry matter [DM]/plant)	9.30	< 0.001	0.15	0.868	0.84	0.501
Crude protein (%)	129.90	< 0.001	0.55	0.603	0.27	0.891
Neutral detergent fiber (%)	21.35	< 0.001	9.85	0.013	0.49	0.745
Acid detergent fiber (%)	11.72	0.002	1.48	0.300	0.13	0.970
Acid detergent lignin (%)	29.47	< 0.001	2.51	0.161	0.56	0.696
Relative feed value	23.35	< 0.001	8.83	0.016	0.37	0.829
In vitro DM digestibility (%)	28.77	< 0.001	4.18	0.073	0.76	0.573

[1]Degrees of freedom for all F tests were 2,10.

Table 3. Nutritive quality values for highbush blackberry (*Rubus argutus*) primary growth exposed to three ozone concentrations from 10 May 2004 to 11 August 2004 and harvested during three periods at 30-d intervals.

	Primary growth			Regrowth	
Nutritive quality	Period 1 ($\bar{x}$ [SE[1]])	Period 2 ($\bar{x}$ [SE])	Period 3 ($\bar{x}$ [SE])	$\bar{x}$ (SE)	Significance[2]
Biomass (g dry matter [DM]/plant)					
Carbon filtered (CF)	68.67 A[3]a[4] (5.13)	78.61 Aa (5.52)	78.03 Aa (5.13)	5.64 (0.87)	NS
Nonfiltered (NF)	64.00 Aa (5.13)	91.18 Ba (5.32)	74.07 Aa (5.521)	4.20 (0.83)	NS
2×	63.32 Aa (5.13)	81.53 Aa (5.32)	74.52 Aa (5.32)	5.28 (1.23)	NS
Crude protein (%)					
CF	17.08 Aa (0.26)	13.04 Ba (1.00)	9.19 Ca (0.56)	8.42 (0.72)	NS
NF	17.28 Aa (0.48)	13.78 Ba (0.12)	9.11 Ca (0.14)	8.31 (0.84)	NS
2×	16.78 Aa (0.15)	12.91 Ba (0.94)	9.38 Ca (0.43)	8.70 (0.38)	NS
Neutral detergent fiber (%)					
CF	21.84 Aa (0.59)	24.89 ABa (0.47)	25.97 Ba (0.70)	26.06 (0.66)	*
NF	21.46 Aa (0.30)	24.40 Aa (0.20)	24.08 Aa (0.46)	24.54 (0.48)	*
2×	22.62 Aa (0.31)	25.15 ABa (1.02)	26.38 Ba (0.92)	25.84 (0.26)	*
Acid detergent fiber (%)					
CF	12.60 Aa (0.44)	14.41 Aa (0.71)	14.79 Aa (0.47)	15.76 (0.34)	NS
NF	12.35 Aa (0.26)	13.93 Aa (0.52)	14.42 Aa (0.27)	14.94 (0.16)	NS
2×	12.96 Aa (0.05)	14.13 Aa (0.60)	15.18 Aa (0.80)	14.83 (0.42)	NS
Acid detergent lignin (%)					
CF	2.56 Aa (0.11)	3.49 ABa (0.22)	3.75 Ba (0.08)	3.49 (0.27)	NS
NF	2.69 Aa (0.07)	3.47 ABa (0.12)	3.91 Ba (0.29)	3.14 (0.22)	NS
2×	2.93 Aa (0.09)	4.25 Ba (0.54)	4.19 Ba (0.26)	3.57 (0.14)	NS
Relative feed value					
CF	337.53 Aa (10.84)	290.61 ABa (7.66)	277.61 Ba (8.77)	273.91 (7.81)	*
NF	343.85 Aa (5.62)	297.67 Aa (3.72)	300.27 Aa (6.59)	293.14 (6.21)	*
2×	324.26 Aa (4.56)	289.20 ABa (13.46)	272.60 Ba (11.63)	278.54 (3.83)	*
In vitro DM digestibility (%)					
CF	89.67 Aa (0.80)	86.65 ABa (1.09)	82.77 Ba (1.05)	—	—
NF	90.08 Aa (0.49)	87.43 ABa (0.69	85.26 Ba (0.39)	—	—
2×	89.87 Aa (0.07)	85.25 Ba (1.29)	84.09 Ba (0.97)	—	—

[1]Standard errors of the mean were calculated from $n = 3$ open-top chambers.
[2]Refers to the statistical difference between samples of regrowth and vegetation in Period 1. NS indicates not significant.
[3]Mean values in a row with different upper-case letters are different ($P < 0.05$) within primary growth based on Tukey-adjusted least-squares means.
[4]Mean values in a column within a period with different lower-case letters are different ($P < 0.05$) based on Tukey-adjusted least-squares means.

Asleson et al. (1996) reported CP concentrations of 4.1–5.8% were adequate for maintenance and 9.9–10.1% for growth of white-tailed deer in south Texas. Although not significant, mean concentrations of lignin in forage samples were 12–22% greater for 2×-treated plants than for CF plants within Periods 1–3. Increases in cell wall constituents, especially lignin that can render parts of the cell wall indigestible, can negatively influence utilization of forages by ruminant herbivores (Van Soest 1994, 1996).

These results suggest that, although blackberry exhibits visible injury from exposure to elevated O_3, it may have sufficient phenotypic variability to attenuate its phytotoxic effects. We did not measure visible injury quantitatively, but it was apparent that blackberry plants in 2× chambers had a greater incidence of purplish stippling of the leaves than the controls, which is indicative of O_3 injury. Because blackberry plants occur in a variety of habitats and environmental conditions, it is possible that the concentrations of O_3 these plants experienced were low enough that a more demonstrable physiological response to fumigation was veiled by phenotypic plasticity. Barbo et al. (1998) found that the cane density of sand blackberry increased with increasing ozone concentrations, despite exhibiting severe visible injury symptoms: Cane density of blackberry represented 33–41% of total canopy cover. Reasons for this are unknown, but may be an effect of both ozone and a reduction in interspecific competition for resources (i.e., blackberry was more tolerant of ozone than other species). Manninen et al. (2003) found that magnitude of response to O_3 fumigation of two wild strawberry (*Fragaria vesca* L.) populations was related to the mean O_3 concentration of a particular population's origin. Blackberry plants occurring in areas with high ambient O_3 concentrations may be adapted to those conditions and respond to fumigation less than plants found at lower O_3 concentrations. Ozone concentrations found in the Auburn area are generally consistent with other rural O_3 averages (US EPA 2001), which probably increases the chance of inducing a response at elevated O_3 concentrations. Ambient air conditions in Auburn of 30–50 ppb with occasional episodes above 80–100 ppb (Muntifering et al. 2000; Chappelka 2002; Powell et al. 2003; Lewis et al. 2006) are consistent with current rural O_3 averages in agricultural and forested areas of the southeastern United States (US EPA 2001). The 2× treatment approximately doubled ambient O_3 levels, and was representative of concentrations observed in the vicinity of large metropolitan areas in the southeastern United States, such as Atlanta and Birmingham (Chameides and Cowling 1995).

However, concentrations recorded for the 2004 growing season were below average for this study area (Muntifering et al. 2000, Powell et al. 2003). Nutritive quality has been reported to be affected in some cases without significant effects on visible injury, growth, or biomass (Krupa et al. 2004; Szantoi et al. 2007). It is also possible that the biennial nature of blackberry would cause it to be more sensitive to O_3 injury during its second year or reproductive phase, a period during which resources need to be shunted toward reproduction and fruiting (Skelly 2000; Chappelka 2002).

We tested forage material available in the context of herbivory because of the importance of nutritive quality to plant selection by browsing herbivores, especially white-tailed deer, in the southeastern United States. The majority of previous O_3 research has dealt with food crops of economic importance and forage crops used in production of livestock for human consumption. Little emphasis has been placed on focusing O_3 research on the effects pollution may have on forage quality for free-ranging wildlife (Krupa et al. 2004). A significant increase in NDF concentration, and corresponding decline in RFV, could decrease the amount of digestible forage a ruminant could consume during a given time period. Muntifering et al. (2006a) found that red (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) exhibited significant increases in lignin concentrations and decreases in digestibility following exposure to elevated O_3, and suggested that these changes in plant composition could result in reduced nutritive quality and digestive efficiency in ruminant herbivores. Digestion of cell wall constituents in low-quality forage is lower and slower than that of high-quality forage. As a compensatory measure, ruminants may vary mean retention time in the gastrointestinal tract so they can digest and assimilate a greater proportion of nutrients in low-quality forages and/or select plant parts of greater nutritive quality (Robbins 1993). White-tailed deer may be able to compensate for poor forage quality by increasing retention time in the rumen and, where quantity is not limiting, selecting high-quality plant parts (e.g., new growth and shoots). However, meeting nutritional requirements could become a challenge if sufficient high-quality forage is unavailable. White-tailed deer typically forage heavily during the spring and summer in order to accumulate fat reserves for the winter and breeding season (Mautz 1978). Reducing forage quality, even marginally, could have a negative effect on energy accumulation and storage. Quantity, quality, and rate of passage of digesta from available forages affect the nutritional status of ruminants. Asleson et al. (1997) found that white-tailed deer fed high-protein (16%) diets gained body mass at a greater rate because of increased feed efficiency, not by increasing intake. On southern ranges, where forage quality declines throughout the summer, white-tailed deer metabolize a major portion of lipid reserves during spring and summer, indicating nutritional stress during those seasons (DeLiberto et al. 1989). Although blackberry in the present study did not exhibit significant declines across most forage quality characteristics, the reductions we observed are potentially of a magnitude that could reduce nutritional efficiency of browsing herbivores, especially during summer, when forage quality normally declines.

IMPLICATIONS

Results were consistent with our hypothesis that nutritive quality should be negatively affected in tandem with visible injury. However, biomass yield of exposed forages did not show similar treatment effects. Our results suggest that highbush blackberry exposed to elevated levels of O_3 exhibits declines in nutritive quality without concomitant declines in biomass yield, which may alter foraging strategies of free-ranging ruminants. As forage quality declines, ruminant and nonruminant herbivores may be forced to alter foraging strategies either by selecting different plants or plant parts. If these changes result in declines in overall nutrient availability or patterns (temporal and/or spatial) of nutrient availability, then animal condition could decline. Natural declines in forage quality have the potential to be compounded in areas where O_3 concentrations reach damaging levels, further stressing grazing and/or browsing herbivores that feed on susceptible plant species. Researchers should begin to direct efforts toward understanding nutritional effects of O_3, which may influence herbivory, in addition to visible injury indices and changes in biomass yield.

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