Efficacy of Rhodamine B as a Fecal Marker for White-tailed Deer

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ABSTRACT

Rhodamine B has been used as a wildlife marker for microherbivores and mesopredators, but has yet to be evaluated for ruminants. Our objective was to determine the efficacy of rhodamine B as a wildlife marker for white-tailed deer (Odocoileus virginianus). We tested the effect of rhodamine B on the production of mold growth, the palatability, lag time, variable concentration effectiveness, and persistence of rhodamine B as a fecal marker in penned white-tailed deer. Liquid rhodamine B was added to corn and alfalfa-based, pelleted deer feed in concentrations of 0.06-oz. and 0.13-oz. of rhodamine B/lb. of grain and either placed in metal containers immediately or allowed a 1-hr drying period before being placed in metal containers. Grain samples that were sprayed with rhodamine and not allowed to dry produced mold quicker than non-marked grain; however, if the dye was allowed a 1-hr drying period immediately after application, then rhodamine B did not enhance mold production. Five deer (2 M, 3 F) were fed for two days a pelleted diet sprayed with a surface coating of 0.00045 oz. of rhodamine B/lb. body weight, and five deer (3 M, 2 F) were fed the same diet but sprayed with a surface coating of 0.0009 oz. of rhodamine B/lb. body weight, then fed a pelleted diet ad libitum thereafter without rhodamine B. Seven deer (n = 10) had feces marked during the first 12 hours and all deer produced marked feces after 36 hours. Rhodamine B persisted as a fecal marker up to 60 hours post-ingestion. White-tailed deer selected against feed that was marked by rhodamine B, but would consume feed that contained the dye. Rhodamine B can be a useful marker of white-tailed deer, especially if one wishes a non-lethal and non-capture method to determine animal movements.

KEY WORDS: Feces, fluorescent dye, marker, Odocoileus virginianus, palatability, rhodamine B, white-tailed deer

Rhodamine B is an industrial and analytical dye that has been used as an external marker to trace animal movements (Clover, 1954; Taber et al., 1956), aid animal identification (Wadkins, 1948), and determine animal use of baits (Farry et al., 1998). Its use as a biological marker has been evaluated in rodents (New, 1958; Gast, 1963), black-tailed jackrabbits (*Lepus californicus;* Evans and Griffith, 1973), mountain beavers (*Aplodontia rufa;* Lindsey, 1983), coyotes (*Canis latrans;* Johns and Pan, 1981; Farry et al., 1998), and birds (Wadkins, 1948; Paton and Pank, 1986). However, to our knowledge, rho-

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damine B has yet to be evaluated as a biological marker for white-tailed deer (*Odocoileus virginianus*).

When mixed with water the dye appears maroon, but fluoresces orange in color under UV light (approximate wavelength 366 nanometers) (Evans and Griffith, 1973; Fisher, 1999). Rhodamine B can be detected visually in concentrations as low as 0.1 part per billion (Gast, 1963; Fisher, 1999).

Lindsey (1983) reported that when ingested, rhodamine B can be used as a short-term marker of the gastrointestinal tract, urine, feces, and blood. Wadkins (1948) used rhodamine B as a short-term external marker of birds by spraying the dye directly onto feathers. Rhodamine B also was used as a long-term marker of claws and growing hair in coyotes (Johns and Pan, 1981).

Potential problems with the use of rhodamine B include reduced palatability of feeds (Webb and Hansen, 1961) and human health concerns (Kawachi et al., 1980). However, Fisher (1999) reported that no epidemiological evidence exists that suggests rhodamine B has carcinogenic, mutagenic, or teratogenic effects.

Our objectives were to determine 1) if mold growth was expedited due to the spraying of rhodamine B on two feedstuffs, 2) the concentration of rhodamine B required to mark feces of white-tailed deer, 3) the lag time required from ingestion of rhodamine B to produce marked feces, 4) the persistence of rhodamine B as a fecal marker in white-tailed deer, and 5) palatability of feed that was surface-coated with rhodamine B to white-tailed deer.

MATERIAL AND METHODS

Mold Growth Trials

Mold growth trials were conducted in the Lehmann Research Laboratory on the campus of Texas A&M University-Kingsville. Rhodamine B powder (Sigma Chemical, St. Louis, Mo.) was dissolved in water. A total of 15 lbs. of whole kernel corn (Wal-Mart, Kingsville, Tex.) and alfalfa-based deer pellets (Purina Mills, Inc., Gonzales, Tex.) was divided into 15 1.0-lb. samples, respectively. Grain samples were initially free from the appearance of mold. Five samples of corn and five samples of pellets were sprayed with a surface coating of liquid rhodamine B to yield minimum dosages of 0.06-oz. and 0.13-oz. of rhodamine B/lb. of grain. Minimum dose of 0.00045 oz. rhodamine B/lb. of animal body mass was needed to produce visible marks in coyote hair (Johns and Pan, 1981). Thus a minimum dosage of 0.06 oz. rhodamine B/lb. of grain was needed to achieve the recommended 0.00045 oz. rhodamine B/lb. of body mass, based on the mean mass of deer from southern Texas (132 lbs.; Cook, 1984) and assuming deer eat 1.0 lb. of rhodamine-dyed feed daily. The remaining five samples of each food type was used as a control. Samples were immediately placed in 1.0 lb. metal containers and sealed with a plastic lid. Metal containers were used to simulate feed being placed in 55 gallon drum feeders. Metal containers were stored at 76°F and 66% RH. Grain samples were checked every 24 hours and inspected for the presence of mold. Trials were arbitrarily discontinued after 20 days if mold was not present. Distributions of residual errors were tested for normality using the Shapiro-Wilk test (SAS Institute, Inc., 1989). Homogeneity of variances among treatments was evaluated with the Bartlett's test (Steel and Torrie, 1980). Because assumptions of parametric tests were satisfied, analysis of variance (SAS Institute, Inc., 1989) was used to test the effect of concentration of rhodamine B on the time required for mold to first appear. Multiple comparisons were made using Tukey's procedure when a significant F-test occurred (Ott, 1993). Tests were considered significant at $P < 0.05$.

A second trial was conducted similar to the one described above, except a 1 hr drying period was allowed before the grain was stored in the metal containers. Trials were arbitrarily discontinued after 45 days if mold was not present.

Fecal Marking Trials

Ten white-tailed deer (5 M, 5 F) were individually housed at the Texas A&M University-Kingsville Captive Deer Facility (Kingsville, Tex.). Individual pens measured 12 H 12 H 8 ft. and consisted of chain link walls, tin roof, and rubber-padded floors. Deer were acclimated to the pens for 1 week prior to the initiation of the experiment. Alfalfa-based, pelleted deer feed (Purina Mills, Inc., Gonzales, Tex.), alfalfa hay, and water were given *ad libitum* during the acclimation period.

Pelleted deer feed was sprayed with a surface coating of liquid rhodamine B as described in the mold growth trials to yield minimum dosages of 0.06-oz. and 0.13-oz. of rhodamine B/lb. of grain (0.00045 oz. and 0.0009 oz. rhodamine B/lb. of body mass, respectively). Five deer (2 M, 3 F) received 1.0 lb. of pelleted feed that contained 0.06-oz. of rhodamine B/lb. of grain and another 5 deer (3 M, 2 F) received 1.0 lb. of pelleted feed that contained 0.13-oz. of rhodamine B/lb. of grain daily for two days. Rhodamine-dyed pellets that were not consumed after 2 days were removed and replaced with non-dyed pellets and alfalfa hay.

We collected feces from each deer at 12 hr intervals during the 48-hr period of feeding white-tailed deer rhodamine-marked feed and the 96-hr period immediately thereafter. Gloves were used to collect feces and were changed between deer pens to avoid cross-contamination. Feces were placed in ziplock bags and labeled with deer number, time of collection, and concentration of rhodamine B feed. Feces were examined for the presence of rhodamine B staining using an ultraviolet light. We recorded the amount of rhodamine B detected in the feces on a subjective scale of 0 to 4. Ratings given were 0 if rhodamine was not detected under the ultraviolet light, 1 if rhodamine was not visible to the naked eye but was slightly detectable under ultraviolet light, 2 if rhodamine was not visible to naked eye but was very noticeable under ultraviolet light, 3 if rhodamine staining was slightly visible to the naked eye, and 4 if rhodamine staining was easily observed by the naked eye.

Assumptions of parametric tests were evaluated as described in the mold growth trials. Analysis of variance (SAS Institute, Inc., 1989) was used to test the effect of rhodamine B concentration on the average ratings of dye detectability. Multiple comparisons were made using Tukey's procedure when a significant F-test occurred (Ott, 1993).

Palatability Trials

Ten white-tailed deer (5 M, 5 F), which were used in the fecal marking trials, were maintained in their individual pens. Palatability trials were conducted using cafeteria style trays containing three compartments. Trays were built to minimize spillage and 20 inch tall dividers were placed between trays to reduce the possibility of deer spilling feed between compartments. Each deer was given 3, 4-lb. rations of pelleted deer feed. Two rations were sprayed with a surface coating of liquid rhodamine B to yield final concentrations of 0.00045 and 0.0009 oz. rhodamine B/lb. body weight (as previously described), respectively, and the remaining ration was used as a control. Rations were placed into randomly selected compartments at the beginning of the trial. Amount of feed remaining in each compartment was weighed at 24-hr intervals after the initiation of the trial and continued for 5 days. At each weighing interval, compartment placement of the rations was re-randomized to avoid the bias of deer that selected food from the same compartment.

Program RODGERS (Krebs, 1989:600-602) was used to calculate the area under the plotted feed consumption versus time curve and the diet preference index for each diet by each deer. Assumptions of parametric tests were evaluated as described in the mold growth trials. Analysis of variance (SAS Institute, Inc., 1989) was used to test the effect of rhodamine B concentration on the preference indices. Multiple comparisons were made using Tukey=s procedure when a significant F-test occurred (Ott, 1993).

RESULTS

Mold Growth Trials

Feedstuffs sprayed with rhodamine B that were not allowed to dry before being placed in metal containers produced mold quicker ($F_{5, 24} = 6.3$, $P < 0.0001$) than feedstuffs not sprayed with the dye (Table 1). Corn tended to mold faster (range = 2 - 9 days) than pelleted feed (range = 4 - 12 days). Feedstuffs not marked by rhodamine B dye did not mold within the 20-day trial.

However, corn sprayed with rhodamine B dye that was allowed a 1-hr drying period did not mold during the 45-day trial (Table 1). Samples of pelleted feed not marked with rhodamine B and samples sprayed with rhodamine B concentrations of 0.06 oz./lb. produced mold within 4 and 5 days, respectively, which produced mold quicker ($F_{5, 24} = 8.8$, $P < 0.0001$) than samples that received rhodamine B concentrations of 0.13 oz./lb. grain or samples of corn at any concentration of rhodamine B (Table 1).

Table 1. Time required for the production of mold on two feedstuffs sprayed with 0.06 and 0.13 oz. rhodamine B/lb. grain and not allowed (A) and allowed (B) a 1-hr drying period before being placed in metal containers.

Feedstuff	Rhodamine concentration (oz./lb.)	N	Time to mold ($\bar{x}$ ± SE, days)	Range	(days)
(A) - *No drying period*[1]					
Corn	0	5	20.0 ± 0.0	A[2]	Never
Pelleted feed	0	5	20.0 ± 0.0	A	Never
Pelleted feed	4	5	10.2 ± 0.7	B	9 - 12
Pelleted feed	8	5	6.6 ± 0.9	C	4 - 9
Corn	4	5	4.4 ± 1.2	CD	3 - 9
Corn	8	5	2.0 ± 0.0	D	2
(B) - *1-Hour drying period*[1]					
Corn	0	5	45.0 ± 0.0	A[2]	Never
Corn	4	5	45.0 ± 0.0	A	Never
Corn	8	5	45.0 ± 0.0	A	Never
Pelleted feed	8	5	29.4 ± 9.6	A	6 - 45
Pelleted feed	0	5	5.0 ± 0.0	B	5
Pelleted feed	4	5	4.0 ± 0.0	B	4

[1] First trial was conducted for 20 days; second trial was conducted for 45 days.
[2] Means within a trial with the same letter are not different ($P > 0.05$).

Fecal Marking Trials

Feces from 3 (60%) and 4 (80%) of the deer fed 0.00045 oz./lb. body weight and 0.0009 oz./lb. body weight, respectively, of rhodamine B were marked within the first 12 hours of exposure to the dye. After 36 and 24 hours of being fed rhodamine-marked feed 100% of deer fed 0.00045 oz./lb. body weight and 0.0009 oz./lb. body weight, respectively, of rhodamine B produced marked feces. Average ratings of dye detectability were greater ($F_{1, 8} = 9.52$, $P = 0.015$) in the 0.0009 oz./lb. body weight concentration of rhodamine B than the 0.00045 oz./lb. body weight concentration only during the 13 - 24 hour period (Table 2). Deer fed rhodamine B for >36 hours produced rhodamine-marked feces that was visible to the naked eye.

Table 2. Average ratings of dye detectability in feces of white-tailed deer fed 0.00045 and 0.0009 oz. of rhodamine B/lb. body weight and the persistence of rhodamine in feces.

Period (hrs)	Average ratings[1]		P-values
	0.00045 oz./lb. body weight	0.0009 oz./lb. body weight	
	Rhodamine in feed[2]		
0 - 12	1.2 ± 0.5	1.8 ± 0.7	0.488
13 - 24	1.6 ± 0.5	3.6 ± 0.4	0.015
25 - 36	3.0 ± 0.4	4.0 ± 0.1	0.056
37 - 48	3.0 ± 0.4	3.4 ± 0.2	0.126
	Rhodamine persistence[3]		
0 - 12	2.6 ± 0.2	3.0 ± 0.3	0.347
13 - 24	2.0 ± 0.4	3.0 ± 0.6	0.233
25 - 36	0.8 ± 0.4	2.0 ± 0.4	0.074
37 - 48	0.2 ± 0.2	0.8 ± 0.4	0.195
49 - 60	0.0 ± 0.0	0.2 ± 0.2	0.347
61 - 72	0.0 ± 0.0	0.0 ± 0.0	1.000

[1]Average ratings were based on 5 white-tailed deer per rhodamine concentration. Ratings given were 0 if rhodamine was not detected under the ultraviolet light, 1 if rhodamine was not visible to the naked eye but was slightly detectable under ultraviolet light, 2 if rhodamine was not visible to naked eye but was very noticeable under ultraviolet light, 3 if rhodamine staining was slightly visible to the naked eye, and 4 if rhodamine staining was easily observed by the naked eye.

[2]Pelleted deer feed sprayed with liquid rhodamine B and fed to white-tailed deer during first 48-hr period.

[3]Time period immediately after feeding white-tailed deer feed containing rhodamine B.

During the rhodamine B persistence phase of the trial (i.e., length of time in which deer continued to produce rhodamine B-marked feces after consumption of rhodamine-marked feed was discontinued), 100% of the deer that were fed 0.00045 oz./lb. body weight and 0.0009 oz./lb. body weight rhodamine produced marked feces up to 24 and 36 hours, respectively, after being taken off of rhodamine-marked feed. Rhodamine staining of feces was no longer detectable in deer that were fed 0.00045 oz./lb. body weight and 0.0009 oz./lb. body weight rhodamine after 60 and 72 hours, respectively. Differences in

rhodamine persistence in feces was not noted ($F_{1, 8} = 4.24$, $P > 0.074$) between the two concentrations of rhodamine B during any 12-hr time period (Table 2).

Palatability Trials

White-tailed deer avoided ($F_{2, 27} = 105.4$, $P < 0.0001$) feed marked with rhodamine B. Deer preferred feed without rhodamine B, then feed with rhodamine B concentrations of 0.00045 oz./lb., followed by feed with rhodamine B concentrations of 0.0009 oz./lb. Average Rodger's indices for white-tailed deer were 1.000 ± 0.00, 0.416 ± 0.08, and 0.025 ± 0.01 for feed containing rhodamine B concentrations of 0 oz./lb., 0.00045 oz./lb., and 0.0009 oz./lb., respectively.

DISCUSSION

Rhodamine B is potentially a very useful, simple to use, and inexpensive bait marker for white-tailed deer. Rhodamine B does not require the capture of animals to produce a mark and it offers a non-invasive and non-lethal means to assess the mark.

Selection criteria for bait markers have included 1) ease of application to the bait material, 2) mark is easily detectable, 3) persistence of mark, 4) no effect on bait acceptance by the target animal, and 5) no adverse health effects (Cowan et al., 1984). When evaluated against these criteria as a bait marker for white-tailed deer, rhodamine B scored favorably in most categories. Rhodamine B is highly soluble in water and is easily mixed with most bait materials. However, if rhodamine B is dissolved in water and then used to surface coat baits, it is advisable to allow the baits to air dry before placing the baits in containers or in storage. Corn and pelleted feed that were sprayed with rhodamine B and not allowed to dry became moldy within one week during our study. Aflatoxins, secondary metabolites of Aspergillus flavus and Aspergillus parasiticus that are produced in moldy grains, could result. Quist et al. (1997) found that white-tailed deer fawns exposed to aflatoxins had reduced body weights and degenerative hepatopathy compared to unexposed fawns. In the present study white-tailed deer that were exposed to rhodamine B for 48-hr produced marked feces that was visible to the naked eye within 36 hours of their initial exposure and the mark persisted for up to three days. Our findings are consistent with other researchers who found that the persistence of rhodamine marking in excreta lasted 1-3 days in mountain beavers (Lindsey, 1983) and 6 - 8 days in black-tailed jackrabbits (Evans and Griffith, 1973). However, white-tailed deer in our study did find rhodamine-marked feed unpalatable. Palatability problems caused by rhodamine B also have been noted in brush-tailed possums (Trichosurus vulpecula; Morgan, 1981), black-tailed jackrabbits (Evans and Griffith, 1973), and laboratory rats (Rattus norvegicus; Webb and Hansen, 1961). Morgan (1981) noted that concentrations <1% rhodamine did not reduce bait palatability. However, Johns and Pan (1981) found that rhodamine concentrations of 0.00045 oz./lb. were required to produce visible marks into keratinous tissue. Therefore, we selected for our study the dosage recommended by Johns and Pan (1981) as the minimum dose of rhodamine to give white-tailed deer, especially if the marking of pelage hairs was desirable. Although white-tailed deer did not prefer feed coated with rhodamine B, they would consume it. Lastly, even though rhodamine B has been described as the most toxic of the xanthene dyes (Smart and Laidlaw, 1977), the lethal concentration values calculated for various species of vertebrates is very high (i.e., >0.008 oz./lb.; Smart, 1984). Fisher (1999) noted that there is no epidemiological evidence of adverse effects of ingestion of rhodamine B by wildlife.

Typically, white-tailed deer movements are studied through the use of radio teleme-

try (Labisky et al., 1999). However, the use of rhodamine B to mark deer offers an inexpensive technique to approximate deer movements (eg., distances deer will travel to and from feeders). Dyes have been used in the past to trace animal movements (Clover, 1954; Taber et al., 1956). In addition, movements could be determined without having to capture the animal, as would be the case with radio telemetry. Also, rhodamine B does not require destroying animals, like does tetracycline to assess marks in bones and teeth (Crier, 1970), but can be assessed within an animal by non-invasive techniques, such as hair clipping. Rhodamine B will persist in hair until the hair is shed during the molt (Fisher, 1999).

REFERENCES

Clover, M. R. 1954. Deer marking devices. California Fish and Game 40:175-181.

Cook, R. L. 1984. Texas. P.457-474 In: L. K. Halls (ed.) White-tailed deer: ecology and management. Stackpole Books, Harrisburg, Penn.

Cowan, D. P., J. A. Vaughan, K. J. Prout, and W. G. Christer. 1984. Markers for measuring bait consumption by the European wild rabbit. J. Wildl. Manage. 48:1,403-1,409.

Crier, J. K. 1970. Tetracyclines as a fluorescent marker in bones and teeth of rodents. J. Wildl. Manage. 34:829-834.

Evans, J., and R. E. Griffith, Jr. 1973. A fluorescent tracer and marker for animal studies. J. Wildl. Manage. 37:73-81.

Farry, S. C., S. E. Henke, A. M. Anderson, and M. G. Fearneyhough. 1998. Responses of captive and free-ranging coyotes to simulated oral rabies vaccine baits. J. Wildl. Dis. 34:13-22.

Fisher, P. 1999. Review of using rhodamine B as a marker for wildlife studies. Wildl. Soc. Bull. 27:318-329.

Gast, J. A. 1963. Rhodamine B dye for studying movements of animals. Ecology 44:611-612.

Johns, B. E., and H. P. Pan. 1981. Analytical techniques for fluorescent chemicals used as systemic external wildlife markers. Am. Soc. Testing Materials, Vert. Pest Control Manage. Materials 3:86-93.

Kawachi, T., T. Yahagi, T. Kada, Y. Tazima, M. Ishidate, M. Sasaki, and T. Sugiyama. 1980. Cooperative program on short-term assays for carcinogenicity in Japan. International Agenc. Res. Cancer Sci. Publ. 27:323-330.

Krebs, C. J. 1989. Ecological methodology. Harper Collins Publ., New York, N.Y., 654pp.

Labisky, R. F., K. E. Miller, and C. S. Hartless. 1999. Effect of Hurricane Andrew on survival and movements of white-tailed deer in the Everglades. J. Wildl. Manage. 63:872-879.

Lindsey, G. D. 1983. Rhodamine B: a systemic fluorescent marker for studying mountain beavers (*Aplodontia rufa*) and other animals. Northwest Science 57:16-21.

Morgan, D. R. 1981. Monitoring bait acceptance in brush-tailed possum populations: development of a tracer technique. N. Z. J. For. Sci. 11:271-277.

New, J. G. 1958. Dyes for studying the movements of small mammals. J. Mammal. 39:416-429.

Ott, L. O. 1993. An introduction to statistical methods and data analysis, 4th ed. Duxbury Press, Belmont, Ca., 1050pp.

Paton, P. W., and L. Pank. 1986. A technique to mark incubating birds. J. Field Orithol.

57:232-233.
Quist, C. F., E. W. Howerth, J. R. Fischer, R. D. Wyatt, D. M. Miller, and V. F. Nettles. 1997. Evaluation of low-level aflatoxin in the diet of white-tailed deer. J. Wildl. Dis. 33:112-121.
SAS Institute, Inc. 1989. SAS/STAT user=s guide. Version 6. SAS Institute, Inc., Cary, N.C., 846pp.
Smart, P. L. 1984. A review of the toxicity of twelve fluorescent dyes used for water tracing. Nat. Speleolog. Soc. Bull. 46:21-33.
Smart, P. L., and I. M. S. Laidlaw. 1977. An evaluation of some fluorescent dyes for water tracing. Water Resourc. Res. 13:15-33.
Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach, 2nd ed. McGraw-Hill Book Co., New York, N.Y., 633pp.
Taber, R. D., A. de Vos, and M. Altmann. 1956. Two marking devices for large land mammals. J. Wildl. Manage. 20:464-465.
Wadkins, L. A. 1948. Dyeing birds for identification. J. Wildl. Manage. 12:388-391.
Webb, J. M., and W. H. Hansen. 1961. Studies of the metabolism of rhodamine B. Toxicol. Appl. Pharmacol. 3:86-95.