NUTRITIVE QUALITY OF EASTERN GAMAGRASS, BIG BLUESTEM, AND

HIGHBUSH BLACKBERRY EXPOSED TO TROPOSPHERIC OZONE

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NUTRITIVE QUALITY OF EASTERN GAMAGRASS, BIG BLUESTEM, AND HIGHBUSH BLACKBERRY EXPOSED TO TROPOSPHERIC OZONE

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John Steven Lewis

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VITA

John Steven Lewis, son of Steven Dell Lewis and Nancy Stamps Lewis, was born July 9, 1979 in Cookeville, TN. He and his sister, Elizabeth Ann (Libby) Lewis were raised in Cookeville. John attended Cookeville High School and graduated with honors in 1998. He attended David Lipscomb University from August 1998 until May of 2000 before transferring to Tennessee Technological University in August of 2000. He graduated with a Bachelor of Science in Wildlife and Fisheries Sciences in December, 2002. After working as a field technician at Palmer Engineering in Winchester, KY for 4 months, he entered the graduate school at Auburn University in May, 2003. He finished the degree requirements for the Master of Science degree in Wildlife Sciences on May 13, 2005.

THESIS ABSTRACT

NUTRITIVE QUALITY OF EASTERN GAMAGRASS, BIG BLUESTEM, AND HIGHBUSH BLACKBERRY EXPOSED TO TROPOSPHERIC OZONE

John Steven Lewis

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Tropospheric (ground-level) ozone (O_3) is a phytotoxic pollutant that has become widespread in industrialized nations of the world. Ozone is produced by the photo-oxidation of hydrocarbons released into the atmosphere by combustion of fossil fuels. Studies have demonstrated that O_3 can be transported from metropolitan areas to rural areas important to agricultural and forestry practices. Many studies regarding O_3 effects have focused on plants important to human food production or agronomic crops of economic importance. However, relatively little is known about effects of O_3 on native vegetation.

I examined effects of O_3 on 2 warm-season grasses indigenous to the Central Plains and eastern U.S., eastern gamagrass (EGG; *Tripsacum dactyloides*) and big bluestem (BBS; *Andropogon gerardii*), in June – Sept. 2003, and highbush blackberry (*Rubus argutus*) in May – Aug. 2004. I fumigated plants with 3 levels of O_3 in a randomized block experiment with 3 replicates for each treatment. Plants were grown

in open-top chambers with introduced air that had been carbon-filtered (CF), characteristic of clean air quality in the U.S.; non-filtered (NF) air, representative of quality in Auburn, AL; and air with double (2X) the ambient concentration of O_3. Because forage quality can be just as important as quantity, I chose to investigate effects on nutritive quality parameters in addition to biomass yield. Mean 12-hr daytime (0900-2100h) O_3 concentrations were 14, 29, and 61 ppb in 2003 and 22, 32, 74 ppb in 2004 for CF, NF, and 2X treatments, respectively. BBS exhibited little response to my treatments. However, for EGG and blackberry I generally observed decreased nutritive quality as evidenced by increased concentrations of fiber fractions (ADF, NDF, and lignin) and decreased crude protein concentration. Regrowth of both EGG and BBS showed no treatment effects which emphasizes the importance of timing of acute O_3 exposures in relation to physiological stage of plant development. Decreases in nutritive quality parameters observed for EGG and blackberry may have implications affecting plant selection by herbivores. Other grass species have also shown negative O_3-treatment response of similar magnitude that could affect utilization by grazing herbivores.

I also calculated *in vitro* dry-matter digestibilities (IVDMD) for O_3-treated highbush blackberry to determine differences in bovine and deer inocula donors. No differences in mean IVDMD were found between bovine and deer inocula. However, significant variation was found between the 2 bovine donors. Differences in IVDMD for cows were attributed to differences in dietary concentrate and fiber content fed to those animals. My results suggest caution should be taken and donors replicated when seeking suitable domestic surrogates for estimating wild ruminant (e.g. white-tailed deer) IVDMD.

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I. NUTRITIVE QUALITY OF BIG BLUESTEM (*ANDROPOGON GERARDII*) AND EASTERN GAMAGRASS (*TRIPSACUM DACTYLOIDES*) EXPOSED TO TROPOSHPHERIC OZONE

ABSTRACT

Tropospheric (ground-level) ozone (O_3) is a phytotoxic air pollutant widespread in industrialized nations of the world. Ozone is produced by the photo-oxidation of hydrocarbons released into the atmosphere by combustion of fossil fuels. Studies have demonstrated that O_3 can be transported from metropolitan to rural areas important to agricultural and forestry practices. While many reports regarding O_3 effects have focused on vegetation important to human food production or agronomic crops of economic importance, relatively little is known about effects of O_3 on native plant species. I examined effects of ground-level O_3 on 2 warm-season grasses indigenous to the Central Plains and eastern US, eastern gamagrass (EGG; *Tripsacum dactyloides,* Linnaeus) and big bluestem (BBS; *Andropogon gerardii,* Vitman), during the 2003 growing season (June-September). Plants were fumigated with 3 levels of O_3 in a randomized-block experiment with 3 replicates of each treatment. Grasses were grown in open-top chambers with introduced carbon-filtered (CF) air, characteristic of clean air quality in the US; non-filtered (NF) air, representative of quality in Auburn, AL; and air with double (2X) the ambient concentration of O_3. I determined various effects of O_3 on

nutritive quality characteristics in addition to biomass yield. Mean 12-hour (0900 – 2100 hr) O_3 concentrations were 14, 29, and 61 ppb for CF, NF, and 2X treatments, respectively. BBS exhibited little response to my treatments. However, for EGG I generally found decreased nutritive quality as evidenced by increased concentrations of cell wall constituents (ADF, NDF, and lignin) and decreased concentrations of CP. Regrowth of both species exhibited no treatment effects which emphasizes the importance of timing of O_3 exposures in relation to physiological stage of plant development. Decreased nutritive quality parameters observed for EGG may have implications to diet selection and nutrient intake by ruminant herbivores. In addition, range managers can use species-specific information regarding O_3 sensitivity to make decisions about mechanical harvesting or grazing regimes of these forages are growing in areas exposed to elevated O_3 concentrations.

INTRODUCTION

Tropospheric ozone (O_3) is a widespread phytotoxic air pollutant found in all industrialized nations of the world (Chameides et al. 1994). It is created by the photo-oxidation of hydrocarbons and nitrogen oxides that result from combustion processes associated with automobiles, industry, power plants, and other sources of high-temperature combustion of fossil fuels (NCEA 1996). Predictive models indicate that worldwide O_3 concentrations, if left unchecked, will increase at a rate of ~0.5% to 2% / year over the next 50 years (Vingarzan 2004). Once thought to be confined to metropolitan areas, O_3 is now known to be transported far from industrial centers to rural areas where much of the world's agricultural and forested lands are exposed to harmful levels of O_3 (Chameides et al. 1994).

During the past two decades (1980 – 2000), overall O_3 concentrations in the US decreased slightly, based on the former 1-hour and the new 8-hour air quality standards (US EPA 2001). However, not all areas of the country have experienced improvement in air quality. In the southern and north-central regions of the US, ambient O_3 levels increased during the 1990s (US EPA 2001). This increase is due to several factors including increased urbanization, favorable climate and dense vegetative cover, which is the main source of organic hydrocarbons (*e.g.*, isoprene) that are precursors for O_3 production.

Rural locales in the South occasionally experience O_3 episodes above 100 ppb (nl l^{-1}), with typical summer daytime levels (0900 – 1600 hr) averaging > 50 ppb (Chameides and Cowling 1995). There are approximately 100 counties in the South currently classified in non-attainment of the 8-hour National Air Quality Standard for O_3 (US EPA 2001). Due to the regional nature of O_3, ecological effects (reductions in growth, productivity, visible injury, etc.) are not limited to these areas, and have been observed in many other localities currently not in non-attainment (Chappelka and Samuelson 1998; Chappelka and Wergowske 1994).

The physiological and chemical responses of vegetation elicited by exposure to elevated O_3 are varied. Ozone can affect plant tissues, resource acquisition, and nutrient distribution that collectively can reduce biomass yield (Kangasjarvi et al. 1994; Kängasjarvi et al. 1994; Krupa and Manning 1988) and cause economic impacts in the millions of dollars (Adams et al. 1989; Adams et al. 1988). Because of their economic importance, O_3 injury to yield and nutritive quality of grain crops and other food crops has been studied extensively. However, only recently has research been directed towards

understanding effects of O_3 on nutritive quality of forage species in relation to ruminant herbivory (Krupa et al. 2004).

Available data indicate that, within the range of ambient O_3 concentrations typically observed in many areas of the US and Europe, forage biomass from managed cool-season grasslands can be decreased on average by approximately 10% (Fuhrer 1997). Muntifering et al. (2000) and Powell et al. (2003) have recently reported that, based on altered plant cell wall chemistry in select warm-season forages, decreases in nutritive quality (for ruminant herbivores) due to O_3 injury could be expected to approach the same order of magnitude as that observed for losses in biomass yield.

Integration of nutritive quality assessment with information on biomass yield is necessary to more fully characterize potential impacts of O_3 on system total productivity (Krupa et al. 2004). If these concepts from managed agricultural systems can be extended and validated experimentally for native grassland and related managed ecosystems, implications regarding plant-herbivore interactions could be enormous.

Production of crops, food animals, wildlife and forests on the same land base, is receiving considerable interest worldwide as a possible solution to the challenge of sustaining forests as a resource for wood products and an instrument of conservation while simultaneously providing more land for increased food production (Byington and Child 1981). Therefore, understanding how anthropogenic disturbances including O_3 influence these ecosystems is of great importance.

I investigated two native, warm-season grass species commonly planted for both their wildlife value and usefulness as forages for livestock production. Big bluestem (BBS; *Andropogon gerardii*, Vitman) and eastern gamagrass (EGG; *Tripsacum*

dactyloides, Linnaeus) were exposed to three levels of O_3 to quantify the impact of this pollutant on nutritive quality of these species. My specific objectives were to determine whether exposure to elevated levels of O_3 influences 1) nutritive quality and 2) above-ground biomass yield of big bluestem and eastern gamagrass.

MATERIALS AND METHODS

SPECIES DESCRIPTION

Eastern gamagrass and big bluestem are native perennial bunchgrasses; however, little is known about their sensitivity to elevated O_3 concentrations. Warm-season grasses such as these have increased in popularity with government agencies that are now using them in restoration and conservation programs such as the Conservation Reserve Program, Wildlife Habitat Incentive Program, Wetland Reserve Program, and the Environmental Quality Incentives Program (Best et al. 1997). Additional uses of native bunchgrasses include applications such as erosion prevention and nutrient absorption in riparian areas (Ball et al. 2002). Giuliano (2002) has shown that integration of warm-season grasses in a managed pasture setting increased diversity and richness of avian species in those fields. Additionally, these grasses are used to support livestock production because palatability and nutritive quality are high during the summer growing season when cool-season grasses such as fescue are at lower levels of production (Ball et al. 2002; Stubbendieck et al. 1997). Consequently, many farmers and range managers are using these perennial forages for livestock production.

O_3 EXPOSURE SYSTEM

The O_3 exposure system used in this study was maintained on a 1.5-ha fenced area on the Auburn University campus (32° 36' N, 85° 30' W) in Lee County, Alabama. The

exposure system consisted of nine open-top chambers (OTC), each 4.8 m high and 4.5 m in diameter. Each aluminum-framed chamber was surrounded with clear plastic which was double-layered and perforated at the bottom for introduction of air by fans (Heagle et al. 1989). The floor of each chamber was sprayed with glyphosate (Roundup®) and mulched with straw to reduce weed growth. Both grass species were seeded in cone-tainers between 16 – 21 April 2003 at the Plant Sciences Research Center greenhouse, a facility of the Alabama Agricultural Experiment Station located on the Auburn University campus approximately 1 km from the O_3 treatment site. Plants were thinned to two/cone-tainer 6 May and then to one plant /cone-tainer on 20 May. Plants were watered daily and fertilized once after the final thinning with a commercial fertilizer (4.5g 15:16:17 of N:P_2O_5:K_2O per 3.8 L of water). Plants were transferred to pots (5.68 L) filled with a Norfolk sandy loam soil (Fine-loamy, kaolinitic, thermic, Typic Kankiudults) on 2 June. Fifteen pots of each species were placed in each chamber and arranged by species into two triangular sections of equal surface area. Plants were allowed to acclimate to chambers for 1 week prior to the beginning of fumigation on 9 June. Plants were irrigated twice daily (1100 and 1600 h) for 10 minutes/watering episode regardless of ambient rainfall, and fertilized once during the acclimation period with 3 g of controlled-release fertilizer (14-14-14 of N: P_2O_5: K_2O). 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 d^{-1} (0900—2100 h), 7 d wk^{-1}. Fans were turned off from 2300 – 0500 hours to permit natural dew formation within the chambers. Ozone concentrations were monitored using an US EPA approved instrument (Thermo Environ. Instr., Inc., Hopkinton, MA). Monitoring is time-shared so

that each monitoring port is read two times per hour. Instruments were periodically calibrated according to US EPA quality assurance guidelines.

STUDY DESIGN

To determine how O_3 affected the nutritive quality of my forages, I fumigated plants with three different O_3 levels from 9 June until 4 September 2003. Treatments were carbon-filtered air (CF; $n = 3$), ambient air (AA; $n = 3$), and two times ambient conditions (2X; $n = 3$). The CF treatment removed ~50% of the ambient ozone, reducing levels to ~14-16 ppb, representing a pristine environment (Lefohn et al. 1990). Ambient air conditions in Auburn of ~30-50 ppb with occasional episodes above 80-100 ppb (A.H. Chappelka, personal communication) are consistent with current rural ozone averages in agricultural and forested areas of the southeastern US (US EPA 2001). The 2X treatment approximates a doubling of ambient O_3 levels, and is representative of concentrations observed in the vicinity of large metropolitan areas in the Southern US, such as Atlanta and Birmingham (Chameides and Cowling 1995).

Five plants of each species were harvested in each period from all nine chambers. In a few cases, I could only harvest 4 plants/chamber due to mortality. Three primary harvests (P1, 8 July; P2, 7 Aug.; P3, 4 Sept.) and two regrowth harvests (R1, 8 Aug.; R2, 5 Sept.) were performed at approximately 4, 8, and 12 weeks following start of fumigation (early, mid-, and late season). Regrowth forage was harvested twice (at 8 and 12 weeks following start of fumigation) from the same plants harvested after 4 weeks (P1) of fumigation. Each plant was harvested at ~15 cm above ground.

Harvested material was oven-dried at 50^{o}C to a constant weight and above-ground biomass recorded. Plants from each chamber were then pooled and were ground in a

Wiley mill to pass a 1-mm screen. Forage cell wall constituents were sequentially fractionated to NDF, ADF, and lignin according to procedures of Van Soest et. al. (1991) using an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY). Crude protein (CP) concentrations of forage samples were determined using the Kjeldahl procedure [Kjeldahl N × 6.25; (AOAC 1995)], and dry matter (DM) was analyzed according to the Association of Analytical Chemists (1995). Relative feed value [RFV; (Rohweder et al. 1978)] was calculated from concentrations of NDF and ADF using prediction equations of Linn and Martin (1989).

STATISTICAL ANALYSIS

The experimental design was a completely randomized split-plot where species is the split-plot and OTC is the main-plot factor. For analysis of variance (ANOVA) of primary-growth forage data we used PROC GLM (SAS 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 appropriate, I used multiple comparison tests (PROC GLM, least squares means, Tukey adjustment) to determine differences among treatments within period and differences among periods within treatment. Because of physiological changes in growth habit initiated by the first cutting, data from regrowth forages were tested separately from P1 forage, using the REPEATED command in PROC GLM.

RESULTS

METEOROLOGICAL DATA

Mean monthly temperatures for June – September 2003 tended to be lower (-0.72°C June, -1.1°C July, -0.27°C Aug, -1.00°C Sept.) than the 30-year monthly averages (1971

– 2000) for the study area (Table 1). Mean monthly rainfall exceeded 30-year averages by 11.73 cm, 11.99 cm, and 5.82 cm for June, July, and August, respectively. September was 1.75 cm below average in rainfall. Mean 12-hr (0900 – 2100 hr) O_3 concentrations over the entire experiment were 14, 29, and 61 ppb, respectively, for CF, NF and 2X treatments (Fig. 1). Within Period 1, hourly peak O_3 exposures were 43, 71, and 169 ppb for CF-, NF-, and 2X-treated plants. CF, NF, and 2X hourly O_3 peaks occurred at 45, 51, and 112 ppb, respectively for Period 2. Within Period 3, hourly peak exposures were 50, 60, and 113 ppb for CF, NF, and 2X, respectively.

EASTERN GAMAGRASS

Both biomass and crude protein were significantly influenced by period of harvest (Table 2). For all treatments, biomass yield increased (1,266 – 1,433 %) from Period 1-3 and CP decreased from 48 – 66 % during Periods 1-3. While I found no differences among treatments for concentration of CP within any period, I did observe that plants treated with 2X O_3 had greater biomass than those exposed to CF air during Periods 2 and 3 (Table 3).

I found period × treatment interactions for concentrations of NDF, ADF, and lignin, and for RFV. Within Period 3 greater concentrations of NDF (12.97%), ADF (14.61%), and lignin (48.52 %) were observed for 2X plants compared with CF plants: there were no differences among Periods 1 and 2. There was a general tendency for NDF and ADF to increase from Periods 1 – 3, and for lignin to decrease from Periods 1 – 3 for most treatments. RFV was less (16.74%) for 2X plants than CF plants within Period 3 and decreased among all treatments as time progressed.

There was a treatment effect ($F_{2,6} = 5.34$; $P = 0.047$) for regrowth of biomass for EGG. Biomass tended to decline (29.78 – 37.68%) for 2X and NF plants, however, CF plants did not show a similar decline. I found no treatment effect ($P \geq 0.050$) for any other response variable measured for regrowth of EGG.

BIG BLUESTEM

Period of harvest had a significant effect on primary growth of BBS for all variables measured (Table 4). Biomass increased for NF (2,345%) and CF (1,727%) plants, but not 2X-treated plants (Table 5). CP decreased (48.15 – 60.16%) among all treatments from Period 1 –3. I detected no differences for NDF concentrations among any treatments. ADF concentration of CF plants was 28.39% greater for Period 3 than Period 1. Lignin concentrations increased 102.14% between Periods 2 – 3 for CF plants, but, increases were not significant for 2X and NF plants. RFV tended to decline (11.46 – 17.99%) among treatments from Period 1 – 3, but multiple comparisons yielded no differences among periods for any treatment.

There was a period effect ($F_{1,6} = 7.15$; $P = 0.037$) for RFV of BBS regrowth. Marginal period effects were detected for concentration of NDF ($F_{1,6} = 5.52$; $P = 0.057$). However, I found no treatment effect ($P \geq 0.050$) for any response variables measured for regrowth of BBS.

DISCUSSION

Ozone concentrations throughout the 2003 growing season were lower than previously reported (Muntifering et al. 2000; Powell et al. 2003) for my study area, yet, treatment effects were still observed for EGG. I generally found increased concentrations of cell wall constituents (ADF, NDF, and lignin) decreased concentration of CP, and decreased

RFV as exposure time increased. While period effects for concentrations of cell wall constituents, crude protein, and of biomass yield were expected as a result of natural plant development, analysis within periods revealed noteworthy differences among treatments for EGG. Treatment effects within Period 3 were greatest and no general treatment effects within Periods 1 and 2 were observed. Within the third period, treatments were significantly different for all variables in EGG except CP concentration. Plants treated with 2X O_3 had elevated NDF, ADF, and lignin concentrations, as well as greater biomass yield, while values for RFV and CP concentration were less. The observed decreases in CP concentration and RFV and increases in concentration of cell wall fractions for EGG in successive periods are similar to cumulative effects reported for other grass species (Muntifering et al. 2000). However, elevated fiber fraction concentrations and decreased CP concentration and RFV with greater above-ground biomass is a relatively novel finding. Generally, decreases in biomass are observed when O_3-sensitive plants are chronically exposed to O_3 (Pleijel and Danielsson 1997), and nutritive quality characteristics suffer as a result. These results suggest that, at least for EGG, nutritive quality can be affected without a concomitant decline in biomass. Increased biomass may be a short-term stress response resulting in increased photosynthate allocation to shoots (Miller 1988) although it is unlikely that this response could be sustained over a longer exposure period. The lack of response for BBS may be attributed to relatively low O_3 exposures during the 2003 growing season, or, BBS may be less sensitive to O_3 than EGG. The period effects observed for BBS may be explained by development of tissues as result of natural plant development. Few acute exposure events may explain a general lack of treatment difference within Periods 1 and 2.

However, the influence of moderate O_3 exposures over nine weeks was substantial enough to induce a cumulative response by Period 3 for EGG.

A number of factors, including stage of plant development, relative humidity, soil moisture, and concentration gradient of O_3 (Heagle et al. 1988; Pleijel et al. 1996; Runeckles and Krupa 1994; Temple et al. 1985) interact to determine plant response. Both EGG and BBS are C_4 plants and were expected to have intrinsically lower stomatal conductance compared with C_3 plants (Volin et al. 1998). However, by keeping the vegetation well-watered and in a nutrient-rich growth medium, I could have potentially enhanced plant response to O_3 by maximizing gas uptake (Muntifering et al. 2000). It has been acknowledged that native plants may vary in response to O_3 as a result of environmental changes that alter gas uptake in the leaves (Manning 2003). However, I chose to hold fixed as many climatic variables as possible to remove confounding factors.

Timing between O_3 episodes and physiological stage of development of harvested forages is considered to be critical in extent of injury caused by fumigation. Yield losses in crops such as wheat that are allowed to grow until senescence are generally greater than losses in pastures which are continually cut or grazed (Pleijel et al. 1996). As O_3 sensitivity increases with leaf age (Karlsson et al. 1995), it is to be expected that younger leaves will show less of a response to fumigation. My results showing little treatment effect on regrowth support this idea. These observations promote the hypothesis that cutting/grazing coupled with harvest timing and frequency can have effects on biomass yield and nutritive quality of managed forage systems (Ashmore and Ainsworth 1995; Muntifering et al. 2000; Wilbourn et al. 1995). By timing a harvest just prior to an acute,

predictable O_3 episode, range managers could potentially avert damage to the standing crop while decreasing O_3 damage on regrowth material.

Few studies have actually quantified losses attributed to ozone pollution in terms of nutritive quality. Selection of plant parts having greater nutritional value is common among browsing herbivores. Bolsinger (1991) observed ornamental milkweed (*Asclepias curassavica*, L.) exposed to elevated O_3 and found interaction between concentration and exposure time. They concluded that changes in metabolites could interact with plant nutrition in ways that might alter insect herbivory. I believe that a similar interaction could occur in systems with domestic and wild ruminants as well as other mammalian herbivores. In addition, plants high in defensive compounds (i.e., phenolics) and indigestible material (i.e., lignin) will be less likely to be selected by herbivorous species. Because both native herbivores and livestock have been shown to select forage species based on a wide variety of criteria (Robbins 1993; Stephens and Krebs 1986; Van Soest 1994), it is critical that we understand how O_3 influences nutritive parameters of forages.

As intake and rate of passage are limiting factors controlling ruminant digestion, lack of biomass reduction with reduced nutritive value creates a situation whereby the ruminant stomach is filled to capacity without realizing full nutritive potential. Powell et al. (2003) found greater concentrations of fiber fractions and decreased in vitro digestibility in 2X and NF than in CF primary growth of sericea lespedeza (*Lespedeza cuneata*), resulting in approximately 7% decline in nutritive quality. Additionally, they found < 2% decrease in quality of little bluestem (*Schizachyrium scoparium*), but concluded that both species would be affected enough to decrease utilization by ruminants. They found no difference among treatments in regards to DM yield for

primary-growth. I found a 30 % reduction in nutritive quality of eastern gamagrass but found an increase in biomass in Periods 2 and 3, suggesting that ruminants consuming these forages may be receiving less nutrition per unit of biomass consumed than from forages exposed to lower levels of O_3.

CONCLUSIONS

While most studies on native species have focused on visible injury (Bussotti et al. 2003; Chappelka et al. 2003), scientists must become increasingly aware of how O_3 affects nutritive quality of forages. Total biomass will always be important; however, wildlife and domestic livestock select forages and plant parts based on nutritional quality as well as quantity (Robbins 1993; Stephens and Krebs 1986; Van Soest 1994). Nutritive quality affected by O_3 could have ramifications for plant selection by herbivores (Muntifering et al. 2000). Furthermore, range managers can use information on O_3 response of specific forage species by altering their cutting regime to maximize nutritional value while decreasing O_3 damage to susceptible species.

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Figure 1. Mean weekly 12-hr (0900 – 2100) O_3 concentrations for 9 June - 4 September, 2003. Bars represent standard error. Arrows indicate harvest week.

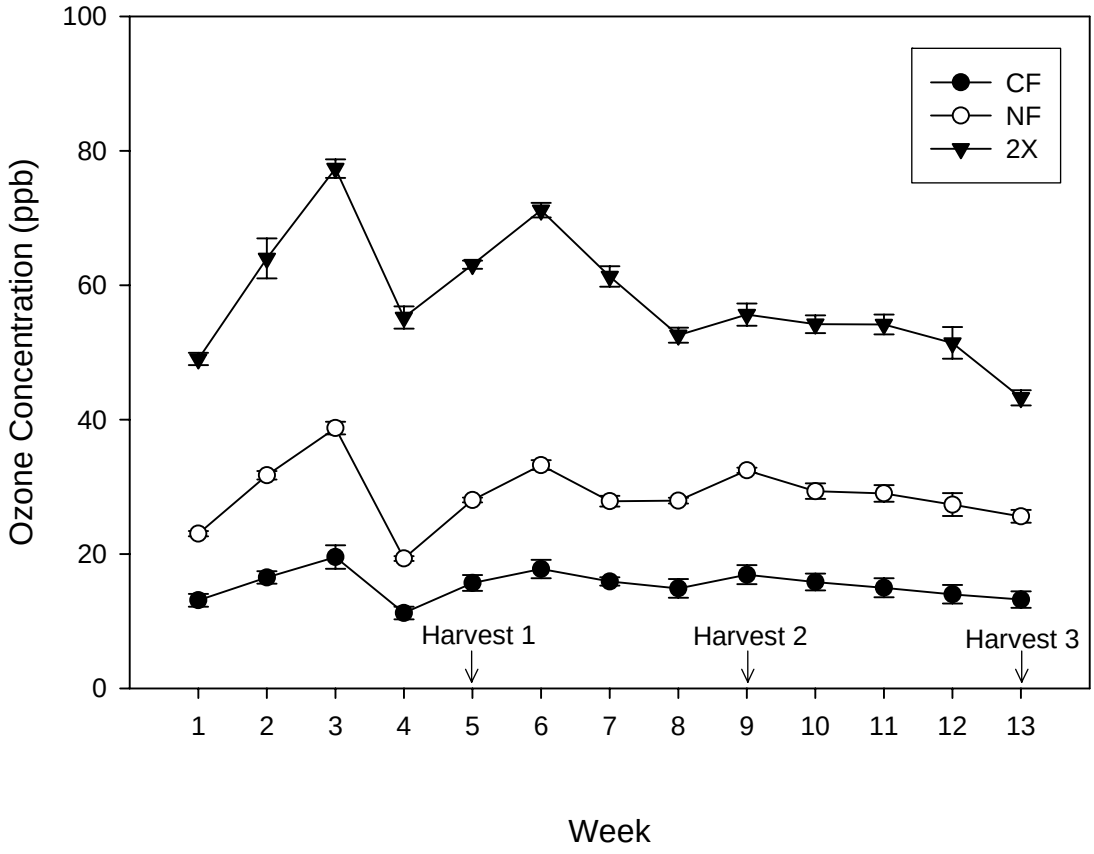
100
80
60
40
20
0
Ozone Concentration (ppb)
CF
NF
2X
Harvest 1
Harvest 2
Harvest 3
1
2
3
4
5
6
7
8
9
10
11
12
13
Week

Table 1. Mean monthly temperature and precipitation for June-September 2003 and 30-year averages (1971 – 2000) for Auburn, AL (Data source: National Climatic Data Center; http://www.ncdc.noaa.gov/oa/ncdc.html).

Month	Air temperature (°C)		Precipitation (cm)	
	2003	30-year mean	2003	30-year mean
June	24.4	25.1	21.8	10.1
July	25.5	26.6	26.5	14.6
August	26.1	26.3	13.5	7.7
September	22.7	23.7	7.7	9.4

Table 2. Period, treatment, and period × treatment main effects for eastern gamagrass (*Tripsacum dactyloides*) primary growth exposed to three ozone concentrations from 9 June – 4 September 2003 and harvested during 3 periods at 30-day intervals.

	Period			Treatment			Period × Treatment		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Biomass (g DM / plant)	2, 10	68.50	<0.001	2, 10	6.66	0.030	2, 10	2.42	0.105
Crude Protein (%)	2, 10	65.32	<0.001	2, 10	1.37	0.323	2, 10	1.28	0.330
Neutral Detergent Fiber (%)	2, 10	67.44	<0.001	2, 10	3.95	0.080	2, 10	11.04	0.001
Acid Detergent Fiber (%)	2, 10	71.48	<0.001	2, 10	4.96	0.054	2, 10	5.02	0.013
Acid Detergent Lignin (%)	2, 10	16.19	<0.001	2, 10	2.72	0.144	2, 10	4.26	0.023
Relative Feed Value (%)	2, 10	77.33	<0.001	2, 10	3.38	0.104	2, 10	7.99	0.002

Table 3. Nutritive quality values for eastern gamagrass (*Tripsacum dactyloides*) primary growth exposed to three ozone concentrations from 9 June – 4 September 2003 and harvested during 3 periods at 30-day intervals.

	Period 1 (30 days)		Period 2 (60 days)		Period 3 (90 days)	
	$\bar{x}$	SE[a]	$\bar{x}$	SE	$\bar{x}$	SE
Biomass (g DM / plant)						
CF	0.90 A[b]a[c]	0.21	7.33 ABa	3.54	13.80 Ba	1.68
NF	1.18 Aa	0.23	11.13 Bab	2.83	16.13 Bab	2.08
2X	1.52 Aa	0.09	17.47 Bb	2.28	23.20 Bb	1.20
Crude Protein (%)						
CF	18.87 Aa	2.01	13.29 ABa	1.27	9.78 Ba	1.65
NF	20.34 Aa	0.86	10.65 Ba	0.22	7.58 Ba	0.72
2X	21.49 Aa	0.27	11.57 Ba	1.63	7.23 Ba	0.38

Neutral Detergent Fiber (%)						
CF	62.76 Aa	0.82	64.41 Aa	1.25	65.24 Aa	1.33
NF	59.96 Aa	0.77	65.85 Ba	0.53	68.22 Ba	0.88
2X	59.68 Aa	0.58	67.26 Ba	1.56	73.70 Cb	1.00
Acid Detergent Fiber (%)						
CF	27.50 Aa	0.41	29.14 Aa	1.21	32.79 Ba	0.89
NF	26.30 Aa	0.70	31.77 Ba	0.30	33.49 Bab	1.41
2X	25.89 Aa	0.28	32.90 Ba	1.06	37.58 Cb	0.70
Acid Detergent Lignin (%)						
CF	2.65 Aa	0.18	1.64 Ba	0.10	1.69 Ba	0.20
NF	2.34 Aa	0.13	1.61 Ba	0.04	1.93 Ba	0.10
2X	2.25 ABa	0.23	1.92 Aa	0.16	2.51 Bb	0.13
Relative Feed Value (%)						
CF	100.07 Aa	1.77	95.73 Aa	3.08	90.45 Aa	2.77
NF	106.20 Aa	2.09	90.64 Ba	1.03	85.71 Bab	2.52
2X	107.18 Aa	1.36	87.66 Ba	3.11	75.31 Cb	1.69

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[a] Standard errors of the mean were calculated from $n = 3$ open-top chambers.

[b] Mean values in a row with different upper case letters are different ($\underline{P} < 0.05$) based on Tukey-adjusted least squares means.

[c] Mean values in a column within a period with different lower case letters are different ($\underline{P} < 0.05$) based on Tukey-adjusted least squares means.

Table 4. Period, treatment, and period × treatment main effects for big bluestem (*Andropogon gerardii*) primary growth exposed to three ozone concentrations from 9 June – 4 September 2003 and harvested during 3 periods at 30-day intervals.

	Period			Treatment			Period × Treatment		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Biomass (g DM / plant)	2, 10	17.24	<0.001	2, 10	0.270	0.771	2, 10	0.75	0.576
Crude Protein (%)	2, 10	35.60	<0.001	2, 10	1.48	0.284	2, 10	0.20	0.932
Neutral Detergent Fiber (%)	2, 10	8.38	0.007	2, 10	2.15	0.179	2, 10	0.31	0.865
Acid Detergent Fiber (%)	2, 10	18.77	<0.001	2, 10	1.85	0.219	2, 10	0.73	0.592
Acid Detergent Lignin (%)	2, 10	14.84	0.001	2, 10	1.33	0.316	2, 10	1.31	0.332
Relative Feed Value (%)	2, 10	14.13	0.001	2, 10	2.04	0.193	2, 10	0.54	0.711

Table 5. Nutritive quality values for big bluestem (*Andropogon gerardii*) primary growth exposed to three ozone concentrations from 9 June – 4 September 2003 and harvested during 3 periods at 30-day intervals.

	Period 1 (30 days)		Period 2 (60 days)		Period 3 (90 days)	
	$\bar{x}$	SE[a]	$\bar{x}$	SE	$\bar{x}$	SE
Biomass (g DM / plant)						
CF	1.16 A[b]a[c]	0.32	10.35 ABa	4.50	21.20 Ba	4.70
NF	0.82 Aa	0.22	9.67 ABa	1.85	20.05 Ba	4.32
2X	0.95 Aa	0.26	12.65 Aa	5.51	12.93 Aa	4.24
Crude Protein (%)						
CF	15.43 Aa	1.43	10.70 ABa	1.18	8.00 Ba	0.89
NF	14.76 Aa	0.22	9.61 ABa	0.87	5.88 Ba	0.92
2X	14.25 Aa	0.74	10.72 ABa	1.70	6.90 Ba	0.81
Neutral Detergent Fiber (%)						
CF	65.75 Aa	0.06	65.68 Aa	0.81	71.92 Aa	2.69
NF	66.46 Aa	0.56	67.67 Aa	0.63	72.36 Aa	1.10
2X	66.04 Aa	0.69	67.42 Aa	1.74	69.46 Aa	1.56

Acid Detergent Fiber (%)						
CF	30.54 Aa	0.13	32.59 ABa	1.25	39.21 Ba	2.46
NF	30.69 Aa	0.86	35.66 Aa	0.42	39.69 Aa	1.68
2X	30.75 Aa	1.21	34.80 Aa	1.76	36.62 Aa	1.74
Acid Detergent Lignin (%)						
CF	1.94 ABa	0.07	1.40 Aa	0.09	2.83 Ba	0.40
NF	1.91 Aa	0.07	1.67 Aa	0.21	2.71 Aa	0.19
2X	1.96 Aa	0.10	1.68 Aa	0.15	1.98 Aa	0.16
Relative Feed Value (%)						
CF	92.12 Aa	0.21	90.01 Aa	2.23	75.86 Aa	5.16
NF	91.00 Aa	1.69	84.04 Aa	1.22	74.63 Aa	2.83
2X	91.52 Aa	2.31	85.49 Aa	3.98	81.03 Aa	3.70

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[a] Standard errors of the mean were calculated from $n = 3$ open-top chambers.

[b] Mean values in a row with different upper case letters are different ($\underline{P} < 0.05$) based on least squares means.

[c] Mean values in a column within a period with different lower case letters are different ($\underline{P} < 0.05$) based on least squares means.

II. NUTRITIVE QUALITY OF HIGHBUSH BLACKBERRY (*RUBUS ARGUTUS*) EXPOSED TO TROPOSHERIC OZONE

ABSTRACT

Tropospheric ozone (O_3) is the most significant phytotoxic pollutant in the United States, and concentrations are expected to rise if current trends continue. To date, the greatest body of research on O_3 has dealt with the effect of this photochemical pollutant on crops important to human food production. Very few studies have examined effects of O_3 on native plant communities, and an even smaller number of studies have investigated consequences of O_3 exposure to native plant nutrition. Nutritive quality is important to both grazing and browsing herbivores, therefore, I examined variables that reflect plant nutrition including fiber fractions, crude protein, and relative feed value. In addition, I recorded changes in biomass over time with varying O_3 concentrations. Because little is known about the effects of ozone on native species, I examined the influence of 3 concentrations of ozone on highbush blackberry (*Rubus argutus*), a browse heavily utilized by white-tailed deer (*Odocoileus virginianus*) in the southeastern US, during the summer of 2004 (10 May – 11 August). I utilized a completely randomized block (3 blocks) design with 3 air treatments within each block. Plants were grown in open-top chambers (OTC) with introduced carbon-filtered (CF) air, characteristic of clean air quality in the US; non-filtered (NF) air, representative of quality in Auburn, AL; and air

with double (2X) the ground-level concentration of O_3. Mean 12-hr daytime (0900 – 2100) O_3 concentrations were 22, 32, 74 ppb for CF, NF, and 2X treatments, respectively. To detect the effect of O_3 on forage quality, I measured nutritive quality parameters as well as above-ground biomass yield. In addition, I determined *in vitro* dry-matter digestibility (IVDMD) using rumen fluid from 2 hunter-harvested, female white-tailed deer. Neutral detergent fiber (NDF) concentration increased 17% from Period 1 – 3 for 2X-treated plants, while relative feed value declined 16% across the same time period for primary growth. Likewise, NDF concentration increased 14% and RFV decreased 14% for secondary growth of blackberry compared to the first primary harvest. Acid detergent fiber (ADF), lignin, and crude protein (CP) concentrations, as well as above-ground biomass and IVDMD exhibited no O_3-treatment response. However, ADF and lignin concentrations increased and IVDMD and CP concentration decreased as plants matured. While O_3 treatments did not illicit a substantial response in nutritive quality, my results suggest ozone can negatively affect plant value by reducing biomass and decreasing nutritive quality parameters, and, most notably, decreasing nutritive quality in the absence of biomass reductions. I hypothesize that reduced nutritive value due to O_3 exposures could influence feeding patterns and selectivity of both wild and domestic herbivores.

INTRODUCTION

Every industrialized nation in the world experiences exposure to elevated tropospheric (ground-level) ozone (O_3) to a variable extent (Chameides et al. 1994). High-temperature combustion of fossil fuels creates hydrocarbons and nitrogen oxides, the primary precursors of O_3; when oxidized by sunlight, these precursors form O_3 (NCEA 1996). It

has been predicted that O_3 levels will continue to increase at rates of ~0.5% to 2%/year for the next 50 years 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 important to human food production (Heck et al. 1988). However, preliminary evidence suggests that O_3 can also dramatically influence native vegetation (Chappelka et al. 2003; Orendovici et al. 2003; Powell et al. 2003). Alterations in biomass production and/or nutrient allocation may directly and indirectly influence wildlife communities and species that are dependent upon 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. 2004). Foliar injury to plant tissues induced by O_3 exposure can cause reductions in biomass yield as well as alter resource allocation and nutritional quality (Kängasjarvi et al. 1994; Krupa and Manning 1988). While the impact of O_3 on economically important crops has been placed in the millions of dollars (Adams et al. 1989; Adams et al. 1988), little has been done to quantify O_3 impacts on native vegetation and subsequent effects on ruminant 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 demands of the wood products industry and requirements of conservationists (Byington and Child 1981). 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 important forage species that may be susceptible to injury from pollutants such as O_3.

Highbush blackberry (*Rubus argutus*) is a principal wildlife browse in the southeastern United States and is consumed by a variety of herbivores including white-tailed deer (*Odocoileus virginianus*) and Eastern cottontails (*Sylvilagus floridanus*). Additionally, the soft mast is consumed by both mammals and a significant number of song and game birds (Miller and Miller 1999). Additionally, 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 or nutritive quality could change utilization of damaged plants by herbivores.

Blackberry species are considered to be very sensitive to O_3. Skelly (2000) identified Allegheny blackberry (*R. allegheniensis*) as a perennial bioindicator for O_3 in open-top chamber investigations in central Pennsylvania. Duchelle et al. (1983) also noted that 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 (*R. cuneifolius*) 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 O_3 concentrations (Chappelka 2002). However, this same study also found fruits were larger and riper in chambers with ambient air and chambers which removed approximately 50% of ambient O_3 concentration indicating a

potential link between fumigation and reproductive quality. Avoidance of damaged fruits by omnivorous or frugivorous animals that consume blackberries could negatively influence seed dispersal of blackberry and cause them to become less prevalent in mixed plant communities. This is of primary importance if that particular plant is integral to meeting the nutritional needs of local herbivores. 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 avoid or change usage patterns of damaged foliage could encounter greater difficulty in meeting nutritional requirements.

While researchers have documented visible injury and reproductive abnormalities in *Rubus* species exposed to ground-level O_3 (Barbo et al. 1998; Chappelka 2002; Chappelka and Wergowske 1994; Evans et al. 1996), effects on nutritive quality and biomass production have yet to be determined. I predicted declines in biomass yield would occur in tandem with declines in concentrations of soluble cell contents, crude protein, and in *in vitro* dry matter digestibility. My specific objectives were to determine if exposure to elevated O_3 levels influenced 1) above-ground biomass and 2) nutritive quality of highbush blackberry (*R. argutus*).

MATERIALS AND METHODS

SPECIES

I examined highbush blackberry because of its foliar sensitivity to O_3 (Duchelle et al. 1983; Jacobson and Hill 1970; Skelly 2000) and its importance as a forage species in natural plant communities (Miller and Miller 1999). Plants were grown from root

cuttings collected in March 2004 from a single stand of blackberry at the Louise Kreher Forest Ecology Preserve (32º 40'04"N, 85º 29'12"W), an Auburn University demonstration forest approximately located 2 km from campus in Lee County, AL. Root cuttings of 15 – 20 cm were placed in 5.68-L pots and filled with a soil-less mix of peat moss (Pro-Mix®) and sand (1:1 by volume). Plants were maintained in the Plant Sciences Research Center greenhouse, a facility of the Alabama Agricultural Experiment Station located on the Auburn University campus approximately 1 km from the O_3 treatment site. Plants sprouted and were allowed to grow unrestricted for five weeks. Pots were transferred to open-top chambers (OTC) on 3 May.

O_3 EXPOSURE SYSTEM

Nine OTC, each 4.8 m high and 4.5 m in diameter, were used as the exposure system. The system was maintained on a 1.5-ha fenced area on the Auburn University campus (32° 36' N, 85° 30' W) in Lee County, Alabama. Each aluminum-framed chamber was surrounded with clear plastic which was double-layered and perforated at the bottom for introduction of air by fans (Heagle et al. 1989). Prior to the start of fumigation, the floor of each chamber was sprayed with glyphosate (Roundup®) and mulched with straw to reduce weed growth. Fifteen 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 one week prior to the start of fumigation on 10 May, and they 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 hr) for 10 minutes/watering episode, irrespective of ambient rainfall. Ozone was generated by passing pure oxygen through a high-intensity electrical

discharge source (Griffin Inc., Lodi, NJ) and applied to the chambers 12 hr d^{-1} (0900 – 2100 hr), 7 d wk^{-1}. Fans were turned off from 2300 – 0500 hr to permit natural dew formation within the chambers. Ozone concentrations were monitored using a US EPA-approved instrument (Thermo Environ. Instr., 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 (3 blocks) in which OTC was the experimental unit. I fumigated blackberry plants with three concentrations of O_3 from 10 May – 11 August 2004. Carbon-filtered air (CF; $n = 3$), non-filtered air (NF; $n = 3$), and twice-ambient conditions (2X; $n = 3$) were the treatments utilized during the experiment. The CF treatment removed ~50% of the ambient O_3, reducing levels to ~ 15-20 ppb, representing a pristine environment (Lefohn et al. 1990). Average ambient air conditions in Auburn of 30-50 ppb (12 h) with occasional episodes above 80-100 ppb (Chappelka, personal communication) are consistent with current rural O_3 averages in agricultural and forested areas of the southeastern US (US EPA 2001). The 2X treatment approximately doubled ambient O_3 levels, and was representative of concentrations observed in the vicinity of large metropolitan areas in the southeastern US such as Atlanta and Birmingham (Chameides and Cowling 1995).

Three primary harvests (P1, 8 June; P2, 8 July; P3, 9 Aug.) and one regrowth harvest (R1, 11 Aug.) were performed at approximately 4, 8, and 12 weeks following the start of fumigation. Five plants were harvested in each of three periods from all nine chambers except where mortality limited harvest to 4 plants. Regrowth forage was

harvested 9 weeks following the P1 harvest. Plants were cut at 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 above-ground biomass was recorded following drying at 50^{o}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 nutritional analyses. I used an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY) to fractionate forage cell wall constituents to NDF, ADF, and lignin following the procedures of Van Soest et al. (1991). Crude protein (CP) concentration of blackberry leaves was determined using the Kjeldahl procedure (Kjeldahl N × 6.25(AOAC 1995). Association of Analytical Chemists (1995) methods were used to assess dry matter (DM) of 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 2 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, bermudagrass, and tall fescue in a matrix of mixed hardwood-pine forest. Does were aged at 4.5 and 2.5 yr using the tooth replacement and wear method of Severinghaus (1949). To maintain anaerobic conditions, deer rumens were double-tied at

both ends and removed immediately after harvest. They were then transferred to a pre-warmed insulated chest and transported to the laboratory. Blackberry leaf material was placed in 4 digestion vessels containing, in duplicate, forages harvested from each of the periods and 2 blanks (3 primary and 1 regrowth harvest; 20 fiber bags/jar). Samples were incubated for 48 hr at 39°C and then rinsed with distilled water. Fiber bags were frozen until neutral detergent extraction was performed. The neutral detergent extraction was completed following Association of Official Analytical Chemists (1995) procedures. Filter bags were dried overnight at 100°C in a drying oven, and then were weighed.

STATISTICAL ANALYSIS

For analysis of variance for primary-growth forage data, I used general linear models procedures [PROC GLM; Statistical Analysis Systems (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 appropriate, I used multiple comparison tests (PROC GLM, least squares means, Tukey adjustment) to determine differences among treatments within period and differences among periods within treatment. I tested for differences between regrowth forage and P1 harvested forage using the REPEATED command of 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. Ambient O_3 concentrations were approximately 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 hr) O_3 concentrations were 21, 19, and 25 ppb for Periods 1, 2, and 3, respectively for the CF treatment (Figure 1). NF-treated forages experienced daytime means of 32, 28, and 37 ppb of O_3, while the 2X treatment produced daily means of 70, 69, and 81 ppb for Periods 1-3.

PRIMARY GROWTH

I 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 effect ($P \geq 0.073$) was found for biomass yield, IVDMD, and CP, ADF, and lignin concentrations (Table 2). Pair-wise comparisons indicated concentrations of NDF were greater ($P \leq 0.043$) in Period 3 than in Period 1 for 2X (16.6 %) and CF (18.9 %) plants (Table 3). Additionally, RFV decreased ($P \leq 0.050$)15.9% and 17.7 % from Period 1 – 3 for 2X and CF-treated plants, respectively. Biomass, NDF, ADF, and lignin concentrations increased ($P \leq 0.002$) from Period 1 – 3, while RFV, IVDMD, and CP concentrations declined ($P < 0.001$) over the same time period.

SECONDARY GROWTH

O_3-treatment effects were detected for NDF concentration and RFV for regrowth of forage material (Table 4). NDF concentration of 2X-treated regrowth forage increased ($F_{2,6} = 6.47$, $P = 0.032$) 14.2% while RFV decreased ($F_{2,6} = 5.02$, $P = 0.052$) 14.1% in relation to primary growth. Across all treatments, NDF, ADF, and lignin concentrations of regrowth tended to be greater ($P \leq 0.003$) compared to Period 1 forage, while RFV, IVDMD, and CP concentrations were likely to decline ($P \leq 0.001$) through time (Table 4).

DISCUSSION

Period effects were significant for all variables measured. This was expected because plant physiology changes as cell-wall fractions increase in maturing tissues (Holechek et al. 2004). I hypothesized *a priori* that concentrations of fiber fractions would increase and 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 quantitative compounds (i.e. lignin and tannins) that can deter herbivory (Briske 1991). I 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, while RFV is an index used to rank forage quality based on NDF and ADF concentrations (Rohweder et al. 1978). Significant variation among plant response to O_3 fumigation within an individual period would also indicate a qualitative change in plant biochemistry in response to O_3 concentration. For example, within Period 3, RFV, CP concentration and above-ground 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.

Crude protein 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. 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 my forage samples were 12 - 22% greater for 2X-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; Van Soest 1996).

These results suggest that blackberry exhibits visible injury, but has adequate phenotypic variability to cope with chronic stressors, such as O_3. While I did not measure visible injury quantitatively, it was apparent that blackberry plants in 2X chambers had a greater incidence of purplish stippling of the leaves, which is indicative of O_3 injury (Chappelka, personal communication). 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 at levels low enough that a more notable physiological response to fumigation was veiled by phenotypic plasticity. Barbo (1996) found cane density of blackberry (*Rubus cunefolius*) represented 33 – 41% of total canopy cover in mixed-plant communities exposed to elevated O_3 concentrations. Manninen et al. (2003) found that magnitude of response to O_3 fumigation of two wild strawberry 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. However, concentrations recorded for the 2004 growing season were below average for this study area.

I 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. 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. Assimilation of cell wall constituents of low quality forage takes longer than if forage quality is high. As a compensatory measure, ruminants may vary MRT so they can digest and assimilate a greater percentage 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 in nature by increasing retention time in the rumen and, where quantity is not limiting, selecting high-quality plant parts (i.e. 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 storage. Quantity, quality, and rate of passage of available forages affect the nutritional status of ruminants. Asleson et al. (1997) found white-tailed deer on 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 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). While these blackberry samples did not exhibit significant declines across most forage quality characteristics, the reductions I observed are potentially of a magnitude that could reduce nutritional efficiency of browsing herbivores, especially during summer when forage quality normally declines.

CONCLUSIONS

Results were consistent with my 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. My results suggest forage quality may decline without concomitant declines in biomass yield, which may alter foraging strategies of free-ranging ruminants. 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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Figure 1. Mean weekly 12-hr (0900 – 2100) O_3 concentrations for 10 May - 11 August, 2004. Bars represent standard error. Dashed lines signify harvest week.

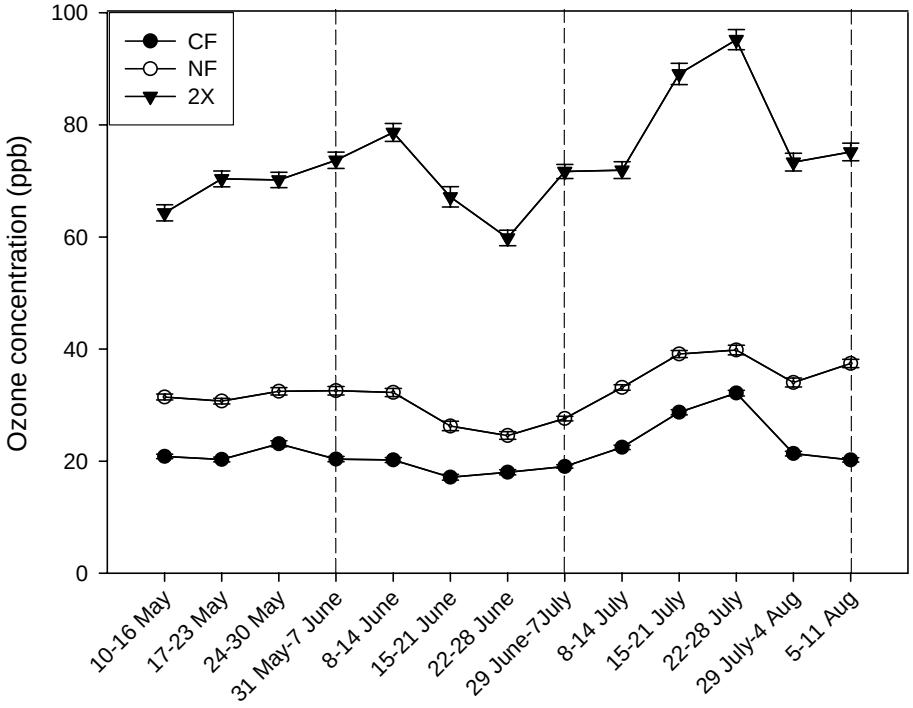
CF
NF
2X
Ozone concentration (ppb)
0
20
40
60
80
100
10-16 May
17-23 May
24-30 May
31 May-7 June
8-14 June
15-21 June
22-28 June
29 June-7July
8-14 July
15-21 July
22-28 July
29 July-4 Aug
5-11 Aug

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

Month	Air temperature (°C)		Precipitation (cm)	
	2004	30-year mean	2004	30-year mean
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

Table 2. Table of main effects for highbush blackberry (*Rubus argutus*) primary growth exposed to 3 concentrations of ozone from 10 May – 11 August 2004 and harvested during 3 periods at 30-day intervals.

	Period			Treatment			Period × Treatment		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Biomass (g DM / plant)	2,10	9.30	<0.001	2,10	0.15	0.868	2,10	0.84	0.501
Crude Protein (%)	2,10	129.90	<0.001	2,10	0.55	0.603	2,10	0.27	0.891
Neutral Detergent Fiber (%)	2,10	21.35	<0.001	2,10	9.85	0.013	2,10	0.49	0.745
Acid Detergent Fiber (%)	2,10	11.72	0.002	2,10	1.48	0.300	2,10	0.13	0.970
Acid Detergent Lignin (%)	2,10	29.47	<0.001	2,10	2.51	0.161	2,10	0.56	0.696
Relative Feed Value (%)	2,10	23.35	<0.001	2,10	8.83	0.016	2,10	0.37	0.829
In Vitro Digestibility	2,10	28.77	<0.001	2,10	4.18	0.073	2,10	0.76	0.573

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

	Period 1 (30 days)		Period 2 (60 days)		Period 3 (90 days)	
	$\bar{x}$	SE[a]	$\bar{x}$	SE	$\bar{x}$	SE
Biomass (g DM / plant)						
CF	68.67 A[b]a[c]	5.13	78.61 Aa	5.52	78.03 Aa	5.13
NF	64.00 Aa	5.13	91.18 Ba	5.32	74.07 Aa	5.52
2X	63.32 Aa	5.13	81.53 Aa	5.32	74.52 Aa	5.32
Crude Protein (%)						
CF	17.08 Aa	0.26	13.04 Ba	1.00	9.19 Ca	0.56
NF	17.28 Aa	0.48	13.78 Ba	0.12	9.11 Ca	0.14
2X	16.78 Aa	0.15	12.91 Ba	0.94	9.38 Ca	0.43
Neutral Detergent Fiber (%)						
CF	21.84 Aa	0.59	24.89 ABa	0.47	25.97 Ba	0.70
NF	21.46 Aa	0.30	24.40 Aa	0.20	24.08 Aa	0.46
2X	22.62 Aa	0.31	25.15 ABa	1.02	26.38 Ba	0.92

Acid Detergent Fiber (%)						
CF	12.60 Aa	0.44	14.41 Aa	0.71	14.79 Aa	0.47
NF	12.35 Aa	0.26	13.93 Aa	0.52	14.42 Aa	0.27
2X	12.96 Aa	0.05	14.13 Aa	0.60	15.18 Aa	0.80
Acid Detergent Lignin (%)						
CF	2.56 Aa	0.11	3.49 ABa	0.22	3.75 Ba	0.08
NF	2.69 Aa	0.07	3.47 ABa	0.12	3.91 Ba	0.29
2X	2.93 Aa	0.09	4.25 Ba	0.54	4.19 Ba	0.26
Relative Feed Value (%)						
CF	337.53 Aa	10.84	290.61 ABa	7.66	277.61 Ba	8.77
NF	343.85 Aa	5.62	297.67 Aa	3.72	300.27 Aa	6.59
2X	324.26 Aa	4.56	289.20 ABa	13.46	272.60 Ba	11.63
In Vitro True 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
2X	89.87 Aa	0.07	85.25 Ba	1.29	84.09 Ba	0.97

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[a] Standard errors of the mean were calculated from $n = 3$ open-top chambers.

[b] Mean values in a row with different upper case letters are different ($\underline{P} < 0.05$) based on Tukey-adjusted least squares means.

[c] Mean values in a column within a period with different lower case letters are different ($\underline{P} < 0.05$) based on Tukey-adjusted least squares means.

Table 4. Nutritive quality values for highbush blackberry (*Rubus argutus*) regrowth exposed to 3 concentrations of ozone from 10 May – 11 August 2004 harvested 60 days subsequent to the first primary harvest (P1).

	$\bar{x}$	SE[a]	Difference from P1
Biomass (g DM / plant)			
CF	5.64	0.87	n.s.
NF	4.20	0.83	n.s.
2X	5.28	1.23	n.s.
Crude Protein (%)			
CF	8.42	0.72	n.s.
NF	8.31	0.84	n.s.
2X	8.70	0.38	n.s.
Neutral Detergent Fiber (%)			
CF	26.06	0.66	>
NF	24.54	0.48	>
2X	25.84	0.26	>
Acid Detergent Fiber (%)			

CF	15.76	0.34	n.s.
NF	14.94	0.16	n.s.
2X	14.83	0.42	n.s.
Acid Detergent Lignin (%)			
CF	3.49	0.27	n.s.
NF	3.14	0.22	n.s.
2X	3.57	0.14	n.s.
Relative Feed Value (%)			
CF	273.91	7.81	<
NF	293.14	6.21	<
2X	278.54	3.83	<

[a] Standard errors of the mean were calculated from $n = 3$ open-top chambers.

III. A COMPARISON OF DEER AND COW INOCULA FOR *IN VITRO* DIGESTION STUDIES WITH HIGHBUSH BLACKBERRY

ABSTRACT

Despite the fact that several researchers have used various domestic sources of inoculum to determine *in vitro* digestibility for wild ruminants, there has been little agreement among results. Typically, several wild ruminants are confined or sacrificed, and a single bovine inocula donor is used to make comparisons among their digestibilities. Wild ruminants are commonly replicated sampling units, but domestic inocula donors are rarely experimentally duplicated. Inoculum source and diet of the study animal are frequently referred to when describing differences of *in vitro* digestibility because differences in digestive physiology and small changes in diet have the potential to alter ruminal microflora populations. I chose to test this hypothesis using inocula from 2 white-tailed deer (WTD; *Odocoileus virginianus*) and 2 cows (*Bos taurus*). I examined a single species, highbush blackberry (*Rubus argutus*), because of its prevalence in the diet of WTD in the southeastern United States. Data from my experiment indicated no difference between bovine and WTD inoculum in ability to digest highbush blackberry. However, mean values for bovine IVDMD were lower in Periods 1 and 2 and greater in Period 3 than IVDMD values for deer. Examination of inocula donors individually

provided greater insight into the differences between donors. Cow 1 was fed bermudagrass hay ad libitum along with a grain concentrate. Cow 2 was free-ranging on annual rye pasture with no concentrate amendment. Cow 1 IVDMD was 12 – 13% greater than Cow 2 within Periods 1 and 2, but IVDMD did not differ within Period 3. I hypothesize that differences in diet resulted in depressed digestion of blackberry for Cow 2 during Periods 1 and 2 because of the lack of highly digestible carbohydrates in her maintenance diet.

My results suggest that diet plays an integral part in determining IVDMD values for highbush blackberry. Using only one inocula donor may give the investigator a false impression about the suitability of an inocula donor for comparative purposes. I advocate duplication of inocula donors and maintaining animals on diets similar to those being tested when determining IVDMD for multiple species.

INTRODUCTION

Accurately and precisely predicting digestibility of forages is an important aspect of assessing food quality for browsing and grazing herbivores. Because nutrition plays an integral role in the health of both wild and domestic animals, it is essential that biologists be able to accurately predict digestibility of forages so that precise assessments of forage quality can be made. While *in vivo* trials are preferred for determining digestibility of forages for a particular plant and animal combination (Palmer et al. 1976), they demand considerable time, and animal maintenance can be expensive and labor-intensive. *In vitro* digestibility trials are popular because of their shorter time demand, ability to test multiple forages with a single inoculum source, and relatively low cost (Campa et al. 1984). However, obtaining inocula from wild ruminants can be challenging (Kim et al.

1996), and stress from capture and/or interactions with anesthetics has the potential to alter rumen microflora which could in turn alter predicted digestibilities.

In vitro dry-matter digestibility (IVDMD) has been used extensively for estimating the potentially available fraction of resources available in ruminant forages (Van Soest 1994). However, many factors can influence repeatability and accuracy of predicted digestibilities. Inoculum source and diet of the study animal are two factors commonly cited when discussing results of *in vitro* trials (Cherney et al. 1993). While several studies have suggested that donor animal (e.g., inoculum source) can affect estimated digestibility of forages (Jenks and Leslie 1988; Robbins et al. 1975), others suggest that more easily managed domestic ruminants can be used to accurately predict digestibility in free-ranging species (Blair et al. 1977; Palmer et al. 1976). However, in the studies I reviewed that compared bovine with white-tailed deer (*Odocoileus virginianus*; WTD, hereafter) inoculum, cattle were never replicated as sampling (experimental) units (Campa et al. 1984; Crawford 1984; Palmer et al. 1976), suggesting that the true variability of cattle inocula used to assess digestibility of wild ruminant forages is unknown. Campa et al. (1984) determined that IVDMD estimates using inocula obtained from domestic animals should only be used to determine comparative intrinsic digestibilities of forages, and not to predict absolute digestibility for wild ruminants. In contrast, Crawford (1984) and Palmer et al. (1976) indicated that bovine IVDMD could be used as a reliable estimator for deer IVDMD, but they did not replicate bovine or WTD donors. Clary et al. (1988) tested *in vitro* digestibilities of 25 forages with 3 wild (*Odocoileus hemionus, Antilocapra americana,* and *Cervus elaphus*) and 3 domesticated ruminants (*Bos taurus, Capra hircus,* and *Ovis aries*). They concluded at

least 2 donor animals should be used and that increasing number of donors is more important than number of subsamples taken from each donor.

Because of the lack of consensus among published data on this topic, I compared white-tailed deer and bovine inoculum for determination of *in vitro* true dry-matter digestibility (IVDMD) of highbush blackberry (*Rubus argutus*), an important browse species for WTD in the southeastern United States. Forage used in this experiment was part of a long-term research project examining effects of tropospheric ozone (O_3) on nutritive quality of native plants. My hypothesis was that inocula donor, as well as growth period, would have significant effects on digestibility of highbush blackberry. The specific objectives of this study were to: 1) compare ability of inocula from WTD and cattle (*Bos taurus*) to digest highbush blackberry, and 2) validate whether stage of maturity affects *in vitro* dry matter digestibility of highbush blackberry.

MATERIALS AND METHODS

Blackberry samples used in the IVDMD analysis were grown in open-top chambers (OTC); (Heagle et al. 1988) with introduced carbon-filtered (CF) air, characteristic of clean air quality in the US; non-filtered (NF) air, representative of quality in Auburn, AL; and air with twice (2X) the ambient concentration of ground-level O_3. Plants were fumigated from 10 May – 11 August 2004. Three harvests (P1, 8 June; P2, 8 July; P3, 9 Aug.) were conducted at approximately 4, 8, and 12 weeks following the start of fumigation. Five plants were harvested in each period from all 9 chambers except where mortality limited harvest to 4 plants. This protocol yielded forage material at 3 different stages of plant growth that could be expected to differ in palatability and digestibility (Gulsen et al. 2004; Short et al. 1974). As plants mature, increasing concentrations of

cell wall constituents such as neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and other structural components increase plant rigidity, but also decrease available cell contents to herbivores (Briske 1991). Only leaf material was used in the *in vitro* analysis, because herbivores commonly select only foliage, not woody stems when browsing. Prior to analyses, forage material was dried to a constant weight at 50ºC, ground in a Wiley mill to pass a 1-mm screen, and stored at room temperature in airtight polyethylene (Nalgene, USA) bottles until analysis.

To determine the effect of rumen inocula donor on IVDMD of blackberry, rumen fluid was collected from 2 WTD and 2 rumen-fistulated dairy cows. Rumen fluid from WTD (D1 and D2, hereafter) was collected from hunter-harvested females on 16 November 2004 and 6 January 2005 at the Piedmont Substation (Camp Hill, AL), a 5,666-ha tract of land owned by Auburn University. Land was mixed pine-hardwood with several tracts of meadow sown in winter wheat, bermudagrass (*Cynodon dactylon*), and tall fescue (*Festuca arundinacea*). Harvested does were aged at 4.5 and 2.5 yr, respectively, using the tooth replacement and wear method (Severinghaus 1949). Immediately following harvest, WTD rumina were double-tied with cotton string at both ends to maintain anaerobic conditions and transferred to a pre-warmed insulated chest in which they were transported to the laboratory. Bovine inocula were obtained from 2 nonlactating, mature, rumen-fistulated Holstein cows (*Bos taurus*) maintained by the Auburn University College of Veterinary Medicine. The first cow sampled (C1, hereafter) was fed bermudagrass (*Cynodon dactylon*) hay ad libitum and approximately 8 kg/day of a grain-concentrate mix containing rolled and cracked corn, cottonseed hulls, hominy feed, soybean meal, cottonseed meal, distillers dried grains, soybean hulls, citrus

pulp and minerals. The second cow (C2, hereafter) was free-ranging on a pasture of annual rye (*Lolium perenne*) and received no grain-concentrate supplement. Both study animals had free access to water. Ruminal fluid was obtained 3 hours postprandial and was siphoned out and transported to the laboratory in a pre-warmed 2 L thermos.

Ruminal contents of both species were strained twice through a double-layer of cheesecloth into a glass vessel fumigated with CO_2. Samples were inoculated within 1 hr following removal of the rumen in the field or 45 minutes following removal from the cow. Analysis following the Goering and Van Soest (1970) modification of the Tilley and Terry (1963) procedure was used. Three digestion vessels contained duplicate incubation bags of forage harvested from each of the periods and 2 blank bags (3 primary harvests; 20 fiber bags/jar). Samples were incubated for 48 hr at 39°C and then rinsed with distilled water. Incubation bags were frozen until neutral detergent extraction was performed following the Goering and Van Soest (1970) modification of the Tilley and Terry (1963) procedure. Bags were dried overnight at 100°C in a drying oven, and then weighed. I also calculated *in vitro* digestibility of NDF (IVNDFD) as $[(NDF_i - NDF_r)/ NDF_i] * 100\%$ where NDF_i was the percent NDF in the introduced sample and NDF_r was the mass of the final NDF residue.

STATISTICAL ANALYSIS

A completely randomized block (3 blocks) experimental design was used with O_3 treatment (3 replications/treatment) as the unit of replication. I used PROC MIXED (SAS 1990) to determine main effects of species, treatments, and periods. Run (species) and OTC (treatments) were identified as the error terms for determining main effects of species and treatments, respectively. The Satterthwaite option [DDFM=SATTERTH;

(Littell et al. 1996)] was used to determine the correct degrees of freedom for tests of interest. When appropriate, I determined differences among treatments and periods and between species using the Tukey adjustment of least squares means.

RESULTS

PRIMARY GROWTH

Differences among treatments ($F_{2,88} = 3.48$, $P = 0.035$; Figure 1) and a period × species interaction ($F_{2,88} = 18.41$, $P < 0.001$) were observed for IVDMD of primary growth of highbush blackberry (Table 1). 2X-treated plants had IVDMD values that were 1.67% less ($P = 0.039$) than those of NF plants, but they did not differ ($P = 0.896$) from CF-treated plants. For blackberry harvested during Periods 1 and 2, deer and bovine IVDMD values were not different ($P \geq 0.906$); however, mean IVDMD values for deer were 4.16% and 2.77% greater, respectively, than cows (Table 2). Within Period 3, mean IVDMD for cows and deer were not different ($P = 0.944$), but deer IVDMD tended to be less than IVDMD of cows. A period × species interaction ($F_{2,196} = 52.92$, $P < 0.001$) was also found for IVNDFD (Figure 3). Deer and bovine IVNDFD did not yield significant differences within any single period ($P \geq 0.844$). Bovine IVNDFD increased ($P < 0.001$) from 37% to 51% from Period 1 – 3, while deer IVNDFD declined ($P < 0.013$) over time. Examination of individual inocula donors within species revealed different results.

Inocula donors (runs) of IVDMD for deer were not different ($F_{1,40} = 0.01$, $P = 0.975$), however, a period effect ($F_{2,40} = 43.85$, $P < 0.001$) was apparent (Figure 2). A 6.5% decline in IVDMD occurred from Period 1 – Period 3 for deer. A period × run interaction ($F_{2,40} = 139.69$, $P < 0.001$) was observed for IVDMD in cows. For C1, IVDMD declined 4.7% from Period 1 – Period 3, while IVDMD for C2 increased 7.5%

over the same time period. Cow 1 IVDMD was 12.2 and 13.9% greater ($P < 0.001$) than C2 IVDMD within Periods 1 and 2, respectively. *In vitro* dry-matter digestibilities did not differ ($P = 0.972$) between bovine inocula donors within Period 3.

DISCUSSION

I found that mean IVDMD and IVNDFD for WTD and bovine inocula donors did not differ significantly within any period. However, mean values for bovine IVDMD tended to be less than those of deer during Periods 1 and 2, and greater in Period 3. In addition, examining individual inocula donors, I discovered that C2 fiber digestibility was lower during Periods 1 and 2, but was not different from that of C1 during Period 3. Blackberry harvested in Period 3 was of lower nutritive quality based on chemical analysis compared with earlier harvest periods (Lewis et al., unpublished data). My results imply that, on average, cows were less efficient in their ability to digest forage material at a younger stage of maturity than deer. Typically, bovines efficiently utilize lower quality, more mature forage because of their large rumen capacity to body size ratio in relation to WTD (Hanley 1982; Hofmann 1989). Although samples were inoculated with equal amounts of rumen fluid from each species and were allowed to incubate for the same amount of time, rumen microfloral populations within cow inocula could be adapted to digesting mature forage more efficiently than deer.

I expected IVDMD to decline over time as plants matured (Briske 1991; Gulsen et al. 2004; Short et al. 1974). Deer and C1 IVDMD conformed to my expectations, but results for C2 were unexpected. I had expected C2 to exhibit greater digestibility during Periods 1 and 2, as had the deer and C1, due to the plants being less mature. I hypothesize that the ruminal microflora of C2 may have been unable to utilize

concentrate as efficiently as C1 because she was fed entirely on pasture grass with no concentrate amendment to her diet. Concentrations of cell wall constituents increased while crude protein concentration declined over time for blackberry (Lewis et al., unpublished data). As the plants matured, their fiber concentration in Period 3 more closely resembled the diet of C2. The amount of concentrate in the diet of C1 resulted in a diet high in intrinsic (potential) digestibility, similar to the diet of browsing deer. The similarity in digestibilities for C1 and deer during Periods 1 and 2 reflect this relationship. Low IVDMD and IVNDFD for C2 within Periods 1 and 2 indicate that ruminal fiber digestion was suppressed in some way. Such depressions in digestibility can be caused by an increase of rapidly fermentable carbohydrates in the diet and lowering of pH in the rumen (Miller and Muntifering 1985). Wedekind et al. (1986) found forage NDF digestibility in adult wethers declined 30% when concentrate in the diet was increased from 0 – 60%. Cow 2 may have experienced a similar negative effect when blackberry foliage from Periods 1 and 2, having lower fiber content and presumably greater non-structural carbohydrate concentration, was introduced to C2 ruminal inocula.

My results suggest that cow inoculum was not suitable universally for estimating digestibility of highbush blackberry by white-tailed deer. Individual cows are used as standards of comparison in most experiments comparing various ruminant digestibilities (Campa et al. 1984; Crawford 1984; Jenks and Leslie 1988; Milchunas and Baker 1982; Palmer et al. 1976). However, my data suggest there may be greater variability between cow inocula than deer inocula when diets are variable. Using a single cow as an inocula donor could give investigators the false impression that all cow inocula can be used as a

surrogate for estimating IVDMD for WTD. This would have been the case had I only used inocula from C1. I found deer inocula to be less variable than cow inocula for highbush blackberry, even though age and diets of deer could not be controlled. While others have found that bovine inocula can be used to accurately predict forage digestibility for white-tailed deer (Blair et al. 1977; Palmer et al. 1976), my data indicate confounding variation between individual animal donors and diet that could produce inaccurate results, and thus caution should be used when using animals other than the species of interest. In addition, replicates should be used for all species being used in an experiment (Clary et al. 1988). While diet is an important aspect to be considered, variation between individual digestive physiologies could also be of significance.

Maintaining an animal on a diet resembling the forage being tested is common practice for domestic- and wild-ruminant nutritionists and my results support this practice. Maintaining a wild ruminant on a standard ration that is similar to the forage of interest would give an estimate of optimal digestibility provided that the ruminal microflora has had time to adapt to the diet being fed. However, free-ranging herbivores utilize a variety of forages on multiple temporal scales (day, month, season, and year). Using rumen contents of free-ranging herbivores would estimate mean digestibility of a particular forage species an animal may encounter out of the many forage species available. Wild ruminants are not kept on strict diets in their native habitats, and it is more biologically relevant to know an estimated digestibility for a forage species given that free-ranging animals are on variable diets. Feeding a standard diet preferred by either deer or cows would place one of the species at a digestive disadvantage (Van Soest 1996) by altering rumen microfloral composition in a way that is not biologically

realistic. Replication of animals occupying habitats which contain the plant species of interest is the most logical way to control for variation in the diets of free-ranging ruminants.

CONCLUSIONS

Using inocula from donors other than the species of interest can yield misleading estimates of *in vitro* digestibility if applied to other animal species. Replication of all species used during *in vitro* trials is the only way to be sure of differences in estimates of digestibility. This study suggests that bovine inocula can not be used universally as an accurate predictor of IVDMD for WTD without careful consideration of the diets being fed and the forage of interest. Variation between animals of the same species may be too great for bovine inocula to be used as an indicator of digestibility of highbush blackberry for WTD unless diets are fixed. Rumen microflora populations are highly variable and small changes in diet composition (i.e. increases in readily available carbohydrates) have the potential to negatively influence fiber digestion in the rumen (Miller and Muntifering 1985). Additionally, maintaining study animals on a standard diet can give the investigator an estimate of optimal digestibility, but biological relevance must always be considered.

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Table 1. Table of main effects for *in vitro* true digestibility of highbush blackberry exposed to 3 ozone concentrations from 10 May – 11 August 2004 and harvested during 3 periods at 4-week intervals using white-tailed deer and cow inoculum.

	df [a]	*F*	*P*
Treatment (TRT)	2,88	3.48	0.035
Period (PER)	2,88	13.44	<0.001
Species (SPP)	1,2	0.07	0.812
TRT × PER	4,88	0.84	0.503
TRT × SPP	2,88	0.16	0.848
PER × SPP	2,88	18.41	<0.001
TRT × PER × SPP	4,88	0.07	0.992

[a] Degrees of freedom were determined using Satterthwaite's approximation

Table 2. Pooled treatment means of period × species values for *in vitro* dry-matter digestibility of highbush blackberry (*Rubus argutus*) primary growth exposed to 3 ozone concentrations from 10 May – 11 August 2004 and harvested during 3 periods at 4-week intervals.

	Period 1		Period 2		Period 3	
Species	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Deer	89.88	0.27	86.45	0.55	84.04	0.44
Cow	86.29	1.21	84.12	1.38	87.19	0.35

Figure 1. Mean *in vitro* dry-matter digestibility for CF, NF, and 2X-treated highbush blackberry harvested 10 May – 11 August 2004 at 4-week intervals. Bars represent standard error.

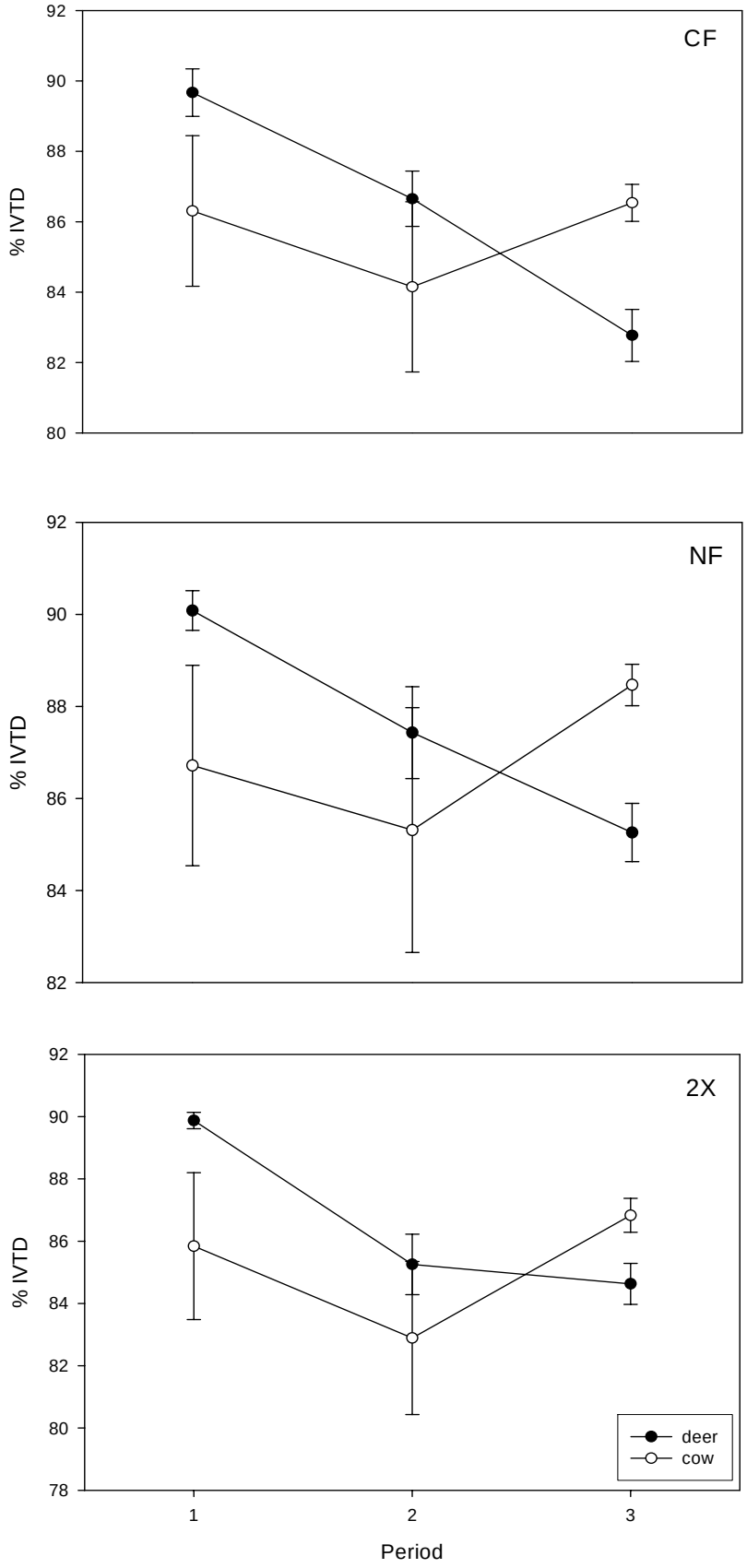

CF
NF
2X
% IVTD
92
90
88
86
84
82
80
78
1
2
3
Period
deer
cow

Figure 2. Mean *in vitro* dry-matter digestibility of NF-treated highbush blackberry harvested 10 May – 11 August 2004 at 4-week intervals for individual runs. Bars represent standard error.

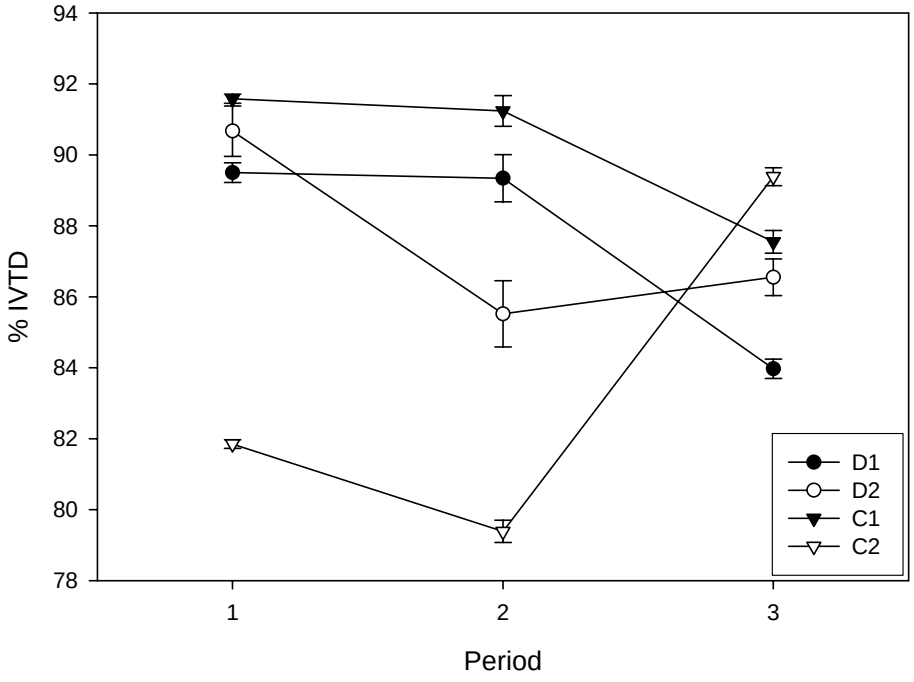
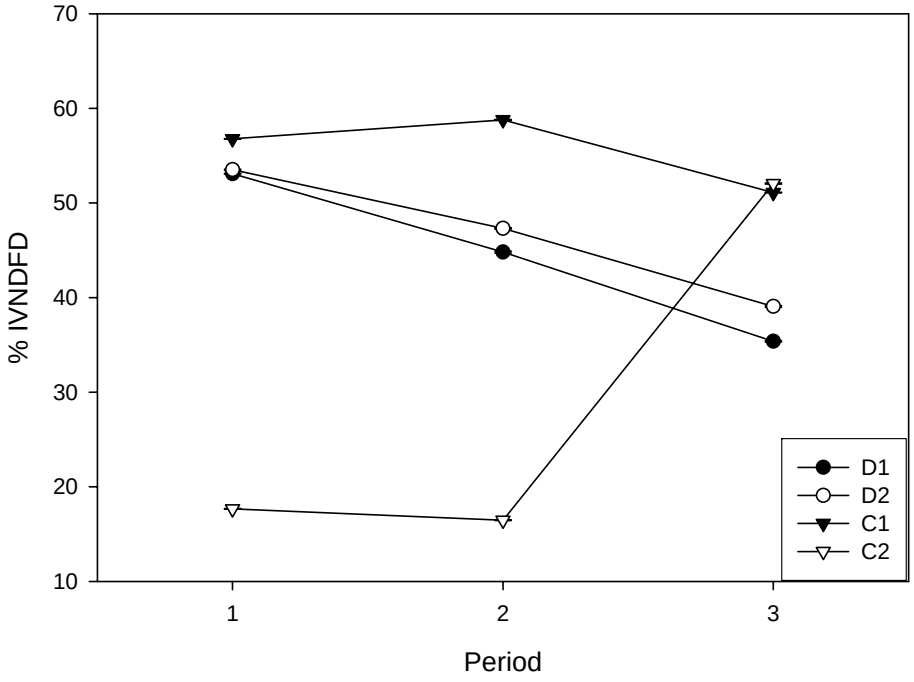

94
92
90
88
86
84
82
80
78
% IVTD
1
2
3
Period
D1
D2
C1
C2

Figure 3. Mean *in vitro* digestibility of neutral detergent fiber from highbush blackberry harvested 10 May – 11 August 2004 at 4-week intervals for individual runs. Bars represent standard error.


70
60
50
40
30
20
10
% IVNDFD
1
2
3
Period
D1
D2
C1
C2