Evaluation of Field Applications of GonaCon™ and DiazaCon™ in Reducing Gray Squirrel Fecundity

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Introduction

The eastern gray squirrel (*Sciurus carolinensis*) is one of the most common wildlife species in urban and suburban communities within the eastern United States. Because of their relative adaptability and lack of predators in urban environments, their numbers have increased in communities across their natural range. With this increase in numbers there has also been a corresponding increase in human-squirrel conflicts. Most notably is the damage gray squirrels are causing to trees and shrubs in managed landscape settings. Gnawing and stripping of tree bark by gray squirrels has become a serious problem in communities where squirrel populations are unusually high. For example, recent observations by landscaping crews on Clemson University's main campus have documented over 100 mature trees killed directly by gray squirrels gnawing and bark stripping, and an additional 100 trees severely damaged by squirrels (Bill Carson, personal communication). An outside arborist consultant estimated the loss of one mature tree from gray squirrels on Clemson's campus to be $13,275.00. Using these estimates, damage caused by gray squirrels on Clemson's campus may exceed $1.3 million dollars. These findings are also consistent with other studies that have shown significant losses of trees due to barking stripping by gray squirrels (Lurz et al. 2001). Unusually high gray squirrel populations in urban environments are also known to damage ornamental shrubs and herbaceous plantings, short out electrical power transformers, cause structural damage to buildings, and increase the likelihood of transmittable diseases and parasites among local squirrel populations.

Efforts to control gray squirrel numbers and their associated damage have been limited to exclusion techniques, habitat modification, repellents, trapping, shooting, and recreational hunting. Unfortunately in some situations, these alternatives are not effective, practical, or socially acceptable as tools for controlling gray squirrel numbers and associated problems in urban and suburban communities. This project proposes a potentially new approach to reduce gray squirrel reproduction and maintain squirrel population numbers at acceptable levels by field testing two experimental contraceptives on gray squirrels. The first contraceptive is GonaCon™, which is an

immunocontraceptive vaccine that shows a potential to render gray squirrels infertile after one injection. The second contraceptive is DiazaCon™, which is an oral contraceptive requiring multiple feedings and ingestion over the life of gray squirrels.

Mode of Action of GonaCon™ and DiazaCon™ (USDA National Wildlife Research Center)

GonaCon™

GonaCon™ is an immunocontraceptive vaccine that induces the immune system to generate antibodies to native ("self") gonadotropin releasing hormone (GnRH). This is accomplished by binding GnRH to a foreign protein. Because the animal's immune system has not been previously exposed to the foreign protein, it generates antibodies to both the foreign protein and to GnRH.

The hypothalamus releases GnRH which then travels to the anterior pituitary, stimulating the release of leuteinizing hormone (LH), and to a lesser extent, follicle stimulating hormone (FSH). These two hormones then trigger the release of testosterone, estradiol, and progesterone from the testes and ovaries. Testosterone is necessary for breeding behavior and the production of sperm. Estradiol plays a crucial role in egg development and quality, whereas progesterone is needed for ovulation and maintenance of pregnancy.

Antibodies to GnRH bind native GnRH as it leaves the hypothalamus, thus preventing it from binding to receptors in the anterior pituitary. As a result, no LH or FSH is released from the pituitary. Without the stimulus of LH and FSH, the testes and ovaries do not produce testosterone, estradiol, or progesterone, thus preventing the production of sperm and eggs. GonaCon™ has been used on a variety of mammals, both large and small. Recently, a single-shot vaccine has been developed that is efficacious for $\geq$ 2 years (Miller et al. 2003). The GnRH immunocontraceptive vaccine has been successfully used on rats (*Rattus norvegicus*) and California ground squirrels (*Spermophilus beecheyi*) for $\geq$ 2 years (Miller et al. 1997, Nash et al. 2004). Using only a single shot, its effects typically last $\geq$ 2 years which means it could render a rodent permanently infertile due to the short life span of rodents. There is no danger to nontargets because the vaccine is injected directly into the target animal. The vaccine consists of proteins; therefore, a secondary consumer could not be contracepted as proteins are broken down in the stomach. Although research continues to develop an oral GonaCon™ vaccine, animals must currently be captured and injected by hand.

DiazaCon™

DiazaCon™ is an oral contraceptive that inhibits the production of cholesterol. Its chemical structure is identical to cholesterol with the exception of two nitrogen substitutions at the 20th and 25th hydrocarbons. Because of this substitution, the liver cannot effectively eliminate DiazaCon™ as it does cholesterol and DiazaCon™ persists in the liver over several months.

The side chain of cholesterol is cleaved to form the precursor hormone pregnenolone. Pregnenolone is the parent compound for all steroid hormones. In the body, pregnenolone is altered to form testosterone, estradiol, and progesterone. These hormones are necessary for sperm and egg production. The nitrogen substitutions at the 20th and 25th hydrocarbon prevents the cleavage of cholesterol to form pregnenolone. As a result, plasma cholesterol concentrations decrease and desmosterol concentrations increase. Desmosterol is the immediate precursor to cholesterol in the cholesterol synthesis pathway and cannot be transformed into pregnenolone. Without sufficient cholesterol, the body “protects” itself by conserving the existing cholesterol for functions necessary for survival. Because reproduction is not necessary for an individual animal to survive, the reproductive system is shut down.

DiazaCon™ has been used on a variety of birds and small mammals. Preliminary laboratory tests with eastern gray squirrels were promising, and a breeding trial is ongoing. A treatment period of 5-10 days is generally required, and the contraceptive effects last up to four months depending on the species. Because DiazaCon™ is an oral bait, there are risks to nontargets that may feed on treated bait. These can be partially mitigated by the type of bait used, placement of bait, and the design of bait stations that primarily allow only the target animal to feed. DiazaCon™ may also be associated with some secondary effects because of its persistence in the liver. Research is currently being conducted to determine the magnitude of the effect. Unlike GonaCon™, animals do not have to be captured to deliver DiazaCon™.

Comparison of GonaCon™ and DiazaCon™

	GonaCon™	***DiazaCon™***
Delivery mode	Injection	Oral
Length of treatment	once	5-10 days
Longevity of effects	2-4 years	$\geq$ 4 months
Sex affected	Both	Both
Reversibility	Usually*	Yes
Animal capture required?	Yes	No
Toxicity/health effects	Minor	Only with overdose
Nontarget hazards	No	Yes
Secondary effects	No	Yes

*May last the lifetime of shorter lived species, making contraception permanent.

Objectives

- To evaluate the effectiveness of GonaCon™ in reducing gray squirrel reproduction in urban/suburban settings.
- To evaluate the effectiveness of DiazaCon™ in reducing gray squirrel reproduction in the field.

Methods

GonaCon™ Study

A treated and control study site (each 10 acres or less) will be located in a mixed hardwood (*Quercus* spp., *Carya* spp.) stand on Clemson University's main campus and Experimental Forest in Clemson, South Carolina. The two sites will be located $\geq$ 3.5 km apart, as the average dispersal range for gray squirrels is ≤ 3.2 km (Cordes and Barkalow 1972). Sites will be randomly assigned as either a control or a treated site. Because each site will be $\leq$ 10 acres, an Experimental Use Permit from the Environmental Protection Agency is not required.

Wooden box traps baited with corn, sunflower seeds, or peanut butter and rolled oats will be used to capture squirrels. Traps will be placed in the shade (hidden from public view) and set at dawn and closed at dusk each day. Traps will be checked 2 times each day: early morning and late evening before sunset. Handling of squirrels will be faciliated by using a conical cloth bag placed over the entrance of traps that allows squirrels to move from the trap directly into the restraint. This will minimize the risk of escape and injury, reduce the amount of handling time, and allow for normal respiration. Estimated handling time per squirrel is approximately 10 minutes. Anticipated mortality of captured squirrels is expected to be close to zero. If injury occurs to captured

squirrels, they will be treated by the attending veterinarian and released. Trapping will be conducted under a South Carolina Department of Natural Resources collection permit.

Eighty gray squirrels will be trapped before the March 2008 breeding season on both the control and treated areas (40 squirrels/area) to determine general health conditions, sex, and age. The general health status of each individual animal will be determined by visually examining each animal. Each animal will be examined for the presence of external parasites, and any abrasions or other injuries will be noted. The overall appearance of the coat will be noted, along with any hair loss not due to molting. The general demeanor of the squirrel when handled will also be recorded (e.g. fast/slow respiration, calm/agitated). The sex of the animal will be determined by visual examination of the external genitalia. Even as juveniles, male squirrels have visible scrotum, distinguishing them from females (Dimmick and Pelton 1996, Ferryman et al. 2006; see appendix).

During trapping 40 adult squirrels will be administered the GonaCon™ vaccine by injection and released. GonaCon™ vaccine (1000 μg GnRH-Blue protein conjugate/mL) will be prepared according to SOP BT 016.02. Both male and female squirrels at the treated site will be injected with 0.4 mL of the vaccine intramuscularly in the thigh (SOP BT 004.00). GonaCon™ contains 1000 μg GnRH-blue protein conjugate/mL; therefore, each 0.4 mL dose contains 400 μg GnRH-blue protein conjugate. Control squirrels (40) will be injected with 0.4 mL saline-AdjuVac™ intramuscularly in the thigh. Both males and females will be treated in March. Vaccines will be administered at two sites in the thigh (0.2 mL at each site).

Blood samples will be collected in the spring 2008 from $\geq$ 40 trapped and tagged squirrels vaccinated with GonaCon™ on both the control and treated sites. Blood will be collected from either the lateral femoral vein or the saphenous vein according to SOP FP 030.00. Plasma will be analyzed for progesterone and testosterone concentrations using radioimmunoassay (RIA) according to SOP BT 025.00.

Additional measures of fertility will be evidence of lactation in females and scrotal pigmentation in breeding males. Lactation will be assessed according to the appearance of the teats. Lactating females will have swollen teats with little or no hair covering them. Breeding males will typically have a gray or black pigmented scrotum with little hair covering them and enlarged testes. Nonbreeding males typically have a

pink pigmented scrotum with hair regrowth evident and small flaccid testes (Pudney 1976, Webley et al. 1985, Ferryman et al. 2006).

DiazaCon™ Study

A treated and control study site (each 10 acres or less) will be located in a mixed hardwood (*Quercus* spp., *Carya* spp.) stand on Clemson University's Experimental Forest in Clemson, South Carolina. The two sites will be located ≥ 3.5 km apart, as the average dispersal range for gray squirrels is ≤ 3.2 km (Cordes and Barkalow 1972). Sites will be randomly assigned as either a control or a treated site. Because each site will be ≤ 10 acres, an Experimental Use Permit from the Environmental Protection Agency is not required.

Bait stations consisting of 4-6 inch diameter plastic PVC pipes 12 inches long will be mounted to select trees distributed across the treatment study area. The bait stations are expected to reduce nontarget hazards. Prebaiting will occur for 2 weeks to acclimate squirrels to bait stations. The type of bait to be used will be determined by bait preference trials which are currently being conducted at Clemson University, SC. After the 2 week baiting period, DiazaCon™ coated bait will be placed in the feeder stations for 2 weeks. DiazaCon™ bait will be manufactured according to SOP BT 030.00 to contain 0.23% DiazaCon™ (w/w) except that rolled oats may not be used. In addition, the bait will also contain Rhodamine B, an inert, nontoxic, and persistent dye. The dye will serve as a biomarker on the teeth to determine which squirrels have consumed treated DiazaCon™ bait (Fisher 1999). Bait will be placed in the stations early in the morning. Prior to sunset, a cap will be placed over the PVC tube to reduce the feeding of southern flying squirrels which are primarily nocturnal. The amount of bait placed in each station, and the amount of bait left at the end of the 2 week period in each station will be recorded. DiazaCon™ will be administered for 2 weeks at the end of February, and for 5 consecutive days in April.

Blood samples will be collected in the spring 2008 from ≥ 40 trapped and tagged squirrels on both the control and treated sites. Blood will be collected from either the lateral femoral vein or the saphenous vein according to SOP FP 030.00. Plasma will be analyzed for progesterone and testosterone concentrations using radioimmunoassay (RIA) according to SOP BT 025.00. Plasma samples will also be analyzed for desmosterol and non-esterified cholesterol by the Analytical Chemistry Project using the

procedures outlined in “Determination of Desmosterol and Free Cholesterol in Prairie Dog, Rat, and Brown Tree Snake Plasma” (method 98A; Johnston et al. 2003). All plasma samples will be stored at -20 to -70° C until analysis.

Additional measures of fertility will be evidence of lactation in females and scrotal pigmentation in breeding males. Lactation will be assessed according to the appearance of the teats. Lactating females will have swollen teats with little or no hair covering them. Breeding males will typically have a gray or black pigmented scrotum with little hair covering them and enlarged testes. Nonbreeding males typically have a pink pigmented scrotum with hair regrowth evident and small flaccid testes (Pudney 1976, Webley et al. 1985, Ferryman et al. 2006).

Age Determination and Marking

A combination of pelage characteristics and body weight should be used to more accurately assign an age class to individuals. Although scrotal appearance can be used to aid in aging male gray squirrels, the appearance of subadult and adult males with regressed testes can often be confusing (Hoffman and Kirkpatrick 1956). Skeletal and tooth characteristics can be used to accurately age gray squirrels, but these methods require euthanizing the animal. Age classes for the purpose of this study are defined as follows: *juvenile* – 0-6 months of age, *subadult* – 6-12 months of age, and *adult* - > 12 months of age.

The appearance of scrotum or teats can be used to help separate gray squirrels into these age classes. Juvenile males (3-4 months) will have hair covering the scrotum with only light pigmentation. Subadult males (8-9 months of age) will have furred and pigmented scrotum. Adult males (> 1 year) will have furless, pigmented scrotum. The scrotum may become less pigmented during periods of testicular quiescence (Ferryman et al. 2006). Nonbreeding, juvenile females will have very small, pink teats that can only be seen by moving the fur. Adult, breeding females will have teats that can easily be seen through the fur, and may have small pigmented areas of gray/black (Dimmick and Pelton 1996). In lactating females, most of the hair will be absent from the teats.

Tail pelage characteristics can also be used to separate gray squirrels into age classes (Sharp 1958, Dimmick and Pelton 1996). Juveniles do not have short secondary hairs appressed (laying flat) along the ventral side of the tailbone, making the outline of the tailbone easily visible. In addition, there are 2-3 dark bands running through the reddish fur parallel to the tailbone on either side (Figure 2). These bands

are most easily seen in the mid-region of the tail. Subadults have short secondary hairs appressed along the lower third of the ventral side of the tail. The outline of the tailbone is still visible. Like juveniles, subadults have 2-3 dark bands running parallel to either side of the tail. Adults have short secondary hairs appressed along the entire length of the ventral side of the tail, obscuring the outline of the tailbone. The dark, parallel bands present in juveniles and subadults are not visible in adults.

During the winter months, pelage can also be used to assign age classes when the fur in the rump region is separated and laid flat (Barrier and Barkalow 1967, Dimmick and Pelton 1996). The area just adjacent to the mid-dorsal stripe must be used as all age classes have black-tipped hairs in the mid-dorsal stripe regardless of season. Juveniles will have no yellow prebasal band in the black underfur and guard hairs will be tipped with black (Figure 3). Subadults will have either no yellow prebasal band in the underfur or an indistinct prebasal band. Guard hairs will be tipped with white. Adults will have a distinct yellow prebasal band in the underfur and white-tipped guard hairs. During summer months, all age classes have black-tipped guard hairs.

The following table of weights can generally be used to aid in assigning individuals to age classes (Uhlig 1955, Koprowski 1994, Dimmick and Pelton 1996). Squirrels will be weighed using a spring scale according to SOP FP 029.00.

Age	Weight
8 weeks	141.5 g
10 weeks	198.1 g
14 weeks	311.3 g
16 weeks	367.9 g
18 weeks	396.2 g
Subadult (6-12 months)	396.2-710.0 g
Adult (> 12 months)	396.2-710.0 g

Squirrels will be ear-tagged for easy recognition. Ear tags will be positioned such that they are attached where the cartilage is the thickest. Pliers will be used to attach ear tags, and numbers will be positioned dorsally. In addition, PIT tags may also be inserted to serve as a second identifier in case ear tags are lost. Prior to inserting the PIT tag, scan the tag to make sure it is working properly and to verify the number. Pinch the dorsal skin between the scapula to form a “tent”. Place the needle at the base of the tent ≤ 45° from the dorsal midline and insert it until the needle tip can be felt against the skin on the side opposite the injection site. Depress the plunger to insert the PIT tag,

making sure the needle has not gone all the way through the skin. As the needle is withdrawn, use the fingers to pinch off the injection site, ensuring that the PIT tag does not fall out. Scan the animal to verify the PIT tag was inserted properly.

Gray Squirrel Density Estimates

Gray squirrel population densities will be estimated on the study and control sites during early winter prior to the initiation of contraceptive treatments and at the end of the study. Although density estimates are not an accurate metric to access the effects of contraceptive treatments, they are important parameters to document during the study.

Transect counts of squirrel observations/nests will be used to determine the numbers of squirrels per acre (Hein 1997). Transect lines will be $\geq$ 140 m, and will be positioned to avoid buildings, roads, and paralleling sidewalks. Four transects will be laid out using an all-terrain measuring instrument. Intervals of 20 m will be measured along the length of the transect, and will be marked using a flagged 16-penny nail and painting the vegetation around the nail with fluorescent orange paint. Each line will be surveyed five times, and surveys will begin between sunrise and mid-morning. Transect lines will be walked at approximately 1 km/hr (100 m/ 6 min). Clusters or aggregations of squirrels will be defined as squirrels that are ≤ 2 m apart. The area around the transect line will be scanned on both sides of the transect line, above the transect line in the canopy, and in front of the observer. When a squirrel is observed, a visual landmark will be used to mark a squirrel's position or the center of a cluster of squirrels. The position will immediately be physically marked with flagging, and the perpendicular distance to the transect line will be measured and recorded. When squirrels are observed overhead, a visual marker directly below the squirrel will be used to mark the position. That position will then be used to obtain the perpendicular distance back to the transect line. Once the measurements are taken, the survey will resume along the transect line. Surveys will not be conducted when there is precipitation and/or the wind is blowing > 10 km/hr.

Statistical Analyses

This experimental design is completely randomized. Data will be analyzed using analysis of variance (ANOVA).

List Number and Title of Standard Operating Procedures (SOPs) Guidelines to be Used

AC/CO 002.00	Animal Handling to Maintain Secure Identification
AC/CO 016.00	Animal Quarantine Procedures at Fort Collins
AD 004.01	Archiving Studies
AD 007.01	Final Reports
AD 008.01	Personnel Qualification Records
AD 011.02	Data Recording and Error Correction
AD 012.02	Test, Control, and Reference Substance Chain of Custody
BT 004.00	Injection Procedure for Immunizing Animals with Immunocontraceptive Vaccines
BT 016.02	Manufacture of GonaCon™ Immunocontraceptive Vaccine Using the Microfluidics™ M110L High Pressure Homogenization Instrument and/or the Manual Hand Method
BT017.00	ELISA Procedure to Assess Antibody Titers in Plasma or Serum
BT 025.00	Use of Radioimmunoassay (RIA) to Assess Reproductive Hormone Concentrations in Plasma or Serum
BT 026.00	Purification of Antibodies From Plasma or Serum Using the Pierce NAb Spin Kit
FP 029.00	Use of a Spring Scale for Body Mass Measurements
FP 030.00	Collection and Processing of Blood Samples from Prairie Dogs and Similar Size Mammals
HS 004.00	Personal Protective Equipment

Methods for which no SOP is available – procedures are detailed in the Methods section

Trapping gray squirrels with wooden box traps
Sexing gray squirrels
Aging gray squirrels
Determination of the general health status of gray squirrels
Ear tagging gray squirrels for individual recognition
Injection of PIT tags for individual recognition of gray squirrels
Assessing the breeding status of male and female gray squirrels
Conducting transect counts for gray squirrel density estimation

List of Records to be Maintained

Aging records
Animal weights
Antibody purification records
Breeding status records
Density estimates
Distance sampling records
ELISA records and results
GonaCon™ chain of custody records
GonaCon™ manufacturing records

Health status records
RIA records and results
Sexing records
Squirrel blood chain of custody records
Squirrel blood collection records
Squirrel vaccination records
Statistical records
Tagging (ear tags and PIT tags) records
Transect line information (distance and location)
Trapping records

Permits/Certifications Obtained

Clemson University Animal Use Protocol (AUP)
Clemson University IBC (Hazardous Chemicals) Protocol
USFWS Section 7 Consult
SC Pesticide Regulatory
SCDNR Scientific Collecting Permit
Zoonotic & Hantavirus Training
Enrollment in Medical Surveillance Program

Cooperators

- Mr. Noel Myers, South Carolina State Director, USDA Wildlife Services.
- Dr. Kathleen Fagerstone, Research Project Manager, USDA Wildlife Serviced, Fort Collins, CO
- Dr. Christi Yoder, Reproductive Physiologist, USDA Wildlife Services, Fort Collins, CO
- Ms. Catherine Bens, Quality Assurance Officer, USDA Wildlife Services, Fort Collins, CO
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- Mr. Tom Swayngham, Regional Wildlife Biologist, South Carolina Department of Natural Resources
- Mr. Derrell Shipes, Chief of Statewide Projects, Research & Surveys with the South Carolina Department of Natural Resources

Literature Cited

Bier J., and F. S. Barkalow, Jr. 1967. A rapid technique for aging gray squirrels in winter pelage. Journal of Wildlife Management 31:715-719.

Dimmick, R. W., and M. R. Pelton. 1996. Criteria of sex and age. Pages 169-214 *in* Research and Management Techniques for Wildlife and Habitats. T. A. Bookhout, ed. Allen Press, Inc., Lawrence, Kansas.

Emmons, G. T., E. R. Rosenblum, J. N. Peace, J. M. Malloy, D. L. Doerfler, I. R. McManus, and I. M. Campbell. 1982. Effects of 2025 diazacholesterol on cholesterol synthesis in cultured chick muscle cells: A radiogas chromatographic and mass spectrometric study of the post-squalene sector. Biomedical Mass Spectrometry 9:278-285.

Fisher, P (1999) Review of using Rhodamine B as a marker for wildlife studies. Wildlife Society Bulletin (27) (2); 318-329

Ferryman, M., B. A. Mayle, and G. W. Morgan. 2006. Visual method for evaluating the state of sexual development in male gray squirrels (*Sciurus carolinensis*). Reproduction, Fertility and Development 18:383-393.

Fisher, P. 1999. Review of using Rhodamine B as a marker for wildlife studies. Wildlife Society Bulletin 27:318-329.

Hein, E. W. 1997. Demonstration of line transect methodologies to estimate urban gray squirrel densities. Environmental Management. Vol. 21, No. 6, pp. 943-947.

Hoffman, R. A., and C. M. Kirkpatrick. 1956. An analysis of techniques for determining male squirrel reproductive development. Transactions of the North American Wildlife Conference 21:346-355.

Johnston, J. J., M. J. Goodall, C. A. Yoder, C. A. Furcolow, D. A. Goldade, B. A. Kimball, and L. A. Miller. 2003. Desmosterol: a biomarker for the efficient development of 20,25-diazacholesterol as a contraceptive for pest wildlife. Journal of Agricultural and Food Chemistry 2003 Jan 1:51(1):140-5.

Koprowski, J. L. 1994. Sciurus carolinensis. Mammalian Species 480:1-9.

Lurz P.W.W, Rushton S.P, Wauters L.A., Bertolino S., Currado .I, Mazzoglio P, Shirley, M.D.F. 2001. Predicting gray squirrel expansion in North Italy: a spatially explicit modeling approach. Landscape Ecology. 16: 407-420

Miller, L. A., B. E. Johns, D. J. Elias, and K. A. Crane. 1997. Comparative efficacy of two immunocontraceptive vaccines. Vaccine 15:1858-1862.

Nash, P. B., D. K. James, L. T. Hui, and L. A. Miller. 2004. Fertility control of California ground squirrels using GnRH immunocontraception. Proceedings of the Vertebrate Pest Conference 21:274-278.

Nixon, C. M., and M. W. McClain. 1975. Breeding seasons and fecundity of female gray squirrels in Ohio. Journal of Wildlife Management 39:426-438.

Pudney, J. 1976. Seasonal changes in the testes and epididymis of the American gray squirrel (*Sciurus carolinensis*). Journal of Zoology 179:107-120.

Sharp, W. M. 1958. Aging gray squirrels by use of tail-pelage characteristics. Journal of Wildlife Management 22:29-34.

Uhlig, H. G. 1955. Weights of adult gray squirrels. Journal of Mammalogy 36:293-296.

Webley, G. E., G. S. Pope, and E. Johnson. 1985. Seasonal changes in the testes and accessory reproductive organs and seasonal and circadian changes in plasma testosterone concentrations in the male gray squirrel (*Sciurus carolinensis*). General and Comparative Endocrinology 59:15-23.

Yoder, C. A., W. F. Andelt, L. A. Miller, J. J. Johnston, and M. J. Goodall. 2004. Effectiveness of twenty, twenty-five diazacholesterol, avian gonadotropin-releasing hormone, and chicken riboflavin carrier protein for inhibiting reproduction in Coturnix quail. Poultry Science 83:234-244.

Waters, R. 2006. Gray squirrels. Alabama Cooperative Extension Service Technical Note.