ABSTRACT

AYERS, CHRISTOPHER RYAN. Effects of Mowing on Anthraquinone for Deterrence of Canada Geese and Survey of Canada Goose Fecal Contaminants. (Under the direction of Christopher Shannon DePerno and Christopher Elliott Moorman.)

Resident Canada goose (*Branta canadensis*) populations have increased in urbanizing regions of the eastern United States, where man-made ponds and lakes surrounded by managed turfgrass offer ideal habitats. High concentrations of geese in urbanizing areas may cause feces accumulation, outbreaks of zoonotic diseases, eutrophication of adjacent waterways, and spread of turfgrass weeds. Although repellents effectively deter geese from turfgrass areas, frequent mowing (e.g., 2 – 3 times/week) may impact their long-term efficacy. My objective was to evaluate the effect of 2 different mowing schedules on the longevity of Flight*Control*® PLUS (FCP), an anthraquinone based avian digestive irritant. From June 2007 to October 2008, I conducted 4, 30-day experiments of repellent efficacy on free-ranging geese at 8 sites. Sites were divided into 4, 0.1-ha plots, each randomly assigned a unique treatment of the repellent (treated or untreated) and mowing frequency (4-day or 8-day). Each experimental session consisted of a 7-day pretreatment period of baseline observations and 30 days of post-treatment observations. Goose droppings were collected daily from transects in each plot, and percent of grass with FCP remaining was measured daily. Also, I tested 234 goose droppings for *Giardia lamblia* using a ProSpect *Giardia* EZ Microplate Assay, measured total Kjeldahl nitrogen (TKN) and phosphorus (TP) in 304 fecal samples, and planted 127 droppings to evaluate plant germination in a greenhouse and potential for weed dispersal via feces.

Over the 30-day period, goose use of FCP treated plots was lower than on untreated plots and goose use and FCP coverage was similar between treated plots mowed every 4 and 8 days. Further, the average FCP coverage on grass blades in treated plots decreased from 95% to 10%. None of the fecal samples tested positive for *Giardia*. The average amounts of TKN and TP in fecal samples were 24.2 mg/g (range = 12.6 – 55.7) and 3.6 mg/g (range = 1.4 – 8.3) of dry matter, respectively, with an average of 4,318.0 g/ha/day deposited by ≈42 geese. Four (3.1%) fecal samples germinated plants: Pennsylvania smartweed (*Polygonum pennsylvanicum* L.), annual bluegrass (*Poa annua* L.), and 2 *Kyllinga* spp.

Flight*Control*® PLUS effectively repelled Canada geese, but longevity of the chemical may depend on keeping treated blades alive and under mowing height. I recommend identifying areas of high goose concentration and using FCP when geese are most prevalent. Transmission of *G. lamblia* by Canada geese does not appear to be a high risk. If geese test positive for *Giardia* sp., trophozoites should be collected to identify species. Resident Canada goose droppings at our study sites contribute 17 – 31% of recommended TKN and 17 – 38% of recommended P in lawn fertilization rates. Resident Canada goose fecal nitrogen and phosphorus deposition could degrade water quality in areas adjacent to goose concentrations. Although *Kyllinga* spp. and annual bluegrass are turfgrass weeds, the low percentage of germinations indicates little risk of their dispersal by resident Canada geese.

Effects of Mowing on Anthraquinone for Deterrence of Canada Geese and Survey of Canada Goose Fecal Contaminants

by
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BIOGRAPHY

Christopher Ryan Ayers was born in Richmond, Virginia on December 3rd, 1980 to Nancy and Richard Ayers. Chris grew up in Powhatan county, Virginia where he learned to appreciate country living and the outdoors. He spent a great amount of time working and playing outside including activities to become an Eagle Scout. Family vacations around the United States and abroad instilled in him a love of traveling that remains today. After graduating from Powhatan High School (1999), Chris attended Virginia Tech for a bachelor's degree in fisheries and wildlife science (2003) and a master's degree in secondary science education (2004). He then taught middle school science for a year in Hawaii and another in Maryland before returning to North Carolina State University for a master's degree in fisheries and wildlife science in 2006. Chris began a doctorate program in fisheries and wildlife science at Mississippi State University in 2009. He hopes to combine his mother's talent as a teacher and his father's passion and curiosity for biology to educate himself and others in the future.

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Chapter 1

EFFECTS OF MOWING ON ANTHRAQUINONE FOR DETERRENCE OF CANADA GEESE

Abstract

Resident Canada goose (*Branta canadensis*) populations have increased in urbanizing regions of the eastern United States, where man-made ponds and lakes surrounded by managed turfgrass offer ideal habitats. High concentrations of geese in these areas may cause feces accumulation, outbreaks of zoonotic diseases, and eutrophication of adjacent waterways. Although repellents effectively deter geese from turfgrass areas, frequent mowing (e.g., as in corporate parks and golf courses) may influence the efficacy of repellents. We tested the effect of 2 different mowing schedules on the longevity of Flight*Control*® PLUS (FCP), an anthraquinone based avian digestive irritant. From June 2007 to October 2008, we conducted 4, 30-day experiments of repellent efficacy on free-ranging geese at 8 sites. Sites were divided into 4, 0.1-ha plots, each containing a unique treatment of the repellent (treated or untreated) and mowing frequency (4-day or 8-day). Each experimental session consisted of a 7-day pretreatment period of baseline observations and 30 days of post-treatment observations. Goose droppings were collected daily from transects in each plot, and percent of grass with FCP remaining was measured daily. On average, goose use of treated plots was lower than on untreated plots for 30 days. Over the 30 day period, goose use and FCP coverage was similar between treated plots mowed every 4 days and those mowed every 8 days and the average FCP coverage on grass blades in treated

plots decreased steadily from approximately 95% to 10%. Flight*Control*® PLUS effectively repelled Canada geese, but longevity of the chemical may depend on keeping treated blades alive and under mowing height.

In the mid-20th century, restrictive waterfowl hunting regulations, increases in suitable habitat, and relocation programs contributed to a rise in resident Canada goose (*Branta canadensis*) populations, especially in suburban areas (Conover and Chasko 1985, Ankney 1996). Like many wildlife species, Canada geese have adapted to living in close proximity to humans. While there may be many stresses, hunting and often predation are reduced in suburban areas compared to more rural landscapes (Ditchkoff et al. 2006). Suburban areas (e.g., golf courses, parks, corporate facilities, and residences) typically contain ponds or lakes surrounded by managed turfgrass, which provide excellent habitat for geese (Conover and Chasko 1985). However, because of feces build-up, the aggressive behavior of Canada geese during nesting and flightless periods, and damage to turfgrass, novel approaches to goose reduction in suburban areas are needed (Ankney 1996, Loker et al. 1999).

Hunting can aid in controlling goose populations in rural areas, but is illegal in most suburban and urban areas (Conover and Chasko 1985). Other lethal methods, including oiling or addling eggs and euthanizing birds captured during the summer molt period, may be effective at reducing populations of geese in suburban and urban areas (Gosser et. al. 1997). However, acquiring depredation permits and assistance for removals requires demonstrating

that non-lethal methods of controlling geese have been attempted (U.S. Fish and Wildlife Service 2007). Further, lethal methods of goose control have varying levels of opposition or support from the general public (Conover and Chasko 1985, Loker et al. 1999).

Non-lethal methods for goose management include habitat manipulation, visual and auditory disturbances, dog harassment, obstructions to water, and chemical deterrents (Gosser et. al. 1997, Castelli and Sleggs 2000). Habitat manipulation and visual and auditory disturbances require maintenance and may be undesirable for aesthetic or functional reasons (Conover 1992), and use of chase-dogs requires continuous oversight and may be cost prohibitive (Castelli and Sleggs 2000).

Previous studies have investigated the efficacy of non-lethal chemical repellents as deterrents of nuisance geese (Conover 1985). For example, Methiocarb (Conover 1985), dimethyl and methyl anthranilate (Cummings et al. 1991, 1995, Belant et al. 1996), and lime (Belant et al. 1997) have had mixed results as Canada goose deterrents, depending on application method and active ingredients. Anthraquinone (AQ) has been shown to be an effective avian deterrent (Avery et al. 1997, Dolbeer et al. 1998), especially when combined with a plant-growth suppressant (Blackwell et al. 1999).

Although AQ has proven effective on captive geese, it has not been tested under natural environmental conditions in habitats occupied by free-ranging Canada geese. Because free-ranging geese are mobile, they have options for foraging locations, which may influence the efficacy of turf-applied chemical deterrents. Additionally, the longevity of turf-applied chemical repellents may be altered by mowing frequency as any chemical on grass

above mowing height will be removed. Although, Blackwell et al. (1999) postulated that higher rates of mowing would remove turf-applied chemicals faster than less frequent rates of mowing, to our knowledge, mowing frequency has not been evaluated for its effect on turf-applied goose repellents. Therefore, our objective was to determine the efficacy and longevity of a rainfast AQ-based avian repellent, Flight*Control*® PLUS (FCP; Arkion® Life Sciences LLC, New Castle, Delaware), as a deterrent of free-ranging resident Canada geese under two mowing frequencies. Flight*Control*® PLUS is intended for use on managed turfgrass areas and the manufacturer recommends reapplication after 2 or 3 mowings.

Study Area

We conducted the study at 8 sites in the Triangle region (Raleigh, Durham, and Chapel Hill) of North Carolina. Sites included corporate facilities, suburban neighborhoods, parks, a greenway, a college pond area, and a cattle facility. Two of the sites were dominated by bermudagrass (*Cynodon dactylon* L.), and 6 of the sites were dominated by tall fescue (*Schedonorus phoenix* [Scop.] Holub.). Each site had at least 0.4 ha of grass adjacent to or nearby a pond or lake with daily use by geese during early summer, early fall, or both.

Methods

We arranged our experiment in a randomized complete block design, with 8 sites (i.e., blocks) each containing 4, 0.1-ha treatment combinations (Ott and Longnecker 2001). At every site, we randomly assigned each 0.1-ha plot to one of the four treatment combinations: (1) treated with FCP and mowed every 4 days (T4); (2) treated with FCP and mowed every 8 days (T8); (3) untreated and mowed every 4 days (U4); and (4) untreated and mowed every 8

days (U8). The schedules represent commonly used mowing frequencies on corporate, residential, and recreational sites during healthy growth periods (Christians 2004). We mowed the 2 sites dominated by bermudagrass at 5.08 cm and the 6 sites dominated by tall fescue at 8.89 cm as recommended by site managers. We conducted four 37-day field sessions (June/July 2007, September/October 2007, June/July 2008, and September/October 2008), which represented the summer molting phase and the full-plumage phase. We randomly rotated the treatment combinations to each of the plots over the course of the 4 sessions.

We mowed all plots 8 days before repellent application and the 2 mowing schedules were then maintained until the end of the post-application observation period. We recorded goose use of all sites on each of the test plots for a 7-day baseline period prior to repellent application. After the final baseline observations, we mowed all sites and applied FCP to the two treated plots at the maximum recommended rate of 9.5 L/ha using a CO_2 pressurized ATV mounted 3.05-m10- boom sprayer or a Solo® backpack sprayer. Daily, during the baseline and post-treatment periods, we counted and removed goose droppings along a permanent 2-m × 21-m transect in each plot. We measured daily blade coverage of FCP visually in treated plots by estimating the proportion of live grass blade surface that had FCP (i.e., spots) remaining in a random ≈103-cm^2 patch.

For each session, we conducted an analysis of covariance (ANCOVA) to compare goose use of plots, using PROC MIXED in SAS 9.1 (SAS Institute Inc., Cary, North Carolina). We used the number of droppings each day after FCP application as the

dependent variable and the baseline number of daily droppings for each transect as a covariate. The 30-day post-treatment period was divided into 4 week-long (7, 7, 7, and 9-day) periods. Independent variables included FCP treatment, mowing frequency, site, post-treatment week, the interaction between FCP treatment and mowing, and the interaction between treatment and post-treatment week. We used treatment, mow, and site as class variables and week as a continuous variable. We considered site a random effect.

For all sessions combined, we conducted an analysis of variance (ANOVA) to test chemical longevity on treated plots, using PROC MIXED in SAS 9.1 (SAS Institute Inc., Cary, North Carolina). The dependent variable was percent of grass blade still containing FCP spots and the independent variable was mowing schedule of the treated plot.

We removed daily records with zero fecal droppings at a site from the analysis because we assumed geese did not use those sites on those days. We did not include 2 of the 8 sites in the fall 2007 ANCOVA analysis because there were no geese present. In fall 2008, we did not use one site because of construction and did not include another in the ANCOVA because no geese were present. All methods were approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol # 08-012-O).

Results

In summer 2007 ($F = 20.79$, $df = 752$, $P < 0.0001$), fall 2007 ($F = 7.23$, $df = 456$, $P = 0.0074$), and summer 2008 ($F = 28.50$, $df = 714$, $P < 0.0001$), plots treated with FCP had less goose use than untreated plots (Table 1.1; Fig. 1.1). Averaged for all 4 sessions, goose use on treated plots in the first week post-treatment was 70% lower than use on untreated plots,

59% lower in week two, 57% lower in week 3, and 41% lower in week 4 (Fig. 1.2). However, throughout the study, goose use was present on treated and untreated plots and never averaged zero droppings for any week during any session (Fig. 1.2).

Goose use was higher on plots mowed every 4 days than on plots mowed every 8 days in summer 2007 but lower on plots mowed every 4 days in the other 3 sessions (Table 1.1). However, mowing frequency did not affect treatment efficacy as goose use of T4 and T8 plots was similar in all sessions (Fig. 1.1). Conversely, mowing frequency did affect goose use of untreated plots and the number of droppings on U4 was 33% lower than on U8 in summer 2007, 120% higher in fall 2007, 35% higher in summer 2008, and 86% higher in fall 2008 (Fig. 1.1).

The average FCP coverage on grass blades was similar during the entire post-treatment period on T4 and T8 plots ($F = 0.01$, $df = 1560$, $P = 0.9314$), and we observed a steady decrease in coverage from ≈95% to 10% over the 30-day post-treatment period (Fig. 1.3).

Goose use during the baseline period affected goose use during the post-treatment period for the 2007 and 2008 summer sessions, but not during the 2007 and 2008 fall sessions (Table 1.1). The average daily numbers of geese observed at each site were 41 and 38 during summer 2007 and summer 2008, respectively, and 53 and 35 during fall 2007 and fall 2008, respectively, but the level of goose use at sites based on fecal counts was lower on all plots in the fall sessions than in the summer sessions (Fig 1.2). With 42 geese/site/day and chemical application once/month, our results indicate dropping concentrations in grazing

areas of similar sites to those in this study would be ≈0.26 droppings/m^2/day in early summer and ≈0.06 droppings/m^2/day in early fall (Fig. 1.1).

Discusssion

One application of FCP consistently reduced use by free-ranging resident Canada geese for the 30-day post-treatment observation period. Similarly, previous studies demonstrated that AQ was an effective avian repellent (Avery et al. 1997, Dolbeer et al. 1998, Blackwell et al. 1999). However, repellent efficacy lasted longer (≥30 days) in our study than the 6 days shown in Dolbeer (1998). Blackwell et al. (1999) determined that a predecessor of FCP, which was not rainfast, combined with a plant-growth suppressant was effective for their entire 22-day observation period. The extended efficacy of the repellent was attributed to the growth suppressant, but the relationship was not directly tested (Blackwell et al. 1999). The application rates of AQ product used by Dolbeer et al. (1998) and Blackwell et al. (1999) were 4.5 L/ha and 2.3 L/ha respectively, and were lower than the rate we used (9.5 L/ha). Because the current formula of FCP is rainfast, the concentration of application should not affect its longevity on the plant, but perhaps could have a stronger repellency effect on geese. In the studies by Dolbeer et al. (1998) and Blackwell et al. (1999), geese were captive and remained near treated areas. Conversely, geese in our study were free-ranging and able to move away from areas treated with FCP, especially during the fall sessions when full plumage allowed flight. Upon moving to alternative feeding locations, geese may avoid treated sites for longer periods than in captive studies.

Mowing frequency had no effect on treatment efficacy or coverage of FCP on grass blades in treated plots. We suggest that mowing removed more untreated grass than treated grass and that most treated grass was removed by senescing of leaves. Mowing reduced the amount of foliage on a given plant; less foliage reduced the shading and subsequent senescing of lower blades (Emmons 2008). Because new untreated blades of grass grow above older treated blades, FCP likely was removed by the senescing and shedding of older treated blades that remained in the shade of newer blades for an extended period (Emmons 2008). Conversely, re-exposure of older treated blades by the mowing removal of younger untreated blades allowed sunlight to reach the treated blades. When treated blades received sunlight and continued to grow, the treated portions may rise above mowing height, depending on leaf growth angle and growth rate (Emmons 2008). To maximize the longevity of FCP efficacy, treated blades need to stay alive but remain below the mowing height as long as possible. Hence, a plant growth regulator can be used to limit the amount of treated grass growth above mowing height and reduce the shading of treated grass by untreated blades (Blackwell et al. 1999). Also, methods of encouraging horizontally growing grass blades may reduce the amount of treated blade removed by mowing (Emmons 2008). Sheffer et al. (1978) noted that lower mowing height (range = 1.3 – 5.1 cm) resulted in more horizontal leaf angles, but less is known about the effect of mowing frequency on blade angle.

Inconsistent differences between goose use of U4 and U8 plots may have resulted from a number of random effects including differences in daily rainfall between 2007

sessions ($\bar{x}$ = 0.30 cm) and 2008 sessions ($\bar{x}$ = 0.48 cm) (including 30 days prior to each session). Blackwell et al. (1999) detected no preference by geese for short grass (4.2 ± 0.7 cm) over tall grass (17.4 ± 3.3 cm); however, we documented higher use on U4 plots than on U8 plots for 3 of the 4 sessions, possibly indicating goose preference for the shorter grass in the more frequently mowed plots. During the very dry summer of 2007, use on U8 was higher than use on U4. The slower grass growth during the dry period may have allowed other factors (e.g., turf damage or disturbance from excessive mowing) to influence goose use of untreated plots, while the remaining sessions were wetter allowing grass to become taller and less palatable in U8 plots (Conover 1991).

Management Implications

Managers must decide if 40 - 70% reduction in goose use each month is a sufficient reduction in fecal nuisance. Although FCP application reduced feces concentrations in treated areas, additional goose presence following treatment may not be acceptable. Also, goose numbers and environmental conditions will vary from site to site, leaving managers the task of having to identify an application rate and total treated area that is not cost prohibitive. The suggested retail price of FCP is $240.00/gal. If FCP is applied once/month at the recommended rate of one half to one gal/acre, the cost of FCP use would be $120.00 - $240.00/acre/month. Generally, repellents should be applied in early spring before nesting occurs to prevent geese from becoming established at a site for breeding and molting (Gosser et al. 1997). However, prior to treatment, we recommend that site managers and homeowners identify areas and times of year of highest goose use. Treating more of the

turfgrass at a site or applying FCP more often may further increase efficacy by reducing free-ranging Canada goose use. Seasonal changes in goose mobility may create differences in efficacy of FCP applications during different times of year. Also, mowing more frequently than every 4 days, as is the case in some parts of golf courses, may affect FCP efficacy. However, if the growth rate and angle of grass blades can be regulated to limit growth of treated blades above mowing height, then more frequent mowing should not have negative effects on FCP longevity.

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Table 1.1. Estimates of variable effects on Canada goose use of 0.1-ha plots during 4 experimental sessions. Negative treatment effect indicates goose use on treated plots lower than goose use on untreated plots. Negative mowing effect indicates lower goose use on plots mowed every 4 days than on plots mowed every 8 days. Testing was conducted during summer and fall, 2007 and 2008 at 8 sites in the Raleigh-Durham-Chapel Hill area of North Carolina, USA.

Experimental Session	Variable			
	Treatment	**Mowing**	**Baseline**	**Week**
Summer 2007†‡	-16.6088	-4.7236	0.3758	1.3352
	<0.0001	0.0189	0.0015	0.0395
Fall 2007	-7.9483	3.3495	0.2189	0.1387
	0.0074	0.0142	0.2009	0.8048
Summer 2008†‡	-15.8598	2.5248	0.6604	-0.0926
	<0.0001	0.0549	<0.0001	0.8675
Fall 2008‡	1.3554	1.989	0.2145	1.6347
	0.5477	0.0203	0.1024	<0.0001

† Significant treatment*mow interaction ($P < 0.05$)

‡ Significant treatment*week interaction ($P < 0.05$)

FIGURES

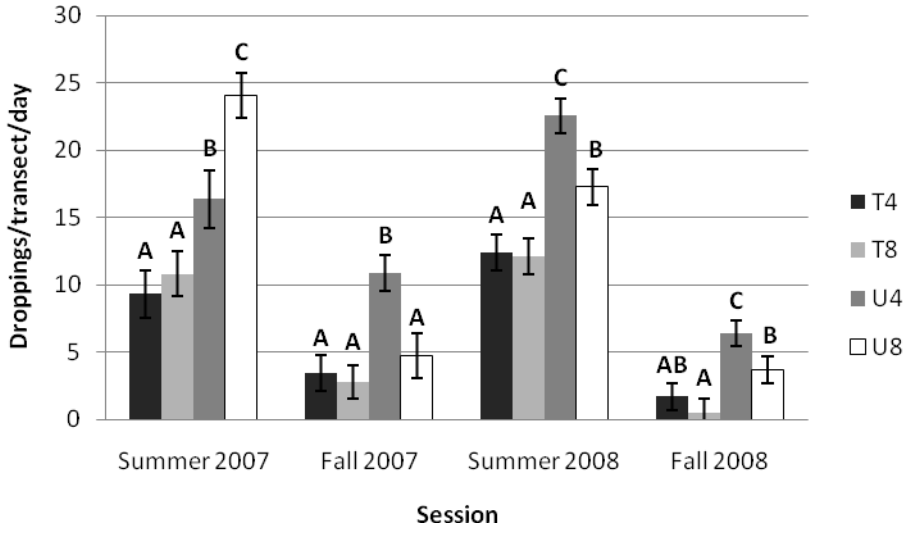

Figure 1.1. Daily Canada goose droppings on 2-m × 21-m transects during the 30-day period after application of FCP goose repellent. T = treated with FCP, U = untreated, 4 = mowed every 4 days, and 8 = mowed every 8 days. Different letters represent significantly different means at $\alpha = 0.05$. Testing was conducted during summer and fall, 2007 and 2008 at 8 sites in the Raleigh-Durham-Chapel Hill area of North Carolina, USA.

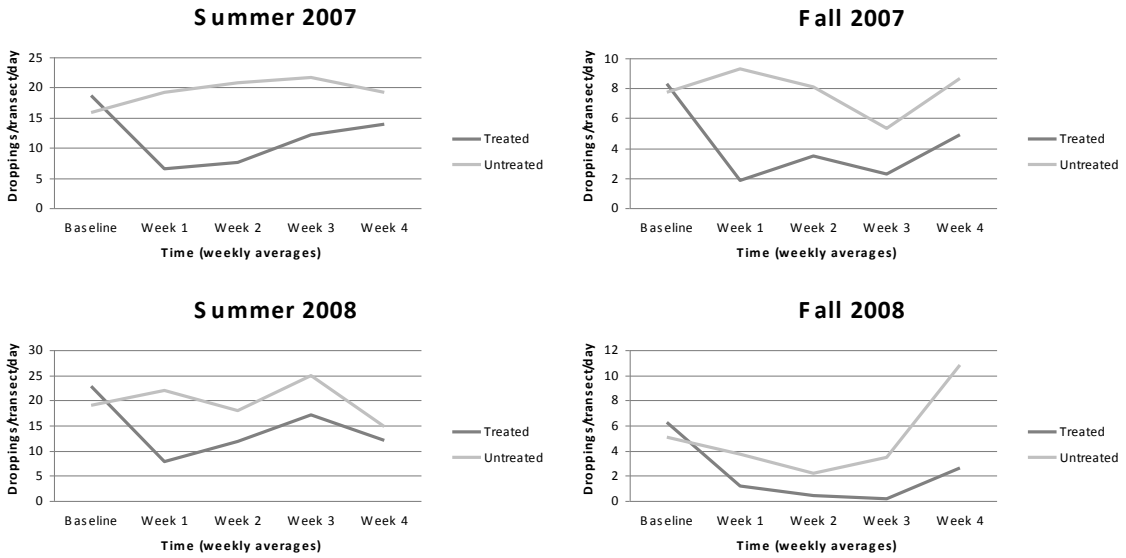

Figure 1.2. Average number of daily Canada goose droppings during 4 sessions before and 4 weeks after FCP application. Weekly values are averages of daily values for that session. Testing was conducted during summer and fall, 2007 and 2008 at 8 sites in the Raleigh-Durham-Chapel Hill area of North Carolina, USA.

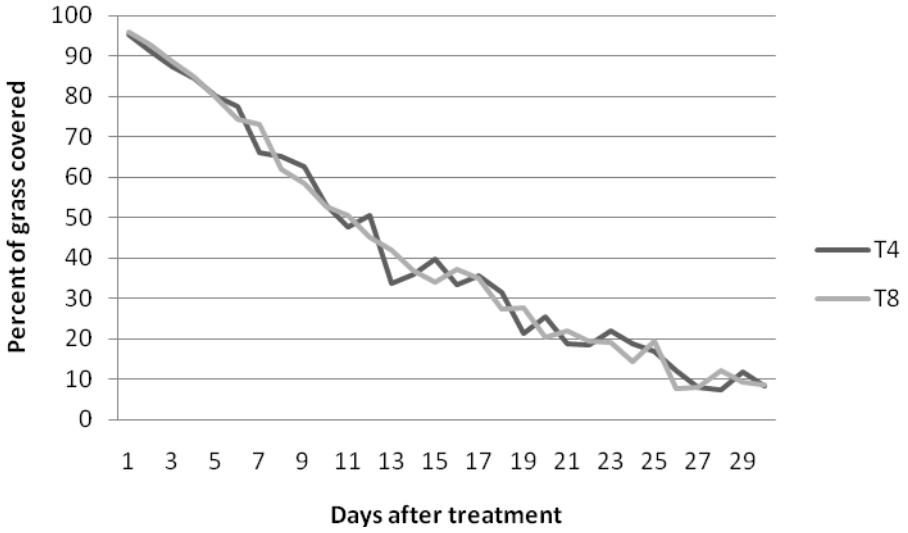

Figure 1.3**.** FCP blade coverage on treated turfgrass plots mowed every 4 days (T4) and treated plots mowed every 8 days (T8) over a 30-day post-treatment period. Testing was conducted during summer and fall, 2007 and 2008 at 8 sites in the Raleigh-Durham-Chapel Hill area of North Carolina, USA.

Chapter 2

SURVEY OF CANADA GOOSE (*Branta canadensis*) FECES FOR PRESENCE OF *Giardia lamblia* IN THE NORTH CAROLINA TRIANGLE

Abstract

As resident Canada goose (*Branta canadensis*) populations increase throughout North America, so do the health and environmental risks associated with goose feces. Goose feces may be a conduit for transmitting giardiasis to humans, which is caused by the intestinal protozoa *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*). We surveyed fecal droppings from free-ranging resident Canada geese for *G. lamblia* at 9 sites in the Triangle area (Raleigh, Durham, and Chapel Hill) of North Carolina. A total of 234 fecal samples were tested using the ProSpect *Giardia* EZ Microplate Assay with no positive results.

Populations of resident Canada geese (*Branta canadensis*) have increased in North America over the past 50 years, especially in suburban areas (Conover and Chasko 1985, Ankney 1996). Corporate and residential growth has generated an increased number of artificial ponds and lakes surrounded by maintained turfgrass, which create suitable habitat for resident (non-migratory) Canada geese (Conover and Chasko 1985). High concentrations of resident geese in suburban areas, particularly where people are frequent visitors, increase the possibility of human and pet contact with goose feces (Graczyk et al. 2008). Also, geese commonly use areas adjacent to waterways that are secondary sources of drinking water or

recreational areas for people (Conover and Chasko 1985). Increased exposure to goose feces may potentially lead to the transmission of infectious diseases to wildlife, livestock, pets, and people (Graczyk et al. 2008).

Migratory Canada goose feces have been shown to contain *Cryptosporidium parvum, Escherichia coli, and Giardia* spp. (Graczyk et al. 1998, 2008, Kassa et al. 2001). Giardiasis is a common waterborne disease of humans caused by the protozoan, *G. lamblia* (Hamnes et al. 2006, Savioli et al. 2006), also known as *G. intestinalis* and *G.duodenalis*. There is no formal agreement on nomenclature, so all three species are interchangeably recognized in the scientific literature (Adam 2001). The life cycle for all *Giardia* spp. has two stages. First, the cyst is shed by an infected host and can persist for a prolonged time in a variety of environments. Second, the trophozoite, which emerges from the cyst under acidic conditions present in the gastrointestinal tract, is the vegetative form that replicates in the small intestine and contributes to the clinical signs of diarrhea and malabsorption (Adam 2001, Thompson 2004).

Giardia spp. have been detected in fecal samples of many mammalian (Heitman et al. 2002, Thompson 2004, Applebee et al. 2005) and avian species (Box 1981, Erlandsen et al. 1990, Upcroft et al. 1997, Filippich et al. 1998, Franssen et al. 2000, Kassa et al. 2001, Kuhn et al. 2002, Majewska et al. 2009). Historically, taxonomic classification of *Giardia* spp. has been based on morphology and host susceptibility. Taxonomy is expected to change with ongoing molecular studies, but currently six species are recognized: *G. lamblia*, *G. agilis*, *G. muris*, *G. microti*, *G. psittaci*, and *G. ardeae* (Cacciò et al. 2005). The latter two species are

found in birds: *G.psittaci* causes diarrhea in parakeets (Panigrahy et al. 1978, Scholtens et al. 1982) and *G. ardeae* has been detected in feces of herons, egrets and ibis (Erlandsen et al. 1990, Kulda and Nohýnková 1995, McRoberts et al. 1996). Attempts to transmit *G. ardeae* to mammals and *G. lamblia* to birds were unsuccessful (Erlandsen et al. 1991). However, viable trophozoites from a clinically ill, wild-caught sulphur-crested cockatoo (*Cacatua galerita*) were propagated in culture and transmitted to laboratory raised mice (Upcroft et al. 1997). These trophozoites were morphologically indistinguishable from *G. lamblia* and bore no resemblance to *G. psittaci*, suggesting that under certain conditions birds can be biologically competent vectors for *G. lamblia*. Further, detection of *Giardia* cysts in waterfowl feces raises concern that birds may act as mechanical vectors for *Giardia* transmission to susceptible human hosts (Graczyk et al. 2008, Majewska et al. 2009).

Methods

Our objective was to investigate the presence of *Giardia* spp. in free-ranging, resident Canada geese in the Triangle region (Raleigh, Durham, and Chapel Hill) of North Carolina. During each summer and fall 2007 and 2008, we twice collected goose feces from 9 sites. These 9 sites were selected for use in a separate study of resident Canada goose behavior and included 3 corporate facilities, 2 parks, a suburban residence, a greenway, a college, and a dairy cow farm (Ayers 2009). The average daily number of geese at each site ranged from 10 – 62. Each collection was conducted at least one week apart and 4 - 10 fresh fecal samples were collected, placed into separate plastic sealable bags, immediately placed on ice, and stored in a freezer until testing. All samples (n = 234) were tested within 2 weeks of

collection using the ProSpecT® *Giardia* EZ Microplate Assay (Remel Inc., Lenexa, KS, USA), which uses a monoclonal antibody to detect *Giardia* Specific Antigen (GSA 65, sensitive to 15.6 ng/ml). Each plate was read visually and spectrophotometrically at 450nm. Initially, 16 samples were positive spectrophotometrically, but lacked positive visual evidence (yellow color) and were retested. Because the test kit was designed for human fecal samples, a fecal interference test was conducted to ensure that avian feces did not interfere with the testing accuracy. Fifty and 25 µl of the positive control provided with the test kit were added to diluted goose feces, which had previously tested negative, for a total volume of 100 µl. These dilutions were then run in parallel with equal amounts of positive control diluted to 100 µl in buffer. These tests were conducted at the North Carolina State University College of Veterinary Medicine. Also, to supplement results from the ProSpecT® *Giardia* EZ Microplate Assay, 30 additional samples (6 from 5 of the test sites) were tested for the presence of *Giardia* cysts by immunofluorescence microscopy using AquaGlo™ G/C Comprehensive Kit (Waterborne Inc., New Orleans, LA, USA) that uses a monoclonal anti-GSA 65 antibody.

Results and Discussion

None of the 234 samples tested positive by enzyme immunoassay. The average optical density of the samples, after subtraction of the negative control value ($\bar{x} = 0.0639$), was -0.0008 (range -0.0310 – 0.0380), which fell below the 0.05 minimum value of a positive test. All retests were visually and spectrophotometrically negative. For the fecal interference test, after the subtraction of the negative control value, the optical densities for

the 50 and 25µl positive controls were 1.816 and 0.898, respectively, compared with 1.651 and 0.811 for positive controls diluted in goose feces. Also, none of the samples (n = 30) tested with AquaGlo™ G/C reagent were positive for *Giardia* cysts.

The ProSpect® test kit used in this study was developed to recognize a 65 kD glycoprotein present on trophozoites and cysts of *G. lamblia* (Rosoff and Stibbs 1986). Subsequently, Stibbs (1989) demonstrated the anti-GSA 65 monoclonal antibody cross-reacted with cysts of *G. muris* and also reacted with cysts of *G. microti* (a.k.a. *G. ondatrae*) from naturally infected muskrats (*Ondatra zibethicus*) as well as detected cell-free antigen in muskrat feces by antigen-capture ELISA (H. Stibbs, unpublished data). This monoclonal antibody was used to detect *Giardia* in the feces of Canada geese within a 20-mile radius of Toledo, Ohio (Kassa et al. 2001), but failed to detect *G. ardeae* in the feces of a heavily infected white stork chick (*Ciconia ciconia*; Franssen et al., 2000).

To detect *Giardia,* an enzyme immunoassay was selected for this survey study because of ease of use, relatively inexpensive cost, and demonstration of high specificity and sensitivity in different susceptible hosts (Johnston et al. 2003, Mekaru et al. 2007, Rimhannen-Finne et al. 2007). Commercially available assays for *Giardia* are designed to detect *G. lamblia* that causes clinical disease in humans. Based on genotyping, there are multiple host-restricted assemblages of *G. lamblia*, which causes considerable confusion about the epidemiology of *G. lamblia* (Monis et al. 2009). Assemblages C and D appear restricted to dogs, E to livestock, F to cats, G to rats, and assemblages A and B are present in humans and detected in a wide variety of mammals and considered zoonotic (Cacciò et al.

2005; Monis et al. 2009). However, conventional diagnostic tests were not designed to distinguish amongst the assemblages of *G. lamblia*.

Earlier studies evaluating *Giardia* in waterfowl used immunofluorescent (IF) antibodies that recognized antigenic determinants on the cyst wall. These immunoassays again were developed to detect *G. lamblia*. Use of immunofluorescent antibodies in combination with multiplexed fluorescence *in situ* hybridization (FISH) using oligonucleotide probes specific for *G. lamblia* demonstrated that waterfowl carry *G. lamblia* cysts (Majewska et al. 2009). In another study, cysts detected in waterfowl feces as *G. lamblia* by conventional IF antibodies were grouped to assemblages A and B by PCR sequencing and Loop mediated isothermal amplification (LAMP; Plutzer and Tomor, 2009).

The potential for zoonotic transmission of *G. lamblia* genotypes associated with humans by waterfowl exists, but the relative risk and importance of such transmission remains to be elucidated (Hunter and Thompson 2005, Monis et al. 2009). Initial studies of migratory Canada geese from nine sites in the Chesapeake Bay area of Maryland indicated that all sites were positive for *Giardia* at an average concentration of 405 cysts/g of fecal material (Graczyk et al. 1998). Similar concentrations of *Giardia* cysts were detected in 18 of 69 fecal samples of hunter-killed wild ducks, primarily mallards (13/51), along the Rio Grande River near Las Cruces, New Mexico (Kuhn et al. 2002). A study in Poland that used FISH in combination with IF antibodies and included a total of 499 samples from free-ranging, captive, and domestic avian species detected 26 *G. lamblia* positive birds (Majewska et al. 2009). Twenty-two of these were free-ranging waterfowl, including 7/32

mallard ducks (*Anas platyrhynchos*), 10/34 graylag geese (*Anser anser*), 1/72 common merganser (*Mergus merganser*) and 4/34 mute swans (*Cygnus olor*). A survey of 16 urban sites (22 composite samples) with Canada geese around Toledo, Ohio detected only 2 positive locations (3 positive samples) using the ProSpect® test to detect GSA 65 (Kassa et al. 2001). The results of these studies suggest that migratory waterfowl are at greater risk of exposure to *Giardia* than resident populations and indicate the risk of exposure varies by geographic location.

Management Implications

The North Carolina Triangle is relatively urban and affluent, and the opportunity for geese to be exposed to a fecal-borne infectious agent of humans is minimal. However, additional research is necessary to determine presence of *Giardia* in resident populations of Canada geese throughout the country. Although commercially available enzyme immunoassays are a relatively inexpensive and quick way to detect *Giardia* in avian feces, further research is necessary to determine which genetic assemblages occur in waterfowl and whether birds actually contribute to zoonotic infections. In urban settings, monitoring resident Canada geese and other wildlife for *G. lamblia* assemblages A and B might indicate areas that may compromise ecosystem and human health.

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Chapter 3

WEED DISPERSAL AND NUTRIENT LOADING FROM RESIDENT CANADA GOOSE FECES IN TURFGRASS SYSTEMS

Abstract

Populations of resident Canada geese have increased in the United States in recent decades raising concerns about turf damage and fecal contaminants. High populations of geese can lead to feces accumulation in areas adjacent to surface waters, creating concern about aquatic eutrophication locally and downstream. Further, turf managers and livestock farmers work to keep their facilities free of noxious or toxic weeds that geese potentially disperse. We investigated the prevalence of viable seeds and nitrogen and phosphorus content in resident Canada goose droppings. We placed 127 fresh individual droppings in seedling trays of potting soil and allowed 30 days to germinate in an irrigated greenhouse. Then, trays were cold stratified for 30 days and returned to the greenhouse for an additional 30 days. Also, we tested 304 fecal samples from 8 sites for total Kjeldahl nitrogen (TKN) and total phosphorus (TP). Out of 127 droppings planted, 4 plants germinated (3.1%): Pennsylvania smartweed (*Polygonum pennsylvanicum* L.), annual bluegrass (*Poa annua* L.), and 2 *Kyllinga* spp. The average amounts of TKN and TP in fecal samples were 24.2 mg/g (range = 12.6 – 55.7) and 3.6 mg/g (range = 1.4 – 8.3) of dry matter, respectively. The results indicate that resident Canada geese in suburban and urban areas are not frequent vectors of

viable seeds, but do have potential to contribute a significant amount of nutrients to adjacent surface waters.

Populations of resident Canada geese (*Branta canadensis*) have increased in North America over the past 50 years, especially in suburban areas (Ankney 1996, Conover and Chasko 1985). Population growth has resulted from an increase in suitable habitats (e.g., golf courses, parks, corporate facilities, airports, and residences), which often contain ponds or lakes surrounded by managed turfgrass (Conover and Chasko 1985). Concentrated goose grazing and fecal build-up can create a variety of management challenges (Conover and Chasko 1985). For example, high concentrations of goose grazing could lead to weed dispersal in areas of managed turfgrass or livestock pastures. Turfgrass managers work to eliminate unwanted plants from turf, and livestock managers must protect livestock from toxic and malnourishing plants invading pastures (DiTomaso 2000, Emmons 2008). Additionally, excessive fecal buildup could create aquatic eutrophication of adjacent and downstream surface water (Correll 1998).

Resident Canada goose movements vary widely, ranging from <1 km - 109 km within a given year (VerCauteren and Marks 2004). During these movements, geese may translocate nutrients and viable weed seeds (Conover and Chasko 1985, Best and Arcese 2009). Endozoochory, the process of ingesting and dispersing viable seeds, has been recorded in mammals (Howe and Smallwood 1982, Myers et al. 2004, Williams et al. 2008) and birds (Best and Arcese 2009, Clausen et al. 2002, Meisenburg and Fox 2002, Soons et al.

2008). Soons et al. (2008) demonstrated that mallards (*Anas platyrhynchos* L.) could disperse viable seeds of 19 different aquatic plant species. Also, viable seeds have been detected in the droppings of migratory Canada geese (Best and Arcese 2009, Neff and Baldwin 2005), but research on viable weed dispersal by resident Canada geese in suburban areas is lacking.

Eutrophication is as a main cause of decreased water quality in coastal and inland waters (Melesse et al. 2008, Heisler et al. 2008). Nitrogen and phosphorus are the two most common causes of harmful algal blooms, which can cause declines in aquatic biodiversity (Correll 1998). In many urban and suburban habitats, goose feces are deposited on hard surfaces or lawns directly adjacent to open surface water (Conover and Chasko 1985). If runoff is not filtered or retained, the nutrients from fecal deposits enter the waterway.

Fecal deposits from migratory waterfowl have been identified as potential causes of poor water quality (Manny et al. 1994, Marion et al. 1994, Pettigrew et al. 1998, Post et al. 1998, Kitchell et al. 1999, Olson et al. 2005, Unckless and Makarewicz 2007, Van Geest et al. 2007). However, Pettigrew et al. (1998) and Unckless and Makarewicz (2007) concluded that migratory geese had little or no negative effect on water quality. To our knowledge, our study was the first to focus on nitrogen and phosphorous content of feces from resident Canada geese. Because resident Canada geese spend much of the year in one area, large congregations may lead to higher levels of nutrient loading than documented by previous studies of migratory geese. We measured the amount of total Kjeldahl nitrogen (TKN), a measurement of organic nitrogen and ammonia content (Morgan et al. 1957) and total

phosphorus (TP) in feces deposited by resident Canada geese. .Our objectives were to survey resident Canada goose feces for viable and measure the amount of TKN and TP in goose feces.

Study Area

We collected samples at 8 sites in the Triangle (Raleigh-Durham-Chapel Hill) region of North Carolina. Sites included 3 corporate facilities, a park, a suburban residence, a greenway, a college, and a cattle facility. The residence and one corporate facility were dominated by bermudagrass (*Cynodon dactylon* L.) and the other sites were dominated by tall fescue (*Schedonorus phoenix* (Scop.) Holub.). Each site had at least 0.4 ha of turf adjacent to or nearby a pond or lake and had daily use by geese on a portion of the grounds during early summer, early fall, or both. At least 0.4 ha of turfgrass used by geese was mowed every 4 – 8 days at heights recommended by site managers as part of an ongoing study (Ayers 2009). Six of the 8 sites were sloped toward the pond or lake from goose-grazed lawns, 7 of the 8 sites did not have complete riparian buffers or grass filter strips separating water from goose-grazed lawns, and 3 of the sites had impermeable surfaces (e.g., walking paths and parking lots) interspersed among goose-grazed lawns.

Materials and Methods

Germination Trials. We collected fresh individual goose droppings in March, June, July, and October of 2008. Each dropping was placed on top of a single cell of a seedling germination tray containing Miracle-Gro® potting mix. In each tray, 24-43 cells contained potting mix with a dropping and 7-20 control cells contained only potting soil depending on tray size and

feces collected. Within 24 hours of fecal collection, each tray was placed into an auto-irrigated greenhouse lighted from 0600 – 2000 daily. Trays were monitored weekly and remained in the greenhouse for 30 days before being placed into 4°C refrigeration for cold stratification. After 30 days of cold stratification, the trays were returned to the greenhouse for 30 additional days.

Nitrogen and Phosphorus Content. From each site, we collected multiple fresh goose droppings where available. The sampling periods were 10 – 12 days apart during each summer (June/July) and fall (September/October) of 2007 and 2008. Also, we collected grass samples at the same sites on the same days. Samples were stored in a resealable plastic bag and placed on ice immediately after collection and refrigerated or frozen within 12 hours until testing began. From each of the samples, 3 to 5, 50-g subsamples were used to measure the amount of TKN and TP through a persulfate digestion, ammonia salicylate method using an Auto analyzer III (Environmental Protection Agency 1983). Extrapolated values for fecal deposition per hectare were based on the daily total mass of feces collected on a 2-m X 21-m transect.

Results and Discussion

Germination Trials. We potted 43 goose droppings in March with 7 control cells, 30 in June with 20 controls, 30 in July with 20 controls, and 24 in October with 12 controls. Four (3.1%) dropping cells and zero control cells germinated a plant. One Pennsylvania smartweed (*Polygonum pennsylvanicum* L.) grew before cold stratification in a sample

collected in March. One annual bluegrass (*Poa annua* L.) grew after cold stratification from a sample collected in March. One *Kyllinga* sp. grew before cold stratification in a sample collected in July and one *Kyllinga* sp. grew after cold stratification from a sample collected in October. We were unable to distinguish between *K. brevifolia* L. and *K. gracillima* Rottb. due to lack of seeds for inspection of teeth on the scale keel (Bryson et al. 1997).

Only 2.2% of the total cells and 0% of control cells germinated a plant. The plant species that germinated are common in turfgrass or moist habitats similar to our fecal collection sites (Christians 2004, Cudney et al. 1998, Michigan State University 2005). Also, the cold stratification requirements and seed production periods for the plants we detected correspond with the time of dropping collection and greenhouse germination (Christians 2004, Cudney et al. 1998, Michigan State University 2005). The plants that germinated are not wind or expulsion dispersed and likely would not have come from adjacent experiments in the greenhouse (Christians 2004, Cudney et al. 1998, Michigan State University 2005).

Although three species of plants were germinated in our survey, only annual bluegrass and *Kyllinga* spp. are common weeds in turfgrass systems in the southeastern United States. *Kyllinga* spp. can become a problem in turfgrass systems with wet soils if established and allowed to extend rhizomatous growth (Cudney et al. 1998, McElroy et al. 2005). Annual bluegrass is a common weed in turfgrass systems throughout the world and has been shown in this study and by Best and Arcese (2009) to germinate from seeds in Canada goose feces. Also, annual bluegrass is not a valued plant for livestock pastures in most of the United States (DiTomaso 2000). We detected a very low percentage of viable

seeds in resident Canada goose feces, most likely indicating that geese are not ingesting many seeds from the frequently mowed turf areas in residential areas where they typically feed. However, because annual bluegrass can produce seeds at heights as low as 0.25 cm, it may have relatively high potential to be dispersed from even frequently mowed turf (Christians 2004). Nevertheless, because geese feed primarily on young grass, their feces should not contain high numbers of seeds (Conover 1991). Hence, resident Canada geese feeding primarily on managed turfgrass do not pose high risk of dispersing viable weed seeds.

Nitrogen and Phosphorus Content. We tested 304 fecal samples for TKN and TP content. The average moisture content of fecal samples was 80%. The average amounts of TKN and TP in fecal samples were 24.2 mg/g (range = 12.6 – 55.7) and 3.6 mg/g (range = 1.4 – 8.3) of dry matter, respectively

An average of 4.32 kg/ha/day of dry feces was deposited at our study sites equating to 104.3 g/ha/day of TKN and 15.5 g/ha/day of TP in dry feces deposited by geese. There was a daily average of 42 geese/site, indicating that each goose deposited an average of 103.9 g/ha/day of dry feces, 2.5 g/ha/day of TKN, and 0.4 g/ha/day of TP. Turfgrass fertilizers contain organic sources of N similar to those in animal waste (Christians 2004). The recommended rate of nitrogen application for turfgrass is 222.28 kg/ha/year for bermudagrass and 123.48 – 148.19 kg/ha/year for tall fescue (Bruneau 2001). Our results indicate that 42 geese deposited 17% of the yearly nitrogen application for bermudagrass and

26 – 31% of the yearly nitrogen application for tall fescue (Fig. 1). Recommended turfgrass fertilizers are commonly formulated with a 3:1 or 4:1 ratio of nitrogen to available phosphates, which are 44% elemental phosphorus (Emmons 2008). Based on this percentage, our results indicate that 42 resident Canada geese would deposit the equivalent of 12.85 kg/ha/year of phosphates. Further, the fecal material collected had a nitrogen-to-phosphate ratio of almost 7:1, similar to the 8:1 ratio in waterfowl feces as described by Post et al. (1998). Based on recommended rates, phosphate application should be 55.57 – 74.09 kg/ha/year for bermudagrass and 33.96 – 45.28 kg/ha/year for tall fescue. Our results indicate that resident populations of geese deposit 17 – 23% of the yearly phosphate application for bermudagrass and 28 – 38% of the yearly phosphate application for tall fescue (Fig. 1).

Excess fecal nutrient runoff will increase with fecal deposition on hard surfaces or turf areas directly adjacent to unbuffered surface waters. We recommend creating and/or maintaining riparian buffers between surface waters and turfgrass to reduce runoff and eutrophication. Also, thick hedges, tall trees, and unpalatable ground cover may reduce goose use (Conover 1992). During our study, concentration of Canada goose grazing pressure varied from site to site and should be considered when determining the fecal nutrient deposition/ha and subsequent effects on aquatic eutrophication. Further research should investigate the nutrient absorption from goose feces by turfgrass, the amount of nutrient runoff reaching open surface waters, and the effects of these levels of nutrient runoff on local and downstream water quality.

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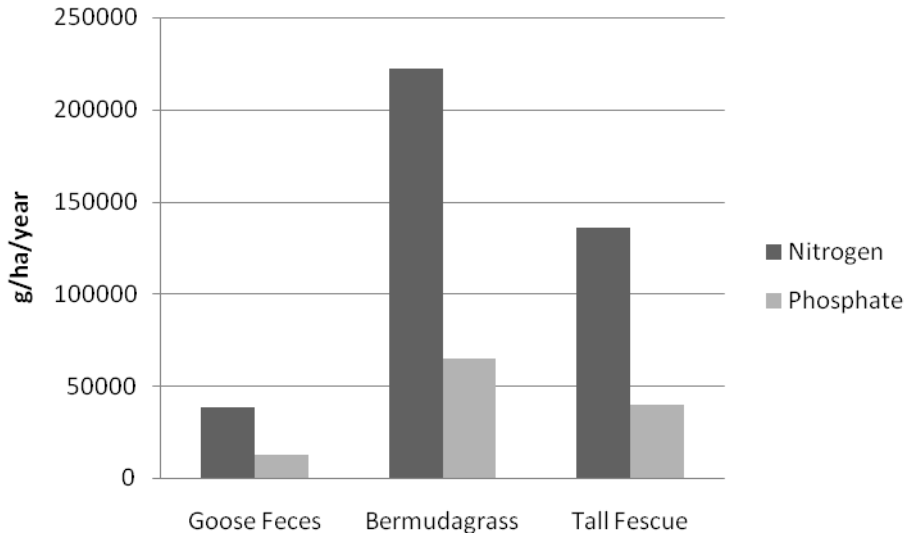

Figure 3.1. Nitrogen and phosphate contributions from ≈42 resident Canada geese/site compared to the recommended nitrogen and phosphate fertilization rates for bermudagrass (*Cynodon dactylon* L.) and tall fescue (*Festuca arundinaceae* L.) in North Carolina.

MANAGEMENT IMPLICATIONS

My results indicate that FCP works to reduce resident Canada goose use of turfgrass areas with no difference between a once/week and twice/week mowing frequency. However, managers must identify an application rate and total treated area that is not cost prohibitive. The suggested retail price of FCP is $240.00/gal. If FCP is applied once/month at the recommended rate of one gal/acre, the cost of FCP use would be $240.00/acre/month. Prior to treatment, we recommend that site managers and homeowners identify areas and times of year of highest goose use. Treating more of the turfgrass at a site or applying FCP more often may increase efficacy by reducing free-ranging Canada goose use. My results indicate that goose use of treated plots remained lower than the baseline even when the chemical coverage on grass went below 50%. Mowing more frequently than every 4 days, as often occurs on golf courses, may affect FCP efficacy. However, if the growth rate and angle of grass blades can be regulated to limit growth of treated blades above mowing height, then more frequent mowing should not have negative effects on FCP longevity.

I was unable to detect *G. lamblia* in free-ranging resident Canada geese in the North Carolina Triangle. Although unidentified species of *Giardia* have been detected in Canada geese, *G. lamblia* has not been identified specifically in their droppings. Additional research is necessary to refine the *Giardia* test kits for avian species and determine which species of *Giardia* are carried and transmitted by Canada geese.

Although 3 species of plants were germinated in our survey, only annual bluegrass and *Kyllinga* spp. are common weeds in turfgrass systems in the southeastern United States.

Because annual bluegrass can produce seeds at heights as low as 0.25 cm, it may have relatively high potential to be dispersed from frequently mowed turf. I detected a very low percentage of viable seeds in resident Canada goose feces, most likely indicating that geese are not ingesting many mature seeds, but instead feeding primarily on immature grass. Hence, resident Canada geese feeding primarily on managed turfgrass do not pose high risk of dispersing viable weed seeds.