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Visitation to Cottonseed Storage Sites by Feral Swine and Evidence of Gossypol Exposure

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From the Field

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Texas ranks first in U.S. cotton production, and southern Texas is a major region of production within the state. Within Kleberg County, for example, approximately 16,147 ha are planted in cotton annually, yielding approximately 68,200 bales, or 15,467 metric tons, of cotton (U.S. Department of Agriculture [USDA] 2009). Cotton producers have discovered new uses for cotton ginned by-products, such as hydro-mulch (Holt et al. 2005) used as a protein supplement for range livestock (DelCurto et al. 2000) and white-tailed deer (*Odocoileus virginianus*; Cooper 2006). Because of this, much of the materials are temporarily stored for later use.

Gossypol is a toxic compound found in cottonseed and other portions of cotton plants, including the leaves, stems, and roots (Berardi and Goldblatt 1980). Monogastric animals tend to be more sensitive to gossypol than ruminants; hence, cottonseed is used widely as a feed ingredient in the dairy and sheep industries. Nevertheless, gossypol can be a concern for ruminant animals that are overfed with cottonseed. Young animals tend to be more susceptible than mature animals (Risco et al. 1992). Swine (*Sus scrofa*) are particularly sensitive to gossypol (Smith 1957). Generally, it is recommended that swine rations not exceed 0.01%, or 100 ppm, of free gossypol (Berardi and Goldblatt 1980, Haschek et al. 1989), although this level can be increased to 200 to 400 ppm with iron or protein supplementation (Berardi and Goldblatt 1980, Haschek et al. 1989). Others have recommended even lower concentrations for swine rations (Penrith et al. 1994).

Aflatoxins may also be present in stored cottonseed. These are secondary fungal metabolites produced by *Aspergillus flavus* or *A. parasiticus* on cereal grains and feedstuffs, including cottonseed, during plant growth or storage (Harvey et al. 1995). Swine are highly sensitive to the toxic effects of aflatoxin (Hoerr and D'Andrea 1983). Though no aflatoxin tolerances have been established for swine, it is recommended that rations not exceed 200 ppb for adult swine (Carson 1986).

The increasing popularity of feeding whole cottonseed as a supplement to livestock and wildlife provides additional avenues for feral swine to consume cottonseed, thereby causing damage (e.g., direct losses and fouling of cottonseed) and possibly toxicosis to the animals. This threat would be intensified during periods of resource scarcity (e.g., drought) when other forages are unavailable. The objectives of our study were to describe our visual and telemetry observations of feral swine visitation to whole cottonseed storage sites to estimate the proportion of feral swine consuming whole cottonseed, and to infer toxicosis using erythrocyte osmotic fragility of feral swine collected within 1.5 km of cottonseed storage sites.

Study area

Our study was conducted on approximately 4,000 ha in Kleberg County, Texas, located within the Eastern Rio Grande Plains. The average daily maximum temperature in July was 35° C and average daily minimum temperature in January was 7.7° C (Griffiths and Bryan 1987). The average annual rainfall from 2000 to 2007 was 75 cm. Our study area was within a transitional zone between expansive agricultural fields to the north and rangelands to the south and included a creek drainage. Two large (5 to 27 ha) areas where whole cottonseed was stored after ginning were included in our study area. These sites had been used for >10 years to store whole cottonseed. Recreational hunting and cattle grazing were the primary land uses within the rangeland portion of the area.

Methods

We trapped and ear-tagged (marked) 76 feral swine on January 11 and 14, 2008. We placed geographic positioning system (GPS) collars with very high frequency (VHF) mortality sensors (Lotek Wireless, Newmarket, Ontario, Canada) on 12 adults (8 boars and 4 sows). We programmed collars to collect locations every 4 hours from Sunday to Thursday and every 15 minutes on Friday and Saturday. For our analysis, we included locations collected during Sunday to Thursday (every 4 hours), to ensure measurement independence. When we detected VHF mortality signals, we located the carcasses via homing (Figure 1) and conducted a field necropsy, including systematic searches of the surrounding areas, to determine the cause of death. We recovered all collars by April 17, 2008, and uploaded location data onto a coverage map of the study area using ArcView® (Environmental Systems Research Institute, Redlands, Calif., 1999). We generated the arithmetic mean of locations and determined distance from this centroid to the 2 cottonseed storage sites using the Animal Movement Extension of ArcView (Hooge and Eichenlaub 1997). We report the percentage of locations occurring within cottonseed storage sites for all feral swine and the distance from centroids to each cottonseed storage site for swine that visited the storage sites and those that did not (Table 1).

We collected blood from feral swine that were located ≤1.5 km from cottonseed storage sites as part of research activities unrelated to this study on February 29, 2008. Additionally, we collected blood from feral swine maintained at the Caesar Kleberg Wildlife Research Institute's Captive Wildlife Research Facility that did not have access to cotton-based forages. We determined plasma gossypol using high-performance liquid chromatography using a slightly modified procedure from that described by Taylor (2003) and Kim et al. (1996). We determined erythrocyte osmotic fragility, a diagnostic of gossypol exposure, following Gray et al. (1993). We compared plasma gossypol concentrations and erythrocyte fragility between field and captive (control) animals with a pooled *t*-test. We used a Type I error rate of 5% for all of our analyses.

We collected samples of cottonseed from various areas at the storage sites where feral

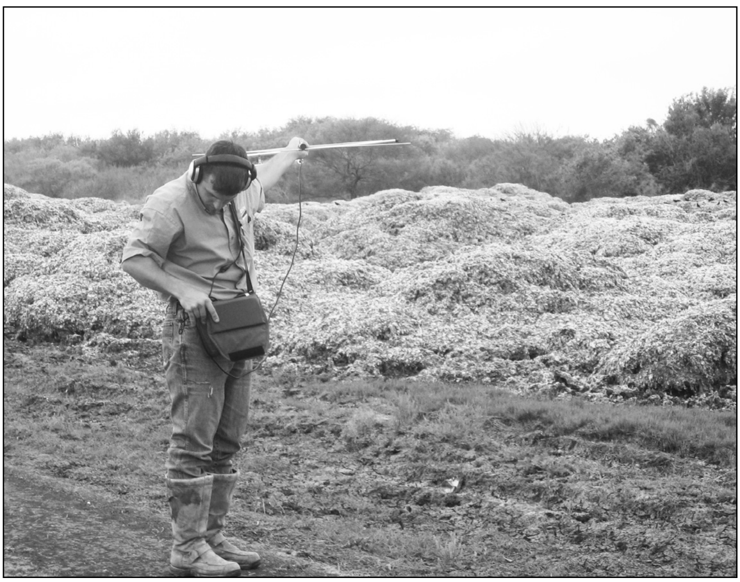

Figure 1. Study personnel using ground-based radio-telemetry to locate feral swine at a whole-cottonseed storage site in Kleberg County, Texas, during February 2008.

Table 1. Location data from feral swine relative to cottonseed storage sites in Kleberg County, Texas from January to April 2008.

Sex	Date recovered (2008)	Fate	No. of locations in site 1	No. of locations in site 2	Total no. of locations	% of total locations[a]	Distance 1 (m)[b]	Distance 2 (m)[c]
M	Feb 11	Died	0	12	113	11	1,370	2,943
M	Feb 22	Died	20	13	159	21	218	2,729
M	March 31	Alive	0	0	90	0	6,521	8,704
M	March 31	Alive	67	2	248	28	276	3,295
M	March 31	Alive	0	0	262	0	3,827	7,363
F	March 31	Alive	0	0	256	0	3,662	7,268
M	March 31	Alive	0	0	269	0	4,382	7,765
M	March 31	Alive	68	0	260	26	138	3,407
F	March 31	Alive	0	0	236	0	2,421	5,783
M	April 5	Alive	110	0	270	41	52	3,692
F	April 16	Alive	71	4	297	25	397	3,277
F	April 17	Alive	68	2	303	23	251	3,124

[a] Percentage of total locations within cottonseed storage sites 1 and 2.
[b] Distance from the centroid to cottonseed storage site 1.
[c] Distance from the centroid to cottonseed storage site 2.

swine had been foraging (evidenced by swine tracks, trails, scats, and rooting in cottonseed material) on February 29, 2008. The white, fuzzy cottonseeds were first separated from debris (soil, gin trash, etc.) and other seeds. The whole seed were then dehulled to yield fiber, hulls, and kernels. The kernels were dried, ground, and analyzed for gossypol as described by Hron et al. (1999). Aflatoxin B1 concentrations of whole cottonseed material were determined by HPLC following the method of Cohen and Lapointe (1981). All capture and handling procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University-Kingsville or the National Wildlife Research Center.

Results

We received a call from a farmer that a collared swine had been shot on February 11, 2008, because it was moribund, displaying clinical signs of lethargy, incoordination, and loss of awareness. Upon inspection of the carcass, we found this animal to show no signs of trauma other than the gunshot wound, suggesting that this animal had not been hit by a motor vehicle. The mortality site of this animal was found 1.2 km from a cottonseed storage site. Our subsequent spatial analysis indicated that during the 29 days that this individual was satellite-monitored, 12 of 113 locations (11%) occurred within one of the cottonseed storage sites.

We received a VHF mortality signal from a collared swine on February 22, 2008. Our analyses indicated that during the 41 days that this individual was satellite-monitored, 33 of 159 locations (21%) were within one of the cottonseed storage sites. A systematic search of the surrounding area found an additional 11 carcasses (9 of them ear-tagged) around the site. Detailed necropsy was not performed on these animals because of the advanced state of decomposition. Furthermore, we observed an untagged juvenile male feral swine on this date displaying similar clinical signs to those described above.

Of the monitored swine, 7 (58%) were found to have visited a cottonseed storage site and five of these animals visited both sites (Table 1). Animals that were found visiting storage sites visited frequently (11 to 41% of the location points). For feral swine visiting Site 1, the mean distance of the location coordinates to the site was 222 m (SE = 48, $n = 6$).

Plasma gossypol concentrations for animals found in close proximity (≤1.5 km) to the cottonseed storage sites averaged 9.23 μg/ml (SE = 1.65, n = 18). Although the range of values was large (0.4 to 27.4 μg/ml), two-thirds of the animals had plasma gossypol concentrations >4.4 μg/ml. For control animals known not to be exposed the cottonseed, plasma gossypol values averaged 0.97 μg/ml (SE=0.08, n = 6), with the greatest value being 1.18 μg/ml. Unsurprisingly, the differences in the mean values were significant (t = -2.84, P = 0.01).

Additionally, we found erythrocyte osmotic fragility for swine in close proximity to cottonseed storage sites were greater than (t = -2.62, P = 0.03) that found in control animals at a saline concentration of 0.65 g/100 ml (Figure 2). Further, we observed direct and indirect evidence of feral swine foraging activities within cottonseed storage sites, including observations of swine at sites (i.e., tracks, trails, scats, and rooting in cottonseed material). In some locations, the foraging activity appeared to have reduced the height of the cottonseed piles by 50 to 70%. The cottonseed sampled from the gins contained a mean concentration of 0.88% (SE = 0.04, n = 3) total gossypol and a mean of 79 ppb (SE = 58, range = 0–348 ppb, n = 6) aflatoxin B1.

Discussion

Whole cottonseed is a high-quality food supplement used for cattle and increasingly for white-tailed deer (Cooper 2006). Previous studies from captive and free-range settings in Texas have found that feral swine and other nontarget animals reduce their visitation to deer feeders and avoid consumption when normal rations were replaced with whole cottonseed (Taylor 2003, Cooper 2006). This provided the basis for recommendations of whole cottonseed as an alternative to whole-kernel corn or high-protein pelleted supplements in intensive deer management programs because swine would not compete with deer for the cottonseed ration (Taylor 2003). Nevertheless, the use of cottonseed and cottonseed meal as a feed ingredient for swine has been studied (Smith and Clawson 1965, Knabe et al. 1979), and incidents of gossypol toxicity in swine have been reported (Haschek et al. 1989, Penrith et al. 1994). It has been observed that animals often need to be conditioned to consume cottonseed (M.C. Calhoun, Texas AgriLIFE Research and Extension Service, personal communication).

Our observation of feral swine visitation patterns to whole cottonseed storage sites appear to be in contrast with the former reports. We observed a high percentage of collared animals (58%) regularly traveling long distances (≤3.2 km) to visit cottonseed storage sites. Furthermore, feral swine that visited cottonseed storage sites spent a substantial portion of time at the sites. The data suggest that these sites were important to the population, presumably because they served as a source of food.

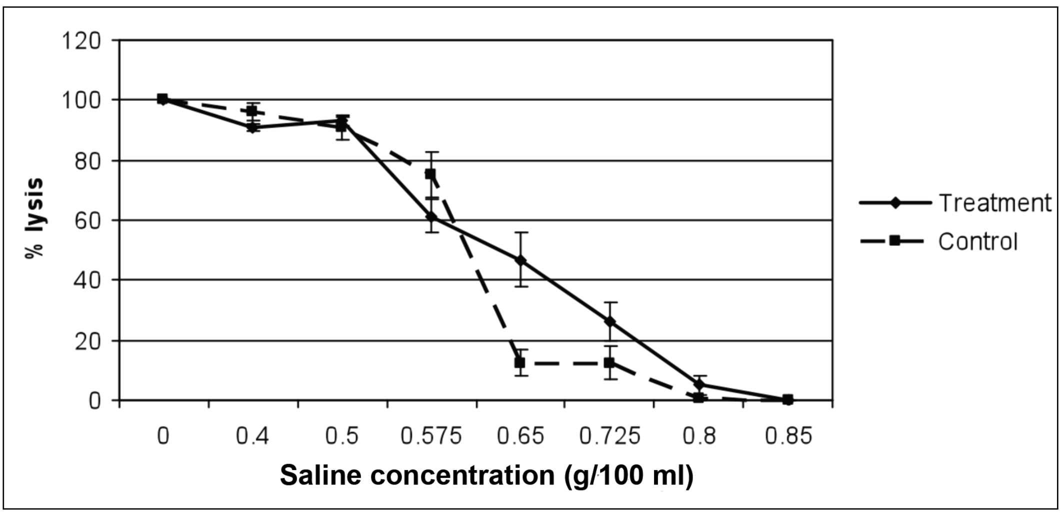

Figure 2. Mean (± SE) erythrocyte osmotic fragility of treatment feral swine (n = 7) in close proximity (≤1.5 km) to cottonseed storage sites and control feral swine (n = 4) that did not have access to cotton-based forages in Kleberg County, Texas, from February to March 2008.

Complementing the visitation data, plasma gossypol concentrations of local feral swine indicated that the animals were consuming cottonseed from these sites. Because these animals were not radio-collared, it is not possible to report on the tendency of these animals to visit the cottonseed storage sites. However, all of the animals tested were located within 1.5 km of a storage site, which was well within the ranging habits of the radio-collared animals. We compared the measured plasma concentrations of these animals with the 8-week data obtained by Taylor (2003) in feeding trials on captive feral swine, and we assumed it to be a steady-state or plateau level. These findings indicated that the average animal may have been consuming 1 to 2% of their diets as cottonseed. At the extreme, the feral swine with the greatest plasma gossypol concentration (27 μg/mL) would indicate an approximate 4 to 5% dietary consumption of cottonseed. Assuming the average kernel gossypol level of 0.9% is all free gossypol and correcting with typical values for kernel moisture (10%) and the fraction of seed that is kernel (50%), a 2% diet of cottonseed translates into a consumed gossypol concentration of approximately 80 ppm, which is in reasonable agreement with most, but not all, acceptable feeding levels. A 4% consumption of cottonseed would be above recommended levels.

Why feral swine were actively visiting and consuming whole cottonseed is unknown. One apparent difference in our situation and the more controlled studies of Taylor (2003) and Cooper (2006) is that existing deer feeders were used in their experiments and neophobia to cottonseed may have influenced their results. The 4- to 8-week duration of these studies may not have been long enough for feral swine to acclimate to the novel supplement. Whole cottonseed has been stored seasonally at these ginning sites over the course of several years, and the local feral swine population may have been well-acclimatized to this food source. A further explanation involves differences in the relative availability of native forages among study sites. For example, Cooper (2006) conducted the 8-week study during a year of above average rainfall, whereas from October 2007 to March 2008 our study site received approximately 5 cm of precipitation. As in Zavala County, Texas (Cooper 2006), Kleberg County (our study area) is semi-arid, and the availability of forage is heavily dependent upon rainfall (Davis 1990). The lack of other available forage in the area may have increased the need of the feral swine to feed on cottonseed.

Our motivation for this report was to observe morbidity and possible toxicosis within a population of feral swine. Specifically, 2 collared feral swine were either shot because they were moribund or their carcasses were discovered within a cottonseed storage site. An additional 9 ear-tagged animal carcasses were discovered at this cottonseed storage site, and a young moribund animal was observed within the site for >1 hour. Other evidence we found of toxicosis were (1) elevated erythrocyte osmotic fragility and plasma gossypol concentrations in treatment feral swine with no visible clinical signs, and (2) samples of cottonseed material from foraging sites containing total gossypol and aflatoxin B1 levels exceeding the recommended ration for swine. Unfortunately, we did not collect tissue samples (e.g., liver) for more definitive diagnosis during our field necropsy from the original moribund animal that was shot. While we believe that complications related to the consumption of cottonseed were occurring within this feral swine population, other possibilities could exist.

Management implications

Our observations that feral swine regularly travel long distances to consume whole cottonseed during periods of resource scarcity presents several challenges for wildlife managers in southern Texas. First, substituting whole cottonseed for grain or pelleted supplemental feed in deer feeders will not deter feral swine from raiding the feeders; under our demonstrated habitat conditions, feral swine will consume whole cottonseed. Second, wildlife managers should expect some removal by feral swine of seasonally-stored cottonseed, particularly during periods of resource scarcity. These losses may become problematic for wildlife managers storing small, unprotected crops for subsequent use. Feral swine use of these temporary sites may be reduced through the use of electrified exclusion fencing (Reidy et al. 2008). Third, feeding a supplement containing a chemical that is largely nontoxic to ruminants, yet potentially lethal to monogastrics, suggests its potential as a feral swine control technique. While reduction in invasive feral swine populations would benefit agriculture and ecosystems (Seward et al. 2004), is it unlikely that gossypol contained within whole cottonseed is suitable for field application or will be approved as a feral swine toxicant by the U.S. Environmental Protection Agency because of sensitivity and specificity issues. Further work is needed in the development of swine-specific toxicants (e.g., see Cowled et al. 2008) and delivery systems before management-appropriate recommendations can be made.

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