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FUSARIUM MYCOTOXINS FROM PEANUTS SUSPECTED AS A CAUSE OF SANDHILL CRANE MORTALITY

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ABSTRACT: An estimated 9,500 sandhill cranes (*Grus canadensis*) died in Gaines County, Texas and Roosevelt County, New Mexico between 1982 and 1987. The predominant clinical sign observed in sick cranes was their inability to hold their heads erect, both while standing and flying. Multiple muscle hemorrhages and submandibular edema were the most common lesions seen at necropsy. Mycotoxins produced by *Fusarium* sp. growing during cold, wet weather on peanuts left in the field after harvest, the predominant foods of the dead cranes at the time of these mortality events, were identified as the most likely cause of this mortality. Rendering moldy peanuts inaccessible to the cranes by conventional tillage resulted in reduced crane mortality in these areas.

Key words: *Fusarium* sp., mycotoxin, peanuts, sandhill cranes, *Grus canadensis*, trichothecene, field study.

INTRODUCTION

The pluvial basins of the Southern High Plains of northwestern Texas and eastern New Mexico are considered critical wintering habitat for more than 400,000 sandhill cranes (*Grus canadensis*) of the midcontinental population in North America (Lewis, 1977; Iverson et al., 1985). These saline lakes within the area serve as valuable roost sites for cranes during the winter months, and as many as 200,000 cranes have been counted on a single lake (Iverson et al., 1985). The cranes also are attracted to this region because of the food provided by grain farming (Iverson et al., 1985).

Aggregations of cranes of this magnitude increase the risk of disease from a variety of causes. Losses have occurred in this crane population from avian cholera and Type C avian botulism, as well as from hailstorms, lightning, high winds (Windingstad, 1988), and lead poisoning (Windingstad et al., 1984). Ingestion of mycotoxins in moldy foods has also been reported (Robinson et al., 1982) or suspected (D. B. Pence, pers. comm.) in several waterfowl and sandhill crane mortalities in Texas and New Mexico. Muleshoe National Wildlife Refuge (NWR) (Muleshoe, Texas 19347, USA) files suggest that moldy peanuts might have been responsible for the death of 174 sandhill cranes near Portales, New Mexico in 1946. Aransas NWR (Austwell, Texas 77950, USA) files implicate aflatoxin, a common mycotoxin, in the death of 30 sandhill cranes feeding on moldy corn at Aransas NWR in southeast Texas in 1979. Aflatoxin was confirmed as the cause of waterfowl deaths in central Texas peanut fields (Robinson et al., 1982) during the winter of 1977–1978. *Fusarium* sp., as well as zearalenone, an estrogenic mycotoxin produced by numerous species of *Fusarium*, was found in the gizzard contents of cranes dying in northern Texas in 1982 (D. B. Pence, pers. comm.).

Recent evidence indicates mycotoxins other than aflatoxins were probably responsible for the sandhill crane deaths near Portales, New Mexico, in 1946, for recent crane deaths there in 1985 and 1987, and for major crane epizootics at a new peanut-growing area in Gaines County, Texas, from 1982 to 1987 (Windingstad et al., 1987).

This paper reviews sandhill crane mortality on wintering grounds since 1982 which we hypothesize to have been caused by mycotoxins produced by *Fusarium* sp.

on moldy waste peanuts under particular climatic conditions. Such losses underscore the importance of understanding and incorporating nonhunting mortality factors in the management of sandhill crane populations.

STUDY AREA

The greatest sandhill crane losses occurred in the Cedar Lake area in Gaines County, Texas (32°49′N, 102°17′W), approximately 120 km southwest of Lubbock (Fig. 1). Cedar Lake is a pluvial basin 3.5 km by 7 km (Reeves, 1966) with a usual water depth of 2 to 10 cm but increasing to a depth of 30 cm in some spots. The lake is highly alkaline, preventing winter freeze-up, and contains sodium sulfate and calcium sulfate (gypsum), both of which are commercially mined at the lake. Cranes use the shallow water and adjacent mudflats for roosting, but apparently they do not drink the alkaline water (Iverson et al., 1985). Instead, they stop at nearby freshwater playas or at springs adjacent to the alkaline lakes to drink on their way to or from feeding areas. Feeding fields contain sorghum, winter wheat or peanuts and are located up to 12 km from the roost. An estimated 25,000 cranes used this roost and adjacent fields in 1985; as many as 45,000 were there in 1987.

Sandhill crane losses also have occurred at the salt lakes in the Portales, New Mexico, area. In January 1985, an estimated 13,000 sandhill cranes were using Little Salt Lake (34°05′N, 103°11′W), a 2 km^2 shallow basin, about 30 km southeast of Portales in Roosevelt County, when mortalities occurred. In January 1987, 15,000 cranes were roosting at Salt Lake (34°05′N, 103°05′W), a 2 km × 5 km basin, 8 km east of Little Salt Lake, when mass mortality occurred. Both of these roosts are alkaline and shallow. Sandhill cranes in the Roosevelt County areas feed on harvested corn in addition to sorghum, wheat and peanuts. Sandhill cranes regularly eat waste peanuts within feeding range of the roost sites in both counties.

Peanuts have been planted in the Portales area for at least 45 yr. About 4,730 ha of peanuts are planted annually in Roosevelt County (Gerhardt and Hand, 1985), although only about 1,620 ha are within feeding range of the salt lake roost sites. Peanuts have been planted only recently in Gaines County, Texas. In 1980, 1,200 ha of peanuts were planted in the entire county, but not in areas commonly used by sandhill cranes. In 1984, peanut production in Gaines County increased to almost 12,000 ha (Findley and Arends, 1984a). Several hundred ha were within the feeding range for cranes using Cedar Lake as their roost site. Harvest of peanuts in this area is generally completed by late December or early January. Wastage, or peanuts missed at harvest, can be as high as 25% depending on weather conditions and harvest techniques (R. M. Windingstad, pers. obs.).

Sandhill cranes were observed in 76 Texas counties in at least one of three midwinter surveys (1982 to 1984); 42 counties in Texas had 405 ha or more of planted peanuts during the same period. Eight counties had both cranes and ≥405 ha of peanuts planted (Fig. 1).

The climate of Gaines and Roosevelt Counties is semiarid and is characterized by cool winters and relatively low humidity. Average annual snowfall is 30 cm in Roosevelt County and 22 cm in Gaines County. Average total precipitation is 39 cm for Roosevelt and 40 cm for Gaines County (Ruffner, 1985). Because of the low precipitation, many crops, including peanuts, must be irrigated and waste crops do not undergo significant microbiological deterioration after harvest, remaining as a food source throughout the winter period if they are not tilled under.

MATERIALS AND METHODS

Federal and state personnel surveyed feeding and roost areas for sick and dead cranes by air and ground in northwestern Texas and eastern New Mexico. Carcasses were collected by personnel on foot or by using all-terrain vehicles.

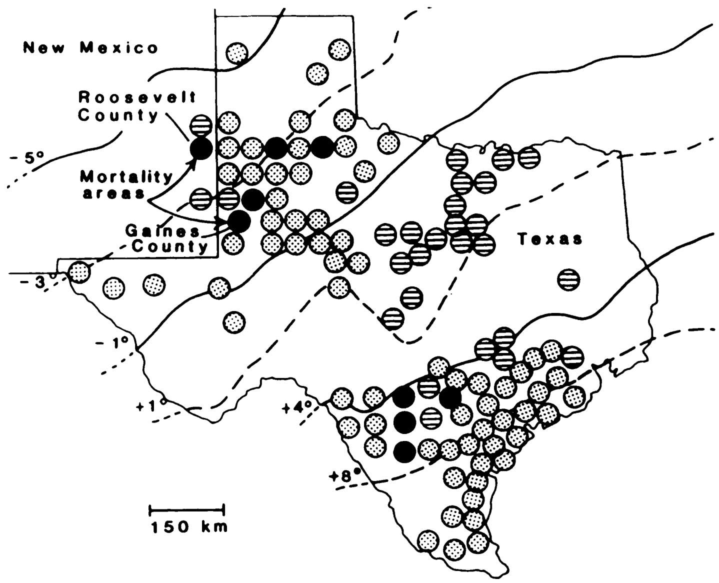

FIGURE 1. Two areas in western Texas and eastern New Mexico known to have experienced sandhill crane mortality from suspected ingestion of toxins produced by fungus growing on peanuts. Also shown is the distribution of planted peanuts and sandhill cranes in mid-winter with mean minimum temperatures for January to differentiate those climatic conditions that are conducive to mycotoxin production. ◎, counties having ≥100 sandhill cranes during at least one of three mid-winter surveys (1982 to 1984); ⊜, counties with ≥400 ha of planted peanuts (1984); ●, counties having both sandhill cranes (≥100) and peanuts (≥400 ha); isotherms for normal daily minimum temperatures (C) January.

Sick cranes were euthanized by cervical dislocation. Cranes found dead and those euthanized were sent on wet ice to the National Wildlife Health Research Center (NWHRC) (Madison, Wisconsin 53711, USA) for necropsy and laboratory analysis. In 1986, 10 carcasses were necropsied in detail in the field at Gaines County. In 1985 and 1986, 26 healthy sandhill cranes in the area adjacent to the peanut fields in Gaines County were killed by shooting and used as controls for laboratory analysis and comparative food habit studies. Tissues (usually liver, kidney, spleen and intestine) were removed at necropsy and submitted to the bacteriology, virology, parasitology, toxicology and histology laboratories at NWHRC for analyses.

Tissues submitted for bacteriological examination (primarily liver, intestine and brain) were cultured aerobically on 5% sheep blood agar plates (BAP) and eosin-methylene blue agar plates (EMB). They were then incubated at 37 C for 24 hr. The API 20E system (Analytab Products, Division of Sherwood Medical, Plainview, New York 11803, USA) was used to identify isolated organisms. Liver, spleen and cloacal swabs were tested and cultured using embryonated chicken eggs and primary duck embryo fibroblasts. Tissues saved at necropsy for microscopic examination were immediately fixed in 10% formalin and stained with hematoxylin-eosin stain. Brain cholinesterase activity was tested according to methods of Hill and Fleming (1982). Arsenic analysis was performed according to Horwitz et al. (1975).

Gizzard contents (peanuts) from carcasses and peanuts collected in Gaines County sent to NWHRC in 1985 were analyzed for aflatoxins, zearalenone and zearalonol at Wisconsin's Central Animal Health Laboratory (CAHL) (Madison, Wisconsin 53705, USA) and the Northern Regional Research Laboratory (Peoria, Illinois 61641, USA) using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Waste peanuts, soil and soil debris were collected randomly in February 1986 in two Gaines County fields where crane mortalities were occurring. These samples were stored dried until they were prepared for culture of fungi and isolation of mycotoxins.

Waste peanuts were cultured on a pentachloronitrobenzene selective medium (Nash and Snyder, 1962; Nelson et al., 1983). ***Fusarium*** spp. that grew from the peanuts were mass transferred to carnation-leaf-water agar (CLA) in petri dishes and to potato-dextrose-agar slants in test tubes (PDA) (Fisher et al., 1982; Nelson et al., 1983). These cultures were grown under light and controlled temperature for 7 to 10 days and identified using the characters described by Nelson et al. (1983). After identification, cultures to be used for further studies were initiated from single conidia and grown on CLA and PDA and lyophilized (Fisher et al., 1982; Nelson et al., 1983).

Fungal cultures were selected for toxicity testing based on reports in the literature of the production of toxins (Marasas et al., 1985) and our best estimate of *Fusarium* sp. capable of producing toxins that might cause signs similar to those observed in sandhill cranes. Approximately 50 g of each fungal culture to be tested was extracted with 100 ml of chloroform-methanol, 2:1 v/v in a Waring Blendor. The extracts were filtered and evaporated to dryness in vacuo at 60 C. Toxicity of the extracts was determined using 1-day-old chickens by the method of Kirksey and Cole (1974). Precautions were taken to remove residual chloroform and methanol (Kirksey and Cole, 1974). The extracts were administered orally via crop incubation (1 ml/chicken) with the native peanut oil as the inert carrier. Chickens were observed for 7 days following administration of the fungal extracts.

A previously undescribed trichothecene, proposed as an isomeric with neosolaniol or iso-neosolaniol, from a *Fusarium compactum* isolate was intubated to three cranes at the Patuxent Wildlife Research Center (Laurel, Maryland 20708, USA); crude extract containing iso-neosolaniol was given to three birds, and crude extract with iso-neosolaniol removed but containing other trichothecenes was given to another three birds. Cranes were given 1.7 ml/kg per day for 3 days and then 3.5 ml/kg of extract on the fourth day. Surviving cranes were administered 7 ml/kg on the fifth day and 14 ml/kg on the sixth day. Three control cranes received the peanut oil carrier only.

A representative sample of waste peanuts (100 g) collected at the site of the intoxication was blended at high speed with chloroform (300 ml) for 3 min in an Ultra Turrax homogenizer (Tekmar Company, Cincinnati, Ohio 45222, USA). After filtration and centrifugation, the chloroform extract was evaporated to dryness to leave 30 g of crude material. A portion of this material (10 g) was dissolved in 60 ml of methanol/water (20/80) and extracted with hexane (3 × 10 ml) which was discarded. The methanol was removed from the aqueous phase on a rotary evaporator, and then the residue (about 50 ml) was extracted with ethyl acetate (300 ml) by adsorption onto a Chemtube (Analytichem International, Harbor City, California 90710, USA). After removal of the ethyl acetate, the residue (4.4 mg) was analyzed directly by TLC and mass spectrometry.

Weather reports for Gaines County were obtained from the National Climatic Center of the National Oceanic and Atmospheric Administration (Asheville, North Carolina 28801, USA; U.S. Department of Commerce 1980/86). Information on peanut production for the area was obtained from Department of Agriculture data (Murfield and Pratt, 1980; Findley and Arends, 1984a, b; Gerhardt and Hand, 1985). Sandhill crane survey information was taken from Texas Parks and Wildlife Department (Austin, Texas 78744, USA) files and reports (George, 1985) and Muleshoe NWR files.

RESULTS

An estimated 9,075 sandhill cranes died at Cedar Lake in Gaines County, Texas, from 1982 through 1987 and another 460 died in Roosevelt County, New Mexico, in 1985 and in 1987 (Fig. 1). Morbidity and mortality were first observed at Cedar Lake in February 1982 when an estimated 650 cranes died. Losses there in 1983 and 1984 were estimated at 1,700 and 700. The most severe losses occurred in 1985 when about 5,000 cranes died in late January and February at Cedar Lake and at feeding fields 12 km west of the roost. In 1986, only 200 cranes died in the vicinity of Cedar Lake, and only 75 cranes succumbed there in 1987. An estimated 225 cranes died in Roosevelt County at Little Salt Lake in 1985, and 235 cranes at Salt Lake in 1987.

The earliest winter mortalities were noted in mid-December 1986; all other losses occurred in mid-January to late February. For those years in which the onset date of mortality was known (1982, 1985, 1986 and 1987): (1) temperatures had dropped to −5 C or below, (2) intermittent freezing and thawing occurred, and (3) snow fell in the affected areas within 15 days before the epizootics began (Fig. 2).

Sick cranes, if able to fly, could do so only with great difficulty. Affected cranes flew with head, neck and sometimes legs drooped perpendicular to the body. On the

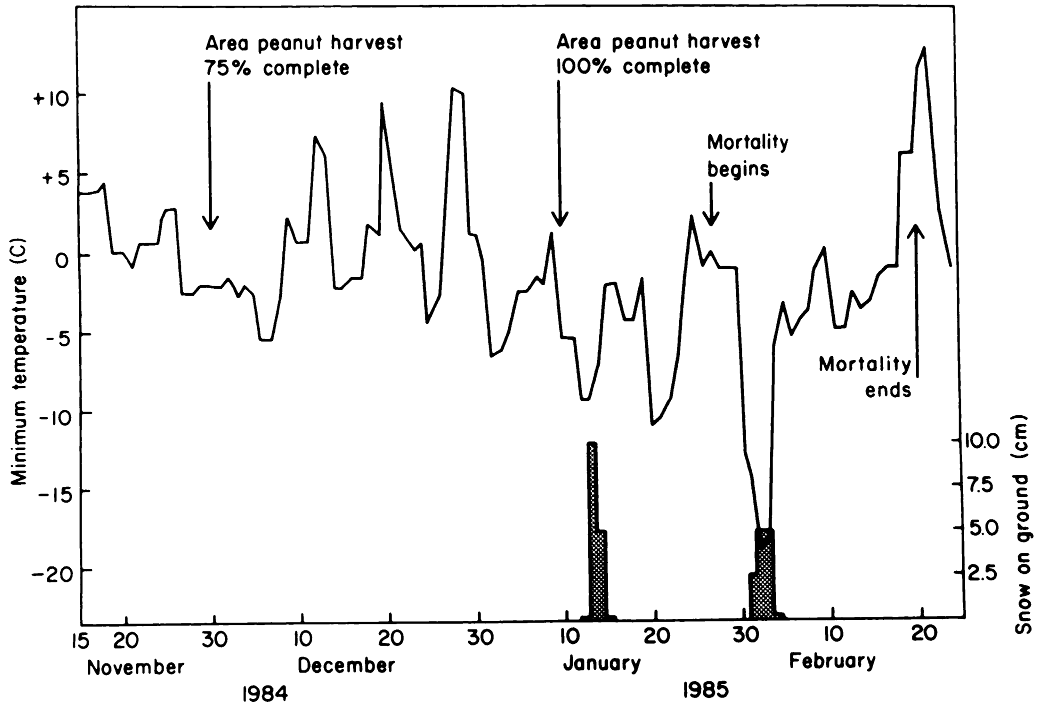

FIGURE 2. Minimum temperatures and snow (shaded bars) on ground before, during and after sandhill crane mortality at Cedar Lake, Texas, 1984/1985.

ground, sick cranes often stood motionless with their heads drooped low to the ground and were easily approached. Two sick cranes that were captured in 1982 recovered after they were given fresh food and water.

In 1986, 10 sick cranes from Gaines County were caught and placed in a pen. Blood was drawn daily for serum chemistries, and the cranes were monitored for 4 days. These cranes showed slight improvement in clinical signs prior to being euthanized for necropsy and histological examination.

Carcasses were heavily scavenged at both areas by coyotes (*Canis latrans*), American crows (*Corvus brachyrhynchos*), and Chihuahuan ravens (*Corvus cryptoleucus*). Forty-one sandhill crane carcasses were examined at necropsy. Less detailed field necropsies were performed on other cranes. Cephalic edema (resulting in swollen heads and necks) and hemorrhagic myositis in the upper leg muscles and breast muscles were seen on some cranes, although these lesions were not seen consistently. A variety of other lesions were found in some birds. Intestines were sometimes congested, swollen and hyperemic. Occasionally, kidneys and livers were swollen and congested. Almost all birds examined were in good body condition, indicating that an acute disease process had occurred. Histological examination showed that vascular changes were evident. Euthanized captive cranes had few lesions, but cephalic edema and muscle hemorrhages were prominent. Detailed gross and histopathologic descriptions as well as serum chemistry changes will be presented elsewhere (T. J. Roffe, R. K. Stroud and R. M. Windingstad, unpubl. data). Of 50 crane carcasses, 21 were males and 29 were females. Only two of 31 cranes necropsied and aged were young of the year; age was not determined on the remaining 10 cranes.

Peanuts were found in 94 % of the gizzards of 120 cranes found dead in Gaines County in late January and early February 1985, and in 96% of 28 cranes that died in the same area in February 1986. Similar prevalences of ingested peanuts as a food item were found in Gaines County and at Salt Lake in January 1987. Peanuts were not found in gizzards of 26 healthy sandhill cranes killed by shooting in sorghum fields adjacent to the Gaines County mortality areas in 1985 and 1986.

Pathogenic bacteria or viruses were not isolated from any of the tissues, nor was any evidence of an infectious disease observed by microscopic examination of the tissues. Brain cholinesterase was not inhibited, indicating that organophosphate and carbamate pesticides were probably not involved. Arsenic concentrations were <0.4 ppm in the four tissues analyzed. Aflatoxins or zearalenone were not found in samples of gizzard contents. Trace levels of zearalonol (3.3 ppm) were found in one sample of peanuts from Gaines County submitted to CAHL.

Analysis of peanuts, soil and soil debris collected at the Gaines County mortality site in February 1986 yielded the following fungi: *Fusarium compactum, F. acuminatum, F. avenaceum, F. moniliforme, F. equiseti, F. proliferatum, F. oxysporum* and *F. solani* (P. E. Nelson, R. J. Cole, T. A. Toussoun, J. W. Dorner and R. M. Windingstad, unpubl. data). All of these, except *F. compactum*, have been reported to be toxigenic (Marasas et al. 1985). *Fusarium compactum* and *F. solani* were the only two species isolated from all the substrates. In tests with 1-day-old chickens 57 % of the *F. compactum* cultures were found to be toxigenic. Cultures of the other *Fusarium* sp. tested were less toxic. Neosolaniol, as well as other unidentified trichothecenes, were found to be produced by two strains of *F. compactum* (Cole et al., 1989). One toxin had not been previously described as naturally occurring. Although this new trichothecene (iso-neosolaniol) was not recovered directly from waste peanuts, it was produced from molds collected from peanuts at the site of the mortality. Other trichothecenes, including neosolaniol, were present in waste peanuts from Cedar Lake (Cole et al., 1989).

None of the sandhill cranes intubated with 1.7 ml/kg of the extracts died in the first 3 days of the dosing experiment, but all three cranes which received iso-neosolaniol at 3.5 ml/kg body weight died within 10 hr. Two cranes intubated with the crude extract died at 6 and 18 hr after initial dose of 3.5 ml/kg body weight, but the third crane died only after being intubated with four times the initial dose and died 60 hr after the initial dose; two of three cranes receiving crude extract minus iso-neosolaniol died at 10 hr and 60 hr, indicating that other mycotoxins produced by *F. compactum* were also lethal. None of the dosed cranes displayed neck and wing droop that was seen in many sick cranes observed in the field. Purified isoneosolaniol as well as the crude extract containing other trichothecenes produced by these strains of *F. compactum* were lethal to all sandhill cranes intubated with these preparations. Isolation and identification of the *Fusarium* sp. and the chemistry of the neo-isosolaniol will be reported elsewhere (P. E. Nelson, R. J. Cole, T. A. Toussoun, J. W. Dorner and R. M. Windingstad, unpubl. data; Cole et al., 1989).

DISCUSSION AND MANAGEMENT IMPLICATIONS

We hypothesize that the most probable cause of the above sandhill crane mortality was trichothecene mycotoxins produced by *Fusarium* sp. on waste peanuts. This is based on (1) the high prevalence of peanuts in gizzards of dead cranes in Texas and New Mexico during several years and no similar mortality elsewhere on the wintering grounds, (2) isolation of toxigenic *Fusarium* sp. from waste peanuts collected from within the area experiencing the mortality and climatic conditions conducive to trichothecene production, (3) recovery of trichothecenes directly from waste peanuts at the site of mortality, (4) experimental toxicity of trichothecenes produced by *F. compactum* strains isolated from the waste peanuts, (5) histopathology consistent with a toxin acting on the vascular system (as it is known to occur for trichothecenes) (Pier, 1973, 1981; Doerr et al., 1974), and (6) no evidence of any other toxins or infectious diseases. Although there is the potential for other mycotoxins, or other unknown mortality factor(s), to be involved in this intoxication, involvement of trichothecenes appears to be paramount. Events described here were similar to other intoxications involving overwintering of grain and production of trichothecenes (Marasas et al., 1985).

In all areas we studied, cold and wet weather conducive to toxin production by *Fusarium* sp. (Joffe, 1962) occurred within 15 days before the morbidity and/or mortality began (temperatures at ≤ -5 C, intermittent freezing and thawing and snow on the ground). Fluctuating cold temperatures and snow during the winter of 1984/1985 (Fig. 2) are representative of climatic conditions that occurred before the epizootics.

Fusarium sp. are extremely common in nature and are worldwide in distribution (Hesseltine, 1977), but without the semiarid climatic conditions found on the Southern High Plains peanuts rapidly deteriorate after harvest and, therefore, are not available as a source of food for sandhill cranes during cold, wet weather. This may explain why losses of sandhill cranes have not occurred in other peanut growing areas in Texas.

The only avian species observed feeding in the peanut fields other than the cranes were Chihuahuan ravens and American crows. The freshwater playas where the cranes drank were used by several hundred ducks, coots and various shorebirds, but few of these were seen at the roosting lakes. Sick or dead birds other than sandhill cranes were not found during our investigations.

Much of the overlap between crane wintering areas and peanut farms lies in the southern part of Texas where minimum temperatures in January average 10 C warmer and rainfall patterns differ from the Gaines County area. There are other counties in northern Texas where peanuts are grown and cranes spend the winter, but the only report of mortality is a single dead crane and 30 sick ones observed during an aerial survey of Mound Lake (33°13′N, 102°05′W) about 60 km north of Cedar Lake on 12 December 1986. Mound Lake is a major crane use area and the habitat there is very similar to Cedar Lake. The lack of significant mortality on other crane roosts may simply reflect greater distance between roost sites and peanut fields. Iverson et al. (1981) found no peanuts in 154 gizzards from sandhill cranes at Rich Lake (33°15′N, 102°11′W) about 10 km northwest of Mound Lake, although some peanuts are grown in that county. In that study, milo accounted for 97% of the aggregate percentage of food found in sandhill cranes and occurred in 63% of the gizzards. Similar findings were noted in our examination of gizzards from healthy sandhill cranes shot in 1985 and 1986 near the mortality site of Cedar Lake with no peanuts found in these cranes. Not all cranes eating peanuts succumbed, but this is not surprising since not all of the peanuts cultured yielded *Fusarium* sp., indicating mycotoxin contamination of peanuts may not have occurred evenly within or between the peanut field(s) being used by cranes.

Reduced losses in Gaines County may have been lessened in 1986 and 1987 because of an active education program for area farmers. Once mycotoxins from waste peanuts were identified as the probable cause of mortality, U.S. Department of Agriculture personnel and Texas Parks and Wildlife officials were able to convince local peanut growers to till waste peanuts before cold, wet weather began. When tilling was not possible in two of the fields in Gaines County in January and February 1986, cranes were dispersed from these two fields with acetylene exploders.

Since none of the cranes experimentally dosed with various extracts of *Fusarium compactum* displayed the neck and wing droop that we saw in the field, we believe additional mycotoxins, perhaps from the other *Fusarium* sp. isolated from peanuts, may have been involved in this morbidity/mortality event. Some physiological response may have occurred in the field that was not present under experimental conditions. Clinical signs exhibited by sick cranes also may have varied depending on the dose of mycotoxin(s) received.

Based on the findings reported here and the similar description of the mortality event involving 174 sandhill cranes found sick and dead in the Portales, New Mexico, area in 1946, we believe that those losses now probably can be attributed to mycotoxins from waste peanuts. Muleshoe NWR records show that a severe snowstorm and temperatures near −20 C preceded the crane morbidity and mortality. Almost 95% of 174 gizzards examined contained peanuts. Descriptions of sick cranes in 1946 were identical to what was observed in mortality reported here: "Stricken birds invariably stand in shallow water with heads hanging pendular and frequently bump or drag on the ground when the birds alight, a pitiful sight to view. Even in flight the most rallied efforts to raise the head fail" (Anonymous, Muleshoe NWR files, 1946, U.S. Fish and Wildlife Service, Muleshoe, Texas 79347, USA).

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