







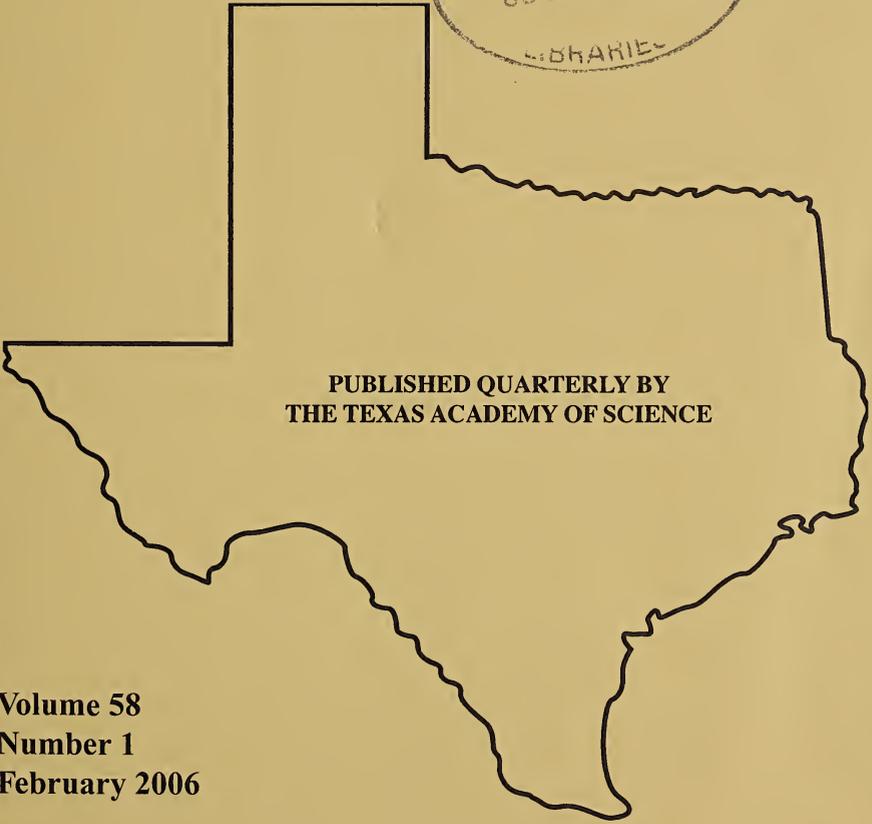


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## A 10 MILLION YEAR OLD ASH DEPOSIT IN THE OGALLALA FORMATION OF THE TEXAS PANHANDLE

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**Abstract.**—A vitric ash bed on the east bank of West Amarillo Creek, Potter County, of the Texas Panhandle occupies a portion of a paleovalley in the Ogallala Formation of late Tertiary age. Major element and selected trace element analyses and comparison of similarity coefficients with other known ashes in the western United States suggest a source in the Twin Falls Volcanic Field of southern Idaho. A K-Ar age determination of 9.5 Ma is consistent with this correlation.

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Late Cenozoic silicic ash fall tephra are well documented across the Great Plains. The youngest of such tephra include the family of “Pearlette” ashes which occur throughout the Great Plains (Izett & Wilcox 1982). The “Pearlette” tuffs were generated by major explosive eruptions in the Yellowstone Plateau Volcanic Field at the active end of the Yellowstone hotspot track between 2.1 Ma and 0.64 years ago (Christiansen 2002). Older late Cenozoic silicic tuffs are also widespread in the Great Plains where they are commonly reported from the middle to late Miocene Ogallala Group (Swineford et al. 1955; Skinner & Johnson 1964; Swinehart & Diffendal 1997). As shown by ongoing research at the University of Utah (Perkins et al. 1995; Perkins 1998; Perkins & Nash 2002) the silicic ash fall tephra in the Ogallala Group are dominantly from eruptions in older Yellowstone hotspot volcanic fields located across southern Idaho.

While there are a number of known occurrences of Pearlette family ash beds in Texas (Izett & Wilcox 1982) the authors are aware of only two reported occurrences of middle to late Miocene age silicic tephra in Texas. These are (1) a late Miocene tephra which entombed the Hemphillian fossils of the Coffee Ranch local fauna in the Texas Panhandle (Voorhies 1990; Passey et al. 2002) and (2) a middle Miocene tephra along the Pecos River in southwestern Texas (Powers & Holt 1993). Analyses done by the junior author indicate that both

of these tephra are from Yellowstone hotspot sources and correlate with known tephra in the Basin and Range sections of Perkins et al. (1998). Thus, the new tephra occurrence reported here is a significant addition to the sparse catalog of known Miocene tephra in Texas.

#### LOCATION AND EXTENT

Detailed geologic mapping along West Amarillo Creek, Potter County, Texas, revealed a small outcrop of volcanic ash on the east bank of the creek and a few hundred meters north of Wildcat Bluff (Fig. 1). The latitude and longitude of the volcanic ash outcrop are 35°14'38" N, and 101° 56'36" W. The volcanic ash is light gray with a maximum exposed thickness for the relatively pure ash of 1.2 m and a maximum horizontal extent of about 12 m (Fig. 2). The average present-day density of the clean ash is 1.7 gm/cc. The base of the clean ash layer is approximately 7 m above the bed of West Amarillo Creek and at the level of the highest and oldest of three fluvial terraces formed by the Creek (Cepeda 2001). The layer of clean ash (Fig. 3), is overlain by two ledges of light pink quartz sandstone of the middle part of the Ogallala Formation. Each of the sandstone beds is approximately 1 m thick and contains a mixture of ash and quartz grains (Fig. 4) suggesting some reworking of the ash deposit and mixing with stream sand during deposition of the Ogallala Formation. Percentage of ash particles in the sandstone decreases with increasing distance above the clean ash layer and is not seen in samples more than 4 m above the ash layer.

The location of the outcrop of ash is within a broad, shallow paleovalley in the Ogallala Formation that trends in a generally northwest to southeast direction. The exact trend of the paleovalley is difficult to determine because erosion has removed the Ogallala Formation to the north and west.

#### MATERIALS AND METHODS

Samples of the clean volcanic ash layer and of the mixed sand/ash beds above this layer were collected at 1 meter stratigraphic intervals. Thin-sections were prepared for each of the samples and these were

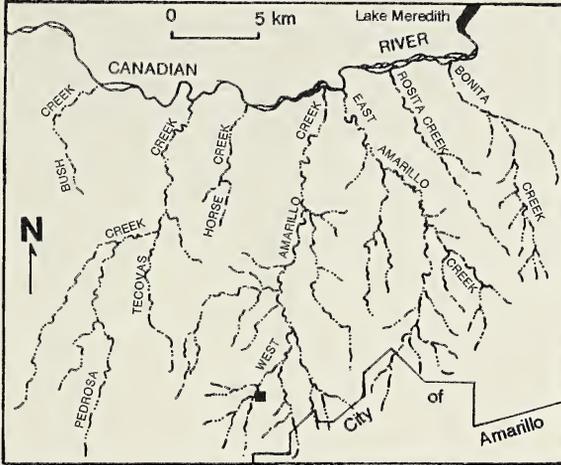


Figure 1. Location Map of the West Amarillo Creek ash locality. The outcrop of volcanic ash is marked by the square just northwest of the city of Amarillo.



Figure 2. View of outcrop of West Amarillo Creek Volcanic Ash. Lowest (white) layer is the 1.2 m thick layer of clean ash. Upper layers in photograph consist of mixed sand, silt and volcanic ash.

used for description and classification of the ash. A 0.5 kg sample was shipped to the United States Geological Survey (USGS) Tephrochronology Laboratory at Menlo Park, California. Volcanic glass was separated from the sample and analyzed by electron-

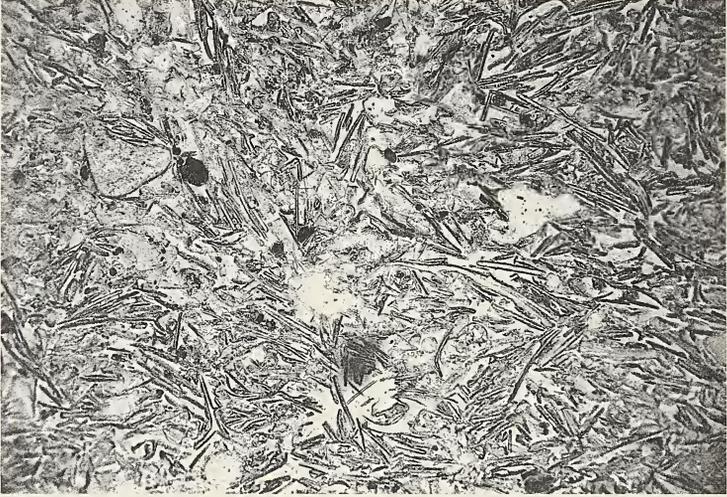


Figure 3. Photomicrograph of the West Amarillo Creek Ash, in plane-polarized light. Width of Field is 4 mm.

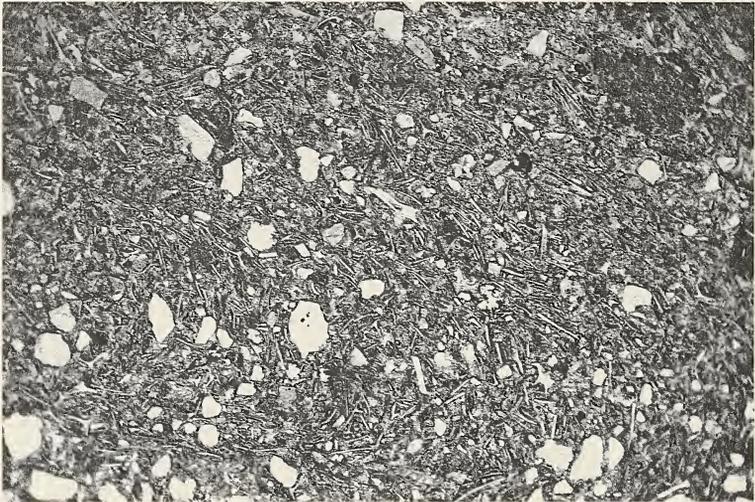


Figure 4. Plane-polarized photomicrograph of sample of mixed quartz sand and glass shards. Width of field of view is 4 mm. Sample collected 2 m. above clean ash layer.

microprobe for major elements. Initial comparison of the analytical results with the USGS database was conducted by Andrei Sarna-Wojcicki. An additional analysis of the volcanic glass by electron microprobe as well as by X-ray fluorescence spectrometry was carried out at the University of Utah. Finally, a glass concentrate from this

ash was prepared and analyzed by a commercial laboratory using the Potassium-Argon method to obtain an age for the sample.

## RESULTS AND DISCUSSION

In thin-section the ash consists of 85 to 90% incipiently to moderately altered, highly elongate glass shards to 0.3 mm in the longest dimension (see Fig. 3). The majority of the shards are bubble wall shards, with a small percentage (<5%) exhibiting bubble wall junctions. The remainder of the ash consists of equidimensional quartz and feldspar microphenocrysts to 0.2 mm in diameter and altered rock fragments with a possible trace of biotite. The ash is classified as a light gray to yellowish gray, silt-sized vitric ash.

The electron microprobe analyses (Table 1) of the volcanic glass shards reveal that the ash is a highly silicic, moderately potassic rhyolite. These compositions are typical of many of the late Cenozoic tephra of western North America. However, the composition of the glass shards of the West Amarillo Creek (WAC) tephra lie within the compositional field of the 15.2 to 7.5 Ma Stage 2 Yellowstone hotspot tephra (Perkins & Nash 2002) and outside of the compositional range of other major sources of middle to late Miocene tephra of the western U.S (Perkins et al. 1998). So the WAC tephra is likely one of the more than 100 tephra erupted during Stage 2 explosive volcanism along the hotspot track.

More particularly the WAC tephra lies within the composition field of the chemical group A of tephra of the Trapper Creek section (Perkins et al. 1998). The Trapper Creek Section lies on the southern edge of the Snake River Volcanic Province in southern Idaho. Fallout tuffs within the Trapper Creek section originated from the nearby Twin Falls volcanic field to the north or from the more distant Bruneau-Jarbidge volcanic field 100 km to the west (Perkins et al. 1995). The group A tephra, with their characteristically high Ba (mostly 1000-1200 parts per million by weight [ppmw]) and low Rb (150-180 ppmw) relative to other stage 2 tephra, occur in the upper part of the Trapper Creek section with most being younger than  $\approx$ 11 Ma. The group A tephra of Trapper Creek are mostly interbedded with ashflow tuffs from the Twin Falls volcanic field of the

Table 1. Major Element Electron Microprobe Analyses of Volcanic glass shards from West Amarillo Creek Ash. USGS analysis at the Tephrochronology Laboratory, Menlo Park, CA. University of Utah analysis by Mike Perkins. Water content calculated from difference between measured and stoichiometric oxygen content assuming all Fe as  $\text{Fe}_2\text{O}_3$ .

Constituent	USGS Raw Probe Data	Recalculated to 100%	Univ. of Utah Raw Probe Data	Recalc. to 100% water-free
$\text{SiO}_2$	70.74	76.45	73.69	76.23
$\text{Al}_2\text{O}_3$	11.35	12.27	11.80	12.21
Fe as $\text{Fe}_2\text{O}_3$	2.20	2.38	2.43	2.51
MgO	0.099	0.11	0.130	0.134
MnO	0.035	0.04	0.040	.041
CaO	0.805	0.87	0.820	0.848
$\text{TiO}_2$	0.309	0.33	0.320	0.331
$\text{Na}_2\text{O}$	2.774	3.00	2.43	2.51
$\text{K}_2\text{O}$	4.222	4.56	4.67	4.83
Cl	—	—	.030	0.031
F	—	—	0.25	0.26
$\text{H}_2\text{O}$	—	—	4.85	
Total	92.531	100.01	101.30	100.00

Yellowstone hotspot, and basal ashfall associated with these  $\approx 10.5$  to 8.5 Ma ashflow tuffs are mostly group A type tuffs. Thus, the  $\approx 10.5$  to 8.5 Ma Twin Falls volcanic field was the most likely source for the WAC tephra.

The correlation to ash of the Trapper Creek Section is the best match if the alkalis (Na and K) are removed from the comparison. Loss of alkalis in volcanic glass by post-depositional alteration and devitrification has been well documented (Noble 1970; Scott 1971). The Trapper Creek Section lies on the southern edge of the Snake River Plain in southern Idaho just north of the boundaries between the states of Idaho, Nevada and Utah. The ashes in this stratigraphic section are believed to be derived from one or more of the Snake River Plain calderas formed by the trace of the Yellowstone hotspot. These calderas are now covered by younger Snake River Plain basalts.

Analysis of the West Amarillo Creek volcanic ash for selected trace elements is shown in Table 2. A similarity coefficient (SC) using the elements Fe, Ca, Ba, Mn, Rb, Sr, Ti, Zr, and Th revealed a

Table 2. Ages and chemical composition of glass by X-ray fluorescence spectrometry for the Wildcat Bluff Ash and 9 to 11 Ma Rhyolitic Ash Beds of the Northern Basin and Range. Data for Northern Basin and Range ash beds from Perkins et al. (1998; 1995) Fe<sub>2</sub>O<sub>3</sub> and CaO given in weight percent, all other data in ppm.

Ash Bed	Age (Ma)	Fe <sub>2</sub> O <sub>3</sub>	CaO	Ba	Mn	Nb	Rb	Sr	Ti	Y	Zn	Zr	La	Nd	Th	Ce
West																
Amarillo Creek	9.5	1.57	0.63	1106	222	39	180	48	1782	NA	46	447	80	61	28	148
Mink Creek	9.24	1.40	0.55	1004	250	46	158	49	1982	57	41	456	74	61	24	138
Sheep Dip	9.46	0.49	0.34	373	309	17	137	59	568	19	17	84	37	27	20	68
Opal Canyon 6	9.52	1.35	0.51	970	212	43	170	42	1546	48	32	389	82	62	26	160
Section 26	9.70	1.44	0.56	971	276	45	160	52	2002	55	39	482	71	60	24	134
Celatron	9.73	0.78	0.54	542	220	30	146	60	897	45	30	175	75	57	20	142
Quarry G 9	9.73	0.72	0.46	566	200	28	146	45	820	43	27	143	74	57	21	144
Hazen	9.81	1.43	0.51	1066	216	40	180	44	1804	59	40	445	74	54	26	140
Opal Canyon 3	10.19	1.57	0.59	1114	232	40	168	48	1785	60	46	449	66	57	26	146
CPT XV	10.45	1.56	0.47	1008	233	45	179	35	1592	64	52	475	81	65	26	160
Opal Canyon 2	10.54	1.65	0.56	1098	257	46	168	50	1635	71	60	449	73	64	26	162
Ibex Peak 19	10.70	1.54	0.54	1130	210	36	168	44	1680	50	45	465	90	50	28	140

good match with either the 10.2 Ma Opal Canyon 3 ash bed of Perkins et al. (1998), or the 10.7 Ma Ibex Peak 19 ash bed of Perkins et al. (1995). The SC values are 0.97 for the match with the Opal Canyon ash and 0.96 for the match with the Ibex Peak ash. Ages and chemical compositions for northern Basin and Range ash beds between 9 and 11 Ma are also given in Table 2 for comparison purposes.

A whole-rock potassium-argon age determination on a sample of the West Amarillo Creek ash yielded an age of  $9.5 \pm 0.3$  Ma. The 9.5 Ma age is not unexpected for a presumed 10.2 to 10.7 Ma ash bed, considering the susceptibility of volcanic glass to loss of argon. A loss of argon from the glass would result in an analytical result younger than the true age of the sample. The analytical results of the age determination are presented in Table 3.

The stratigraphic position of the West Amarillo Creek ash within the late Tertiary Ogallala Formation, its potassium argon age date of  $\approx 10$  Ma, and its major element and trace element composition of the glass shards suggest an origin in the Twin Falls Volcanic Field of southern Idaho. Providing a specific correlation of the WAC tephra to dated group A tephra is problematic. Individual group A tephra are, within analytical uncertainty, all quite similar. In particular individual group A tephra commonly have compositional ranges, and these ranges show considerable overlap between different group A tephra. This overlap is most apparent with electron probe analysis, but overlap also occurs to a lesser degree with the higher precision XRF analyses of group A tephra. When electron probe analysis of the WAC tephra are compared with tephra derived from the Yellowstone hotspot (the sections of Perkins & Nash 2002), 10 or so potential correlations, using methods of Perkins et al. (1998), are found with tephra in the  $\approx 11$  to 9.5 Ma age range.

Trace element comparisons using XRF analyses further the possible correlations. All of the possible correlatives to the WAC ash are within the same general age range. However, one of two tephra, the  $10.74 \pm 0.10$  Ma Ibex Peak 19 tephra and the  $10.18 \pm 0.10$  Ma Opal Canyon 3 tephra of Perkins & Nash (2002), is the most likely

Table 3. Results of Whole-rock Potassium Argon Age Determination. Constants used:  $\lambda_e = 0.584 \times 10^{-10} \text{ yr}^{-1}$ ,  $\lambda_\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$ ,  $^{40}\text{K}/\text{K} = 1.193 \times 10^{-4} \text{ gm/gm}$ . Analysis by Geochron Laboratories, September 1999.

Sample Number	Material Analyzed	$^{40}\text{Ar}$ , ppm	$^{40}\text{Ar}/\text{Total } ^{40}\text{Ar}$	%K	Avg. % K	$^{40}\text{K}$ , ppm	$^{40}\text{Ar}/^{40}\text{K}$
WB-1	Glass conc.	0.002045	0.194	3.079	3.112	3.712	0.000556
	80-200 mesh	0.002082	0.165	3.144			

correlative of the WAC tephra. A specific correlation of the WAC tephra to a specific northern Basin and Range ash bed may be possible, but would require additional analyses beyond the scope of this investigation. It has not yet been determined if this ash is correlative with other  $\approx 10$  Ma ash beds that have been identified in the Great Plains and mid-continent region. Until such correlations are made, the full extent and volume of the ash plume cannot be determined. However, the fine particle size of the glass shards suggests that they rest near the distal end of an ash plume - consistent with the 1250 km distance between the likely source in southern Idaho and West Amarillo Creek.

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## REMOTE MAPPING OF SALT CEDAR IN THE RIO GRANDE SYSTEM OF WEST TEXAS

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**Abstract.**—The Rio Grande is a major source of water for agricultural and municipal uses in Texas and northern Mexico. Water shortages in the Rio Grande have been significantly impacted by the invasion and spread of the invasive shrub saltcedar (*Tamarix chinensis* Lour.). Conventional color aerial photography and videography were acquired simultaneously of the Rio Grande to distinguish saltcedar. The videography was integrated with global positioning system (GPS) and geographic information system (GIS) technologies for detecting and mapping the distribution of saltcedar. Integration of the GPS with the video imagery permitted latitude-longitude coordinates of saltcedar infestations to be recorded on each image. The GPS coordinates on the video scenes depicting saltcedar infestations were entered into a GIS. Distribution maps were developed denoting the locations of saltcedar infestations along the Rio Grande.

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The Rio Grande is one of the longest river systems in the United States. The river extends 3,040 km from its source in the San Juan Mountains of Colorado to the mouth at the Gulf of Mexico on the United States-Mexico border in extreme south Texas (Gilpin 1949). Approximately two-thirds (2,020 km) of the Rio Grande is the effective border between Texas and Mexico (Davis 2002). Construction of dams and reservoirs along the lower Rio Grande for flood control and for agricultural and municipal uses have resulted in losses of much of the natural vegetation (Lonard et al. 2000).

Today, extensive areas along the Rio Grande system in Texas have been invaded by exotic, invasive plant species which have ultimately displaced much of the original native vegetation and contributed to water shortages in the river. Saltcedar (*Tamarix* sp.), waterhyacinth (*Eichhornia crassipes* [Mort.] Solms), and hydrilla (*Hydrilla verticillata* [L. F.] Royle) are major exotic species that have invaded the Rio Grande system of Texas (Davis 2002).

Eight species of saltcedar (*Tamarix* sp.) have been introduced into the United States from Europe, Asia, and Africa for ornamentals, wind-breaks, and erosion prevention of streambanks (Baum 1967). At least five species of saltcedar are found in Texas (Hatch et al. 1990).

Two deciduous saltcedar species (*Tamarix ramosissima* Ledeb. and *Tamarix chinensis* Laur.) are invaders of riparian sites of the southwestern United States (including Texas) and northern Mexico. These two very similar species form dense, low thickets that displace native vegetation, impede water flow, increase sedimentation, use excessive water, and increase soil salinity (Horton & Campbell 1974; Deloach 1990). Saltcedar communities are also much less valuable for wildlife than are the native riparian communities they displace (Kerpez & Smith 1989; Deloach 1990). Research on herbarium specimens and growing plants of *Tamarix chinensis* and *Tamarix ramosissima* has shown that it is difficult to distinguish between the two species (Horton 1977). Molecular research on these two species indicates that some populations are genetically indistinguishable and that there is some evidence of hybridization among several species of saltcedar (Gaskin & Schall 2003). Although *Tamarix chinensis*, *Tamarix ramosissima*, and possible hybrids occur in west Texas, the saltcedar taxon that causes a nuisance in this area is generally referred to as *Tamarix chinensis*.

Riparian zones and other wildland areas are often too large and inaccessible to determine their characteristics by ground surveys. Remote sensing techniques offer potentially timely, cost-effective means of obtaining reliable data for these areas (Tueller 1982). The value of remote sensing for distinguishing among plant species and communities is well established (Carter 1982; Driscoll et al. 1997). Aerial photography, airborne electronic imagery (videography and digital), and satellite imagery have been used to remotely detect weedy species over large and inaccessible areas (Gausman et al. 1977; Tueller 1989; Anderson et al. 1993; Everitt et al. 1995; Lass & Callihan 1997; Ramsey et al. 2002).

Over the past several years remote sensing, geographic information system (GIS), and global positioning system (GPS) technologies have been integrated for detecting and mapping the distribution of noxious plant species (Dewey et al. 1991; Anderson et al. 1993; Everitt et al. 1996). Remote observations in georeferenced formats help to assess the extent of infestations, develop management strategies, and evaluate control measures on noxious plant populations.

Several studies have been conducted using remote sensing techniques to distinguish saltcedar. Everitt & Deloach (1990) described the spectral light reflectance characteristics of saltcedar and demonstrated the application of normal color aerial photography for distinguishing infestations in Texas riparian areas. Everitt et al. (1996) used normal color aerial videography integrated with GPS and GIS technologies to detect and map saltcedar infestations on three river systems in the southwestern United States. More recently, airborne multispectral digital imagery has been used to map saltcedar and other riparian vegetation along the middle Rio Grande River in New Mexico (Akasheh et al. 2004). Anderson et al. (2004) used airborne hyperspectral imagery to assess biocontrol of saltcedar in Nevada and reported limited success in differentiating this species from other riparian vegetation.

The objectives of this study were to use aerial photography and videography, GPS, and GIS technologies for detecting and mapping the distribution of saltcedar infestations along the Rio Grande system in west Texas.

## MATERIALS AND METHODS

This study was conducted along the Rio Grande system on the Texas-Mexico border in west Texas. Aerial photography, airborne videography, and ground truth were conducted for this study.

Conventional color photography and videography were acquired simultaneously of the Rio Grande from Lajitas near Big Bend National Park to near El Paso in west Texas on 10 December 2002. Imagery was obtained at an altitude above ground level of 3,050 m. Kodak Aerochrome conventional color (0.40 to 0.70  $\mu\text{m}$ ) type 2448 film was used with a Fairchild type K-37 large format (23 cm by 23 cm) mapping camera. The camera aperture setting was f8 at 1/250 sec. Conventional color video was acquired with a Canon mini-digital video camera (model GL-1) with a zoom lens (4.2 to 84mm) and a super-VHS recorder.

A Cessna (model 404) airplane, equipped with a camera port in the floor, was used to obtain the aerial photography and videography. The cameras were maintained in nadir position during image acquisition. Imagery was acquired between 1130 and 1300 hours Central Standard Time under sunny conditions.

An Omnistar (model 3000L) differential GPS and Horita (model GPT-50) real-time GPS video/digital captioner/interphaser were integrated with the video system. The GPS acquired the latitude-longitude coordinate data of the aircraft location over the scene of interest, while the video interphaser transferred and superimposed the GPS data at the bottom of the video scene. The accuracy of the GPS was approximately  $\pm 20$  m from the center coordinates of each video scene. Location coordinates of saltcedar were obtained from each video scene and entered into the computer manually. Before the GPS data were obtained from the video scenes, population levels of saltcedar were assigned to each photographic image. Population levels were assigned to the photographic images because they had better spatial resolution than the video scenes. Population levels of saltcedar were accomplished by breaking down the width of stands that grow in corridors along the Rio Grande. Population levels of saltcedar were assigned to each photograph using the following criteria:  $> 120$  m wide, dense; 60 to 120 m wide, moderate; and  $< 60$  m wide, light. The length of the corridor was not considered since most were greater than 0.75 km long. Each video scene of saltcedar covered a linear distance along the river of approximately 2,100 m, whereas the photographs covered a linear distance of approximately 2,500 m. Population density or percent cover of the population levels was not quantitatively estimated. Personal computer MapInfo-GIS software (MapInfo, Inc. 1998) was used to generate regional and detailed maps along the Rio Grande. MapInfo uses StreetWorks which is a street display mapping product that provides coverage of U.S. streets, highways, city and town boundaries, area landmarks, point locations, and water features. StreetWorks is based on U.S. Census Bureau TIGER (Topologically Integrated Geographic Encoding and Referencing) 1995 data that includes street-level detail to the local level. The TIGER map-based system was constructed using USGS 1:100,000 scale digital line graph maps. These maps were produced

to geographically map saltcedar infestations along the Rio Grande using the airborne video survey GPS data.

Ground truth surveys were conducted at sites where aerial photography and videography were obtained. In some instances, ground surveys were done of some sites prior to acquiring aerial imagery. Observational data recorded were plant species and cover. Ground surveys were made only on the U.S. side of the border.

## RESULTS AND DISCUSSION

Figures 1a and 1b show a black-and-white photographic print of a normal color photograph and a normal color analog video image, respectively, of a saltcedar infestation on the Rio Grande north of Candelaria in west Texas. The photograph is a portion of a 23 cm photograph (1:10,000 scale), whereas the video image (3.0 m pixel size) was extracted from a slightly larger video scene. The arrows on the two images point to the orange-brown tonal response of a dense stand of saltcedar. Bare soil and sparsely vegetated areas have white to various light gray tones, shrubs have a dark gray or black image response, and water has light green to dark green tones. Although the video image has coarser resolution than the photograph, saltcedar can be easily distinguished. The GPS latitude-longitude coordinates of the area are displayed at the top of the video image. The distinct image response of saltcedar was due to its yellow-orange to orange-brown late fall foliage color prior to leaf drop. Saltcedar could be readily distinguished in all the normal color photography and videography obtained along the Rio Grande. Saltcedar has higher visible reflectance during this phenological stage that facilitates its detection on normal color photography and videography (Everitt & Deloach 1990; Everitt et al. 1996). Figure 2 shows a GIS map of the four-county area of west Texas where the aerial survey was conducted. The Rio Grande forms the left boundary of the map. The GPS latitude-longitude data provided on the aerial videographic imagery from the December 2002 survey of the Rio Grande have been integrated with the GIS to georeference infestations of saltcedar along the river. Areas with stars represent the densest populations of saltcedar, those with dots were moderate populations, and those represented by triangles

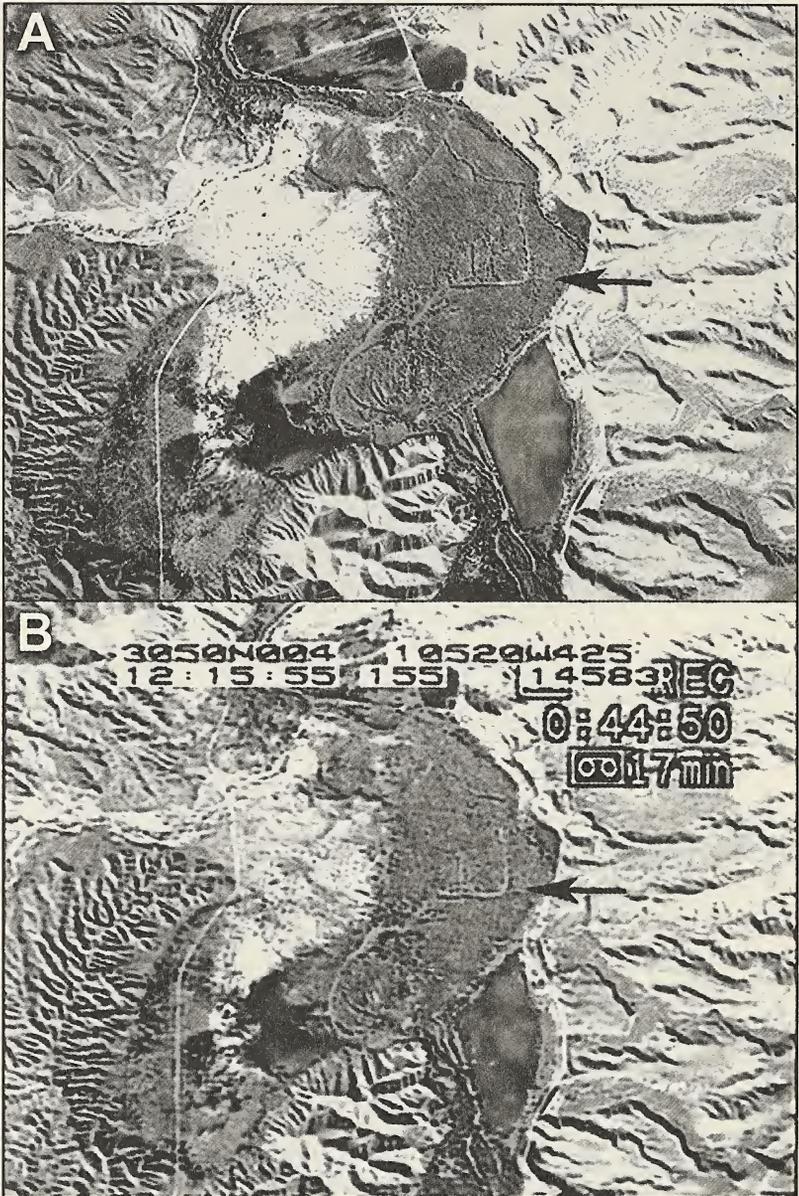


Figure 1. Black-and-white photographic print of normal color photographic (a) and analog videographic (b) images obtained 10 December 2002 of a saltcedar infestation along the Rio Grande north of Candelaria in west Texas. The arrows point to a dense stand of saltcedar. The GPS data is superimposed at the top of the video image.

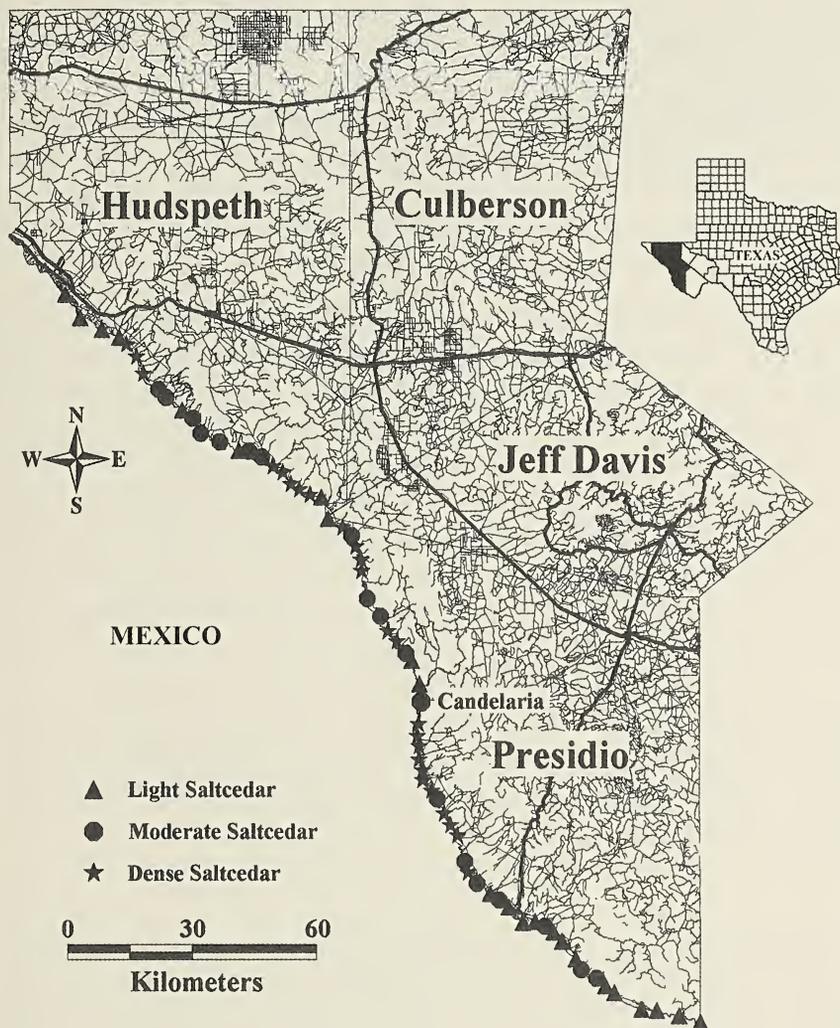


Figure 2. Regional GIS map of a four-county area in west Texas. The Rio Grande forms the western boundary of the map with Mexico. The symbols along the Rio Grande represent GPS latitude-longitude coordinates of saltcedar infestations obtained from the airborne video imagery.

have light populations. Many of the population symbols are stacked on each other because of the small scale of the map. Results indicate that approximately 460 river-km of saltcedar occurred along the Rio

Grande study area. The densest populations occurred in the eastern portion of Hudspeth County and the western part of Presidio County where many of the corridors were  $> 120$  m wide. Ground surveys of the individual sites indicated that saltcedar stands with  $> 25\%$  cover could be distinguished on the imagery; however, those stands with  $> 50\%$  cover had a more pronounced image response. Many small saltcedar plants and some isolated larger plants with sparse canopies could not be distinguished on the imagery.

The 2002 survey map (Figure 2) of saltcedar distribution was similar to a 1994 survey map of the same general area (Everitt et al. 1996). Total river-km of saltcedar was not computed in the 1994 survey, but a qualitative comparison between the two maps is very similar. However, some of the saltcedar density levels differed between the two surveys. This was partially due to changes in plant populations over the eight-year interval between surveys, but was primarily attributed to using different criteria for assignment of population levels in the 1994 survey and acquisition of imagery at a different altitude. The 1994 imagery was obtained at altitudes ranging from 1,050 to 1,500 m, whereas the 2002 survey was obtained at an altitude of 3,050 m. The higher altitude imagery of the 2002 survey provided a much greater horizontal width of coverage of the Rio Grande floodplain and the detection of more saltcedar populations than in the 1994 survey.

### CONCLUSIONS

Results from this study have shown that airborne remote sensing, GPS, and GIS technologies are valuable tools for detecting and mapping saltcedar along the Rio Grande system of west Texas. These findings indicate that approximately 460 river-km of the Rio Grande from Lajitas to near El Paso are infested by saltcedar. This estimate is probably an underestimation of the actual number of river-km since many small saltcedar plants and some individual larger plants could not be distinguished on the aerial imagery.

The integration of airborne videography with GPS technology can serve as a permanent geographically located image database to monitor future contraction or spread of saltcedar over time. The GIS

database can be used to record attribute information for areas of interest. The joint use of these technologies provides important information on the distribution of saltcedar in the Rio Grande system along the Texas-Mexico border. It is anticipated that these technologies can be used for a variety of other natural resource management applications.

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## THE VASCULAR FLORA OF HOWARD COUNTY, TEXAS

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**Abstract.**—Following a comprehensive four-year survey in Howard County, Texas, and the review of three non-comprehensive surveys, a combined checklist of 307 species of vascular plants is reported. This checklist includes 202 genera and 61 families, with Asteraceae (28%), Fabaceae (8%) and Poaceae (6%) the most common. Vegetative associations and common names are also included.

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Howard County is located in the extreme southern part of the High Plains at a junction point with the Edwards Plateau, the Llano Estacado, and the Rolling Plains. The Chihuahuan Desert extends to 50 km west of the county. This makes Howard County unique in both plant and animal diversity (Yanez & Simpson 2001).

Comprehensive county plant surveys have been completed for several counties in the West Texas area; King (1975) in Coke County, Eckhardt (1975) in Tom Green County, Hutchins (1968) in Garza County, and Collins (1966) in Ector and Midland counties. Three non-comprehensive surveys within Howard County have been accomplished. Army surgeon V. Havard (1885) studied the flora while assigned to an outpost at Big Spring and recorded 25 species. Reed (1978) surveyed the 1977 spring flora of Howard County and reported finding 105 species. The Big Spring State Park produced a brochure listing some 62 native plants found inside the park boundaries (Anon. 1992).

## DESCRIPTION OF HOWARD COUNTY

Howard County is located between 32° 00' and 32° 32' N latitudes and between 101° 10' and 110° 42' W longitudes (Anon. 1995). The county is approximately 48 km by 48 km encompassing

2334 sq. km. Elevations range from 670 m to 846 m. Mean annual rainfall is 48.8 cm with a growing season of 217 days (Anon. 2004); the climate is classified as semi-arid (Stoner et al. 1969). January mean minimum temperature is  $-2.2^{\circ}$  C with a July maximum of  $34.4^{\circ}$  C (Anon. 2004).

### GENERAL VEGETATIVE SITES

Using Stoner et al. (1969) and Simpson & Cerda (2004) as a guide, four major vegetative associations were recognized. (1) Cultivated or recently cultivated sites show gradual slope or no slope and recent mechanical tillage. The soil is sandy loam and though spotted through the county, cultivated sites dominate the western half of the county. (2) Rolling-range sites are predominately located in the eastern portion of the county. The sites are characterized by mild to steep slopes with tight clay loam soils. Rolling-range sites are rarely utilized for farming due to slopes and potential erosion. (3) Sites with slopes of steep to vertical were called hillside sites. These sites were typically located through the central portion of the county along a remnant of the Edwards Plateau. The soil at hillside sites is dominated by limestone rock with dense woody vegetation. (4) Riparian areas cover less than 1% of the county and are characterized by temporary streams and man-made lakes and ponds.

### METHODS

The county was divided into 25 zones; with each zone being approximately 9 km by 9 km. Sampling began in March 2001 and continued through June 2004. Most specimens were collected in months May through August. All zones were sampled in at least three locations, usually along the roadside. Soil type and topographic feature were noted and the plants were returned to the lab for processing. Specimens were pressed and dried. Plants were identified and assigned family and species names using Jones et al. (1997). Corrections and verifications were provided by B. L. Turner and A. M. Powell at Sul Ross State University. Specimens

were then mounted and herbarium labels containing collecting information were attached. Herbarium labels included family, species, and frequently used common names (Correll & Johnston 1979; USDA 2004) for reference by gardening and agricultural associations, and soil types and vegetative sites. Labeled specimens were placed in the Howard College Herbarium for teaching and public use. A complete set of voucher specimens was deposited in the Sul Ross State University Herbarium (SRSC). Selected specimens were also placed in the University of Texas at Austin Herbarium (TEX-LL).

#### LIST OF VERIFIED SPECIES

The following list of verified plant species is by no means considered to be a complete list of all species located within Howard County. This list is an attempt to compile the works done on Howard County plants in the past (Havard 1885; Reed 1978; Anon. 1992), combine these reports with the authors' recently collected plants, and produce a list of verified plant species within Howard County.

In the list of verified species, vegetative sites are abbreviated as follows: Cultivated or recently cultivated (C), rolling-range (RR), hill-side (HS), and riparian (R).

#### PINOPSIDA

##### CUPRESSACEAE

*Juniperus pinchotii* Sudw., Red-berry juniper, pinchot juniper (HS).

#### GNETOPSIDA

##### EPHEDRACEAE

*Ephedra antisiphilitica* Berland. ex C. A. Mey., Clapweed, joint-fir, popote, cañatilla (HS, R).

## MAGNOLIOPSIDA (DICOTS)

## ACANTHACEAE

*Dyschoriste linearis* (Torr. & A. Gray) Kuntze, Snake herb (RR, HS).

*Justicia pilosella* (Nees.) Hilsenb., Hairy tube-tongue (RR, HS).

## AMARANTHACEAE

*Amaranthus blitoides* Wats., Prostrate pigweed (C).

*Amaranthus palmeri* Wats., Careless weed (C).

*Guilleminea densa* (Willd.) Moq., Cottonflower (C, RR).

*Tidestromia lanuginosa* (Nutt.) Standl., Espanta vaqueros (C).

## ANACARDIACEAE

*Rhus microphylla* Engelm. ex Gray, Desert sumac (C).

## APIACEAE

*Cymopterus macrorhizus* Buckl., Wild parsley (HS).

*Daucus pusillus* Michx., Rattlesnake-weed (RR, HS).

## ASCLEPIADACEAE

*Asclepias brachystephana* Engelm. ex Torr., Purple milkweed (HS).

*Asclepias engelmanniana* Woods, Engelmann's milkweed (HS, R).

*Asclepias latifolia* (Torr.) Raf., Green milkweed, Texas milkweed (HS, R).

*Asclepias oenotheroides* Schltld. & Cham., Hierba de Zizotes (C).

*Asclepias subverticillata* (Gray) Vail., Silkweed (HS).

*Matelea biflora* (Raf.) Woodson, Two-flower milkvine (HS).

## ASTERACEAE

*Acourtia nana* (Gray) Reveal & King, Desert holly, dwarf desert holly (RR, HS).

*Amblyolepis setigera* DC., Huisache-daisy (C).

*Ambrosia grayi* (A. Nels.) Shinnery, Ragweed (C).

- Ambrosia psilostachya* DC., Western ragweed (RR, R).  
*Amphiachyris dracunculoides* (DC.) Nutt., Prairie broomweed (HS).  
*Aphanostephus ramosissimus* DC., Plains lazy daisy (C).  
*Aphanostephus riddellii* T. & G., Lazy daisy (HS).  
*Aphanostephus skirrhobasis* (DC.) Trel. var. *skirrhobasis*, Lazy daisy (HS).  
*Artemesia ludoviciana* Nutt., Western mugwort, Mexican sagewort (HS).  
*Baccharis salicina* T. & G. Willow, baccharis, groundsel-tree (RR, HS).  
*Baccharis texana* (T. & G.) A. Gray, Groundsel-tree (C, R).  
*Berlandiera lyrata* Benth., Lyre leaf green-eyes (RR, HS).  
*Centaurea americana* Nutt., Basket-flower, American star-thistle (C).  
*Centaurea melitensis* L. Tocalote, Malta star-thistle (C).  
*Chaetopappa ericoides* (Torr.) G. L. Nesom, White aster, rose heath (RR, HS).  
*Cirsium ochrocentrum* Gray, Yellow-spine thistle (RR).  
*Cirsium texanum* Buckl., Texas thistle, plumed thistle (HS).  
*Conyza canadensis* (L.) Cronquist., Horse-weed (RR, HS).  
*Dyssodia acerosa* DC., Dogweed (RR, R).  
*Dyssodia pentachaeta* (DC.), Robins. Parralena (C, R).  
*Dyssodia tenuiloba* (DC.), Robins. Dogweed, fetid marigold (RR).  
*Engelmannia pinnatifida* Gray ex Nutt., Engelmann daisy (RR).  
*Erigeron modestus* Gray, Fleabane (HS).  
*Erigeron strigosus* Muhl., ex Willd. White-top (HS).  
*Erigeron versicolor* (Greenm.) G. L. Nesom, Fleabane (C).  
*Euthamia leptcephala* (T. & G.) Greene, Euthamia, bushy goldendrop (HS).  
*Evax verna* Raf., Rabbit-tobacco, cotton-rose (RR).  
*Florestina tripteris* DC., Sticky palafaxia (C, RR).  
*Gaillardia multiceps* Greene, Onion blanketflower (HS).  
*Gaillardia pinnatifida* Torr., Old red eye (HS).

- Gaillardia pulchella* Foug., Blanket flower, Indian blanket, firewheel (C).
- Gaillardia suavis* (Gray & Engelm.) Britton & Rusby, Blanket flower (HS).
- Grindelia nuda* Wood var. *aphanactis* (Rydb.) G. Nesom, Gumweed, tarweed (C).
- Gutierrezia sarothrae* (Pursh.) Britt. & Rusby, Snakeweed (HS).
- Gutierrezia sphaerocephala* Gray, Roundleaf, snakeleaf (HS, R).
- Haploesthes greggii* Gray, False broomweed, false cotton (HS).
- Helianthus annuus* L., Common sunflower, mirasol (C).
- Helianthus ciliaris* DC., Blue-weed (HS).
- Helianthus petiolaris* Nutt., Prairie sunflower (C, RR).
- Heterotheca stenophylla* (Gray) Shinnery, Golden aster, camphor weed (C, RR).
- Heterotheca subaxillaris* (Lam.) Britt. & Rusby, Camphor weed (C, RR).
- Hymenopappus artemisiifolius* DC., Woolly-white (HS).
- Hymenopappus flavescens* Gray var. *flavescens*, Woolly-white (RR).
- Hymenopappus scabiosaeus* L'Hér., Old plainsman (HS).
- Hymenopappus tenuifolius* Pursh., Slimleaf hymenopappus, chalk hill hymenopappus (HS).
- Hymenoxys odorata* DC., Bitterweed (RR).
- Lactuca serriola* L., Prickly lettuce, wild lettuce, compass plant (C).
- Lygodesmia texana* (T. & G.) Greene, Skeleton plant, skeleton weed, pink dandelion (C).
- Machaeranthera gracilis* (Nutt.) Shinnery, Desert aster (HS).
- Machaeranthera pinnatifida* (Hook.) Shinnery, Lacy tansyaster (HS, RR).
- Machaeranthera tanacetifolia* (Kunth.) Nees, Tahoka daisy (HS, RR).
- Melampodium leucanthum* T. & G., Black-foot (RR).
- Palafoxia rosea* (Bush) Cory, Sand palafoxia (C).
- Parthenium confertum* Gray, Lyreleaf parthenium, Grey's feverfew (RR).

- Psilostrophe tagetina* (Nutt.) Greene, Paper flower (C, RR).  
*Pyrrhopappus pauciflorus* (D. Don) DC., False dandelion (C).  
*Ratibida columnifera* (Nutt.) Wooton & Standl., Mexican hat (HS, RR).  
*Senecio douglasii* DC., Butterweed (HS).  
*Senecio flaccidus* Less., Burweed (RR).  
*Simsia calva* (Gray & Engelm.) Gray, Awnless bush sunflower (RR).  
*Sonchus asper* (L.) Hill, Spiny sow thistle (C).  
*Sonchus oleraceus* L., Sow thistle (C, R).  
*Stephanomeria pauciflora* (Torr.) A. Nels., Wire lettuce (C).  
*Symphotrichum subulatum* (Michx.) G. Nesom, Annual aster (HS).  
*Tetraneris linearifolia* (Hook.) Greene, Four-nerve daisy (HS).  
*Tetraneris scaposa* (DC.) Greene, Four-nerve (HS).  
*Thelesperma filifolium* (Hook.) A. Gray, Green-thread (C).  
*Thelesperma megapotamicum* (Spreng.) O. Ktze., Cota, Indian tea, Navajo tea (RR).  
*Thelesperma simplicifolium* Gray, Greenthread (HS).  
*Tragopogon dubius* Scop., Goat's-beard (C).  
*Tragopogon porrifolius* L. Salsify, oyster plant (C).  
*Trixis californica* Kell. var. *californica*, American trixis (HS).  
*Verbesina encelioides* (Cav.) Gray, Cowpen daisy (HS).  
*Verbesina nana* (Gray) Robins. & Greenm., Crown beard (HS, RR).  
*Vernonia marginata* (Torr.) Raf., Plains ironweed (RR, R).  
*Viguiera stenoloba* Blake, Resin-bush (HS).  
*Xanthisma texanum* DC., Texas sleepy daisy (C).  
*Xanthocephalum* sp. Willd., Snakeweed, Broomweed (HS).  
*Zinnia acerosa* (DC.) Gray, Dwarf zinnia (HS).  
*Zinnia grandiflora* Nutt., Prairie zinnia (RR, R).

## BERBERIDACEAE

- Berberis trifoliata* Moric., Algeritas, agarito (C).

## BORAGINACEAE

- Cryptantha angustifolia* (Torr.) Greene, Frostweed, narrowleaf popcorn flower (HS).  
*Cryptantha cinerea* (Greene) Cronquist var. *jamesii* A. Cronq., James catseye (HS).  
*Cryptantha crassisepala* (T. & G.) Greene, Thick-sepal catseye (C).  
*Cryptantha mexicana* (Brandeg.) I. M. Johnst., Mexican cryptantha (HS).  
*Cryptantha minima* Rydb., Little cryptantha (HS).  
*Cryptantha palmeri* (Gray) Payson, Palmer's grappling-hook (HS).  
*Lithospermum incisum* Lehm. Gromwell, puccoon (HS).

## BRASSICACEAE

- Capsella bursa-pastoris* (L.) Medic., Shepherd's purse (HS).  
*Descurainia sophia* (L.) Webb, Flixweed, herb sophia (C).  
*Dimorphocarpa candicans* (Raf.) Roll., Palmer's spectaclepod (C).  
*Draba cuneifolia* Nutt. ex T. & G., Wedge-leaf whitlow-grass (C).  
*Lepidium virginicum* L., Lentejilla (C, R).  
*Lesquerella fendleri* (Gray) Wats., Bladder pod (RR).  
*Lesquerella gordonii* (Gray) Wats., Gordon's bladderpod (C).  
*Rapistrum rugosum* (L.) All., Annual bastard cabbage (C).  
*Sisymbrium* L., London rocket (HS).

## CACTACEAE

- Epithelantha micromeris* (Engelm.) Weber, Button cactus (HS).  
*Mammillaria heyderi* Müehlenpf., Pin-cushion cactus (HS).  
*Opuntia engelmannii* Salm-Dyck., Engelmann's prickly pear (HS).  
*Opuntia imbricata* (Haw.) DC., Tree cholla, coyonostle, tree cactus (HS).  
*Opuntia leptocaulis* DC., Desert Christmas cactus (RR).  
*Opuntia macrorhiza* Engelm. Plains prickly pear (HS, RR).

## CARYOPHYLLACEAE

*Paronychia jamesii* T. & G., James's nailwort, whitlow-wort (C).

## CHENOPODIACEAE

*Atriplex canescens* (Pursh) Nutt., Saltbush (RR).

*Chenopodium berlandieri* Moq., Pitseed goosefoot (RR).

*Chenopodium incanum* (Wats.) Heller., Goosefoot, pigweed (C).

*Chenopodium pratericola* Rydb., Thickleaf goosefoot (C).

*Kochia scoparia* (L.) Roth, Belvedere (HS).

## CONVOLVULACEAE

*Convolvulus arvensis* L., Field bindweed (C).

*Convolvulus equitans* Benth., Bindweed, possession vine (C, RR).

*Evolvulus alsinoides* (L.) L., Slender dwarf morning glory (HS).

*Ipomoea leptophylla* Torr., Bush morning glory (C).

## CUCURBITACEAE

*Citrullus lanatus* (Thunb.) Matsum. & Nakai., Pie-melon (C).

*Cucurbita foetidissima* Kunth., Buffalo-gourd (RR).

*Ibervillea tenuisecta* (Gray) Small, Cutleaf globe berry (RR).

## CUSCUTACEAE

*Cuscuta leptantha* Engelm., Slender dodder, cuscuta (RR).

## EUPHORBIACEAE

*Croton dioicus* Cav., Grassland croton (RR).

*Croton pottsii* (Kl.) Müll., Arg. Leather-weed (RR).

*Euphorbia albomarginata* T. & G., Spurge (C).

*Euphorbia chaetocalyx* (Boiss.) Tidest., Bristle cup sandmat (HS).

*Euphorbia dentata* Michx., Poinsettia, Spurge (C, RR).

*Euphorbia lata* Engelm., Hoary sandmat (RR).

*Euphorbia longicuris* Scheele, Spurge (RR).

- Euphorbia wrightii* T. & G., Spurge (C).  
*Tragia ramosa* Torr., Noseburn (C).

## FABACEAE

- Acacia greggii* Gray var. *wrightii* (Benth.) Isely, Cat's claw, catclaw (HS).  
*Acacia roemeriana* Scheele, Cat's claw (RR).  
*Astragalus lindheimeri* Engelm. ex Gray, Milk-vetch, loco weed (HS).  
*Astragalus mollissimus* Torr. var. *coryi* I. Tidestr., Woolly loco weed, Texas loco weed, Purple loco weed (RR).  
*Astragalus plattensis* Nutt. ex T. & G., Milk-vetch, Loco weed (HS).  
*Caesalpinia gilliesii* (Hook.) Benth., Yellow bird of paradise, desert bird of paradise (C, R).  
*Dalea aurea* Nutt., Golden dalea (HS).  
*Dalea candida* Michx. ex Willd., Prairie clover, white prairie clover (C).  
*Dalea cylindriceps* Barneby, Andean prairie clover (C).  
*Dalea formosa* Torr., Feather plume, pea bush (C).  
*Dalea nana* T. & G., Dwarf prairie clover (HS).  
*Desmanthus illinoënsis* (Michx.) MacM., Illinois bundleflower (C).  
*Desmanthus obtusus* Wats., Bluntpod, bundleflower (RR).  
*Hoffmannseggia glauca* (Ort.) Eifert, Rush-pea, hog-potato, mesquite weed (C).  
*Medicago minima* (L.) L., Small bur-clover (C).  
*Medicago sativa* L., Bur-clover (RR).  
*Mimosa aculeaticarpa* Ort. var. *biuncifera* (Benth.) Barneby, Catclaw mimosa (C).  
*Mimosa borealis* Gray, Pink mimosa, fragrant mimosa (RR).  
*Mimosa rupertiana* B. L. Turner, Sensitive plant (C).  
*Pediomelum cuspidatum* (Pursh) Rydb., Large bract Indian breadroot (HS).  
*Prosopis glandulosa* Torr., Honey mesquite (C).

*Senna roemeriana* (Sheele) Irwin & Barneby, Two-leafed senna (C).

*Sophora secundiflora* (Ort.) DC., Texas mountain laurel (HS).

## FAGACEAE

*Quercus havardii* Rydb. var. *havardii*, Sand shinnery oak (C).

*Quercus mohriana* Buckl., Mohr's oak, shin oak, scrub oak (HS).

## FUMARIACEAE

*Corydalis aurea* Willd., Scrambled eggs (C).

## GENTIANACEAE

*Centaurium beyrichii* (T. & G.) Robins, Mountain pink (C).

*Centaurium calycosum* (Buckl.) Fern., Buckley centaury, Arizona centaury, rosita (HS).

## GERANIACEAE

*Erodium cicutarium* (L.) L'Hér., Cranesbill, stork's-bill, pin clover (C, RR).

*Erodium texanum* Gray, Stork's-bill (HS).

## HYDROPHYLLACEAE

*Nama hispidum* Gray, Rough nama, sandbells (C).

*Phacelia congesta* Hook, Blue-curls, spike phacelia, spider flower (RR).

*Phacelia popei* T. & G., Scorpion weed (HS).

## JUGLANDACEAE

*Juglans microcarpa* Berland., Little walnut (HS).

## KRAMERIACEAE

*Krameria lanceolata* Torr., Sandspur, trailing ratany (HS).

## LAMIACEAE

*Brazoria scutellarioides* Engelm. & Gray, Texas wisteria (HS).

- Hedeoma drummondii* Benth., Drummond false pennyroyal (HS).  
*Marrubium vulgare* L., Common horehound, marrubio (C, RR).  
*Monarda citriodora* Cerv. ex Lag., Lemon mint, wild bee balm (HS).  
*Monarda punctata* L., Spotted bee balm, horsemint (C, RR).  
*Salvia farinacea* Benth., Mealy blue sage (HS).  
*Salvia reflexa* Hornem., Rocky mountain sage (C, RR).  
*Salvia texana* (Sheele) Torr., Blue sage (HS).  
*Teucrium cubense* Jacq. var. *laevigatum*. (Vahl) Shinners, Cutleaf germander (C, RR).

#### LINACEAE

- Linum berlandieri* Hook, Flax (RR).  
*Linum compactum* A. Nels., Wyoming flax (C).  
*Linum lewisii* Pursh., Blue flax, prairie flax (RR).  
*Linum pratense* (Nort.) Small, Blue flax, meadow flax (HS).  
*Linum rigidum* Pursh., Stiff-stem yellow flax (HS).  
*Linum sulcatum* Ridd., Yellow prairie flax (HS).

#### LOASACEAE

- Mentzelia strictissima* (Woot. & Standl.) Darl., Grassland blazingstar (HS).

#### MALVACEAE

- Abutilon fruticosum* Guill. & Perr., Texas Indian mallow (RR).  
*Callirhoë pedata* (Nutt. ex Hook.) Gray, Poppy mallow (HS).  
*Rhynchosida physocalyx* (Gray) Fryxell, Bladderpod sida (RR).  
*Sphaeralcea angustifolia* (Cav.) G. Don, False mallow, globe mallow (RR).  
*Sphaeralcea coccinea* (Nutt.) Rydb., Scarlet globe-mallow, red false mallow (HS).  
*Sphaeralcea hastulata* Gray, Orange globe-mallow (RR).

## MENISPERMACEAE

*Cocculus carolinus* (L.) DC., Red-berried moonseed, snailseed (C, RR).

## NYCTAGINACEAE

*Acleisanthes longiflora* Gray, Angel trumpets (C, RR).

*Boerhavia linearifolia* Gray, Spiderling (C).

*Mirabilis linearis* (Pursh) Heimerl., Umbrellawort, four-o'clock (C).

*Nyctaginia capitata* Choisy, Scarlet musk-flower (C).

## ONAGRACEAE

*Calylophus berlandieri* Spach., Square-bud day-primrose (HS).

*Calylophus hartwegii* (Benth.) Raven, Evening primrose (C).

*Calylophus serrulatus* (Nutt.) Raven, Yellow sundrop, yellow evening primrose (C).

*Gaura calcicola* Raven & Gregory, Texas butterfly-weed (HS).

*Gaura coccinea* Pursh., Scarlet gaura (C).

*Gaura parviflora* Hook, Velvet weed, willow gaura (RR).

*Gaura sinuata* Sér., Wavy-leafed gaura (C).

*Gaura suffulta* Engelm. ex Gray, Bee blossom, wild honeysuckle, kisses (C, RR).

*Gaura villosa* Torr., Hairy gaura, woolly beeblossom (C).

*Oenothera elata* Kunth., Hooker's evening primrose (HS).

*Oenothera engelmannii* (Small) Munz., Engelmann's evening primrose (C, RR).

*Oenothera grandis* (Britton) Smyth., Showy evening primrose (C).

*Oenothera macrocarpa* Nutt., Big-fruit evening primrose (C).

*Oenothera pubescens* (Willd.) Munz., South American evening primrose (C).

## OROBANCHACEAE

*Orobanche uniflora* L., Naked broomrape (HS).

## PAPAVERACEAE

*Argemone chisosensis* G. Ownbey, Prickly poppy (C).

*Argemone squarrosa* Greene, Hedgehog pricklypoppy (HS).

## PEDALIACEAE

*Proboscidea louisianica* (Mill.) Thell., Devil's claw (C).

## PHYTOLACCACEAE

*Rivina humilis* L., Pigeon-berry, rouge-plant, coralito (C, RR).

## PLANTAGINACEAE

*Plantago helleri* Small, Cedar plantain (HS).

*Plantago patagonica* Jacq., Indianwheat, buckhorn plantain, woolly plantain (C).

*Plantago rhodosperma* Decne, Red-seeded plantain (C).

*Plantago wrightiana* Decne, Wright's plantain (HS).

## POLEMONIACEAE

*Gilia rigidula* Benth., Blue bowls (RR).

## POLYGALACEAE

*Polygala alba* Nutt., White milkwort (C).

*Polygala lindheimeri* Gray, Shrubby milkwort (C).

## POLYGONACEAE

*Polygonum hydropiperoides* Michx., Mild water pepper (C, R).

*Rumex crispus* L., Curley dock, yellow dock (RR).

*Rumex hymenosepalus* Torr., Wild rhubarb, dock (C, R).

*Rumex pulcher* L., Fiddle dock (C, R).

## RANUNCULACEAE

*Anemone tuberosa* Rydb., Tuber-anemone, desert-anemone (HS).

*Clematis drummondii* T. & G., Texas virgin's bower, barbas de chivato, old man's beard (C, RR).

*Delphinium carolinianum* Walt., Prairie larkspur, blue larkspur (HS).

## RHAMNACEAE

*Condalia ericoides* (Gray) M. C. Johnst., Javelina bush (HS).

*Ziziphus obtusifolia* (T. & G.) Gray, Lotebush, gumdrop tree, clepe (RR).

## RUBIACEAE

*Galium proliferum* Gray, Limestone bedstraw (HS).

*Hedyotis nigricans* (Lam.) Fosb., Bluets (HS).

*Hedyotis acerosa* Gray, Bluets (RR).

## RUTACEAE

*Zanthoxylum hirsutum* Buckl., Prickly ash, toothache tree, tickle-tongue (HS).

## SALICACEAE

*Salix amygdaloides* Anderss., Peach-leaved willow (RR).

*Salix nigra* Marsh, Black willow (C).

## SAPINDACEAE

*Sapindus saponaria* L. var. *drummondii* (H. & A.) L. Benson, Western soapberry, wild chinaberry (HS).

*Ungadia speciosa* Endl., Mexican buckeye (HS).

## SCROPHULARIACEAE

*Castilleja sessiliflora* Pursh., Indian paintbrush (HS).

*Penstemon jamesii* Benth., Beard-tongue (RR).

## SOLANACEAE

*Chamaesaracha sordida* (Dun.) A. Gray, False nightshade (HS).

*Lycium berlandieri* Dun., Wolfberry (C).

*Margaranthus solanaceus* Schltl., Netted globe-berry (C).

*Physalis cinerascens* (Dun.) Hitchc., Ground cherry (C).

*Quincula lobata* (Torr.) Raf., Purple ground cherry (RR).

- Solanum elaeagnifolium* Cav., Silver-leaf nightshade (C, RR).  
*Solanum rostratum* Dun., Buffalo bur (RR).

## TAMARICACEAE

- Tamarix chinensis* Lour., Salt cedar (C).

## ULMACEAE

- Celtis laevigata* Willd. var. *reticulata* (Torr.) L. Benson, Netleaf hackberry, Texas sugarberry, palo blanco (C).

## VERBENACEAE

- Aloysia gratissima* (Gill. & Hook.) Tronc., Whitebrush (C).  
*Glandularia bipinnatifida* (Nutt.) Nutt., Dakota-mock vervain, small-flowered verbena (RR).  
*Phyla nodiflora* (L.) Greene, Common frog fruit, cape weed, turkey tangle (C).  
*Verbena bracteata* Lag. & Rodr., Prostrate vervain (C).  
*Verbena plicata* Greene, Fanleaf vervain (RR).

## VISCACEAE

- Phoradendron tomentosum* (DC.) Engelm. ex A. Gray, Mistletoe, injerto (RR).

## VITACEAE

- Cissus incisa* (Nutt.) Des Moul., Marine ivy, ivy treebind (C, RR).

## ZYGOPHYLLACEAE

- Kallstroemia parviflora* Nort., Hairy caltrop (RR).  
*Larrea tridentata* (DC.) Cov., Creosote bush, gobernadora, hediondilla (RR, R).  
*Tribulus terrestris* L., Goat head, puncture weed (C).

## LILIOPSIDA (MONOCOTS)

## AGAVACEAE

- Yucca campestris* McKelvey, Plain's yucca (HS).

*Yucca glauca* Nutt. var. *glauca*, Soap-weed, yucca (C).

#### COMMELINACEAE

*Commelina erecta* L., Widow's tears, hierba del pollo (C).

*Tradescantia occidentalis* (Britt.) Smyth., Prairie spiderwort (RR).

#### CYPERACEAE

*Carex* sp. L., Sedge (R).

*Cyperus retroflexus* Buckl., Oneflower flatsedge (C, R).

#### IRIDACEAE

*Sisyrinchium scabrum* Cham. & Schecht, Blue-eyed grass (HS).

#### LILIACEAE

*Allium canadense* L., Canada garlic (HS).

*Allium drummondii* Regel., Wild onion (HS).

*Allium perdulce* S. V. Fraser var. *perdulce*, Wild onion (HS).

*Cooperia drummondii* Herb., White rain lily (C, RR).

*Nothoscordum bivalve* (L.) Britt., Crow-poison, false garlic (C, RR).

#### POACEAE

*Aristida purpurea* Nutt., Purple three-awn (C, RR).

*Arundo donax* L., Giant reed, Georgia cane, carrizo (RR).

*Bouteloua curtipendula* (Michx.) Torr., Side-oats grama (HS).

*Bromus catharticus* Vahl., Rescue grass (C, RR).

*Cenchrus spinifex* Cav., Grassbur, coast sandbur (C).

*Digitaria cognata* (Schult.) Pilg., Fall witchgrass, Carolina crabgrass (C).

*Echinochloa colona* (L.) Link, Jungle-rice (C).

*Elymus canadensis* L., Canada wild-rye (C, RR).

*Elymus elymoides* (Raf.) Swezey, Squirreltail (HS).

*Eragrostis lehmanniana* Nees., Lehmann lovegrass (C).

*Erioneuron pilosum* (Buckley) Nash, Hairy tridens (C, RR).

- Hesperostipa neomexicana* (Thurb.) Barkworth, New Mexican feather-grass, Needlegrass (HS).  
*Hordeum murinum* L., Barley grass (HS).  
*Hordeum pusillum* Nutt., Little barley (C, RR, R).  
*Melica nitens* (Scribn.) Piper, Three-flowered melic (HS).  
*Nassella leucotricha* (Trin. & Rupr.) Pohl., Needlegrass, Speargrass (C).  
*Panicum antidotale* Retz., Blue panicum (C).  
*Pleuraphis mutica* Buckl., Tobosa (C, RR).  
*Poa* sp. L., Bluegrass (C, RR, HS).  
*Polypogon monspeliensis* (L.) Desf., Rabbitfoot grass (C, R).  
*Schizachyrium scoparium* (Michx) Nash., Little bluestem (HS).  
*Setaria leucopila* (Scribn. & Merr.) Schum., Plains bristle-grass (HS).  
*Sorghum halepense* (L.) Pers., Johnson grass (C).  
*Tridens albescens* (Vasey) Woot. & Standl., White tridens, tule (HS).

## TYPHACEAE

- Typha domingensis* Pers., Southern cat-tail (RR).

## RESULTS

At the conclusion of this study 307 species of vascular plants representing 202 genera and 61 families have been reported from Howard County, Texas. These totals represent the sum of all collecting reported by the authors of this paper and studies prior to March 2001 (Havard 1885; Reed 1978; Anon. 1992). The most common families include 81 Asteraceae (28 %), 23 Fabaceae (8%) and 19 Poaceae (6%).

Of the 307 species of plants documented within Howard County, 12 species were determined to be significant range extensions. Range extensions were determined by using the distributional dot maps found in Turner et al. (2003). Only four of these species are verifiable with voucher specimens. Eight of the species determined to be significant range extensions were found only in the literature

(Reed 1978; Anon. 1992) and where not verified by voucher specimens, but are still listed here for possible future investigators. Those specimens collected during this study from March 2001 to June 2004 are listed as specimens collected. Additional records list those specimens collected prior to this study. All voucher specimens listed may be found in the Sul Ross State University Collection (SRSC).

*Epithelantha micromeris* (Engelm.) Weber.–Turner et al. (2003) record specimens 200 km to the southwest of Howard County from Reeves and Val Verde counties and to the southeast from Bandera County. King (1975) reports collecting this cactus in Coke County 70 km to the southeast.

*Specimens collected.*–M. W. Nickell s.n. (SRSC).

*Monarda citriodora* Cerv. ex. Lag.–Turner et al. (2003) report specimens only from southern Texas along the coast. The Howard County specimens reported are a 725 km extension. Reed (1978) had reported this species from his Coke County survey. Other reports of this plant are from Garza County (Hutchins 1968) to the north, Tom Green County (Eckhardt 1975) to the south and Winkler and Andrews counties (Collins 1966) to the west.

*Specimens collected.*–C. N. Cerda 361 (SRSC).

*Additional records.*–J. D. Reed 202, 236, 283, 302 (SRSC)

*Calylophus hartwegii* (Beth.) Raven.–Reed (1978) reported this species during his Howard County survey. Turner et al. (2003) shows two varieties of this species, *C. hartwegii hartwegii* to the southwest of Howard County and *C. hartwegii maccatii* to the south. An overlap of the two varieties occur in Crocket and Val Verde counties. King (1975) also reports specimens from Coke County 70 km to the southeast. Collins (1966) collected *C. hartwegii hartwegii* and *C. hartwegii fendleri* in his study of the Concho Bluff Ecotone to the west. Hutchins (1966) reported *C. h. fendleri* from Garza County to the north of Howard County while

Eckhardt (1975) reported *C. hartwegii filifolius* and *C. hartwegii pubescens* from Tom Green County to the south.

*Specimens collected.*—J. D. Paredez 040; C. N. Cerda 069, 239, 308, 322, 347; V. Yanez 126, 129 (SRSC).

*Additional specimens.*—J. D. Reed 43, 249 (SRSC).

*Oenothera pubescens* (Willd.) Munz.—Turner et al. (2003) show this species occurring 300 km to the south and southwest of Howard County in Val Verde, Brewster, and Jeff Davis counties.

*Specimens collected.*—V. Yanez 021, 050 (SRSC).

The following eight species are found only in the literature (Reed 1978; Anon. 1992). No voucher specimens of these species have been located, which suggest that they may have been misidentified at the time of reporting or reported without a specimen being collected from Howard County.

*Erigeron strigosus* Muhl ex Willd.—Turner et al. (2003) show the nearest specimen of this species to be found in Taylor County, 200 km to the east of Howard County. Reed (1978) reported collecting specimens from Howard County prior to the current study but no voucher specimen was found.

*Euthamia leptcephala* (T. & G.) Greene.—Turner et al. (2003) show a dividing line of records in eastern Texas ranging from Bowie County south through Lee County to the Gulf of Mexico in Victoria County. Specimens listed in the literature would be 650 km extensions.

*Hymenopappus artemisiifolius* DC.—Turner et al. (2003) show a line of records reaching from Wise County south through Travis County to Jim Hogg County. Specimens listed from Howard County would be 300 km west of this line.

*Machaeranthera gracillus* (Nutt.) Shinn.—Nearest records to Howard County shown by Turner et al. (2003) are from Jeff Davis

and Brewster counties 300 km to the southwest. Reed (1978) had reported collecting this plant during his study. It is possible that a specimen of *Marchaeranthera pinnatifida* var. *pinnatifida*, J. D. Reed 34 (SRSC), collected by him may have been misidentified.

*Trixis californica* Kell.—Turner et al. (2003) show numerous records from Brewster, Jeff Davis and Culberson counties, to the southwest of Howard County. Specimens listed in literature prior to this study would be 300 km extensions.

*Cryptantha angustifolia* (Torr.) Greene.—Turner et al. (2003) record specimens from Brewster and Culberson counties, 300 km to the southwest of Howard County.

*Centrurium beyrichii* (T. & G.) Robins.—Turner et al. (2003) show a north-south line of records 350 km to the east of Howard County. King (1975) reported collecting two specimens from Coke County 70 km to the southeast.

*Linum sulcatum* Ridd.—Turner et al. (2003) show specimens for Jack and Burnet counties 425 km to the east of Howard County.

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EFFECT OF HOST ODOR CUES ON BEHAVIORAL RESPONSES  
OF THE SPIDER WASP, *PEPSIS FORMOSA*  
(HYMENOPTERA: POMPILIDAE)

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**Abstract.**—This study analyzed the ability of females of the spider wasp, *Pepsis formosa* to detect and respond to olfactory cues (treatment odors) associated with two species of theraphosid spider hosts (*Aphonopelma harlingenum* and *A. heterops*), as well as a novel odor associated with a stored grain beetle (*Tenebrio molitor*), a species not likely to be encountered by these wasps. Field-collected adult wasps were tested using choice experiments where they were exposed to a piece of filter paper conditioned with one of these treatment odors versus one sprayed with water (control). Wasps spent significantly more time on paper conditioned with odor cues associated with *A. harlingenum* than they did on paper conditioned with cues from *A. heterops* or *T. molitor*. Data collected at the study site in southern Texas (Hidalgo County), showed that 90.3% of all *P. formosa* larvae were found attached to *A. harlingenum* as compared to only 9.7% for *A. heterops*, despite the fact that the abundance and size of these two theraphosids were similar. Seventy-three percent of all paralyzed *A. harlingenum* found with wasp larvae attached to their bodies were females.

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The ability of many species of animals to detect and respond to olfactory cues associated with predators and prey has been well documented (see reviews by Chapman et al. 1987; Kats & Dill 1998). Regarding the detection of potential prey by arthropod predators or parasitoids (Vinson 1985), a number of different proximal cues are known to affect patch choice, residence time, and assessment of suitability of prey, including visual and tactile cues as well as chemosensory cues associated with prey odors (olfaction).

The types of sensory cues used by arthropod herbivores and carnivores in the detection of plants or prey are diverse and vary according to microhabitat conditions and foraging strategies. Many orthopteran insects and lepidopteran larvae exhibit genetic or cognitive-based responses to specific chemical cues associated with food plants and utilize these cues to make decisions concerning the amount of time to spend in a particular patch before switching to another location (Bernays 1993). With respect to predators, the ability to utilize olfactory cues to detect prey has been demonstrated in many

species, including crustaceans (Lima & Dill 1990; Chivers & Smith 1998), spiders (Foelix 1996), and insects (Bernays 1993; Punzo 1996). For example, the lynx spider *Oxyopes salticus* and wolf spider *Trochosa parthenus*, are capable of detecting odors associated with insect prey species, and will choose substrates associated with the odor of these insects over those that do not (Punzo 2002; Punzo & Kukoyi 1997).

Among parasitoid Hymenoptera, it is well known that many species rely on olfactory cues to identify their hosts after they have been located (Vinson 1985; Vet et al. 1991). In some species, the ability to detect and respond to host-specific odors is associated with a genetically-based (innate), hard-wired behavioral program (Doutt 1959), while host-finding behavior in other species may be affected by experience (learning) at various stages of the life cycle (see review by Turlings et al. 1993).

Spider wasps of the genus *Pepsis* (Hymenoptera: Pompilidae) are common inhabitants of North American deserts (Hurd 1952; Punzo 1994a; 2005). Nectivorous females selectively hunt theraphosid (tarantula) spiders as a food source (host) for their carnivorous larvae (Williams 1956; Punzo 1991). A spider is paralyzed and then dragged backwards over the ground and placed in a nest (Petrunkevitch 1926: 1952). A wasp may use the spider's burrow as its nest, or excavate one of its own (Evans & Eberhard 1970; Punzo 1994b). After depositing a single egg on the abdomen of the spider, the entrance to the nest is sealed and the wasp begins searching for another host (Passmore 1936; Cazier & Mortenson 1964). Although experimental evidence is lacking, it has been suggested that these wasps utilize species-specific olfactory cues to assess the suitability of a host spider (Lucas 1919; Petrunkevitch 1926; Punzo & Garman 1989). It is unclear whether or not pepsine wasps will utilize more than one species of theraphosid spider as a host in areas where two or more theraphosid species occur sympatrically (Punzo 2000; 2005).

*Pepsis formosa* (Say) has a wide geographical distribution and is found throughout most of Texas, westward into New Mexico, Arizona, Nevada and California (Hurd 1952). Previous studies on the biology of *P. formosa* include descriptions of various aspects of its

natural history and ecology (Williams 1956; Cazier & Mortenson 1964; Punzo & Garman 1989; Punzo 2000) as well as a detailed description of hunting behavior exhibited by female wasps from the Big Bend region (Brewster County) of far west Texas (Punzo 1991).

In southern Texas (Hidalgo County), two species of tarantula spiders are found where *P. formosa* occurs: *Aphonopelma harlungenum* (Chamberlin) and *A. heterops* Chamberlin. The purpose of this study was to determine if *P. formosa* can detect and respond to olfactory cues associated with these tarantula spiders, and if one theraphosid species is preferred over the other.

#### MATERIALS AND METHODS

*Subjects.*—All wasps were adult females of *P. formosa* and were collected with sweep nets within a 4-km radius of Elsa, Texas (Hidalgo County, USA) during August, 2003. All species of pepsine wasps collected were identified to species. Because it has been suggested that non-inseminated pepsine females will not hunt (Williams 1956; Punzo 2000), only those females of *P. formosa* who had been observed to mate with males were used in subsequent experiments ( $n = 90$ ). Females were collected immediately after they were observed mating in the field.

Wasps were transported back to the laboratory and placed individually in cylindrical glass containers (40 cm in length, 7 cm in diameter) where they were provided with water ad libitum and fed on a diet consisting of honey mixed with a glucose solution. Wasps were maintained at  $22 \pm 0.2^\circ\text{C}$ , 62-65% relative humidity, and 12L:12D photoperiod regime in Percival Model 85A environmental chambers (Boone, Iowa, USA).

*Examination of hosts and burrows.*—All tarantulas found wandering over the surface of the ground were collected and identified to species, and their body weights and sex recorded. Whenever a tarantula burrow was located, it was excavated to determine if a spider was present, the species of spider, and whether it had been paralyzed and had an egg or larva attached to its abdomen. The sex of spiders was also recorded. All parasitized spiders ( $n = 134$ ) were transported back

to the laboratory and maintained under constant darkness at  $22 \pm 0.2^\circ\text{C}$  and 62-65% RH in environmental chambers. Attached eggs and larvae were allowed to complete development, and after eclosion, parasitoids were identified to genus and species.

*Experimental design and behavioral testing.*—Chemical stimuli (treatments) were obtained from theraphosid spiders (*A. harlingenum* and *A. heterops*) (Araneae: Theraphosidae) as well as from the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Tenebrio molitor* is a pest of stored grains and is not likely to be encountered by these wasps. Both species of spiders were collected from the same areas inhabited by *P. formosa*. Pieces of absorbent filter paper (Whatman No. 612, Carolina Biological Supply Co., Burlington, North Carolina, USA) were conditioned with odors associated with these arthropods by placing paper on the floors of cages that housed spiders and beetles for a period of one week prior to testing.

The response of individual wasps exposed to these three different chemical stimuli was tested. For each trial, half of the floor of a rectangular plastic chamber (30 by 15 by 8 cm) was lined with a piece of paper that was moistened with dechlorinated tap water (control side). The other half (treatment side) was lined with paper that had been conditioned with chemosensory cues associated with *A. Harlingenum*, *A. heterops*, or *T. molitor*. The two pieces of paper were separated by a distance of 2 cm to minimize contamination of chemical stimuli between the two sides. After the pieces of paper were placed on each side of the chamber, they were sprayed with dechlorinated tap water to saturate the papers. This ensured that any differences in the response of wasps to treatment versus control papers could be attributed to chemical cues and not to moisture level. Walls of the chamber were sprayed with Fluon® (Central Scientific, Chicago, Illinois) to prevent wasps from climbing up on them. Observations were made through a one-way mirror to minimize disturbances to test subjects.

At the start of each trial, an individual female wasp was grasped gently with forceps and placed into the center of the chamber. At 30-min intervals, over a 3-hr period, the location of the test subject on the control or treatment paper was recorded. If a wasp was located at the

center of the floor, the position of the head and antennae was used to assign location. The chamber was rotated 180° every 30 min during testing to control for the possibility of any bias in the wasp's orientation in the chamber.

Thirty different wasps in each of the three treatments (90 tests) were tested. Individual wasps were used in only one test. For each trial, the number of times each wasp was located on the treatment side of the chamber out of a possible five observations (one per 30 min for 150 min = five observations) was summed. For each of the three treatments a comparison of whether wasps spent significantly more time than expected on a particular side of the chamber was compared using a Wilcoxon Signed Rank test (Sokal & Rohlf 1995). A similar behavioral bioassay for testing responses of animals to chemical stimuli has been used in studies on a variety of taxa including vertebrates (Chivers & Smith 1998) and other species of invertebrates (Kats & Dill 1998).

## RESULTS

The total number of males and females (spiders within burrows plus spiders wandering at the surface of the ground) of *A. harlingenum* was 252 and 185, respectively, as compared to 238 and 172, for *A. heterops*, indicating that these two theraphosids occur at similar densities at this study site. The number of burrows containing *A. harlingenum* (112) and *A. heterops* (103) was also similar. There was no significant difference between the mean body weights for males and females of *A. harlingenum* (Ms: 6.8g ± 0.8 SE; Fs: 9.8 ± 1.2g) and *A. heterops* (6.6 ± 0.4; 9.7 ± 0.5) (*t* test, *P* > 0.50).

With respect to wasps, *P. formosa* was more common than other pepsine wasps. From a total of 786 adult wasps collected during the course of this study, 532 (67.7%; 284 males, 248 females) consisted of *P. formosa*, 88 (11.1%; 48, 40) were *P. thisbe*, and 166 (21.2%; 89, 77) were *P. cerberus*.

All species of pepsine wasps associated with paralyzed host spiders found in the field are listed in Table 1. Out of 112 parasitized *A. harlingenum* found, 82 (73.2%) were females, as compared to 78 of

Table 1. Species of pepsine wasp larvae associated with tarantula (Theraphosidae) spider hosts in Hidalgo County, Texas. Data represent the number of each species of pepsine wasp found in a total of 215 spider burrows. Two species of theraphosids were found at this study site: *Aphonopelma harlingenum* and *A. heterops*. Paralyzed spiders were excavated from their burrows and the attached wasps eggs or larva allowed to complete development in the laboratory for subsequent identification of emergent adult wasps.

Pepsine wasp	Spider burrows	
	<i>Aphonopelma harlingenum</i>	<i>Aphonopelma heterops</i>
<i>Pepsis formosa</i>	93	10
<i>Pepsis thisbe</i>	11	61
<i>Pepsis cerberus</i>	8	32
Total	112	103

103 (75.7%) for *A. heterops* (Chi Square contingency test:  $X^2 = 57.43$ ,  $P < 0.001$ , and 45.09,  $P < 0.001$ , respectively).

All wasps found at this time of the year were in various larval stages. Of the 103 *P. formosa* larvae, 90.3% were associated with *A. harlingenum* as compared to only 9.7% for *A. heterops* (Table 1). The other two species of pepsine wasps also utilized *A. harlingenum* and *A. heterops* as hosts.

The responses of the 90 female wasps to papers containing various treatment odors are shown in Fig. 1. Wasps spent significantly more time on paper conditioned with the odors associated with *A. harlingenum*, as compared to paper containing olfactory stimuli from *A. heterops* ( $Z = 3.92$ ,  $P < 0.001$ ) or the beetle, *T. molitor* ( $Z = 3.88$ ,  $P < 0.001$ ). In fact, there was no significant difference in the way *P. formosa* responded to odors of *A. heterops* or the novel stimuli associated with the beetle, *T. molitor* ( $P > 0.60$ ).

When wasps were introduced into the test chamber they waved their antennae in the air, and also used them to tap the floor as they moved quickly over the floor of the chamber. When making contact with paper conditioned with odors from *A. harlingenum*, wasps typically exhibited a series of circular movements localized to a particular region of that paper, and their general level of activity increased. Eighty-one of the 90 wasps (90%) were also observed to periodically extrude their stinger, making contact with the floor of the

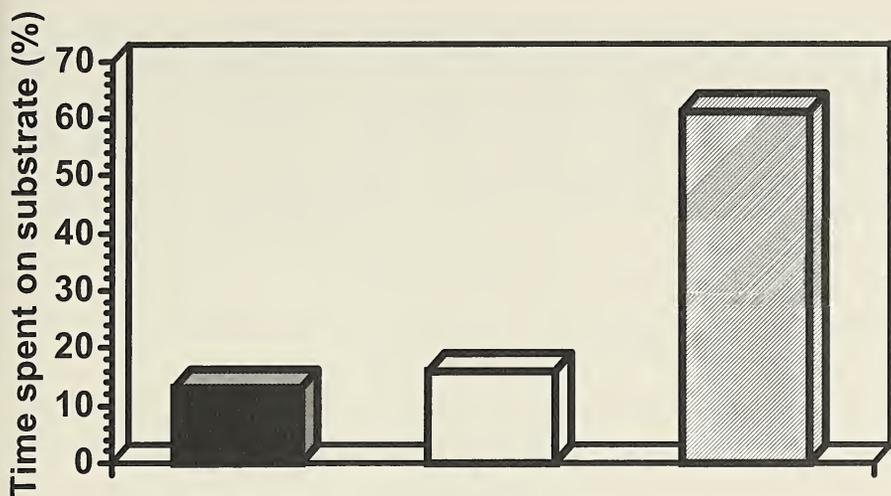


Figure 1. Percent of time adult females of *Pepsis formosa* spent on the treatment side of the test chamber ( $n = 90$ ). Treatment side contained paper conditioned with the odors associated with two species of theraphosid spiders (*Aphonopelma harlingenum*, unshaded bar, middle) and *A. heterops*, (stipled bar on the right), or the mealworm beetle (black bar, on the left), *Tenebrio molitor*.

chamber. In contrast, this behavior was exhibited by only 6 wasps (6.7%) when walking on paper conditioned with odors from *A. heterops* or *T. molitor*.

#### DISCUSSION

Results showed that *P. formosa* was the most common pepsine wasp at this location and that females chose *A. harlingenum* as a host far more frequently than *A. heterops*. Because these spiders were of similar size and present in similar numbers, this difference in host choice must be the result of specific cues associated with these spiders that are used by *P. formosa* in making decisions on host selection, perhaps including olfactory cues, rather than host size or availability. In addition, because the wasps used in these experiments were collected as adults, and the species of host upon which they completed their larval development unknown, no conclusions can be reached concerning the possible role that larval olfactory imprinting may have played in their subsequent choice of hosts as adults.

The majority of spiders paralyzed by *P. formosa* were females. Because of their larger body size, female spiders provide more food for developing wasp larvae. Larvae provided with larger hosts attain a larger adult body size (Lucas 1919; Field 1992). It is known that larger body size in pepsine wasps is correlated with a higher number of eggs produced per female (Evans & Eberhard 1970; Punzo 2000; 2005). However, it is not known whether female wasps reject male spiders in favor of females, choose males as hosts only when female spiders cannot be located, or are opportunistic hunters and select spiders as they are encountered in the field.

In contrast, other species of pepsine wasps that occur sympatrically with *P. formosa* at this location (*P. thisbe* and *P. cerberus*) selected *A. heterops* as a host more frequently than *A. harlingenum*. This type of resource partitioning may reduce competition between this guild of parasitoid wasps that all utilize theraphosid spiders as hosts. Further studies should be conducted to test this hypothesis.

Predators may use a variety of prey-associated cues to locate potential prey, including visual, mechanical, and chemical stimuli (Chapman et al. 1987). Results from this experiment show that wasps were able to detect olfactory cues associated with *A. harlingenum* and spent more time on substrates conditioned with odors associated with this spider than on those containing cues from *A. heterops* or *T. molitor*. This is in agreement with studies on other species of invertebrate predators responding to olfactory cues associated with their prey, including muricid snails (Carriker & Zandt 1972), tiger beetles (Wilson 1978), ants, (Hölldobler & Wilson 1994), and other species of solitary wasps (Evans & Eberhard 1970; Turlings et al. 1993). In the case of arachnids, field-collected lynx and wolf spiders showed a significant preference for substrates containing olfactory cues associated with field crickets, a common prey species (Punzo & Kukoyi 1997).

The ability to detect and respond to stimuli associated with suitable prey will reduce energy and time expended in random search. This should improve hunting success while minimizing the probability of encountering other predators known to capture and ingest pepsine wasps such as roadrunners, grasshopper mice, wolf spiders, solifugids,

and mantids (Punzo 1998; 2000), all of which are found at this study site. Further studies should be conducted on captive-bred wasps that have had no previous experience with theraphosid spiders to determine whether their ability to respond to host olfactory cues is primarily innate or dependent to some degree on adult learning or larval olfactory imprinting.

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DIETARY COMPOSITION OF THE MEXICAN SPADEFOOT TOAD  
(*SPEA MULTIPLICATA*) FROM A SAND DUNE HABITAT  
IN SOUTHWESTERN COAHUILA, MÉXICO

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**Abstract.**—Diet composition of the Mexican spadefoot toad has been poorly studied, especially in the southern part of this species' geographic range. This study reports the diet composition of a population inhabiting a sand dune system in southwestern Coahuila, México. The stomach contents of 43 specimens revealed 24 items, 22 were arthropods and two were vegetal material. Numerically, ants comprized the greater percentage of the stomach contents (49.7%) followed by homopterans (16.7%) and hemipterans (12.8%). Volumetrically, beetles were the most important item (42.4%). There was a linear relationship between SVL and stomach volume, SVL and snout length, and snout length and length of one prey group (Hemiptera). In comparison with northern populations which feed primarily on termites, it is possible that toads in this population will need more than seven feedings to survive for one year.

**Resumen.**—La composición de la dieta del sapo *Spea multiplicata* a sido escasamente estudiada, especialmente en el rango de su distribución más sureño. Este estudio muestra la dieta de una población de *S. multiplicata* en un sistema de dunas en el suroeste de Coahuila, México. Se encontraron relaciones significativas entre la LHC y el contenido volumétrico estomacal, la LHC y la longitud del hocico y la longitud del hocico y la longitud de los hemípteros. El contenido estomacal de 43 sapos mostró 24 categorías, de las cuales, 22 fueron de artrópodos y dos de vegetales. Numéricamente, las hormigas mostraron el mayor porcentaje del contenido estomacal (49.7%) seguido por los homópteros (16.7%) y los hemípteros (12.8%). Volumétricamente, los escarabajos fueron los más importantes (42.4%). En comparación con las poblaciones norteñas, las cuales se alimentan básicamente de termitas, es posible que la población estudiada requiera de más de siete alimentaciones para sobrevivir un año.

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Although moisture conditions of North American deserts are not favorable for a wide diversity of amphibians as are tropical regions, there are many species particularly adapted to tolerate droughts and the high temperatures of the arid environments (Bentley 1966;

Mayhew 1968; Rubial et al. 1969). Due to the high permeability of the skin and to the susceptibility to desiccation under arid conditions, the annual activity period of the amphibians like *Spea multiplicata* (formerly *Scaphiopus multiplicatus*) depends upon the availability of moisture; consequently, the daily activity tends to be concentrated at night (Degenhardt et al. 1996). This toad exhibits a generalist diet, however the composition varies geographically (Dimmitt & Rubial 1980; Punzo 1991; Degenhardt et al. 1996). The groups most consumed by this amphibian are termites, beetles, orthopterans, ants, and spiders (Dimmitt & Rubial 1980; Punzo 1991; Degenhardt et al. 1996). Likewise, Dimmitt & Rubial (1980) suggest that *S. multiplicata* require at least seven feedings to accumulate sufficient amounts of fat to survive for 12 months. However, because the diet composition of this toad varies among localities and because this species inhabits many habitat types, the number of feedings required to survive one year could change geographically depending on the availability of food resources and the major groups consumed (Degenhardt et al. 1996).

There are few studies concerning food patterns on desert toads; particularly for *S. multiplicata*. Populations in Texas represent the southernmost location where diet composition of this toad has been studied (Anderson et al. 1999; Whitaker et al., 1977; Punzo 1991), and dietary patterns of Mexican populations of *S. multiplicata* are unknown. Furthermore, it is important to know how the sand dune vegetation can influence diet composition of this toad in comparison with other populations. This study provides the diet composition of a population of the Mexican spadefoot toad in a sand dune habitat in southwestern Coahuila, Mexico.

#### STUDY SITE

The climate of the sand dunes in southwestern Coahuila is dry and very warm. Mean annual precipitation vary between 200 to 300 mm, occurring primarily during July to September. Mean annual temperature is about 22°C. December and January are the

coolest months, and July and August the warmest (García 1981). The collecting site is near the lowest part of the Aguanaval River which has water only in rainy years (INEGI 1988).

Mean altitude of the area is 1100 m. Vegetation is xerophitic (Redowsky 1978) with a greater abundance of creosote bush (*Larrea tridentata*) and desert seepweed (*Suaeda nigrescens*) and lesser abundance of mesquite (*Prosopis glandulosa*) and Christmas cactus (*Cylindropuntia leptocaulis*). The soil is basically SiO<sub>2</sub> sand forming dunes of varying elevation.

#### METHODS AND MATERIALS

Stomach contents of 43 spadefoot toads (*Spea multiplicata*) from the herpetological collection of the Facultad de Ciencias Biológicas of the Universidad Autónoma de Nuevo León, México were analyzed. The specimens were collected in November, 2003 in the sand dunes region of Viesca, Coahuila.

During November 2003 a large number of spadefoot toads were observed in the sand dune system of Viesca, Coahuila. Although this toad is basically nocturnal (Degenhardt et al. 1996), the toads at Viesca were active during the morning before 09:00 hrs (at an air temperature lower than 23.8°C and a relative humidity of 43.3%). However, by afternoon activity had ceased. By sunset (after 19:00 hrs) a few isolated active toads were seen, when the air temperature had dropped to 22.0°C and the humidity increased to 36.5%. Active toads were seen under different kind of plants, however, conglomerated groups (by the morning) were frequently observed in depressions shaded partially by grasses with damp substrates.

Toads were preserved in alcohol (70%) three hours after been collected and sacrificed by cooling. Snout-vent length (SVL) and snout length (SL) of each toad were measured with a caliper to the nearest 0.01 mm. A Pearson's regression analysis was applied to determine any relationship between these two variables and

between SVL and the stomach volume. Stomach contents ( $S$  = number of stomachs containing item  $i$ , where  $i$  = prey species) were examined under a stereomicroscope. Individual prey items ( $n$  = number of prey items) were identified and percentage of stomachs with that item  $i$  ( $S\%$ ) was calculated. The volume of food items ( $V$ ) of each taxonomic category in a single stomach was estimated by using the length and width (0.01 mm) of intact preys with caliper. Prey volume was calculated as an ellipsoid ( $V = 4/3 \pi (w/2)^2 (l/2)$ ), where  $w$  is prey width and  $l$  is prey length (Punzo 1991; Oliveira-Mesquita & Rinaldi-Colli 2003).

The sum of the above three relative abundance measures (in percentage) for item  $i$  was used to estimate the importance value (Acosta 1982; Oliveira-Mesquita & Rinaldi-Colli 2003).

Niche breadth for pooled stomachs was determined from numeric and volumetric percentages of preys using the Shannon Index ( $H' = -\sum p_i \log^2 p_i$ ) (Pianka 1973; Oliveira-Mesquita & Rinaldi-Colli 2003). The Shannon Index was preferred instead of the Simpson index ( $D$ ) commonly used in lizard studies (Pianka 1973) because the former is less sensitive to the frequency of dominant prey items, and unlike  $D$ , the casual ingestion of a prey item by an opportunistic predator does not disturb  $H'$  (May 1975).

For the main prey items identified in the stomach contents, the relationship between toad SVL and the largest body length of the prey were examined using Pearson's regression analysis (Gadsden & Palacios-Orona 1997; Ramírez-Bautista & Lemos-Espinal 2004). Statistical analyses were performed with SPSS Ver. 10 considering a  $P < 0.05$  to assess statistical significance. Values are showed as mean  $\pm 1SE$ .

Specimens examined are deposited in the Herpetological Collection of the Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas. UANL-6696-6738.

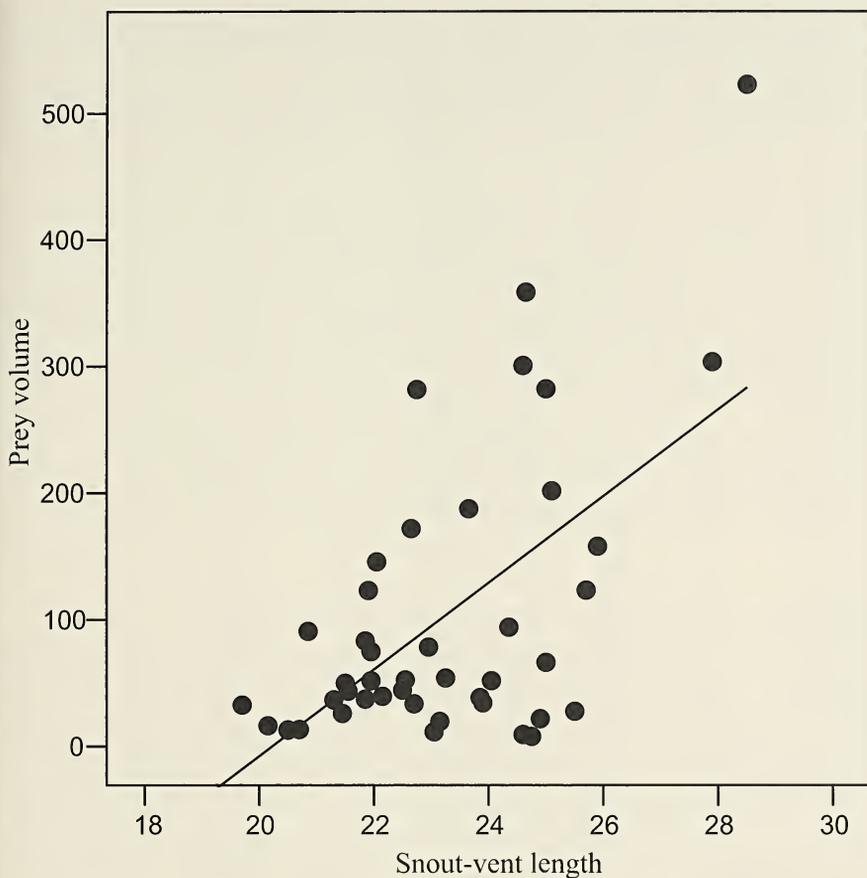


Fig. 1. Relationship between snout-vent length and prey volume of *Spea multiplicata* from the sand dunes of Viesca of southwestern Coahuila México.

## RESULTS

The mean SVL of the toads was  $23.22 \pm 0.29$  mm, and the mean snout length was  $7.82 \pm 0.11$  mm, both variables were related significantly ( $R = 0.76$ ;  $F_{1,41} = 56.51$ ;  $P = 0.000$ ). The mean stomach volume was  $102.67 \pm 113.46$  mm<sup>3</sup> and there was a significant linear relationship between SVL and the prey volume ( $R = 0.58$ ;  $F_{1,41} = 21.42$ ;  $P = 0.000$ ) (Fig. 1).

Table 1. Contents of the stomachs of *Spea multiplicata* ( $n = 43$ ). S = number of stomachs containing item  $i$ ; S% = percentage of stomachs with item  $i$ ;  $n$  = number of prey items; N% = percentage of items in total sample; V = volume in  $\text{mm}^3$ ; V% = percentage of total volume represented; VI = importance value and VI/3 = standardized importance value. Formicidae is excluded from the order Hymenoptera.

Item	S	S %	N	N %	V	V %	VI	VI/3
Acari	12	4.13	18	0.78	3.81	0.08	4.99	1.66
Araneae	11	3.79	22	0.96	42.28	0.97	5.72	1.9
Chilopoda	1	0.34	1	0.04	1.7	0.03	0.41	0.13
Coleoptera	33	11.37	113	4.94	1834.75	42.49	58.8	19.6
Coleoptera larvae	4	1.37	6	0.26	6.75	0.15	1.78	0.59
Collembola	11	3.79	28	1.22	10.09	0.23	5.24	1.74
Diptera	9	3.1	14	0.62	17.18	0.37	4.09	1.36
Diptera larvae	12	4.13	21	0.91	11.36	0.26	5.3	1.76
Formicidae	43	14.82	1137	49.75	256.22	5.93	70.5	23.5
Hemiptera	35	12.06	294	12.86	346.63	8.21	33.13	11.04
Homoptera	37	12.75	382	16.71	483.42	11.19	40.65	13.55
Hymenoptera	6	2.06	6	0.26	29.57	0.68	3	1
Isoptera	9	3.1	72	3.15	14.93	0.34	6.59	2.19
Lepidoptera	10	3.44	12	0.52	29.03	0.67	4.63	1.54
Lepidoptera larvae	23	7.93	47	2.05	1157.56	26.81	36.79	12.26
Neuroptera larvae	4	1.37	6	0.26	7.82	0.18	1.81	0.6
Plants	3	1.03	3	0.13	19.1	0.44	1.6	0.53
Pseudoscorpionida	3	1.03	5	0.21	2.77	0.06	1.3	0.43
Psocoptera	1	0.34	6	0.26	1.8	0.04	0.64	0.21
Scorpionida	2	0.68	2	0.08	7.08	0.16	0.92	0.3
Seeds	15	5.17	84	3.67	20.82	0.48	9.32	3.1
Solifuga	2	0.68	2	0.08	8.47	0.19	0.95	0.31
Thysanoptera	3	1.03	3	0.13	0.65	0.01	1.17	0.39
Tysanura	1	0.34	1	0.04	1.01	0.02	0.4	0.13
Total	290	99.85	2285	99.89	4314.8	99.99	299.88	99.82

Snout length was related only with hemipteran length ( $R = 0.30$ ;  $F_{1,73} = 7.47$ ;  $P = 0.008$ ), and not with ant ( $R = 0.041$ ;  $F_{1,284} = 0.47$ ;  $P = 0.492$ ), beetle ( $R = 0.136$ ;  $F_{1,76} = 1.43$ ;  $P = 0.235$ ), homopteran

( $R = 0.058$ ;  $F_{1,171} = 0.57$ ;  $P = 0.451$ ) or larval lepidopteran lengths ( $R = 0.85$ ;  $F_{1,44} = 0.32$ ;  $P = 0.572$ ). Twenty-four items consumed by *S. multiplicata* were identified; of which 22 were arthropods (four in larval stages) and two were vegetal material (Table 1).

Numerically, the family Formicidae (separate from Hymenopteran *per se*) represents the greatest percentage of the stomach contents (49.7%) followed by the order Homoptera (16.7 %) and Hemiptera (12.8%). Volumetrically, coleopterans were the most important item (42.4%) followed by lepidoptera larvae (26.8%) and homopterans (11.1%). On the other hand, the greater importance values associated with the five orders stated above (Table 1), indicate that these are the insect groups widely consumed by *S. multiplicata* in the southwestern region of the sand dune habitat in Coahuila.

The low amount of vegetal material found in the stomachs suggests that plants are not an important component in the diet of this toad (Whitaker et al. 1977), and may have been consumed inadvertently while capturing animal prey items (Table 1). The Shannon-Index for the numeric data was  $H' = 2.48$  with an evenness of  $E_H = 0.78$ . The index for the volumetric data was  $H' = 2.35$  with an evenness of  $E_H = 0.73$ . The SVL has a significant relationship with the prey length for prey of the order Hemiptera, but SVL was not correlated with prey length in the other groups of insects (Table 2).

## DISCUSSION

Dimmitt & Rubial (1980) indicate that *S. multiplicata* in San Simon Valley Arizona, feeds basically on termites and beetles. Conversely, Punzo (1991) showed that *S. multiplicata* fed on beetles, orthopterans, ants, spiders, and termites (comprising 93.8% of its diet) in western Texas. This current study shows that *S. multiplicata* in southwestern of Coahuila feeds on ants, homopterans, and hemipterans as the numerically most common food items, but that volumetrically, beetles represented more than 40%

Table 2. Relationship between the snout-vent length of *Spea multiplicata* and the length of the main insect prey items.

	<i>R</i>	<i>r</i> <sup>2</sup>	<i>F</i>	<i>df</i>	<i>P</i>
SVL vs Hemiptera	0.32	0.1	8.81	1, 73	0.004
SVL vs Formicidae	0.05	0.003	0.84	1, 284	0.35
SVL vs Coleoptera	0.20	0.04	3.19	1, 76	0.69
SVL vs Homoptera	0.03	0.001	0.15	1, 171	0.69
SVL vs Lepidoptera larvae	0.17	0.02	1.30	1, 44	0.26

of the stomach content followed by larvae of lepidopterans and homopterans. These results coincided with the observed by Anderson et al. (1999), where a population of *S. multiplicata* (from southern high plains of Texas) feeds on beetles of the Carabidae family. These diet preferences probably reflect that in sand dune systems, homopterans, lepidopterans and coleopterans are uncommon among the perennial plants during drought periods, but these kinds of arthropods are particularly abundant in shaded areas with grass after the rains and when water ponds begin to dry up.

Dimmitt & Rubial (1980) suggest that the Arizona population requires seven feedings to accumulate the necessary fat to survive 12 months considering a diet made up mainly of beetles and alate termites. However, termites are more digestible and have higher lipid and caloric contents than most of the insects consumed by this amphibian (Fast 1964). If it is considered that the population of *S. multiplicata* studied here feeds on less nourishing insect species (mainly ants, homopterans and hemipterans) than termites, the number of feedings they require to survive one year could be greater than the San Simon Valley population. However, body size of this population was less than that estimated by Dimmitt & Rubial (1980) and K. Pfennig (pers. comm.) for populations of this toad in Arizona. This could also compensate and influence the number of total feedings to survive one year.

Brown (1976) found that the western population of *S. multiplicata* has a mean SVL of 46.9 mm and the Arizona's population reaches 40 mm (K. Pfennig, pers. comm.). The studied population has the smallest size (23 mm) which probably was represented by young adults. However, although this population was represented by adults, it is possible that size and collection date of toads may have an influence on diet composition. Likewise, the insects more commonly eaten by this population could reflect environmental arthropod availability and not a preference for the more nutritious orders. Dietary breadth of the Mexican population (24 items consumed) was similar to the population of Texas (20 items) studied by Anderson et al. (1999). These also suggest that food availability in dune systems could be similar to the southern high plains of Texas, or that diet preferences of this toad can be comparable in a wide geographic range. Differences in diet composition of spadefoot toads among the populations studied could be related to dissimilarity in habitat composition, habitat use, size and collection season.

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REPRODUCTIVE CYCLE OF THE SPOTTED SAND LIZARD,  
*PEDIOPLANIS LINEOCELLATA* (SQUAMATA: LACERTIDAE)  
FROM SOUTHERN AFRICA

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**Abstract.**—The reproductive cycle of the spotted sand lizard, *Pedioplanis lineocellata*, is described from histological examination of gonadal material. Reproduction is seasonal and mainly confined to summer. Males have a testicular cycle in which spermiogenesis occurred primarily in December-February. Females with enlarged ovarian follicles (> 4 mm length) occurred January-February and October. The presence of females with corpora lutea from a previous clutch and yolk deposition for a subsequent clutch suggests more than one clutch can be produced in the same reproductive season. Mean clutch size for 22 females with enlarged ovarian follicles was  $6.8 \pm 2.0$  SD, range: 2-11. Two eggs is a new minimum clutch size and 11 is a new maximum clutch size for *P. lineocellata*. The smallest reproductively active male measured 42 mm SVL and the smallest reproductively active female measured 50 mm SVL.

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The spotted sand lizard, *Pedioplanis lineocellata*, occurs in the western half of the African subcontinent including parts of Namibia, Botswana and Republic of South Africa, but is absent from regions of deep sand like the Namib and central Kalahari deserts (Branch 1998). It occurs in varied habitats including karroid veld, mesic thicket and arid and mesic savannah (Branch 1998); it is a sit and wait predator (Pianka 1971). Anecdotal information on its reproduction appeared in Fitzsimons (1943); Pianka (1971; 1986); De Waal (1978); Auerbach (1985); Baard (1987); Rogner (1997) and Branch (1998). The purpose of this paper is to present additional information on the reproductive cycle of *P. lineocellata* from a histological examination of gonadal material from museum specimens. Information on the reproductive cycle of an organism is needed to understand the evolution of life-history strategies. Such data are also useful in formulating conservation policies.

## MATERIALS AND METHODS

One-hundred twelve female (mean snout-vent length, SVL = 56.0 mm  $\pm$  3.1 *SD*, range = 48-63 mm) and 167 male (mean SVL = 57.2 mm  $\pm$  4.5 *SD*, range = 42-67 mm) *P. lineocellata* were examined from the herpetology collection of the Natural History Museum of Los Angeles County, Los Angeles, CA. Lizards were collected during 1969-1970. The left testis and epididymides were removed from males and the left ovary was removed from females for histological examination. Enlarged follicles (> 4 mm length) were counted but not examined histologically. Oviductal eggs were previously removed for an ecological study (Pianka 1986). Tissues were embedded in paraffin, sectioned at 5  $\mu$ m and stained with Harris' hematoxylin followed by eosin counterstain. Testes slides were examined to determine the stage of the spermatogenic cycle and epididymides were examined for the presence of sperm. Ovary slides were examined for the presence of yolk deposition or corpora lutea. The relationship between body size (snout vent length, SVL) and clutch size was examined by linear regression analysis and male and female *P. lineocellata* mean body sizes and mean egg clutch sizes from Botswana and the Republic of South Africa were compared with unpaired *t* tests using InStat (vers. 3.0b, Graphpad Software, San Diego, CA).

*Material examined.*—Specimens of *Pedioplanis lineocellata* from the Republic of South Africa (Northern Cape Province) and Botswana (Kgalagadi Province) examined from the herpetology collection of the Natural History Museum of Los Angeles County, Los Angeles (LACM).

## REPUBLIC OF SOUTH AFRICA

31 km N, 100 km E Upington (28°13'S, 22°16'E). LACM 79158, 79159, 79160, 79161, 79164, 79166, 79170-79173, 79178, 79179, 79184, 79187, 79190, 79192-79194, 79196, 79200, 79207, 79210, 79211, 79231, 79234, 79256, 79297, 79302, 79305, 79314-79316, 79321.

120 km N, 54 km W Upington (27°22'S, 20°43'E). 78740-78742, 78745-78749, 78752-78771, 78773-78777, 78779, 78780, 78782, 78784-78788, 78790-78792, 78794-78797, 78799, 78801-78803, 78809, 78810, 78820, 78874, 78904, 79019.

24 km N, 53 km E. Upington (28°17'S, 22°05'E). 84085, 84086.

Kalahari-Gemsbok National Park (25°45'S, 20°44'E). 138978-138980, 138983.

29 km S, 40 km E Rietfontein (27°00'S, 20°27'E). 78923, 78924, 78926-78949, 78951, 78952, 78954-78958, 78960-78965, 78968-78970, 78972, 78973, 78974, 78975, 78977, 78978, 78979, 78980, 78981, 78982, 78985, 79428.

Kalahari-Gemsbok National Park, 1 km W Kameel Sleep (23°45'S, 20°44'E). 79324-79328, 79330-79333, 79335, 79337, 79342, 79344-79354, 79356, 79358, 79369.

121 km N, 16 km E Upington (27°22'S, 21°25'E). 79446-79450, 79459, 79597.

129 km N, 65 km W Upington (27°17'S, 21°54'E). 79020-79024, 79026, 79027, 79029-79034, 79036-79040, 79042-79044, 79047, 79049, 79050, 79055-79057, 79059, 79060, 79062, 79064, 79066, 79067, 79069, 79079, 79084-79086, 79135, 79138, 79139, 79142, 79146, 79147.

## BOTSWANA

11 km S. Tsabong (26°08'S, 22°28'E). 79712-79714, 79718-79722, 79725-79738, 79740, 79741, 79743-79750, 79755, 79756, 79759, 79761, 79763-79766, 79768-79770, 79773, 79774, 79816, 79817, 79829, 79834, 79845, 79846, 79848, 79851, 79852.

## RESULTS

The mean of the male *P. lineoocellata* sample was significantly larger than that of the female sample ( $t = 2.44$ ,  $df = 277$ ,  $P = 0.0151$ ). Seasonal changes in the testicular cycle are presented in

Table 1. In the regressed testis, the germinal epithelium is exhausted and the predominant cells are Sertoli cells and spermatogonia. In testes undergoing recrudescence there is a renewal of the germinal epithelium for the next period of spermiogenesis. Primary and secondary spermatocytes are the predominant cells; some spermatids, but no spermatozoa may be present. During spermiogenesis the seminiferous tubules are lined by clusters of spermatozoa and metamorphosing spermatids and the epididymides are packed with sperm.

The main period of spermiogenesis (sperm formation) occurs from December-February (summer) (Table 1). During this time 123/126 (98%) of males were undergoing spermiogenesis and sperm was present in the epididymides. Males with regressed testis were present in February-April and September. Testes in recrudescence (recovery) were present in September-November (spring). The smallest reproductively active male (spermiogenesis in progress) was from September. It measured 42 mm SVL (LACM 79816). Another male measuring 43 mm SVL from October was also undergoing spermiogenesis.

Data on the seasonal ovarian cycle is presented in Table 2. The primary period of ovarian activity occurred during summer (January-February) when 82/97 (85%) females were reproductively active (i.e., early yolk deposition, enlarged follicles or corpora lutea). The period of ovarian activity apparently occurs from October-February as one female with enlarged follicles (> 4 mm length) was collected in October (Table 2). The presence of early yolk deposition for a subsequent clutch and corpora lutea from a previous clutch in the same female suggests that female *P. lineoocellata* may produce two clutches in a reproductive season. The smallest reproductively active female (corpora lutea present) measured 50 mm SVL (LACM 79234). Mean clutch size for 22 sets of enlarging ovarian follicles (> 4 mm diameter) from *P. lineoocellata* females from South Africa and Botswana was  $6.8 \pm$

Table 1. Monthly distribution of reproductive conditions in seasonal testicular cycle of 167 *Pedioplanis lineoocellata*. Values are the numbers of males exhibiting each of the three conditions.

Month	<i>n</i>	Regressed	Recrudescence	Spermiogenesis
January	64	0	0	64
February	42	3	0	39
March	2	1	0	1
April	5	5	0	5
September	6	1	4	1
October	19	0	10	9
November	9	0	5	4
December	20	0	0	20

Table 2. Monthly distribution of reproductive conditions in seasonal ovarian cycle of 112 *Pedioplanis lineoocellata*. Values shown are the numbers of females exhibiting each of the four conditions.

Month	<i>n</i>	Inactive	Early yolk deposition	Enlarged follicles (>4 mm length)	Corpora lutea	Corpora lutea yolk deposition
January	50	9	28	10	3	0
February	47	6	25	11	3	2
April	1	1	0	0	0	0
October	3	2	0	1	0	0
November	4	4	0	0	0	0
December	7	5	2	0	0	0

2.0 *SD*, range 2-11 (Table 3). There was no statistical difference between clutch sizes from these two areas ( $t = 0.010$ ,  $df = 20$ ,  $P = 0.99$ ). The clutch size of two from LACM 79726 collected in January represents a new minimum clutch size for *P. lineoocellata*. The clutch size of 11 (LACM 78975) from February is a new maximum clutch size for *P. lineoocellata*. Linear regression analysis for 22 gravid females revealed that the relation between body size (SVL) and clutch size was not significant ( $r = 0.36$ ,  $P = 0.100$ ). Clutch sizes are presented in Table 3.

Table 3. Clutch sizes for 22 *Pedioplanis lineocellata* estimated from counts of enlarged follicles > 4 mm length from southern Africa; Kgalagadi Province (Botswana), Northern Cape Province (Republic of South Africa).

Month	SVL (mm)	Clutch size	Province	LACM #
January	55	4	N. Cape	79170
January	52	4	N. Cape	79184
January	60	7	N. Cape	79022
January	58	7	N. Cape	79030
January	59	8	Kgalagadi	79736
January	57	9	Kgalagadi	79730
January	58	7	Kgalagadi	79735
January	54	2	Kgalagadi	79726
January	59	7	Kgalagadi	79765
January	52	9	Kgalagadi	79738
February	59	7	N. Cape	79447
February	58	7	N. Cape	78802
February	60	9	N. Cape	78981
February	58	7	N. Cape	78973
February	55	5	N. Cape	78982
February	58	11	N. Cape	78975
February	60	8	N. Cape	78965
February	58	7	N. Cape	78961
February	58	6	Kgalagadi	79774
February	59	7	Kgalagadi	79765
February	63	6	Kgalagadi	79747
October	58	5	N. Cape	84086

## DISCUSSION

*Pedioplanis lineocellata* males have a seasonal testicular cycle in which spermiogenesis occurs primarily during summer. Recrudescence (recovery and renewal of germinal epithelium) occurs in spring. The ovarian cycle of *P. lineocellata* was also seasonal with ovarian activity occurring in October-February (spring-summer). More than one egg clutch may be produced in the same reproductive season. Nkosi et al. (2004) reported that reproduction in the congener *Pedioplanis burchelli* similarly occurred in spring-summer and that some females may produce two clutches per reproductive season. As with *P. lineocellata*, clutch

size was not correlated with snout-vent length. Similarly, reproduction in *Pedioplanis namaquensis* occurred mainly in summer, clutch size was also not correlated with snout-vent length and multiple egg clutches were possible (Goldberg 2006).

Baard (1987) reported that mating in captivity for *P. lineocellata* occurred in October. Attempts to deposit eggs were made in November which concurs with that reported by Rogner (1997) and Branch (1998) that females lay 4-8 eggs in that month; De Waal (1978) reported 4-8 eggs being deposited in February. Presumably eggs may be deposited from November-February. Fitzsimons (1943) reported that *P. lineocellata* laid about six eggs in early summer; Auerbach (1985) reported about six eggs are laid. Pianka (1986) reported an average clutch size ( $n = 123$ ) of  $6.9 \pm 2.0$  *SD* eggs which compares to the value presented herein ( $6.8 \pm 2.0$  *SD*).

The lacertid lizard, *Meroles cuneirostris* from Namibia, exhibited a testis cycle similar to *P. lineocellata* wherein spermiogenesis occurred in spring-summer followed by autumn regression (Goldberg & Robinson 1979). Females of *M. cuneirostris* contained oviductal eggs over a five month period (September-March) and may produce two clutches per year (Goldberg & Robinson 1979). In contrast, male *Meroles anchietae* exhibited continuous spermiogenesis throughout the year, while females were capable of year-round reproductive activity (Goldberg & Robinson 1979). This continuous reproductive activity may be the result of *M. anchietae* eating seeds as well as insects thus having access to a continuous supply of food (Goldberg & Robinson 1979). Further histological examination of seasonal gonad samples from other lacertid species from southern Africa would be required to ascertain variations in reproductive cycles of these species.

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ECTOPARASITES OF BURROWING OWLS  
(*ATHENE CUNICULARIA HYPUGAEA*)  
WINTERING IN SOUTHERN TEXAS

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**Abstract.**—Fifteen Western Burrowing Owls (*Athene cunicularia hypugaea*) were captured over two winters (2001-2003) in southern Texas and examined for ectoparasites. Four of the 15 owls (27%) harbored feather lice, and the maximum number of lice found on any individual was  $\leq$  three. Two species of feather lice were found: *Colpocephalum pectinatum* occurred on three of the owls, and *Strigiphilus speotyti* was found on four owls. No fleas or other ectoparasites were found on any of the Burrowing Owls. The low diversity and numbers of ectoparasites suggest that ectoparasites are not threatening the health of wintering Burrowing Owls in southern Texas.

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Little is known about the winter ecology of the Western Burrowing Owl (*Athene cunicularia hypugaea*), a declining subspecies in North America (Wellicome & Holroyd 2001; Klute et al. 2003). The Western Burrowing Owl is mostly migratory, breeding in the south-central Canadian prairies and in desert or prairie habitats of the western United States and north-central Mexico. The winter range includes parts of the southwestern United States (including southern Texas) and Mexico. Loss of habitat, especially the conversion of grassland for agricultural production, has contributed to the decline of this subspecies (Klute et al. 2003). The Western Burrowing Owl is endangered in Canada, threatened in Mexico, and is considered to be a Bird of Conservation Concern in the United States by the U. S. Fish and Wildlife Service (Klute et al. 2003).

Burrowing Owls nest in abandoned burrows of prairie dogs (*Cynomys* sp.), ground squirrels (*Spermophilus* sp.), land tortoises (*Gopherus* sp.), foxes (*Vulpes* and *Urocyon* sp.), badgers (*Taxidea taxus*), skunks (*Mephitis*, *Spilogale*, and *Conepatus* sp.), and armadillos (*Dasypus novemcinctus*) (Fisher 1974), or they may nest in other natural (e.g., rock crevices) (Rich 1984) or artificial burrows (Smith & Belthoff 2001a). Parasites associated with mammals inhabiting the burrows have received attention due to the risk of sylvatic plague (from infection by the bacterium *Yersinia pestis*) carried by

fleas (Tyler & Buscher 1975). In much of southern Texas, however, mammal burrows are scarce due to widespread cultivation. Many wintering Burrowing Owls roost at road culverts or in a variety of other manmade or natural cavities. Of 46 Burrowing Owl roost sites in southern Texas, only 11% were classified as natural burrows (Williford et al. 2006).

Arthropods, especially various species of mites, are common in raptor nests (Philips & Dindal 1977). Heavy infestations of avian ectoparasites can produce severe reactions in their hosts, sometimes even causing death. Ectoparasites have been identified on adult and nestling Burrowing Owls during the breeding season (Hubbard 1968; Thomsen 1971; Tyler & Buscher 1975; Philips & Dindal 1977; Clayton 1990; Baird & Saunders 1992; Smith & Belthoff 2001b), however, nothing is known about the occurrence of ectoparasites on Burrowing Owls wintering in southern Texas. The objectives of this study were to identify and determine the relative abundance of ectoparasites present on Burrowing Owls wintering in southern Texas.

#### MATERIALS AND METHODS

Fifteen Burrowing Owls were captured with noose traps at their roost sites in Nueces, Jim Wells, and San Patricio counties, in southern Texas, during the winters of 2001–2002 and 2002–2003. Of the 15 owls captured, 13 were roosting at road culverts in agricultural areas. The remaining two owls were roosting in grassland habitats at structures other than culverts (an artificial burrow and an eroded space under a concrete ledge). One owl was captured twice, once during each of the two winters of the study.

Each captured owl was examined for visible ectoparasites by searching approximately 2 cm<sup>2</sup> of skin and all parts of feathers (within the 2 cm<sup>2</sup>) in each of the following regions frequently known to harbor parasites: upper leg, crown, under-wing, and vent. Occasionally, an ectoparasite was found crawling on the hands of the examiner while the owl was being searched. Parasites were preserved and stored in 70% ethanol for later identification.

Parasites were examined using compound and dissecting microscopes with bright-field illumination. To aid identification, digital images were also captured with Miotis Images software (2000: Version 1.3), using a National digital camera mounted to a dissecting

compound microscope. Photographs were sent to experts on Burrowing Owl ectoparasites for identification.

## RESULTS

Ectoparasites were found on four (27%) of the 15 Burrowing Owls. Two of the three genera of chewing feather lice (ITIS 2004) known to parasitize owls (Clayton 1990) were found on Burrowing Owls wintering in southern Texas. A total of eight ectoparasites representing two species of feather lice were found on the Burrowing Owls. Four *Colpocephalum pectinatum* (Order: Phthiraptera, Suborder: Amblycera, Family: Menoponidae) were collected from three owls, and four *Strigiphilus speotyti* (Order: Phthiraptera, Suborder: Ischnocera, Family: Philopteridae) were found on three owls. Both species were found on two Burrowing Owls. Of the four owls with lice, the maximum number of lice found per bird was three. The owl that was captured twice (each of the two winters) had the same number (one) and species (*S. speotyti*) of louse found upon each capture. No fleas, ticks, mites, or wingless dipterans were found on any of the Burrowing Owls captured.

## DISCUSSION

The captured owls were, at most, only lightly infected with lice. It is unknown how these results compare to numbers of lice on museum specimens. However, results from this study are similar to at least two other reports. Smith & Belthoff (2001b) collected eight *S. speotyti* from 11 adults and four broods of Burrowing Owls in southwestern Idaho. Thomsen (1971) stated that some of the Burrowing Owls examined in California carried "a few" *C. pectinatum* lice. Based on Smith's (1999) classification, all of the Burrowing Owls captured in southern Texas had an infestation level of "low" (five or fewer individual ectoparasites). In contrast, over 40 lice were found on a single Burrowing Owl in southwestern Idaho (Smith 1999). This infestation may have caused reproductive failure of this particular owl.

Population-level effects of ectoparasites on Burrowing Owls have not been established. Nesting Burrowing Owls may be more susceptible to ectoparasites than wintering Burrowing Owls. Underground burrows create a favorable environment for ectoparasites, because burrows maintain moderate temperatures and high humidity

(Kennerly 1964). Eveleigh & Threlfall (1976) suggested that birds nesting in colonies are more susceptible to infestation due to frequent contact between individual birds. Although not considered a colonial nesting species, Burrowing Owl use of prairie dog burrows creates a loose “colony” by bringing nesting pairs and broods of Burrowing Owls into close proximity to one another. Dependence upon mammal burrows for nests also makes Burrowing Owls vulnerable to fleas (James & Harwood 1969), since fleas can transfer to owls from burrowing rodents (Philips & Dindal 1977). Burrowing Owl nests may harbor at least 39 different arthropod species, a minimum of 15 of which are fleas (Philips & Dindal 1977). Indeed, of six published studies on Burrowing Owl ectoparasites, at least four found lice, and all but one (Tyler & Buscher 1975) found fleas (Hubbard 1968; Thomsen 1971; Philips & Dindal 1977; Baird & Saunders 1992; Smith & Belthoff 2001b). In Idaho, 143 fleas were collected from 11 adult Burrowing Owls and four broods, whereas only eight lice were collected from the same owls (Smith & Belthoff 2001b). Although fleas were much more common on breeding owls in Idaho, only a sample of ectoparasites were collected from each owl captured, so the total number of fleas and lice on owls in Idaho actually was higher than indicated (B. W. Smith, pers. comm.).

Perhaps the most significant finding in this study is that no fleas were found on any of the 15 owls examined from southern Texas. Although temperatures below 18°C are considered less favorable for egg-laying among many flea species (James & Harwood 1969), the weather in southern Texas often exceeds 18°C during winter. The lower temperature limit for development (egg to adult) of the cat flea (*Ctenocephalides felis*) is 13°C, with an upper relative humidity limit of 92% (Silverman et al. 1981). Adult *Pulex irritans*, a flea often found on breeding Burrowing Owls (Thomsen 1971; Philips & Dindal 1977; Baird & Saunders 1992; Smith & Belthoff 2001b) and other burrowing animals (Baird & Saunders 1992) can survive for 125 days with no food at 7-10°C (James & Harwood 1969). In Corpus Christi, the mean winter temperature (December - January) during this study was 14.2°C with relative humidity ranging from 67%-87% (National Weather Service 2002-2003). Both temperature and relative humidity measures during this study were within the limits for the development of at least these two species of fleas.

The absence of fleas on captured burrowing owls may be attributable to the fact that 13 of the 15 owls captured (87%) were roosting at road culverts. Since mammals in the area are not known to regularly use road culverts as burrow sites, the culverts probably did not harbor large flea populations. Moreover, unlike nesting Burrowing Owls, wintering Burrowing Owls do not roost inside their burrows. Most Burrowing Owls in southern Texas roost at the entrance to the burrow and enter the burrow's interior only briefly for protection from inclement weather or to avoid avian predators.

Wide dispersal on the winter range may also greatly limit the incidence of ectoparasite transfer between Burrowing Owls, as winter roost sites in southern Texas are scattered throughout open areas. It is not uncommon to drive > 1 km to observe two Burrowing Owls.

Although sample size is small, the low numbers of lice and the total lack of fleas in this study of Burrowing Owls wintering in southern Texas indicate that the winter habits (i.e., use of widely dispersed road culverts instead of natural mammal burrows) may be advantageous in avoiding ectoparasites, especially fleas.

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BREEDING ECOLOGY OF RADIO-TAGGED  
WHITE-WINGED DOVES (*ZENAIIDA ASIATICA*)  
IN THE COASTAL BEND REGION OF TEXAS

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**Abstract.**—The primary objective of this study was to measure nesting parameters and productivity of White-winged Doves in the Coastal Bend region of Texas. Forty adult White-winged Doves (*Zenaida asiatica*) were surgically implanted with subcutaneous radio transmitters in the field at the capture site and immediately released following recovery from anesthesia. Radio-marked doves were monitored for up to 96 days. Twenty-six individuals made at least one nesting attempt, 10 made two attempts, and three made three attempts. Mean nesting success rates for individuals was 93%, 16%, and 6% for first, second, and third nesting attempts, respectively. Overall nest success was 60%. This is the first study on White-winged Doves using subcutaneous radio-transmitters implanted in the field.

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White-winged Doves (*Zenaida asiatica*) in Texas have been undergoing an unprecedented northward range expansion over the past 30 years (George et al. 1991; Schwertner et al. 2000). Concurrent with range expansion has been a strong trend toward establishment of urban, year-round populations (Small & Waggerman 1999). Prior to 1980, the number of White-winged Doves in Texas north of the Lower Rio Grande Valley (LRGV) was considered negligible (Cottam & Trefethen 1968; George et al. 1994). Understanding the ecology, particularly nesting ecology, of these relatively new populations will aid in management of the overall population of this species (Small et al. 1989).

Hayslette & Hayslette (1999) documented breeding of White-winged Doves in the Coastal Bend of Texas outside their traditional breeding period of mid-May to mid-August. However, nesting birds were not marked and individual variation in nesting success and productivity could not be quantified. Recent studies have indicated that radio telemetry is a feasible means for studying doves in the U.S. (Schulz et al. 1998, 2001; Small et al. 2004).

## METHODS AND MATERIALS

All doves used in this study were handled in accordance with guidelines established by U.S. Government's Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training and methods were approved by Texas State University—San Marcos Animal Care and Use Committee, protocol number 5QEKCT.

White-winged Doves for radio-tagging were captured in Kingsville, Texas between 19 May and 9 June 2000 using standard wire funnel traps (92 by 60 by 15 cm) (Reeves et al. 1968). Kingsville has an estimated 27,000 breeding pairs of White-winged Doves (Wagberman & Lyon 2001). Ground reconnaissance was used to identify aggregations of White-winged Doves and establish trap sites. Based on these observations three trap sites were established on the Texas A&M University-Kingsville campus and four trap sites in neighborhoods < 1.5 km directly west of campus, encompassing an area 16 km<sup>2</sup>. The study area included all area within the incorporated city limits of Kingsville. White-winged Doves concentrated in residential areas and on the campus of Texas A&M University—Kingsville. These areas consistently exhibited similar characteristics of > 50% of Rio Grande ash (*Fraxinus berlandieriana*), oak (*Quercus virginiana*), hackberry (*Celtis pallida*), and mesquite (*Prosopis glandulosa*). Canopies were predominately open with an open understory consisting of turf grass lawns. Maximum canopy height was 15 m.

Seven trap sites were established and baited with a 3:1 mixture of commercial chicken scratch and black oil sunflower seeds (Purina Corp, St. Louis, MO). Four traps were used to capture White-winged Doves. Traps were rotated among trap sites to reduce trapping pressure at any one location. Traps were checked for captures every 90 min.

Transmitters used in this study were obtained from Advanced Telemetry Services (ATS, Insanti, MN). Transmitters weighed 3.5 g ( $\leq 2.5\%$  dove mass), measured 25 by 12 by 6 mm, and had a 15.2 cm

antenna. Transmitters had a manufacturer's estimated operational life of 120 d. Mean unobstructed line of sight signal distance for transmitters placed 400 cm above the ground was 0.52 km ( $n = 40$ ,  $SE = 0.01$ ) as measured with an omni-directional antenna.

Forty adult White-winged doves were surgically implanted with subcutaneous radio transmitters and released at the trap sites (see Small et al. 2004). No reliable methods for aging White-winged Doves exist other than determining adults from hatching year birds. Estimated average handling time was about 15 min. Sex was determined using cloacal characters (Miller & Wagner 1955). Radio-tagged White-winged Doves were tracked from 19 May to 23 August 2000. An omni-directional antenna mounted on the roof of a vehicle was used to detect transmitter signals in a general area and a directional H-antenna located individuals to a specific site. Specific location points ( $\pm 3$  m) were determined using Global Positioning Systems (Garmin eTrexVista, Garmin Ltd., Olathe, KS) and classified as nest site or non-nest site. Locations were plotted on aerial photos, however, the number of unique points was too small to reliably estimate nesting home ranges.

Radio tagged White-winged Doves were randomly placed into five groups of eight birds each for tracking. Tracking was conducted daily from 0800 h to 1700 h with each member of a group tracked to a source location a minimum of twice every 14 days. Tracking times for individual doves in a group was rotated by 1 h each tracking period and tracking of groups was rotated weekly so each individual was tracked at varying times during ensuing days. This allowed us to collect location information across a range of diel and temporal periods for each individual.

Three hours of searching time was allocated for each group during a normal rotation. If an individual was not located within the initial rotation time, an extra hour of searching was conducted. If the individual was still not located, an extra hour of searching was conducted at night. If the dove was not located following an additional three days of searching, it was considered lost and removed

from the search rotation, although its frequency was included while scanning for other individuals during night monitoring.

Doves located to nests were monitored every fourth day and nest status assessed using a mirror mounted on an extendable pole (Parker 1972). Species of nest tree, tree height, and nest height to the nearest 0.1 m were recorded. Differences in nest tree species and nest height were compared using goodness-of-fit tests with significance established as  $\alpha \leq 0.05$  (Zar 1998).

Nesting success was calculated based on exposure days by the Mayfield method (Mayfield 1961; 1975) using a 14 d incubation period and 10 d nestling period established *a priori*. The Mayfield method was chosen because similar studies on White-winged Dove nesting success used this method or a variation of it (Hayslette et al. 1996; Hayslette & Hayslette 1999; Small et al. 2005) thus allowing for comparison. Hatch rate (which requires disturbing nesting individuals) was not measured due to concern of its potential influence on next success. Standard errors and 95% confidence intervals for nesting success were calculated (Johnson 1979). Because both parents participate in egg and nestling care with at least one adult present at all times (Cottam & Trefethen 1968), nests were considered active if at least one adult was present. In instances when no adult was present a mirror mounted on an extendable pole (Parker 1972) was used to verify that the nest was inactive (i.e., eggs or nestlings were no longer present). The likelihood of eggs surviving to hatching was considered equal to nestlings surviving to fledging because young are altricial and completely helpless (Small et al. 2005). Nests in which at least one fledgling survived to day 10 post-hatching were designated as successful. Nesting success was based on, and compared to, nest attempts. Nesting success was considered different if 95% confidence intervals did not overlap.

## RESULTS

Mean surgery times (not including anesthesia inducement and recovery) was 8.04 min ( $n = 40$ ,  $SE = 0.42$  min, range = 4.88 – 15.45 min). Anesthesia inducement was standardized at 3 min for all

individuals. Post surgery recovery was varied and not recorded because in most cases implant surgery on another individual was begun as soon as the previous surgery was completed. However, estimated handling time was about 15 min and did not exceed 25 min.

Individual White-winged Doves were monitored for a minimum of 27 and maximum of 96 d ( $n = 38$ ,  $\bar{x} = 68.11$  d,  $SE = 3.12$ ). Doves were tracked to known locations 488 times ( $n = 38$ ,  $\bar{x} = 12.84$ ,  $SE = 0.85$ ). Thirty-nine nests were located using radio telemetry. No implanted doves in this study were observed to have formed pair bonds with each other. Doves lost to domestic cat (*Felis catus*) predation ( $n = 2$ ) were excluded from analysis because time of mortality could not be definitively determined. Nineteen (73.1%) of 26 males and 7 (58.3%) of 12 females were observed nesting at least once. Of these, 9 (47.4%) of 19 males and 1 (14.3%) of 7 females that attempted a first nesting were observed making at least one more attempt. Of doves observed attempting 2 nestings, 3 (33.3%) of 9 males and no females ( $n = 1$ ) made a third nesting attempt. There was a significantly greater proportion of males observed nesting (19) than females (7) ( $\chi^2 = 5.54$ ,  $P < 0.025$ ). There was no difference in proportion of second attempts between males and females ( $\chi^2 = 0.01$ ,  $P = 0.40$ , Fisher's exact test) (Sokal & Rolf 1995).

Nests occurred in Rio Grande ash (12), oak (21), and hackberry (6). There was no significant difference among nesting tree species. Mean ( $SE$ ) tree height, nest height, and nest height/tree height were 11.32 (0.31), 7.46 (0.32), and 0.66 (0.02), respectively.

Daily nesting success was 93% for first nest attempts, 16% for second nest attempts, 6% for third nest attempts, and 60% for all nest attempts combined. Nest success was significantly different between first and second nest attempts (Table 1). Six nests failed in the incubation stage and three nests failed in the brooding stage, all the result of abandonment for unknown reasons. No nests were reused, however, three doves re-nested in the same tree as the first nesting.

Table 1. Mayfield method nest success indices (standard error) and 95% confidence intervals by nest attempt for White-winged Doves in Kingsville, Texas, 2000.

Nest Attempts	No. Exposure Days	No. Nests	No. Nests Failed	Nest Success (SE)	95% CI
1st	324	26	1	0.928 (0.003)	0.925 – 0.932
2nd	81	10	6	0.158 (0.029)	0.129 – 0.187
3rd	18	3	2	0.059 (0.074)	0.000 – 0.133
All	423	39	9	0.597 (0.007)	0.590 – 0.604

## DISCUSSION

Previous studies of White-winged Dove nesting have been based on observations of unmarked individuals making comparisons between nesting attempts impossible because individuals could not be identified (Small et al. 1989; Hayslette & Tacha 1996). The use of radio telemetry in this study allowed for comparisons between nesting attempts by known individuals to be made.

Historically, White-winged Doves in the LRGV have relied heavily on anacua (*Ehretia anacua*), Texas ebony (*Pithecellobium flexicaule*), Mexican ash (*Fraxinus berlandieriana*), and, to some extent mesquite (*Prosopis glandulosa*), as well as citrus for nesting (Cottam & Trefethen 1968, Schwertner et al. 2002). Tree species used for nesting differed from those traditionally used by White-wing Doves in the LRGV despite the presence of all of the traditional nest tree species in the study site (40 km<sup>2</sup>).

Although most reports of White-winged Dove nesting maintain that one or two clutches are attempted each breeding season (Cottam & Trefethen 1968, George et al. 1994), anecdotal evidence has suggested a greater number of attempts, particularly in urban, resident populations. Greater than two nesting attempts were, however, definitively determined to occur in this study and a subsequent study (Schaefer et al. 2004).

Daily nest success was similar to previous reports in urban, resident populations (Hayslette & Hayslette 1999; Small et al. 2005). A significantly greater proportion of males were found nesting than females, but the proportion that re-nested was not different between the sexes. This is likely because, in birds, there is generally a greater investment in time and energy expenditure for nesting by females (Whittow 2000). These differences may also suggest previously unknown gender differences, including a violation of the assumption of monogamy (Schwertner et al. 2002) in White-winged Doves. Categorization of doves as monogamous as defined by Lack (1968) and Wittenberger & Tilson (1980) is tenuous, and the term “apparent monogamy” is used for Columbids (Gowaty 1985). In addition, Blockstein & Westmoreland (1993) concluded that monogamy in the family Columbidae is based on few, mostly captive species with known exceptions. This study suggests that White-winged Dove natural history parameters in recently colonized populations differ greatly from White-winged Doves in their historic breeding range. Further research is warranted to determine how traditional and recently established populations differ in parameters not addressed in this study.

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## GENERAL NOTES

PARASITES OF THE BRAZILIAN FREE-TAILED BAT,  
*TADARIDA BRASILIENSIS* (CHIROPTERA: MOLOSSIDAE),  
FROM SOUTHWESTERN ARKANSAS**Chris T. McAllister, Charles R. Burseay and Nixon Wilson***Department of Biology, Angelo State University  
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The Brazilian free-tailed bat, *Tadarida brasiliensis* (I. Geoffroy) is one of the most widely distributed bat species in the Western Hemisphere (Wilkins 1989). In Arkansas, this bat has been reported from at least 14 counties of the state, mostly in the Interior Highlands of the Ouachitas, the West Gulf Coastal Plain, and the Delta or Mississippi Alluvial Plain (Sealander & Heidt 1990). This medium-sized molossid bat roosts in buildings, the undersides of bridges, and other man-made structures.

Although information is available on helminth parasites of this bat from the southern United States, particularly in Louisiana (Martin 1976), New Mexico (Cain 1966), Oklahoma (Nickol & Hansen 1967), and Texas (Jameson 1959; Ubelaker 1970; Martin 1976; Specian & Ubelaker 1976; Ritzi et al. 2001), nothing has been published on any parasites of these bats from Arkansas. This study presents data on some parasites of a small sample of *T. brasiliensis* from southwestern Arkansas.

On 29 January 2005, 10 adult *T. brasiliensis* were collected by hand from a nuisance roost in downtown Ashdown, Little River County, Arkansas (33°67'N, 94°13'W). Bats were returned alive to the laboratory and processed within 24 hr. Specimens were euthanized by cervical dislocation or exposure to ether and the

pelage brushed for ectoparasites in an enamel dissecting pan. Mites were preserved in 70% ethanol and examined as temporary mounts on microscopic slides. The entire gastrointestinal tract (including the liver and gall bladder), coelomic cavity, kidneys, urinary bladder, and reproductive organs were examined for helminth parasites. Feces from the rectum were collected and placed in individual vials containing 2.5% aqueous potassium dichromate and examined by light microscopy after flotation in Sheather's sucrose solution (sp. gr. 1.18) (Todd & Ernst 1977). Intact cestodes were relaxed in cold tap water overnight, transferred to 70% ethanol for fixation, stained with acetocarmine, and mounted entire in Canada balsam. Nematodes were placed in a drop of glycerol on microscopic slides and identifications were made from these temporary mounts. Helminth voucher specimens were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA as follows: *Vampirolepis decipiens* (USNPC 95784), *Molinostrongylus delicatus* (USNPC 95785). Mite specimens were deposited in the Florida State Collection of Arthropods, Gainesville, Florida, U.S.A. Host voucher specimens were deposited in the Texas A&M University-Texarkana Collection of Vertebrates (TAMUT-CV, Texarkana, Texas, U.S.A.).

All 10 bats (100%) harbored parasites, including 10 infested with numerous immature and adult mites, *Chiroptonyssus robustipes* (Ewing), a single host (10%) with eight tapeworms, *Vampirolepis decipiens* (Diesing) in the small intestine, and seven bats (70%) infected with 35 nematodes (mean intensity = 4.5, range 3-12), *Molinostrongylus delicatus* (Schwartz) Travassos in the lower gastrointestinal tract. None of the bats harbored trematodes or coccidian oocysts in the feces.

Although no coccidian parasites (eimerians or isosporans) were isolated from this small sample of *T. brasiliensis*, that result may not be too surprising given previous surveys. Indeed, Duszynski et al. (1988, 1999) and Scott & Duszynski (1997) were unable to find coccidia in a total of 41 bats from New Mexico and Mexico, and

McAllister et al. (2004) did not report coccidia in 10 *T. brasiliensis* from Oklahoma. For some unknown ecological reason, perhaps certain genera of bats in the family Molossidae may not be as suitable hosts of coccidia as are those of the family Vespertilionidae. Furthermore, in his summary of the coccidia of bats, Duszynski (2002) lists coccidians from 23 vespertilionid species compared to only five molossids.

Albeit this sample size is relatively small, not finding trematodes in this bat species is somewhat surprising. Ubelaker (1970) summarized the trematodes reported from *T. brasiliensis* and listed 10 species, six of which are Neotropical and also present in the Nearctic, but only within the range of *T. brasiliensis*. In addition, Guzmán-Cornejo et al. (2003), who examined 98 *T. brasiliensis mexicana* from arid regions of central and northeastern Mexico, reported three digenean trematodes infecting their host sample. As further noted by Ubelaker (1970) the influence of migration on the trematode fauna of bats has not been investigated and, most notably, this bat is a strong migrant.

The macronyssid mite, *C. robustipes* appears to be a very common ectoparasite of *T. brasiliensis*. In the United States, there are numerous records of this mite from this host in Oklahoma (Radovsky 1967) and Texas (Randolph & Eads 1946; Eads et al. 1957; Jameson 1959; Radovsky 1967; Whitaker & Easterla 1975; Dooley et al. 1976; Ritzi et al. 2001), as well as Alabama, Arizona, California, Florida, Georgia, and Kansas (Radovsky 1967; Durden et al. 1992; Sparks et al. 2003). Other reports of the mite from *T. brasiliensis* outside the United States are discussed by Radovsky (1967). More recently, Guzmán-Cornejo et al. (2003) reported *C. robustipes* from *T. brasiliensis mexicana* from Mexico. To the author's knowledge, this is the first report of this mite on any host from Arkansas.

The cestode, *V. decipiens* has been reported previously from *T. brasiliensis* from Florida (Foster & Mertens, 1996). This study

documents this parasite species from Arkansas for the first time. Interestingly, McAllister et al. (2005) previously reported an immature *Vampirolepis* from Rafinesque's big-eared bat, *Corynorhinus rafinesquii* from another site in Little River County, about 35 km northwest of the Ashdown site noted herein.

The nematode, *M. delicatus* is a relatively common roundworm of *T. brasiliensis*. The species has been previously reported in *T. brasiliensis* from Florida (Foster & Mertens 1996), New Mexico (Cain 1966), Oklahoma (Nickol & Hansen 1967), and Texas (Jameson 1959; Martin 1976). This parasite has also been reported from the black mastiff bat, *Molossus ater* in Mexico (Cain & Studier 1974). This study reports this nematode from Arkansas for the first time.

In summary, this study documents three new geographic records for parasites from the Brazilian free-tailed bat. This report represents the fourth paper on parasites of bats from Arkansas (see McAllister et al. 2001; 2004; 2005). Additional surveys on parasites of other species of bats of the state are recommended to add to the growing knowledge of this important aspect of their ecology.

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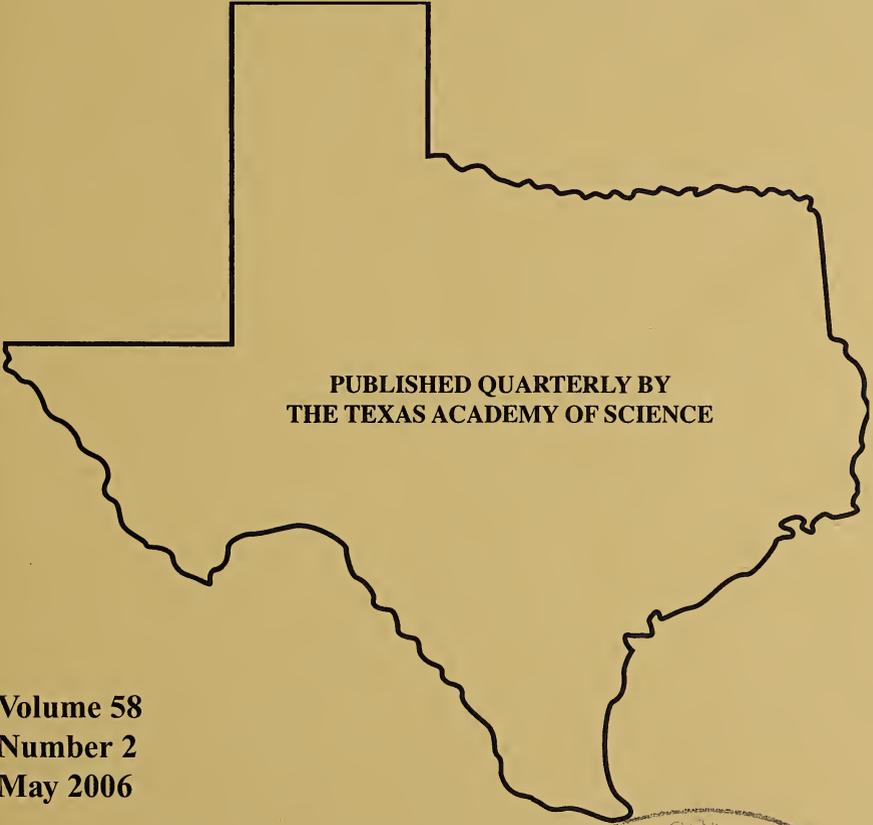
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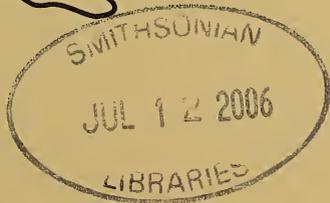
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RODENT COMMUNITIES OF NATIVE WOODLAND,  
REPLANTED, AND SECONDARY SUCCESSION SITES  
IN THE LOWER RIO GRANDE VALLEY, TEXAS

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**Abstract.**—Wildlife habitat is rapidly disappearing in the Lower Rio Grande Valley of Texas and is in critical need of protection. United States Fish and Wildlife Service plans call for protection of 53,420 ha in the area, with the Rio Grande serving as the major corridor linking tracts of native and restored vegetation. Species richness, diversity, density, biomass and similarity of rodent communities were compared among a native woodland, a replanted field, and an unaided secondary succession site to obtain information on the efficacy of vegetation efforts in promoting rodent community diversity. Species diversity varied from  $H' = 0.0$  to 0.65 depending on habitat, grid and season. Species richness ranged from 5 to 9 among the three habitats and a total of 10 species was captured. Density for all species combined ranged from 269 to 388 rodents/ha. *Sigmodon hispidus*, *Peromyscus leucopus*, and *Liomys irroratus* were the most abundant rodents in the native woodland and replanted habitats where they together comprised 88% and 90%, respectively, of the individuals captured. *Mus musculus* replaced *P. leucopus* in abundance in the unaided succession habitat. Biomass was greatest where *S. hispidus* was most abundant. Community similarity was greater between the two successional habitats than either was to the native woodland. The native woodland had fewer species but greater evenness than either successional habitat. The replanting technique used by the United States Fish and Wildlife Service in a formerly cultivated field produced greater diversity of rodents in less time than unaided secondary succession of a fallow field. Variation in application of planting techniques can produce significant differences in vegetation and rodent communities on small replanted areas.

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The Lower Rio Grande Valley (LRGV) of Texas is both a political unit and a biogeographic unit (Judd et al. 2002). As a political entity, it includes the four southernmost counties in the state, i.e., Cameron, Hidalgo, Starr and Willacy. Biogeographically, it corresponds closely with the Matamorán District of the Tamaulipan Biotic Province (Blair 1950). This 1.2 million ha region exhibits great biodiversity. More than 500 vertebrate and

170 woody species occur in the LRGV (Judd et al. 2002) and 67 species are listed as threatened or endangered (Jahrsdoerfer & Leslie 1988). Because of its high biodiversity, large number of threatened and endangered species, large number of neotropical species that reach the northern limit of their distribution in the LRGV (Blair 1950; Oberholser 1974; Lonard & Judd 1993), and small amount of native habitat remaining (Purdy 1983; United States Fish and Wildlife Service 1985; Jahrsdoerfer & Leslie 1988), state and federal governments and non-profit organizations are purchasing lands for preservation and revegetation.

It is estimated that less than 5% of the habitat that originally covered the LRGV still remains (Purdy 1983; Jahrsdoerfer & Leslie 1988). Indeed, the United States Fish and Wildlife Service (USFWS), Texas Parks and Wildlife Department (TPWD) and Texas Nature Conservancy have identified the LRGV as an area where wildlife habitat is rapidly disappearing and in dire need of protection. In 1979, the Lower Rio Grande Valley National Wildlife Refuge (LRGVNWR) was established to implement a USFWS Land Protection Plan that calls for protection of 53,420 ha in the LRGV with the Rio Grande serving as the major corridor linking tracts of native and restored vegetation (USFWS 1985). When completed, the Lower Rio Grande Wildlife Corridor will extend 240 km from the mouth of the river in Cameron County to Falcon Dam in Starr County. The refuge currently consists of over 138 tracts comprising about 31,697 ha. To date, about 4,047 ha have been replanted.

Lands acquired for the corridor usually are fields that have been in cultivation. Left alone, these abandoned fields undergo secondary succession. The first plants to become established typically are herbaceous annuals (Vora & Messerly 1990). In time, these colonizing species are gradually replaced by woody species. The rate at which succession proceeds depends, in part, on the ability of mid- and late-successional species to disperse to a site and successfully compete with colonizing species that are already

established. Revegetation projects based on the Facilitation Model of succession (Connell & Slatyer 1977) attempt to accelerate succession by introducing climax species into an area (Judd et al. 2002).

Due to conversion of many riparian areas of the southwestern USA to urban and agricultural uses, restoration projects are becoming common. Still, there is a paucity of information regarding the affects of restoration efforts on rodent communities (Anderson 1994; Ellis et al. 1997; Ellison & Van Riper 1998; Patten 1997). Judd et al. (2002) and Sternberg (2003) have assessed success of TPWD and USFWS revegetation efforts in accelerating plant succession and in achieving similar composition and structure as native woodlands, but there is no published information on animal communities of replanted tracts in the LRGV.

This study compares species richness, diversity, density, biomass and similarity of rodent communities among native woodland, replanted field and unaided secondary succession sites in a riparian area. It provides information on the efficacy of revegetation efforts at promoting rodent community diversity in the LRGV by investigating the null hypothesis: there are no significant differences in rodent species richness, diversity, density or biomass at native woodland, replanted field, and unaided secondary succession sites.

#### MATERIALS AND METHODS

*Study area.*—The study sites are located approximately 6 km south of Weslaco, Hidalgo County, Texas, and about 1 km north of the Rio Grande. The McManus Unit of the Las Palomas Wildlife Management Area is 22 ha of relatively undisturbed, thorn woodland managed by TPWD. Coma (*Sideroxylon celastrina*) and cedar elm (*Ulmus crassifolia*) are the dominant trees (Sternberg 2003). Coma and granjeno (*Celtis pallida*) are the dominant shrubs and crucita (*Chromolaena odorata*) is the dominant species in the ground layer. There is a dense cover of trees and shrubs at this site (Sternberg 2003).

The La Coma tract, managed by the USFWS, includes a 14.99 ha farm field that was replanted in October 1995 with 7,948 seedlings of 31 native shrub and tree species. Seedling density was 592 individuals/ha and plant survivorship was estimated at 85% in July 1996. Sternberg (2003) gives the number of seedlings planted for each of the 31 species. Tenaza (*Havardia pallens*) was the most abundant with 925 individuals. Tepeguaje (*Leucaena pulverulenta*) and jara dulce (*Baccharis neglecta*) were dominant trees at the replanted site and jara dulce was the dominant shrub when the area was sampled from November 1999 to January 2000. Guineagrass (*Urochloa maxima*) and jara dulce were the dominant species in the ground layer. Cover provided by trees and shrubs was markedly less than at the adjacent native woodland.

The La Coma tract also includes an unaided secondary succession site of about 55 ha. It is a field that was in agricultural production until 1985 when USFWS purchased the land and allowed it to go fallow. Mesquite (*Prosopis glandulosa*), retama (*Parkinsonia aculeata*) and lotebush (*Ziziphus obtusifolia*) were the only trees present at this site and cover provided by trees and shrubs was 6.5 to 19.8 times less than at the native woodland (Sternberg 2003). Mesquite was the dominant shrub and guineagrass was the dominant species in the ground layer (Sternberg 2003).

*Rodent trapping.*—Two 25-trap grids were established in each habitat. An edge grid was located within 50 m of the border of a given habitat and an interior grid was placed randomly within a site, but more than 30 m distant from the edge grid. Sherman live-traps (8 cm W by 9 cm H by 23 cm L) were set at 10-m intervals and baited with rolled oats. On 13 December 1998, a temperature decrease of 17.8°C from 21.7°C to 3.9°C killed nine trapped Mexican spiny pocket mice (*Liomys irroratus*). Thereafter, cotton bedding was added to the traps (Schweiger et al. 2000) and peanut butter was mixed with rolled oats to prevent additional mortality, when temperature was forecast to be lower than 15°C. No additional deaths of trapped Mexican spiny pocket mice occurred.

Rodents were trapped every other week in each of the habitats. Trapping commenced on 1 November 1998 and ended on 1 December 1999. Thus, each grid was trapped 25 times. Edge and interior grids were trapped on separate, but consecutive nights with the starting order alternating among trapping periods. Traps were set at dusk, checked at dawn and left closed and in place between trapping periods.

Rodents were weighed to the nearest 0.5 g using a Pesola<sup>®</sup> spring balance, marked uniquely by toe-clipping and released at the point of capture following guidelines of the *Ad Hoc* Committee for Acceptable Field Methods in Mammalogy (1987). Individuals were identified to species, gender, and age using criteria in Genoways (1973), Batzli (1977), Holbrook (1979), Davis & Schmidly (1994), McMurry et al. (1994) and Schmidt & Engstrom (1994).

*Analyses.*—Species richness, species diversity, and evenness were compared between edge and interior grids of a given habitat using the seasonal values for these parameters as samples in Student *t*-tests. Species richness, species diversity, and evenness were compared among habitats and seasons using two-way analysis of variance (*ANOVA*). Student *t*-tests were used to identify significant differences in pair-wise comparisons of the parameters between habitats.

Species richness was the count of species present. Species diversity was assessed using the Shannon diversity index based on  $\log_{10}$  (Brower et al. 1998; Krebs 1999). For seasonal comparisons, Winter was 14 November 1998 to 1 March 1999; Spring 2 March to 2 June 1999; Summer 3 June to 2 September 1999; and Fall 3 September to 13 November 1999. The last trapping period was included in the annual data, but was omitted from seasonal comparisons so that each season would have six trapping periods.

Similarity of rodent communities was compared between habitats using the Percent Similarity test (Brower et al. 1998).

Density is reported as numbers per hectare. The Jolly-Seber method (Krebs 1999) was used to estimate total rodent density and density of three species (i.e., Mexican spiny pocket mouse, white-footed mouse [*Peromyscus leucopus*] and hispid cotton rat [*Sigmodon hispidus*]) that were recaptured frequently enough to permit calculation of the Mean Maximum Distance Moved (MMDM). MMDM was added to the periphery of a grid to calculate species-specific grid size for each of the three species. Density of all rodents combined was compared among habitats using the edge and interior grids within a habitat as samples in a one-way *ANOVA*. Student *t*-tests were used in pair-wise comparisons to assess differences in the density of Mexican spiny pocket mice, white-footed mice, and hispid cotton rats among habitats.

Biomass was the sum of the average weights of individuals of a given species captured at least three times on a given grid. After applying the MMDM to the species-specific grid size, biomass per hectare for each species was calculated. Defined in this way, biomass estimates were not possible for six species represented by very few individuals and captures. Total biomass of resident rodents was compared among habitats using grids within a habitat as samples in a one-way *ANOVA*.

## RESULTS

*Species richness, evenness, and diversity.*—A total of 3,750 trap-nights resulted in 2,122 captures of 923 individuals. Species richness per grid ranged from 5 to 9, and 10 species were captured among all grids (Table 1). Grids in the same habitat generally had similar species richness, but edge grids in the replanted and unaided succession habitats had two more species than the interior grids. Edge grids in the replanted and unaided succession habitats had four (44.5%) more species than either grid in the native woodland. At the native woodland, three species (hispid cotton rat, white-footed mouse, and Mexican spiny pocket mouse) accounted for 88.0% of the individuals captured. At the replanted site these three species accounted for 90.3% of the individuals captured. At the

Table 1. Comparison of species richness and total number of individuals captured on edge and interior grids at native woodland, replanted, and unaided succession sites in Hidalgo County, Texas.

Species	Native Woodland		Replanted		Unaid. Succession	
	Edge	Interior	Edge	Interior	Edge	Interior
<i>Sigmodon hispidus</i>	38	24	114	34	125	142
<i>Peromyscus leucopus</i>	39	41	35	88	2	3
<i>Liomys irroratus</i>	45	33	19	16	16	7
<i>Mus musculus</i>	0	0	8	5	7	14
<i>Neotoma micropus</i>	8	16	1	0	1	0
<i>Rattus rattus</i>	3	3	4	3	1	3
<i>Oryzomys couesi</i>	0	0	4	3	5	3
<i>Reithrodontomys fulvescens</i>	0	0	2	2	1	3
<i>Chaetodipus hispidus</i>	0	0	0	0	1	0
<i>Oryzomys palustris</i>	0	0	1	0	0	0
Total individuals	133	117	188	151	159	175

unaided succession site, hispid cotton rat, Mexican spiny pocket mouse, and house mouse (*Mus musculus*) comprised 93.1% of the individuals captured. Thus, house mice replaced white-footed mice in abundance in the unaided succession habitat.

Species richness, species diversity, and evenness were compared between edge and interior grids to determine if there was significant variation between the grids of a habitat on an annual basis (Table 2). There was no significant difference in species richness between grids of the native woodland ( $t = 0.658$ , 6 *df*,  $P > 0.5$ ), replanted ( $t = 0.918$ , 6 *df*,  $P > 0.2$ ), or unaided succession ( $t = 0.343$ , 6 *df*,  $P > 0.5$ ) habitats. Likewise, there was no significant difference in species diversity between grids of the native woodland ( $t = 0.934$ , 6 *df*,  $P > 0.2$ ), replanted ( $t = 0.934$ , 6 *df*,  $P > 0.2$ ), or unaided succession ( $t = 0.389$ , 6 *df*,  $P > 0.5$ ) habitats. Similarly, evenness did not exhibit significant variation between grids of the native woodland ( $t = 0.723$ , 6 *df*,  $P > 0.4$ ), replanted ( $t = 0.625$ , 6 *df*,  $P > 0.5$ ) or unaided succession ( $t = 0.634$ , 6 *df*,  $P > 0.5$ ) habitats.

Table 2. Comparison of species richness (R), evenness (J'), and Shannon-Wiener diversity (H') of rodents on edge and interior grids from Winter, Spring, Summer, and Fall at native woodland, replanted, and unaided succession habitats in Hidalgo County, Texas.

Parameters	Native Woodland		Replanted		Unaid. Succession	
	Edge	Interior	Edge	Interior	Edge	Interior
Winter						
R	5	5	7	6	7	6
J'	0.8186	0.7107	0.7153	0.6956	0.4704	0.5657
H'	0.5722	0.4968	0.6460	0.5413	0.4248	0.4402
Spring						
R	5	5	6	6	2	4
J'	0.9114	0.8350	0.7203	0.5830	0.2109	0.3282
H'	0.6370	0.5836	0.5035	0.4537	0.0635	0.1976
Summer						
R	4	5	6	5	4	1
J'	0.9552	0.8258	0.5409	0.6029	0.4830	0
H'	0.5751	0.5772	0.4209	0.4214	0.2908	0
Fall						
R	4	4	4	3	4	4
J'	0.7158	0.8053	0.7086	0.6747	0.4263	0.3596
H'	0.5004	0.4849	0.4266	0.3219	0.2567	0.2165

Species richness was relatively stable among seasons at the native woodland and most variable among seasons in the unaided succession habitat (Table 2). There was significant variation in species richness among seasons and habitats, and due to interaction (Table 3). Between habitat differences were due to values in spring and summer (Table 2). The mean for these two seasons combined was significantly greater for the replanted habitat ( $n = 4$ ,  $\bar{x} = 5.75$ ,  $SD = 0.5$ ) than either the native woodland ( $n = 4$ ,  $\bar{x} = 4.75$ ,  $SD = 0.5$ ) ( $t = 2.829$ , 6 *df*,  $P < 0.05$ ) or the unaided succession habitat ( $n = 4$ ,  $\bar{x} = 2.75$ ,  $SD = 1.5$ ) ( $t = 3.795$ , 6 *df*,  $P < 0.01$ ). Species richness of the native woodland also was greater in spring and summer than in the unaided succession habitat ( $t = 2.530$ , 6 *df*,  $P < 0.05$ ).

Table 3. Two-way *ANOVAs* for species richness, species diversity and evenness. Source = source of variation, *df* = degrees of freedom, *SS* = sums of squares, *MS* = mean square, *F* = *ANOVA* value, *P* = probability level. Each is a Model I *ANOVA*.

Parameter	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Species	Seasons	3	16.34	5.45	7.26	<0.005
Richness	Habitats	2	7.59	3.80	5.06	<0.05
	Interaction	6	14.40	2.40	3.20	<0.05
	Error	12	9.00	0.75		
Species	Seasons	3	0.087	0.029	5.07	<0.025
Diversity	Habitats	2	0.430	0.215	37.72	<0.001
	Interaction	6	0.099	0.016	2.88	>0.05
	Error	12	0.069	0.006		
Evenness	Seasons	3	0.028	0.009	0.35	>0.75
	Habitats	2	0.895	0.447	16.76	<0.001
	Interaction	6	0.119	0.020	0.74	>0.50
	Error	12	0.320	0.027		

Differences in the number of individuals captured per species were reflected in evenness values (Tables 1 and 2). Evenness was greatest in the native woodland and lowest in the unaided succession habitat where hispid cotton rats alone accounted for 79.9% of the individuals captured. The only source of significant variation for evenness was among habitats (Table 3). Comparison of mean annual values showed that the native woodland ( $n = 8$ ,  $\bar{x} = 0.8222$ ,  $SD = 0.084$ ) had a significantly greater mean than the replanted ( $n = 8$ ,  $\bar{x} = 0.6548$ ,  $SD = 0.698$ ) ( $t = 4.326$ , 14 *df*,  $P < 0.001$ ) and unaided succession habitats ( $n = 8$ ,  $\bar{x} = 0.3555$ ,  $SD = 0.180$ ) ( $t = 6.639$ , 14 *df*,  $P < 0.001$ ). Furthermore, the replanted habitat had significantly greater mean annual evenness than the unaided succession habitat ( $t = 4.382$ , 14 *df*,  $P < 0.001$ ).

Species diversity was consistently lower at the unaided succession site than at the replanted or native woodland habitats (Table 2) and markedly so in spring, summer and fall. Variation was significant among habitats and seasons (Table 3). There was

no consistent pattern of seasonal variation in species diversity. Species diversity was greatest in spring in the native woodland and in winter in the replanted and unaided succession habitats. Species diversity was least in fall in the native woodland and replanted habitats, and in spring in the unaided succession habitat. Mean annual species diversity was significantly greater in the native woodland ( $n = 8$ ,  $\bar{x} = 0.5534$ ,  $SD = 0.054$ ) than in the replanted ( $n = 8$ ,  $\bar{x} = 0.4669$ ,  $SD = 0.097$ ) ( $t = 2.212$ , 14  $df$ ,  $P < 0.05$ ) or unaided succession habitats ( $n = 8$ ,  $\bar{x} = 0.2362$ ,  $SD = 0.155$ ) ( $t = 5.478$ , 14  $df$ ,  $P < 0.01$ ). The replanted habitat also had significantly greater mean annual species diversity than the unaided succession habitat ( $t = 3.571$ , 14  $df$ ,  $P < 0.01$ ).

*Community similarity.*—Information needed for calculating Percentage Similarity values is provided in Table 1 and the resulting values are compared between the two grids of the same habitat and among grids of differing habitats in Table 4. Edge and interior grids of the native woodland and unaided succession habitats had a high degree of similarity (86.3% and 91.2% respectively), but the grids of the replanted habitat had only a modest degree of similarity (59.6%). Unaided succession grids showed lower similarity with native woodland grids than did the replanted grids (Table 4). The replanted edge grid had greater similarity with both the unaided succession grids than it did with the replanted interior grid.

*Density.*—Estimates of total rodent density ranged from 262 to 387 rodents per ha and the 95% confidence intervals were broad (Table 5). Variation in total rodent density among habitats was not significant ( $F = 0.178$ , 2 & 3  $df$ ,  $P > 0.75$ ). Only five white-footed mice and 23 Mexican spiny pocket mice were captured on the unaided succession grids (Table 1), and there were too few recaptures of these individuals to permit calculation of density estimates in this habitat. There was no significant difference in the density of white-footed mice in the native woodland and replanted habitats ( $t = 0.811$ , 2  $df$ ,  $P > 0.49$ ), but the density of Mexican spiny pocket mice was significantly greater ( $t = 8.732$ , 2  $df$ ,  $P < 0.02$ ) in

Table 4. Comparison of community similarity (%) among grids. NWE = Native Woodland Edge, NWI = Native Woodland Interior, RE = Replanted Edge, RI = Replanted Interior, USE = Unaided Succession Edge, USI = Unaided Succession Interior.

Grids	NWI	RE	RI	USE	USI
NWE	86.3	59.9	64.4	41.2	27.9
NWI		51.8	68.1	33.1	27.9
RE			59.6	80.1	75.1
RI				40.4	36.2
USE					91.2

Table 5. Comparison of Jolly-Seber density estimates (number/ha) among three habitats in Hidalgo County, Texas. Each habitat had an edge and interior grid. Density estimates are the means of 23 trapping periods between 14 November 1998 and 1 December 1999. Ninety-five percent confidence intervals for the estimates are in parenthesis. An asterisk (\*) indicates there were too few captures to calculate a density estimate.

Category	Native Woodland		Replanted		Unaided Succession	
	Edge grid	Interior grid	Edge grid	Interior grid	Edge grid	Interior grid
Total rodents	322 (122-414)	270 (108-1052)	388 (255-1084)	263 (185-734)	311 (175-585)	269 (171-538)
<i>Liomys irroratus</i>	94 (69-118)	90 (52-128)	19 (3-36)	2 (0-5)	*	*
<i>Peromyscus leucopus</i>	81 (59-103)	71 (45-97)	64 (30-99)	193 (169-217)	*	*
<i>Sigmodon hispidus</i>	81 (43-118)	47 (34-60)	230 (188-272)	27 (13-41)	258 (171-345)	203 (162-245)

the native woodland (Table 5). *ANOVA* of hispid cotton rat density among the three habitats indicated no significant variation ( $F = 1.863$ , 2 & 3 *df*,  $P > 0.25$ ), but *t*-tests revealed that the density of hispid cotton rats was significantly greater ( $t = 5.150$ , 2 *df*,  $P < 0.05$ ) in the unaided succession than in the native woodland.

*Biomass.*—Total rodent biomass ranged from 3.2kg/ha on the replanted interior grid to 12.1kg/ha on the replanted edge grid (Fig. 1). The variation in total rodent biomass between the two replanted site grids was greater than that among all the other grids and as a

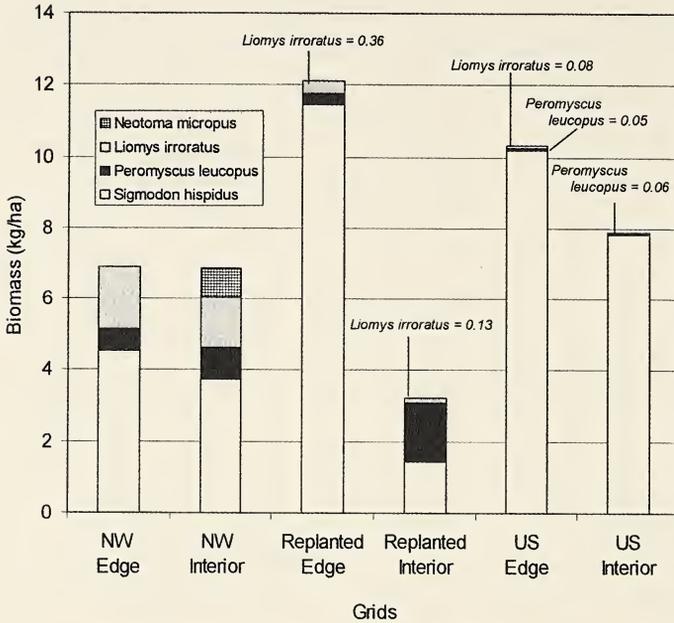


Figure 1. Resident rodent biomass (kg/ha) for each grid and habitat. Areas of the grids (1,600 m<sup>2</sup>) have been adjusted based on the Mean Maximum Distance Moved for *Sigmodon hispidus*, *Liomys irroratus*, and *Peromyscus leucopus*. Biomass of *Neotoma micropus* was not based on Mean Maximum Distance Moved as this species was not captured often enough to make the estimates reliable. Abbreviations are as follows: NW = native woodland, US = unaided succession.

consequence the variation among habitats was not significant ( $F = 0.120, 2 \text{ \& } 3 \text{ df}, P > 0.75$ ). Both grids of the unaided succession habitat had greater rodent biomass than the native woodland grids and the replanted interior grid. This was due to the abundance of hispid cotton rats on the unaided succession grids. Similarly, the high biomass on the replanted edge grid was a result of the high abundance of hispid cotton rats there. The low total biomass of the replanted interior grid was due to the low numbers of hispid cotton rats and the high abundance of the white-footed mouse, which is a relatively small rodent. An adult hispid cotton rat weighs about 110g while an adult white-footed mouse weighs approximately 22g. Thus hispid cotton rats are about five times heavier than white-footed mice.

## DISCUSSION

The null hypothesis that there were no significant differences in rodent species richness, species diversity, density, or biomass among the three habitats was falsified for species richness, species diversity and density for two of the three most abundant species. However, the findings reported here do not necessarily extend to all riparian woodland communities of the Lower Rio Grande Valley because Lonard & Judd (2002) reported that at least three different riparian communities exist between the mouth of the river and Falcon Dam. The habitats investigated in this study were stages in succession of the same Mid-Valley riparian woodland community.

Maintaining and restoring species diversity is a major management objective of the USFWS and it was a point of interest in this study. Two lines of evidence suggest that the replanting technique used by USFWS achieves greater rodent species diversity in less time than unaided secondary succession of fallow fields. First, the replanted habitat had 10 fewer years for development (replanted in 1995) than the unaided secondary succession (fallow since 1985) yet it had significantly greater rodent species diversity. Second, there was no significant difference in rodent species diversity of the replanted habitat and the native woodland that had been undisturbed for more than 40 years. It is important to note that the replanted site had only three years of progress when this study was begun, so it was far from being "restored" (i.e., at or near climax).

The replanted and unaided succession habitats had identical species richness (nine species). Thus, the difference in species diversity of the two habitats was due to greater evenness in the replanted habitat. There are no published studies of rodent species diversity in the LRGV of Texas, and Blair (1952) is the only author to provide information on rodent species richness in LRGV woodlands. The same seven species that he captured were all captured in this study.

Two studies report rodent species diversity at sites in southern Texas north of the LRGV. Nolte & Fulbright (1997) found rodent species diversity values ranging from  $H' = 0.39$  to  $0.79$  in mesquite grasslands 280 km north of the LRGV in San Patricio County, and Windberg (1998) reported a rodent species diversity of  $H' = 0.63$  in Webb County 230 km northwest of the LRGV. Rodent species diversity in this study varied from  $H' = 0.0$  to  $H' = 0.65$  depending on habitat and season. However, most values ranged from  $H' = 0.20$  to  $H' = 0.65$  and, consequently, were relatively similar to those reported by Nolte & Fulbright (1997) and Windberg (1998).

There are no studies reporting rodent species diversity of replanted sites in southern Texas, but Joule & Cameron (1974) found a rodent species diversity of  $H' = 0.40$  in coastal prairie south of Houston where hispid cotton rats were the dominant species. The unaided secondary succession habitat in this study was principally grassland where hispid cotton rats were the dominant species, but it achieved a species diversity of this magnitude only in winter. Grasses also dominated the replanted edge grid (Sternberg 2003) and hispid cotton rats were the dominant species. It had  $H'$  values above 0.40 in all four seasons. Likewise, the native woodland had rodent species diversity values above 0.40 in all seasons.

Greater species richness in the replanted and unaided secondary succession habitats than in the native woodland may have been due to greater density of grasses in the successional habitats. Greater grass cover might have allowed the presence of species such as fulvous harvest mouse (*Reithrodontomys fulvescens*), Coues' rice rat (*Oryzomys couesi*) and marsh rice rat (*Oryzomys palustris*) which require habitats with dense grass cover (Davis & Schmidly 1994).

The comparatively low similarity of rodent communities of the edge and interior grids of the replanted habitat was likely due to difference in the vegetation of the two grids and the numbers of cotton rats and white-footed mice on the grids. The replanted edge

grid was dominated by grasses in the ground layer (Mean Percent Cover [MPC] = 73.1) while the replanted interior grid was dominated by jara dulce in all three vegetation layers, i.e., tree (MPC = 61.5), shrub (MPC = 58.6) and ground (MPC = 22.1) layers (C. Best pers. comm.; Sternberg 2003). Hispid cotton rats were numerically dominant on the edge grid where grasses dominated the ground cover and white-footed mice were numerically dominant where the woody jara dulce was the principal vegetation. Likewise, the greater similarity in the rodent communities of the unaided succession and the replanted habitat than either was in comparison to the native woodland was likely due to the presence of grassland habitat in the two successional communities and the low abundance of grass in the native woodland. Absence of jara dulce on the edge of the replanted habitat was probably due to a delay in the plowing of this area prior to planting seedlings. Thus, unlike the interior area of the habitat, a bed was not prepared on the edge of the habitat for dispersing jara dulce seeds (Sternberg 2003). Clearly, small variations in revegetation techniques can lead to significant differences in rodent communities. The differences may be temporary, however, as the rodent community of the replanted habitat is likely to change as the vegetation develops.

Seasonal variation in habitat use by rodent species is well known (Flehart et al. 1972; Larrison & Johnson 1973; Whitford 1976; Kitchings & Levy 1981; Turner & Grant 1987; Foster & Gaines 1991; Heske et al. 1997; Hanley & Barnard 1999). Thus, seasonal variation in species diversity, such as is reported here, is expected. Some species such as house mice were present on a grid only for several trapping periods and never seen again on the same grid. This may have been due to a dispersal event resulting from crop harvesting of nearby agricultural fields. Clearly, studies conducted for less than a year will likely yield biased estimates of species diversity and richness.

Hispid cotton rat density is correlated positively with grass cover (Cameron 1977; Cameron & Kincaid 1982; Kincaid et al. 1983; Turner & Grant 1987). High hispid cotton rat density also occurred in this study on the unaided succession grids and the replanted edge grid where grass cover was abundant. Cameron (1977; 2003) reported mean Minimum Number Alive values of 6 and 15 hispid cotton rats per hectare (respectively) in eastern Texas, while grids in this study supported hispid cotton rat densities two to 40 times greater.

Similarly, estimates of white-footed mouse density in the native woodland (71 to 81/ha) of this study were markedly greater than mean estimates reported by Wilkins (1995) for a wooded area of east central Texas (16.6 to 32.2/ ha). Wolf & Batzli (2004) suggest that forest edges have lower habitat quality for white-footed mice due to higher predation. Interior grids in this study had higher densities of white-footed mice, Mexican spiny pocket mice, and hispid cotton rats in all habitats except for the hispid cotton rat in the replanted edge grid.

Density of the Mexican spiny pocket mouse has not been reported previously. Estimates of density for this species here are limited to the native woodland and replanted habitats as Mexican spiny pocket mice were few in number and not recaptured frequently enough to yield reliable density estimates for the unaided succession habitat.

Total rodent community biomass has not been reported previously for any habitat in southern Texas. Values obtained in this study ranged from 3.2 to 12.1kg/ha depending upon habitat and grid, and all but one grid (replanted interior) had total rodent biomass above 6.5kg/ha. These values were markedly greater than the 0.45 to 0.90kg/ha reported by Henke & Bryant (1999) for rodent communities in west Texas and the value of 4.7kg/ha reported by Grant et al. (1985) for east central Texas. Clearly, the habitats of subtropical LRGV support high rodent biomass.

The purpose of the LRGV reforestation effort is to establish a wildlife corridor along the Rio Grande from its mouth to Falcon Dam. Wildlife monitoring on remnant native woodlands should be initiated to provide baseline information on species distribution and density. Also, future revegetation efforts in the LRGV should establish control areas that are contemporaneous with the planted areas but that are not planted. Doing so will facilitate comparisons of the success of species recruitment to the two treatment areas and the development of communities on them so that the efficacy of revegetation efforts can be assessed.

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*IN VITRO* AND *IN VIVO* STUDIES OF THE EFFECTS OF ALUMINUM  
ON WHEAT (*TRITICUM AESTIVUM* L.) ROOT CELL WALL  
PEROXIDASES

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**Abstract.**—The subject of this study was aluminum (Al) and its effect on wheat cell wall peroxidases. Isolated cell wall peroxidases were assayed using *o*-dianisidine, guaiacol and syringaldazine. Neither guaiacol peroxidase (guaiacol-POD) nor syrin-galdazine oxidase (syr-oxidase) activities were affected by Al when the enzymes were assayed *in vitro*. On the other hand, tissue printing techniques revealed that Al enhanced syr-oxidase activity in both the Al-tolerant cultivar Atlas 66 and the Al-sensitive cultivar Tam 105 intact roots. This study also examined whether or not Al changed the lignin content in the cell walls of both wheat cultivars. The lignin content remained unchanged upon Al treatments. However, the Al-sensitive cultivar Tam 105 roots had 2 to 5 times more lignin than the Al-tolerant cultivar Atlas 66. The results of this study suggest that Al affects the activity of the wheat root tip cell wall peroxidases *in vivo* and that lignification is not an early response to Al stress.

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Aluminum (Al) is the third most common element in the earth's crust (Foy et al. 1978). At alkaline, neutral or mildly acidic pH, Al is mostly found in the form of insoluble aluminosilicates or oxides. As soils become more acidic, phytotoxic forms of Al are released from these insoluble complexes and dissolved in the soil solution surrounding the plant roots (Kochian 1995). Therefore, the productivity of crops on acid soils has been greatly affected worldwide by Al toxicity.

The mechanisms of Al toxicity and Al tolerance remain elusive despite extensive investigations in this field. There are a number of hypotheses in the literature regarding the mechanisms of Al toxicity and Al tolerance but none has been substantiated unambiguously (Foy et al. 1978; Taylor 1988; Kochian 1995; Matsumoto 2000).

The site of Al toxicity (Ryan et al. 1993) and Al accumulation (Rincón & Gonzales 1992; Samuels et al. 1997) has been localized

to the root apex. The major symptom of Al toxicity is a rapid inhibition of root growth. Root cell elongation, and not cell division, is initially inhibited within 1-2 h of exposure to Al. Over longer periods of time (>24 h) both cell division and cell elongation are inhibited (Kochian 1995). Inhibition of root cell elongation may be caused by an Al-induced inhibition of cell wall biosynthesis (Bennet et al. 1985; 1987; Puthota et al. 1991), disruption of the cytoskeleton (Blancaflor et al. 1998), lipid peroxidation (Cakmak & Horst 2001; Yamamoto et al. 2001), oxidative stress (Yamamoto et al. 2002; 2003), changes in cell wall structure (e.g., enhanced lignification, polymer cross-linkage, and callose deposition), disruption of  $\text{Ca}^{2+}$  homeostasis (Jones et al. 1998; Zhang & Rengel 1999), etc.

Some plant species show reduced Al toxicity symptoms when grown in soils with high Al concentrations. Several hypotheses have been proposed on how plants avoid the deleterious effects of Al (Kochian 1995; Matsumoto 2000). Plants may avoid toxicity by restricting accumulation of Al in their tissues and by detoxifying intracellular Al. Reduced Al accumulation may be accomplished by increased organic acid (e.g., malate, citrate, oxalate, etc.) and phosphate efflux from the roots into the rhizosphere (Pellet et al. 1996; Ryan et al. 2001; Kochian 2004). Organic acids and/or phosphate bind Al thereby reducing the chemical activity of Al and rendering Al non-toxic.

Al-stimulated organic acid efflux has been documented in wheat (Delhaize et al. 1993; Ryan et al. 1995), corn (*Zea mays* L.) (Pellet et al. 1995), snapbeans (*Phaseolus vulgaris* L.) (Miyasaka et al. 1991), soybean (*Glycine max* L.) (Yang et al. 2000; Silva et al. 2001; Yang et al. 2001), buckwheat (*Fagopyrum esculentum*) (Zheng et al. 1998) and *Cassia tora* (Ma et al. 1997). Moreover, Ryan et al. (1997) demonstrated the presence of Al-stimulated anion channels in the plasma membrane using patch-clamp techniques on wheat protoplasts isolated from 2 to 3 mm root tips, the site of Al toxicity. Furthermore, Sasaki et al. (2004) cloned and

characterized a wheat gene, *ALMT1* (aluminum-activated malate transporter) encoding a transmembrane protein likely to be involved in the Al-induced malate efflux in wheat roots. In addition, transgenic barley expressing *ALMT1* not only showed Al-activated malate efflux but also displayed higher Al-tolerance than wild type plants (Delhaize et al. 2004). These results strongly support that *ALMT1* gene is involved both in Al-tolerance and Al-activated malate efflux. Internal tolerance may be also achieved by Al binding to organic acids (Ma et al. 1998) and to intracellular chelators and by sequestering Al in organelles such as the vacuole (Cuenca et al. 1991).

While Al mainly accumulates in the cell walls of roots (Zhang & Taylor 1989; 1990; 1991; Samuels et al. 1997), the precise role of cell walls in Al toxicity or tolerance is not known. Aluminum may bind to cell wall components, which may change cell wall elasticity and plasticity. For instance, Ma et al. (2004) reported that the viscosity and elastic extensibility of the cell wall of root apices of the Al-sensitive wheat cultivar Scout 66 were reduced by growth-inhibitory concentration of Al.

Premature lignification of the cell walls has been suggested as a mechanism by which Al inhibits root cell elongation (Sasaki et al. 1996). Formation of lignin occurs in the cell walls through a dehydrogenative polymerization of monolignols, which are transported from the cytoplasm (Boerjan et al., 2003). *In vitro* assays and the expression of peroxidases (PODs) and laccases in lignifying tissues strongly suggest involvement of these enzymes in the dehydrogenation of monolignols to monolignol radicals (O'Malley et al. 1993; Boerjan et al. 2003). It has been hypothesized for a long time that polymerization of monolignol radicals is non-enzymatic. However, the discovery of the dirigent protein, a glycoprotein, argues against this hypothesis (Davin & Lewis 2000; Boerjan et al. 2003).

A POD specific to lignification has not been identified yet; however, Kay & Basile (1987) showed a correlation between iso-

peroxidases and developmental events in tobacco 'epidermal' explants. Furthermore, MacAdam et al. (1992a; 1992b) found that the activities of both ionically bound and soluble apoplastic PODs increased in the leaf elongation zones of tall fescue inhibiting cell elongation. Anderson et al. (1995) reported that nine POD isoenzymes were induced in maize seedling mesocotyls during chilling acclimatization. Localization of two of the POD isoenzymes in the cell wall and the increased lignin content in mesocotyls suggest a possible involvement of these enzymes in the process of lignification.

Moreover, expression and enzymatic activities of PODs change as plants respond to environmental cues involved in growth and development. In maize seedlings, phytochrome regulates the content of an anionic isoperoxidase in the cell walls within minutes of red light irradiation (Kim et al. 1989). Chen et al. (2002b) showed an increase in the activity of anionic and cationic PODs and reduction in growth of mungbean hypocotyls upon light irradiation. As PODs, especially the anionic, are most likely involved in lignification, the enhanced lignin synthesis in the hypocotyl region and subsequent inhibition of hypocotyl growth by light is attributed to the increased activity of the anionic PODs.

Peroxidases may be also involved in the formation of cross-links among the various cell wall polymers. These cross-links involve oxidative coupling of polysaccharides and glycoproteins by phenolic groups of feruloyl or *p*-coumaroyl or isodityrosine bridges, and of the monolignols (Cassab & Varner 1988).

Peroxidase activities change in response to environmental stresses. For example, chilling temperature (Anderson et al. 1995) and toxic metals affect POD activities and expression of POD genes (Cakmak & Horst 1991; Ezaki et al. 1996; Richards et al. 1998; Ezaki et al. 2000). Cakmak & Horst (1991) showed that Al increased POD activity and decreased catalase activity in soybean

root tips. Decreased catalase activity may lead to generation of oxygen free radicals with consequent tissue damage.

Sasaki et al. (1996) showed that Al induces deposition of lignin in wheat roots. As Al caused severe and irreversible changes in the cell wall, Al-induced lignification could be closely related to the inhibition of cell wall elongation. However, Sasaki et al. (1996) could not demonstrate Al-induced lignification was the primary cause of cessation of root growth because in their study, lignification was examined 48 h after the Al treatment and Al-induced inhibition of root growth begins within 1-2 h after addition of Al (Ownby & Popham 1989).

Initial Al-induced inhibition of cell elongation in wheat root tips may be mediated by an enhanced activity of cell wall PODs involved in lignification and/or cross-linking of the cell wall constituents. The enhanced activity of PODs may be due to a direct activation and/or elevated gene expression upon Al exposure. It is also possible that Al-induced inhibition of cell elongation in wheat root tips is due to an increased activity of cell wall PODs involved in the formation of reactive oxygen species (ROS). Yamamoto et al. (2002; 2003) reported that Al at growth-inhibitory concentrations triggers the formation of ROS in cultured tobacco cells and in pea roots and the increased levels of ROS correlated with the disruption of the normal activities of mitochondria (Yamamoto et al. 2002).

The objective of this investigation was to study whether or not Al affects the activity of the wheat (*Triticum aestivum* L.) root tip cell wall peroxidases.

### Materials and Methods

*Plant material.*—The Al-tolerant cultivar (cv.) Atlas 66 seeds (Cargill Hybrid, Fort Collins, Colorado) and the Al-sensitive cv. Tam 105 seeds (Texas Foundation Seed Service, Vernon, Texas)

were used in this study. Seeds were germinated and seedlings were grown hydroponically as described by Samuels et al. (1997).

*Isolation of wheat cell wall proteins.*—Root tips (0-5 mm) from 5-d old seedlings of the Al-sensitive cv. Tam 105 were cut and frozen in liquid nitrogen. Wheat cell wall proteins (WCWP) were extracted as described by McQueen-Mason et al. (1992) with some modifications (Javed 1998). Wheat root tissue was pulverized in liquid N<sub>2</sub> using a mortar and pestle. Approximately 50 mL of buffer A (20 mM NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>, 2 mM EDTA, pH 4.5) was added to the frozen, pulverized tissue and thawed on ice. The tissue was homogenized in an Omni mixer homogenizer pulsing for 15 sec intervals every 5 sec for five times. The homogenate was vacuum filtered through six layers of miracloth and a filter paper and the filtrate was discarded. The residue was washed five times with buffer A and placed in a beaker containing buffer B (20 mM HEPES, 1 M NaCl, 2 mM EDTA and 3 mM freshly added Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, pH 6.8; 1 mL g<sup>-1</sup> tissue). The cell walls were kept refrigerated overnight in buffer B. The extract was vacuum filtered as above into a cold flask. Proteins were precipitated from the filtrate with NH<sub>4</sub>SO<sub>4</sub> (0.4 g mL<sup>-1</sup>) at 4°C with constant stirring. The precipitate was pelleted by centrifugation (Beckman Centrifuge, Model J2-21) at 20,000g for 20 min at 4°C and the pellets were washed three times with buffer C (50 mM NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>, pH 4.5) and then resuspended in 4 mL of buffer C. The resuspended pellets were pooled and concentrated using microconcentrator Centriplus 3 tubes in a refrigerated Beckman tabletop centrifuge at 3,838g and 4°C for 3 h. The protein concentration was determined using a Commassie Protein Assay (Pierce) with BSA as a standard.

*Peroxidase assay.*—Peroxidases (PODs) present in the wheat cell wall protein extract were assayed using the artificial POD substrate *o*-dianisidine (Fry 1988) and the natural substrates guaiacol and syringaldazine. The activities were compared to that of purified horse radish peroxidase (PHRP). The standard conditions for the spectrophotometric assays of various substrates in a total volume of

one milliliter were as follows: 13.3 mM guaiacol or 0.8 mM *o*-dianisidine, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM H<sub>2</sub>O<sub>2</sub> or 0.1 mM syringaldazine in DMSO, 1.5 mM H<sub>2</sub>O<sub>2</sub>, 37.5 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 4.7) (Fry 1988; Brownleader et al. 1995; Peyrano et al. 1997). Substrate solutions were made fresh for each experiment.

Aluminum chloride (AlCl<sub>3</sub>) was added to give final concentrations (in µM) of 0, 5, 10, 25, 50, 75, 100, and 150. Reactions were started by adding 30 µL of PHRP (15 ng protein mL<sup>-1</sup>) or 30 µL of WCWP (225 ng protein mL<sup>-1</sup>) and incubated at room temperature for 30 min. The oxidation of *o*-dianisidine, guaiacol, and syringaldazine was followed by monitoring absorbencies at 420 nm, 470 nm, and 550 nm, respectively using a Beckman DU-640 spectrophotometer.

*Tissue printing.*—After treatments, root tips (0-15 mm) were excised and blotted on absorbent paper. The root tips were then pressed on a nitrocellulose transfer membrane (MSI 0.45 µm, Fisher Scientific) for 20 sec using a gloved finger. In most cases, successive imprints (up to 3) of the same set of roots were obtained. The membranes were then incubated in chloronaphthol (0.01% H<sub>2</sub>O<sub>2</sub> in 4-chloro-1-naphthol solution), guaiacol (13.3 mM) or syringaldazine (0.1 mM) substrate mixtures for 10 minutes in the dark. The membranes were rinsed in distilled water and photographed using a 35 mm Ektachrome T64 slide film and a Pentax P-Z-70 camera.

*Lignin extraction.*—Root tips (0-8 mm) were excised from control and Al-treated 5-d old seedlings. Lignin extraction from root tissue was performed as described by Bruce & West (1989) with modifications (Javed 1998). Briefly, 1 g of tissue was obtained from control and Al-treated root tips and kept in ice-cold CaCl<sub>2</sub>. The tissue was drained and homogenized in a mortar and pestle at room temperature; after homogenization 20 mL of absolute MeOH was added. The homogenate was further homogenized in an Omni mixer homogenizer at top speed for 2 min. The homogenate was

vacuum-filtered over 5.5-cm fiberglass filters (Whatman GF/A). The residue was placed in an oven at 65 to 70°C for 24 h. The dried residue was used for lignin determination. After adding 2 N HCl (0.10 mL mg<sup>-1</sup> dried tissue) and thioglycolic acid (0.01 mL mg<sup>-1</sup> dried tissue), the samples were placed in a boiling water bath in screw capped glass test tubes and shaken initially for 2 to 3 min to hydrate the dried residue; after 4 h, the tubes were cooled to room temperature and the contents transferred to polypropylene centrifuge tubes and centrifuged at 30,000g for 10 min at room temperature. The supernatant was discarded and the pellets were rinsed once or twice with H<sub>2</sub>O. To extract lignin thioglycolate, the pellets were resuspended in 0.5 N NaOH (0.10 mL mg<sup>-1</sup> dried tissue) and the centrifuge tubes were covered with parafilm, and agitated gently on a clinical rotator at slow speed for 18 h at 25°C to 28°C. The samples were centrifuged at 30,000g for 10 min and the supernatant transferred to 15 mL polypropylene conical tubes. Concentrated HCl (0.02 mL mg<sup>-1</sup> dried tissue) was added to the supernatant, which was then aliquoted into 1.5 mL Eppendorf tubes and incubated at 4°C for 4 h. The samples were then centrifuged in an Eppendorf centrifuge at 15,800g for 10 min and the supernatant discarded. The resulting pellet was either left in a freezer (-20°C) overnight or quick frozen in an ultra cold freezer (-70°C) for 30 to 60 min before drying in a vacuum concentrator for 1 to 2 h. The dried pellets were dissolved in 0.5 N NaOH and the  $A_{280}$  was measured. The amount of lignin was calculated from the absorbance at 280 nm using a specific absorbance coefficient of 8.0 L g<sup>-1</sup> cm<sup>-1</sup> determined from a linear calibration curve with commercial alkali lignin (0-250 µg; Aldrich, Steinheim, Germany).

All treatments were in replicates of five and experiments were repeated at least twice, otherwise indicated in the figure legends. Chemicals were purchased from Sigma, St. Louis MO, unless otherwise stated.

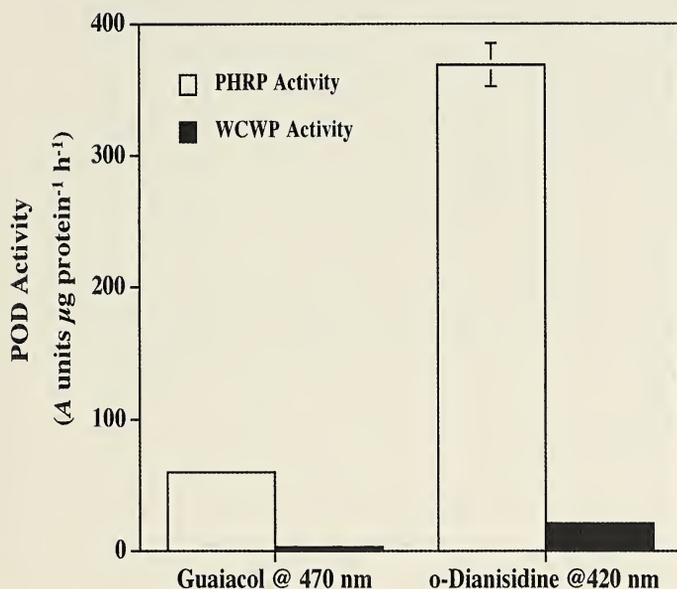


Figure 1. Activity of cell wall peroxidases in the wheat cell wall protein (WCWP) extract and of purified horseradish peroxidase (PHRP) in the presence of either guaiacol or *o*-dianisidine. Enzyme assays were conducted as described in Materials and Methods. Values are Means  $\pm$  SD of five replicates from one typical experiment. This experiment was repeated three times with similar results. The standard deviation bars are not shown when too small.

## RESULTS

*In vitro peroxidase assays.*—The presence of various peroxidases (PODs) in the wheat cell wall (WCW) crude extract from the AI-sensitive cv. Tam 105 root tips was demonstrated by following the oxidation of *o*-dianisidine, and guaiacol and compared the activities to that of purified horse radish peroxidase (PHRP) towards the same substrates. Both PHRP and WCW POD activities towards the artificial substrate *o*-dianisidine were 6 and 9 times higher than those towards guaiacol, respectively (Fig.1).

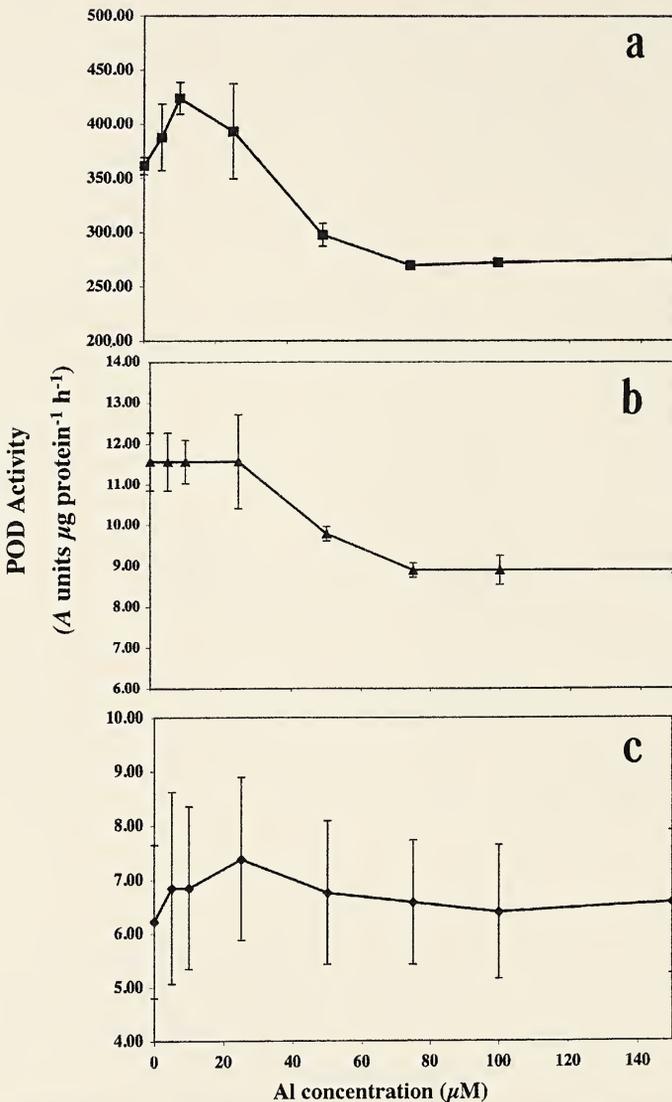


Figure 2. Effect of Al on the activity of PHRP and peroxidases in the WCWP extract. Enzyme assays were conducted as described in Materials and Methods. (a) PHRP activity using *o*-dianisidine. Values are Means  $\pm$  SD of five replicates from one typical experiment; the experiment was repeated twice. (b) Peroxidase activity in the WCWP extract using *o*-dianisidine. Values are Means  $\pm$  SD of five replicates from one typical experiment; the experiment was repeated twice. (c) Guaiacol-POD in the WCWP extract. Values are Means  $\pm$  SD from two experiments.

The optimum pH for guaiacol-POD in the WCW extract was determined using 10 mM  $\text{NaH}_2\text{PO}_4$  at the following pH: 4.2, 4.5, 4.7, and 5.5. The activity of the guaiacol-POD increased as the pH of the assay medium increased from 4.2 to 5.5 (results not shown). To investigate the effect of Al on cell wall POD's, further assays were performed at pH of 4.7 as the phytotoxic Al species form at pH below 5 (Foy et al. 1978; Kochian 1995).

PHRP activity assayed with *o*-dianisidine and in the presence of low Al concentrations, increased 9% to 12% above the 0  $\mu\text{M}$  Al control, and it decreased 21% to 34% at Al concentrations higher than 50  $\mu\text{M}$  (Fig. 2a). Peroxidase activity in the WCW extract assayed with *o*-dianisidine did not change at concentrations of Al below 50  $\mu\text{M}$ , but it decreased at Al concentrations higher than 50  $\mu\text{M}$  (Fig. 2b). Guaiacol-POD activity was highly variable (Fig. 2c) and the effect of Al was not statistically significant as demonstrated by one-way *ANOVA* ( $n = 10$ ;  $P > 0.10$ ).

Syringaldazine-oxidizing peroxidase (syr-oxidase) is reported to be specifically involved in the lignification of plant cell walls (Goldberg et al. 1985; Christensen et al. 2001). If Al injury leads to an early lignification of the wheat roots, then the activity of this enzyme may be stimulated by Al. Figure 3 illustrates the effect of incubation time and Al on the activity of syr-oxidase. The activity of the enzyme increased 6-fold from 0 to 5 minutes both in the presence and in the absence of Al. At 15 minutes, the activity of syr-oxidase was 50% of the activity at 5 minutes.

*In vivo detection of wheat cell wall peroxidase by tissue printing techniques.*—Intact roots of 5-day old seedlings of both cultivars were treated with 50  $\mu\text{M}$  Al for 3 h and tissue prints were obtained and treated as described in Materials and Methods. Figure 4 shows tissue prints from Atlas 66 and Tam 105 roots incubated with syringaldazine reaction mixture. In the absence of  $\text{H}_2\text{O}_2$ , no staining of the roots was observed (not shown). Syr-oxidase activity increased in the Al-treated roots of both cultivars, mainly in the 0-8 mm root tip region compared to the control. It is worth

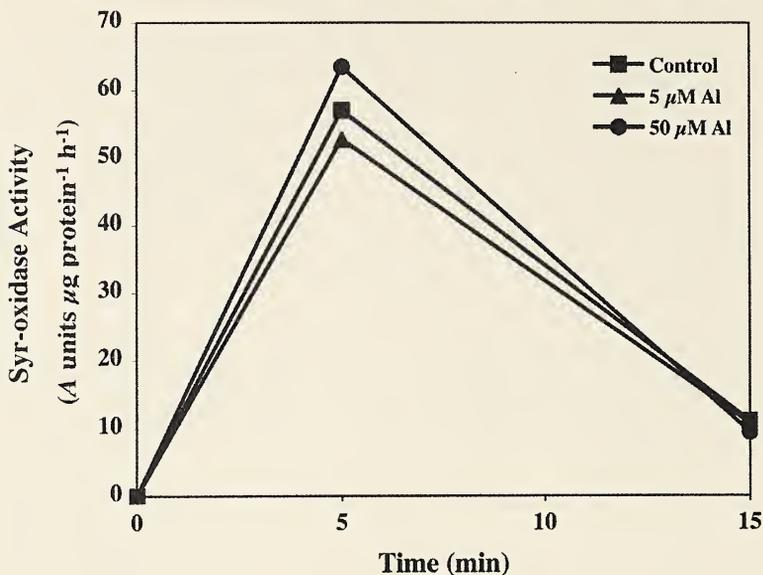


Figure 3. Effect of incubation time and  $\text{AlCl}_3$  on the activity of syringaldazine oxidase in the WCWP extract. Enzyme assays were conducted as described in Materials and Methods. Values are Means  $\pm$  SD of three replicates from one typical experiment. The experiment was repeated twice and results were similar.

noting that the root cap and the root tip region at the boundary of root cap in all root tissue prints showed syr-oxidase activity. However, no differences in enzyme activity in the root cap and the boundary were visualized upon Al treatments.

*Effect of Al on lignin content.*—To test whether or not the Al-induced increased syr-oxidase activity revealed by the tissue prints from the Al-treated roots of Atlas 66 correlated with an increased lignification, the lignin content in these roots was determined. Lignin was extracted from controls and Al-treated roots of both wheat cultivars as described in Materials and Methods. The UV absorption spectrum of  $40 \mu\text{g mL}^{-1}$  LTGA from root tissue of the Al-tolerant cv. Atlas 66 shown in Figure 5 is very similar to that of castor bean LTGA (Bruce and West 1989). The  $A_{280}$  of  $40 \mu\text{g mL}^{-1}$  wheat LTGA was 0.550.

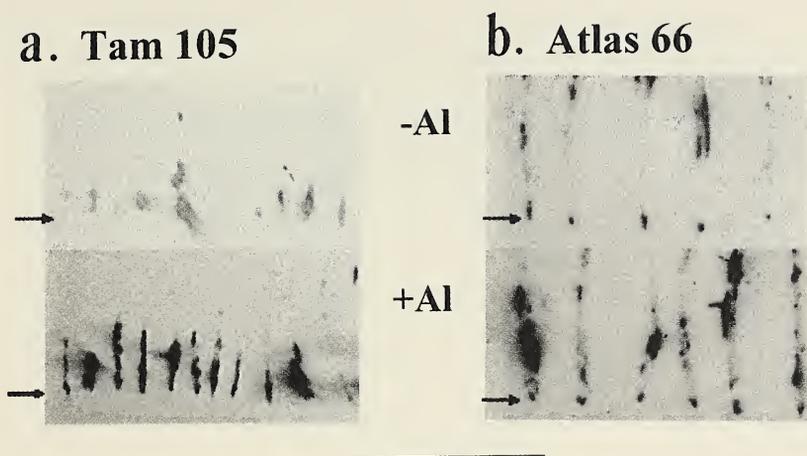


Figure 4. Effect of  $\text{AlCl}_3$  on the activity of syringase in wheat roots as visualized by tissue printing techniques. Fifteen millimeters root tips (including the cap) were excised from untreated and Al-treated seedlings and tissue prints of the roots were obtained as described in Materials and Methods. (a) Tam 105; (b) Atlas 66. Bar, 8 mm; arrow, root tips. This experiment was repeated three times and results were similar.

Table 1 compares the lignin content of both Al-tolerant and Al-sensitive cultivars at different concentrations of Al and at different treatment times. There was no significant difference in the amount of lignin extracted from Al-treated and untreated Atlas 66 root tips at 3 h or 24 h. Similarly, when Tam 105 roots were treated with either 10  $\mu\text{M}$  or 50  $\mu\text{M}$  Al for 3 h, there was no significant difference in the amount of lignin extracted.

#### DISCUSSION

The purpose of this study was to investigate the effect of Al on the activity of wheat root tip cell wall peroxidases (PODs). Both *in vitro* and *in vivo* protocols were applied. The results shown in Figs. 1, 2, and 3 demonstrated the presence of PODs in the wheat cell wall extract. The cell wall PODs oxidized the artificial substrate *o*-dianisidine as well as the natural substrates guaiacol and syringaldazine.

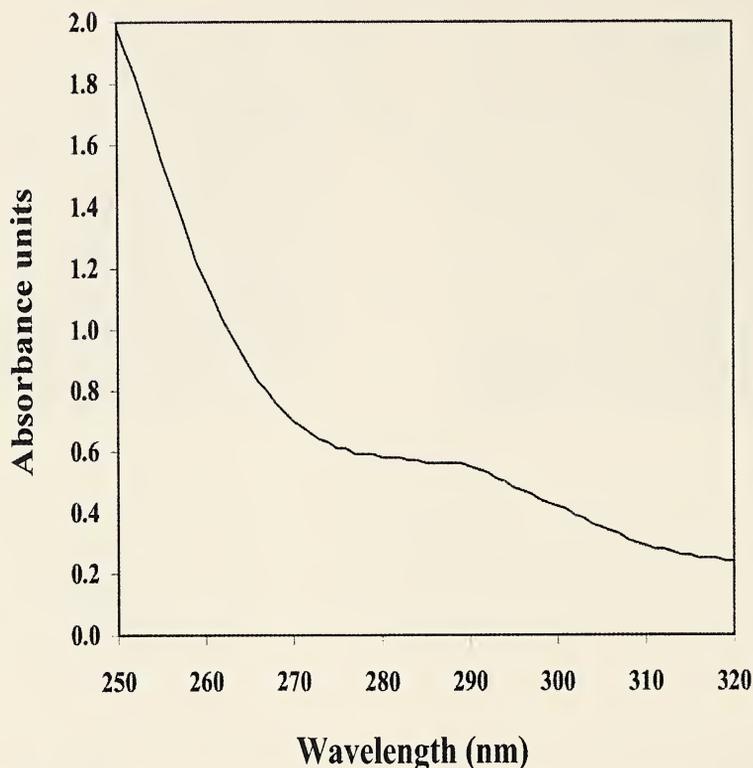


Figure 5. UV absorption spectrum of 40 µg mL<sup>-1</sup> lignin thioglycolic acid (LTGA) extracted from the root tissue of the Al-tolerant cultivar Atlas 66.

Among the cell wall bound PODs, syringaldazine oxidase (syr-oxidase) is reported to be specifically involved in the final step of the polymerization of the lignin monomers. Syr-oxidase activities have been detected in lignifying cell walls of phloem fibers and xylem (Imberty et al. 1985). The enzyme requires H<sub>2</sub>O<sub>2</sub> in *in vitro* assays but the enzyme activity can be detected in *in vivo* assays in the absence of exogenous H<sub>2</sub>O<sub>2</sub>. Hydrogen peroxide may come from the oxidation of NADH by PODs associated with cell walls (Gross et al. 1977).

Table 1. Lignin content in the root tissue of the Al-tolerant cultivar Atlas 66 and the Al-sensitive cultivar Tam 105. Intact roots were treated with different concentrations of  $\text{AlCl}_3$  for different time intervals at room temperature. Root segments of 0-8 mm were excised and 1 g of the root tissue was subjected to lignin extraction as described in Materials and Methods. UV absorbance was measured at 280 nm and the concentration of lignin was estimated. Mean  $\pm$  SD of two separate experiments.

Cultivar	Al concentration $\mu\text{M}$	Duration of Al treatment h	Lignin $\mu\text{g mg}^{-1}$ DW
Atlas 66	0	3	$4.13 \pm 1.1$
		24	$2.50 \pm 0.7$
	50	3	$3.00 \pm 0.9$
		24	$3.00 \pm 0.9$
Tam 105	0	3	$9.50 \pm 1.4$
	10	3	$11.00 \pm 2.8$
	50	3	$9.25 \pm 3.7$

Syringaldazine oxidase is strongly bound to lignifying cell walls and it has a high affinity for syringaldazine as compared to other substrates such as guaiacol (Goldberg et al. 1983). The strong association of syr-oxidase with cell walls was shown when 80% of the activity stayed with the cell wall after treatment with NaCl for 30 min (Goldberg et al. 1985). Syringaldazine can be used *in vivo* to detect isoperoxidases involved in lignification due to the high enzyme-substrate specificity. Syringaldazine is also oxidized by syr-oxidase isolated from non-lignifying walls *in vitro* (Goldberg et al. 1985). Recently, cDNA of the syr-oxidase has been characterized in differentiating poplar (*Populus trichocarpa*) xylem (Christensen et al. 2001).

While Al did not affect the activity syr-oxidase *in vitro* (Fig. 3), Al increased the activity of syr-oxidase *in vivo* in both the Al-sensitive cv. Tam 105 and the Al-tolerant cv. Atlas 66 (Fig. 4), however, the increased activity did not correlate with an increased

lignin content in the roots during short-term exposures to Al, 3 h and 24 h (Table 1). It is worth noting that the lignin content of Al-sensitive cultivar Tam 105 roots was 2 to 5 times higher than that in the Al-tolerant cultivar Atlas 66 roots. These results are in agreement with those reported by Sasaki et al. (1996) in that lignification is not an early response to Al stress. Al-induced lignification may be a secondary response to long-term Al exposures (Sasaki et al. 1996). However, it is possible that Al increases the content of lignin in the root cells but the extraction technique used in this study is not sensitive to detect small changes. It is also possible that syr-oxidase is involved in cross-linking cell wall polymers leading to deformed, thick and brittle roots. Another possibility is that syr-oxidase in the presence of Al may mediate the formation of reactive oxygen species (ROS) leading to growth inhibition. Cross (2005) observed that Al caused formation of ROS in wheat root tips of both cultivars Tam 105 and Atlas 66 within 24 h of Al treatment.

Aluminum affects expression of POD genes. Ezaki et al. (1996) observed that Al induced or repressed expression of several soluble POD isozymes in cultured tobacco cells but it did not alter the gene expression or activity of cell wall bound PODs. Similarly, Richards et al. (1998) reported that Al enhanced the synthesis of POD mRNA and of other oxidative stress genes (SOD, GST, and blue-copper-binding protein) and inhibited the expression of catalase mRNA in *Arabidopsis thaliana* roots.

Ezaki et al. (2000) showed that Al stress related genes expressed in *Arabidopsis thaliana* confer tolerance to Al. Transgenic lines *Arabidopsis* expressing separately an *Arabidopsis* blue-copper-binding protein (*AtBCB*), the tobacco genes: anionic POD (*NtPOX*), glutathione *S*-transferase, and GDP-dissociation inhibitor, and the yeast heat shock protein *HSP150* showed less accumulation of Al in the roots and less Al-induced oxidative damage.

Inhibition of the activity of cell wall PODs by Al may prevent normal formation of secondary cell walls in differentiating cells, which in turn would weaken the roots leading to susceptibility to pathogen invasion and mechanical injury. It was shown that Al inhibited the *in vitro* activities of both PHRP and WCWP (Figs. 2a & 2b) when *o*-dianisidine was used as a substrate. However, the effect of Al on the *in vitro* activity of wheat cell wall guaiacol-POD was not statistically significant (Fig. 2c). Inhibition of POD by Al has been shown in other experimental plant models. For instance, de Souza et al. (1999) showed that high concentrations of Al (250  $\mu$ M; 80 min treatment) significantly decreased POD activity in the first 6 mm of the root apex in the sensitive inbred line of maize but not the tolerant inbred line. Also, López-Serrano & Barceló (1996) reported that metals such as Al, La<sup>3+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup> inhibited PODs secreted into the medium by grapevine cultured cells.

The results from the *in vitro* syr-oxidase assays are difficult to interpret because the assays were performed in a NaH<sub>2</sub>PO<sub>4</sub> buffer at pH 7.0 (Fig. 3). At this pH, Al precipitates as Al(OH)<sub>3</sub> species are formed (Martin 1988). In other words, the effect of Al on syr-oxidase may be underestimated. If the assays were performed at a low pH, syringaldazine precipitated and was unstable. Nevertheless, it appears that Al did not affect the *in vitro* activity of syr-oxidase (Fig. 3).

On the other hand, the *in vivo* studies using tissue printing techniques, revealed that Al stimulated syr-oxidase (Fig. 4) and guaiacol-POD (Javed 1998) in both the Al-sensitive cv. Tam 105 and the Al-tolerant cv. Atlas 66. However, it appears that in wheat roots stimulation of peroxidases is a response to metal stress. La<sup>3+</sup> (50  $\mu$ M) increased syr-oxidase activity to the same extent as Al did (not shown). Increased peroxidase activity has been observed upon Cu<sup>2+</sup> and Cd<sup>2+</sup> treatments (Chen et al. 2002a; Chaoui et al. 2004). Chen et al. (2002a) showed that Cu<sup>2+</sup> concentration as low as 1  $\mu$ M inhibited growth of radish roots. In addition, cationic and anionic

peroxidase activities increased upon  $\text{Cu}^{2+}$  treatment, which correlated with increased lignin content in the root tissue.

In summary, the results of this study suggest that (1) Al stress affects the activity of wheat root tip peroxidases *in vivo* especially that of syr-oxidase and guaiacol-POD; and (2) the increased syr-oxidase activity by Al does not correlate with early lignification of the tissue. Further studies are needed to elucidate the role of syr-oxidase and guaiacol-POD in the mechanisms of metal stress.

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ENTEROCOCCI SPECIES IN GULF COAST  
MARINE WATER SAMPLES AS MEASURED BY  
THE ENVIRONMENTAL PROTECTION AGENCY METHOD 1600

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**Abstract.**—Enterococcus density, as determined by the United States Environmental Protection Agency Method 1600, is used as an index of fecal pollution in marine waters. To determine the range of fecal and non-fecal enterococci species captured by the assay, seawater samples were collected in 2004 at six locations along twelve miles of southeast Texas coastline over a five month period. Using USEPA Method 1600, ninety one confirmed enterococcus isolates were recovered. Differential sugar and sugar alcohol fermentation patterns were used to identify the species of each isolate. Fecal enterococci species (*E. faecalis*/*E. avium*, *E. faecium*, *E. gallinarum*) accounted for only 52 percent of the confirmed enterococcus isolates. Another 45 percent of the isolates were environmental enterococci (*E. raffinosus*, *E. mundtii*, *E. hirae*, *E. dispar*, *E. durans*, *E. malodoratus*, *E. pseudoavium*). Three percent of the isolates could not be identified as a known species. These data show that the current method used to measure enterococcus density in sub-tropical marine waters fails to differentiate between fecal and environmental enterococcus species. Therefore, the USEPA Method 1600 may yield erroneous data concerning recreational water quality.

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Based on data from multi-year, multiple location studies of marine waters near New York City and Boston (Cabelli et al.), a linear relationship was established between enterococcus density and the frequency of swimming associated gastroenteritis per 1000 persons (Salas 1989). Based on these data, the USEPA adopted enterococcus density as the primary fecal indicator in marine waters (Anonymous 1997). To standardize methodology, the agency validated a membrane filter assay (USEPA Method 1600) that confirmed the presence of enterococcus in water samples (Anonymous 1997).

It is known that 16 different species of enterococci can be found in coastal waters (Clesceri et al. 1998). Only four species (*E. faecalis*, *E. faecium*, *E. avium* and *E. gallinarum*) are commonly found in the feces of warm blooded animals (Clesceri et al. 1998;

Watanabe et al. 1981). Other species are often found in the environment and may not be associated with fecal pollution (Ashbolt et al. 1997; Fujioka & Hardina 1995; Lopez-Torres et al. 1987).

Because of the non-selective nature of the USEPA Method 1600, it is possible that the method fails to discriminate between fecal and non-fecal, environmental enterococci in sub-tropical marine waters. This study was undertaken to determine the range of fecal and non-fecal enterococcus species isolated in the USEPA Method 1600.

### MATERIALS AND METHODS

Seawater samples were provided by the Jefferson County Beach Watch Program over a five month period (January to May) in 2004 from six sampling stations along 12 miles of McFaddin and Sea Rim State Park beaches in southeast Texas. Water samples (100 mL) were collected one foot from the sand or sediment layer in water approximately three feet deep. The USEPA Method 1600 was used to confirm the presence of enterococcus species in the water samples (Anonymous 1997). Initially, samples were filtered through a 0.45 micron membrane filter. The filter membrane was then transferred to a plate containing Enterococcus Indoxyl- $\beta$ -D-Glucoside Agar (mEIA), an enrichment media for marine enterococcus, and incubated at 45°C for 24 hours. Colonies with a blue halo were considered to be putative enterococcus species.

Putative enterococcus colonies were transferred to brain heart infusion (BHI) broth tubes and incubated (24 hours for broth / 48 hours for slants) at 35°C. Gram stains were used to assess the purity of each isolate. In confirmation tests, an aliquot of each isolate was then transferred to: (1) Bile Esculin Agar (BEA) and incubated at 35°C, (2) BHI broth and incubated at 45°C and (3) BHI broth with 6.5 percent NaCl and incubated at 35°C. Following incubation for 48 hours, all tubes were examined for bacterial

growth. If growth occurred in all three media, the isolate was considered a confirmed enterococcus isolate.

Confirmed enterococci isolates from the water samples were sub-cultured in BHI broth at 37°C for 24 hours. One percent solutions of 11 different sugars or sugar alcohols were prepared in phenol red broth base media and filter sterilized. Duplicate 150 µL aliquots of the 11 different solutions were added to 0.2 mL, 96 well U-bottom microtest plates. The sugars included: D-Xylose, L-Rhamnose, Sucrose, Lactose, Melibiose, Raffinose, Melezitose, Glycerol, Adonitol, Sorbitol and Mannitol. Three microliter samples of each confirmed enterococcus isolate were added to the duplicate wells. Included in each experiment were positive (*E. faecalis*) and negative controls. The plates were incubated at 37°C for 24 to 48 hours. A positive reaction was recorded when the sugar fermentation changed the pH indicator from red to yellow.

Sugar utilization patterns were recorded and compared to known fermentation patterns of enterococcus species in the Bergey's Manual of Determinative Bacteriology (Holt et al. 2000). To reduce the possibility of erroneous identification at the species level, fermentation patterns between two species must have differed by, at least, two sugars, and  $\geq 80$  percent of the strains within a given species must utilize a specific sugar.

## RESULTS

Over the five months of sample collection, 91 isolates were confirmed as enterococcus species using Method 1600. Based on patterns of sugar utilization, fecal enterococci comprised only 52 percent of the confirmed isolates. Because of variability in the utilization of L-Rhamnose and Adonitol, *E. faecalis* and *E. avium* species could not be clearly delineated. In the data analysis, these isolates were combined into the *E. faecalis*/*E. avium* group. The majority of the remaining isolates (45 percent) were considered to originate in the environment. Three percent of the enterococcus isolates could not be identified as a known species using conventional sugar utilization patterns (Figure 1).

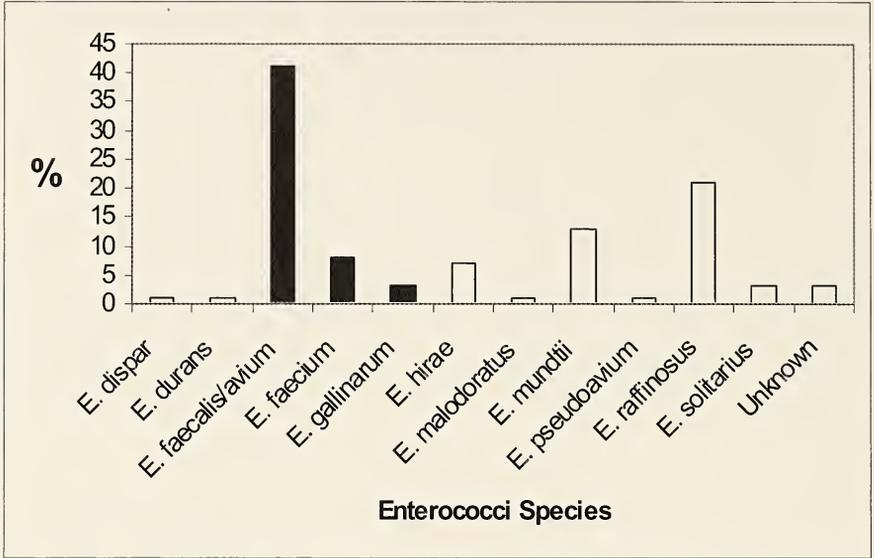


Figure 1. Percentage of fecal and non-fecal enterococcus species ( $n=91$ ) isolated using USEPA Method 1600 for enterococcus density. Black columns represent fecal enterococci species. White column represent non-fecal, environmental enterococci species.

## DISCUSSION

Since only 52 percent of the total enterococci were of fecal origin, the data show that the current USEPA method for determining enterococcus density may yield false positive numbers for human fecal enterococci in marine waters of southeast Texas. Further, the data also suggest that an assessment of fecal pollution in sub-tropical marine water may require additional testing to discriminate between fecal and environmental enterococci.

Although previous studies (Svec & Sedlacek 1999) also reported similar percentages of fecal and environmental enterococci in surface water samples, it is difficult to compare data from different studies. It has been difficult to clearly identify enterococcus species because of the lack of an acceptable, standardized identification protocol. This study utilized a standardized biochemical key to

classify enterococcus to the species level (Holt et al. 2000). The identification scheme takes into consideration the reclassification of *Enterococcus* and *Streptococcus* genera (Holt et al. 2000) and the variability in sugar utilization among enterococcus strains. The data also show that *E. avium* and *E. faecalis* cannot be clearly delineated by sugar utilization patterns. Other assays such as polymerase chain (PCR) amplification of 16S rRNA must be used to differentiate *E. faecalis* from other species (Harwood et al., 2004).

The source of the environmental enterococci found in the water samples is unknown. However, environmental enterococci generated in tidal saltwater marshes have been reported to significantly impact surf zone water quality measurements (Grant et al. 2001).

This study demonstrated that USEPA Method 1600 captures both fecal and a wide range of non-fecal enterococcus species. The data also suggests that enterococci species found in sub-tropical marine waters cannot be assumed to be fecal in origin until adequate testing of the isolates confirms such an assumption. Differentiation between fecal and environmental enterococci densities may reduce the frequency of poor water advisories issued along the Texas Gulf Coast.

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THE USE OF ODORS IN BURROW SELECTION  
BY THE WHIPTAIL LIZARD *ASPIDOSCELIS LAREDOENSIS* B  
(SQUAMATA: TEIIDAE)

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**Abstract.**—Whiptail lizards, genus *Aspidoscelis*, are terrestrial lizards that use burrows as overnight retreats. When a whiptail lizard encounters a burrow entrance, it inserts its head inside and rapidly flicks its tongue to determine if the burrow is already occupied. An empty but recently occupied burrow would still have the odors of the recent occupant in it; these odors may be detected by the investigating whiptail and may cause it to reject the burrow. This hypothesis was tested by performing burrow choice tests using one of the members of the Laredo striped whiptail complex, *Aspidoscelis laredoensis* B. Individual lizards were given a choice between a clean (no odor) burrow and a lizard-odor burrow that had been recently occupied by either a Texas spotted whiptail (*A. gularis*) or another *A. laredoensis* B. In all tests, *A. laredoensis* B showed no avoidance of, or preference for, lizard-odor versus clean burrows, suggesting that odor alone is not a factor this species uses to choose a burrow.

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Whiptail lizards, genus *Aspidoscelis* (formerly *Cnemidophorus*, Reeder et al. 2002), family Teiidae, are ground-dwelling lizards found throughout Texas (Dixon 2000). When active, they employ a “wide-foraging” search tactic (Huey & Pianka 1981) of wandering over a large area, inserting their noses and flicking their tongues into small holes and crevices and under objects to locate their arthropod prey (Milstead 1957; Scudday & Dixon 1973). At the end of their daily activity period, whiptail lizards retire to burrows in the ground that they either dig themselves or appropriate from other small animals (Kennedy 1968; Leuck 1982; Walker et al. 1986). When a whiptail lizard locates a burrow, it inserts its nose into the burrow entrance and flicks its tongue a few times before entering (Rybiski & Paulissen 1995). Sometimes, however, after investigating a burrow entrance, a whiptail lizard will reject the burrow and move off to find another (Rybiski & Paulissen 1995). One reason a whiptail lizard might do this is that the burrow is already occupied. Anecdotal observations of several species of

whiptail lizards suggest they are reluctant to enter an occupied burrow or rarely share burrows with other lizards (Fitch 1958; Hardy 1962; Kennedy 1968; Paulissen 2001). Behavioral studies on captive whiptail lizards suggest that many species do not share burrows readily (Leuck 1982; Paulissen 2002), though *Aspidoscelis tesselata* and *A. neotesselata* (formerly “triploid *Cnemidophorus tesselatus*”, Walker et al. 1997) are exceptions to this general rule (Leuck 1982). Therefore it is reasonable to conclude that the investigative behaviors a whiptail performs prior to entering or rejecting a burrow are to ascertain if the burrow is already occupied. Presumably, if a burrow is occupied, the investigating lizard will detect the occupant and move on to another burrow.

Whiptail lizards have an excellent sense of smell that they use to locate prey and mates (Simon 1983; Cooper 1997), so it is reasonable to hypothesize that the sense of smell is important in helping a whiptail determine if a burrow is occupied or not. However, if a burrow was recently occupied by a lizard but is empty at the moment a whiptail lizard investigates it, the odors left by the recent occupant will still be present in the burrow. If odors in a burrow are the only sensory cue that a whiptail lizard uses to determine if a burrow is occupied, then a whiptail investigating an empty but recently occupied burrow may incorrectly ‘conclude’ that the burrow is occupied and reject it. If on the other hand a whiptail lizard uses sensory cues in addition to (or instead of) odors, then the investigating whiptail lizard should correctly determine that the recently occupied burrow is empty and choose it for its own. Subjecting whiptail lizards to a simple choice test, in which individual lizards are made to choose between a clean (no odor) burrow and a recently occupied burrow with lizard odors, should be able to distinguish between these alternatives.

This report presents the results of such a test conducted on individuals of one of the species of the all-female, parthenogenetic *Aspidoscelis laredoensis* (Laredo striped whiptail) complex. The *A. laredoensis* complex consists of two species: *A. laredoensis* A (=LAR-A), the form originally described by McKinney et al.

(1973), and *A. laredoensis* B (=LAR-B) which was discovered in 1984 (Walker 1987) but has not been given a formal Linnaean name (see Paulissen & Walker 1998 for a review of nomenclature). This study focuses on *A. laredoensis* B. In Texas, it is found in the counties bordering the Rio Grande from Del Rio to just west of Brownsville, plus a few outlying areas (Abuhteba et al. 2001); it commonly co-occurs with the bisexual Texas spotted whiptail, *A. gularis* (Paulissen et al. 1992). It is a small species, snout-to-vent length 65-75 mm, ground-dwelling, and feeds on small arthropods (especially termites, Paulissen et al. 1988). Individuals of *A. laredoensis* B investigate burrows by inserting the head into an entrance and flicking the tongue before either entering or rejecting the burrow. Furthermore, laboratory studies showed that they rarely entered occupied burrows (Paulissen 2002). Therefore, this species is ideal to test the hypothesis that whiptail lizards use odors alone as the cue to determine if a burrow is occupied.

#### MATERIALS AND METHODS

*Housing of captive lizards.*—Individuals of *A. laredoensis* B and both sexes of *A. gularis* were collected from various sites in Cameron and Hidalgo counties in Texas in June 1990, 1991, and 1992. The lizards collected in 1990 were transported to a lab room at Slippery Rock University where they were housed individually in 10-gallon terraria (50 by 26 by 31 cm) provided with a sand substrate, a 10 cm by 10 cm flat piece of cardboard under which a lizard could dig a “burrow”, a food dish kept supplied with mealworms, and a water dish that was checked frequently and refilled as needed. The terraria were kept in a room with overhead lights controlled by a timer to provide a photoperiod of 14:10 L:D every 24 hours. Each terrarium was equipped with a 60 Watt heat lamp that was switched on five hours per day to provide lizards the opportunity to bask. Temperature in the lab room was always between 25 and 30°C. Five *A. laredoensis* B females, two *A. gularis* males, and five *A. gularis* females were used in the first series of tests conducted from September 1990 through April 1991.

Lizards collected in 1991 and 1992 were transported to a lab room at McNeese State University and again were housed individually in 10-gallon terraria. The only difference was that the room used at McNeese was cooler than the one used at Slippery Rock, so additional heat was provided by 250 watt infrared lamps hung from the shelves holding the terraria. These infrared lamps were switched on for five hours a day and warmed the room to 30–33°C during those hours. Eight *A. laredoensis* B females, eight *A. gularis* males and two *A. gularis* females were used in the second series of tests conducted July 1992 through April 1993. Two of the *A. laredoensis* B used in this round of the study were collected in June 1991 and held in captivity until used in 1992; all other lizards used in this segment of the study were collected in June 1992.

*Experimental design.*—All tests were conducted using a 91 by 45 by 54 cm high glass terrarium provided with a substrate of about 3 cm of clean sand. Initially the terrarium was divided into 45 by 45 by 54 cm halves by a 50 cm tall piece of cardboard buried into the sand to prevent lizards from burrowing from one half to the other. Each half was provided with a water dish, a food dish containing mealworms, and a 10 by 10 cm piece of cardboard under which a lizard could dig a “burrow” to use as a retreat. An overhead light set to the same photoperiod as the lizard heat lamps was suspended 40 cm above the sand over each half of the divided terrarium to provide equal light and heat to the two halves. A lizard was placed in one half of the divided terrarium for a 48 hour conditioning period; the other half was left empty except for the food and water dishes (sides were chosen at random by coin flip). During the conditioning period, the lizard established a burrow under the cardboard retreat in its half of the terrarium leaving its odors in the burrow; this burrow is hereafter known as the “lizard-odor burrow”. The cardboard retreat in the unoccupied half is hereafter known as the “clean (no odor) burrow”. After the conditioning period was over, the conditioning lizard, the food and water dishes, and the cardboard partition were removed and a single *A. laredoensis* B was introduced. This test lizard was free to move from one half of the

terrarium to the other and eventually chose to retreat for the night under either the lizard-odor burrow or the clean burrow. Two-tailed binomial tests were used to evaluate if *A. laredoensis* B showed a significant avoidance of, or preference for, the lizard-odor burrow. Binomial tests were also run to check if *A. laredoensis* B showed a significant preference for either the right or the left half of the terrarium.

Three sets of trials were run on each of the 13 *A. laredoensis* B: (1) using an *A. gularis* male as the conditioning lizard; (2) using an *A. gularis* female as the conditioning lizard; and (3) using another *A. laredoensis* B as the conditioning lizard. The order in which the trials were conducted was varied among the 13 test lizards. Due to the accidental death of one *A. laredoensis* B,  $n = 12$  for the sets of trials involving *A. gularis*.

A limited fourth set of trials was run on five of the eight *A. laredoensis* B tested at McNeese State University. An *A. laredoensis* B was placed in one half of the divided terrarium and allowed to establish a burrow during the 48 hour conditioning period. This lizard was temporarily removed, the partition, food and water dishes removed, and then the conditioning lizard was returned to the undivided terrarium as the test lizard to see if it would choose its own (lizard-odor) burrow or the clean burrow. Results were again evaluated using a binomial test.

## RESULTS AND DISCUSSION

Analysis of all trials combined revealed that the *A. laredoensis* B test lizards chose the left side burrow 19 times and the right side burrow 18 times indicating the test lizards had no significant side preferences in this experiment ( $P$  of binomial test = 0.85). With respect to choice of burrow based on odor, *A. laredoensis* B exhibited neither a significant avoidance of, nor a significant preference for, the lizard-odor burrow as its overnight retreat (Table 1). Regardless of the identity of the lizard that conditioned the lizard-odor burrow, *A. laredoensis* B chose its burrow essentially at random. This suggests that odors of burrows alone are not a factor,

Table 1. Number of trials in which individuals of *Aspidoscelis laredoensis* B chose the Lizard-Odor Burrow versus Clean Burrow in burrow choice tests. The Lizard-Odor Burrow was conditioned with the odors of the Conditioning Lizard for 48 hours before the test; the Clean Burrow had no lizard odors. The *P* value is the result of a two-tailed Binomial Test.

Conditioning Lizard	Lizard-Odor Burrow Chosen	Clean Burrow Chosen	<i>P</i>
<i>A. gularis</i> male	7	5	0.58
<i>A. gularis</i> female	8	4	0.27
<i>A. laredoensis</i> B female	7	6	0.79

or at least are not the only factor, that *A. laredoensis* B uses in choosing a burrow.

The five trials in which an *A. laredoensis* B was made to choose between its own burrow and a clean burrow showed that test lizards chose their burrow twice and the clean burrow three times (*P* of binomial test = 0.69). This suggests *A. laredoensis* B does not prefer to use a previously occupied burrow, a result that agrees with a lab study that showed *A. laredoensis* B more often chose another lizard's burrow than its own as an overnight retreat (Paulissen 2002).

The results of this study, the lab study of Paulissen (2002), and field observations present the following overview of burrow choice in *A. laredoensis* B. When a lizard is seeking an overnight retreat and encounters a burrow entrance, it investigates the burrow by inserting its head in the entrance and flicking the tongue to discover if the burrow is already occupied. If the burrow is occupied, the lizard detects the occupant and moves off to locate another burrow. However, if the burrow is empty, the lizard moves into the burrow and claims it as its own, even if the burrow was recently occupied by another lizard and still bears the odors of the previous occupant. Clearly burrow odors alone do not convince an *A. laredoensis* B that a burrow is occupied and do not dissuade it from using a burrow. Furthermore, individuals of *A. laredoensis* B show no attachment to the burrows they used previously, being just as likely (or perhaps even more likely) to choose a clean burrow or another lizard's burrow than the one they used the night before. This is not surprising for a lizard that is not territorial and which wanders over a wide area during its

daily activity period, reducing the likelihood that it will be near the burrow it used previously. Since all other whiptail species are also non-territorial and wide foragers (Wright & Vitt 1993; Martins 1994), they may also be expected to show little preference for burrow choice (other than avoiding burrows that are already occupied) and little burrow site fidelity.

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MULTI-SCALE ASYNCHRONY AND SPATIAL STRUCTURING  
OF MESOPREDATOR ABUNDANCE TRENDS  
IN CENTRAL TEXAS, 1978–2003

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**Abstract.**—Trends in mesopredator abundance were examined at the regional, county, and local level in central Texas, 1978-2003, using data collected during annual spotlight surveys conducted by Texas Parks and Wildlife Department biologists. At the regional level, only raccoon (*Procyon lotor*) and gray fox (*Urocyon cinereoargenteus*) abundance exhibited significant trends. However, at the county and local level, ringtail (*Bassariscus astutus*), Virginia opossum (*Didelphis virginiana*), and skunks (*Mephitis mephitis* and *Conepatus mesoleucus*) also exhibited significant trends in abundance. Further, for all species except raccoon, direction of the trend varied among counties and survey routes, and among spatial scales. Results suggest that spatial asynchrony in mesopredator abundance trends may cause smaller-scale dynamics to be masked at broader scales. This could hide ecologically important effects. Thus, where sufficient data are available, abundance of mesopredator and their effects on other components of the ecosystem should be monitored and evaluated at multiple spatial scales.

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Mammalian carnivores are important components of terrestrial ecosystems (Estes 1996). In central Texas, most species of large carnivores (e.g., black bear [*Ursus americanus*] and gray wolf [*Canis lupus*]) were extirpated or greatly reduced in abundance during historic times (Davis & Schmidly 1994). However, the mesopredator community has remained relatively robust (Goetze 1995).

This paper focuses on mesopredators, mammalian carnivores of the orders Didelphimorphia and Carnivora generally weighing < 20 kg. Mesopredators can significantly influence other wildlife populations including neotropical songbirds (Heske et al. 2001), game birds (Miller & Leopold 1992; Rollins & Carroll 2001), small mammals (Henke & Bryant 1999; Hansson 2002), and reptiles (Christiansen & Gallaway 1984). They also may directly or

indirectly affect humans by causing property damage (Conover 2001) or by transmitting infectious diseases and parasites (Davis et al. 1981).

Public perception in central Texas is that some mesopredator species, especially raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*), have increased in abundance during recent years. If true, then increasing mesopredator abundance could have serious ecological effects. The initial objective, then, was to identify long-term trends in mesopredator abundance across central Texas from 1978 through 2003. Population trends that emerge at such broad scales, however, may not be representative of population dynamics at smaller spatial scales, because ecological patterns tend to be scale-dependent (Wiens 1989). Population changes at finer scales might be of different magnitudes, or even move in opposite directions, than those at broader scales. Such spatially structured asynchronous dynamics might partially or completely cancel each other and become masked when viewed at broad scales allowing potentially important ecological effects to go undetected. Therefore, carnivore population data were examined at three scales (regional, county, and more local) to identify population trends and possible spatial structure and asynchrony in mesopredator abundance.

#### STUDY AREA

Analysis was performed on mesopredator data collected in 38 central Texas counties (Fig. 1). The study region encompassed the Edwards Plateau as well the southern portion of the Cross Timbers ecological regions (Gould 1975). Topography ranged from rolling to steep, with mainly shallow rocky soils. Historically, the region was a grassland or open savannah, but woodlands and brushlands presently dominate (Wills 2005). Most of the region was rural, although three major cities occur at the periphery (Austin, Travis County; Del Rio, Val Verde County; San Angelo, Tom Green County).

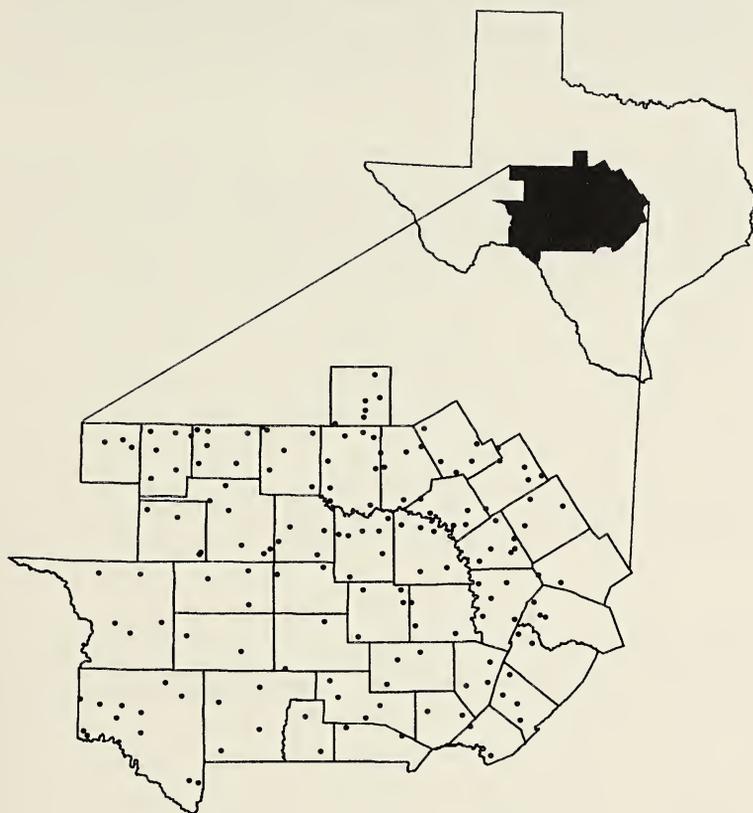


Figure 1. County map illustrating the central Texas study area. Circles indicate locations of the 192 spotlight-survey routes where mesopredator data were collected, 1978–2003.

## METHODS

*Data collection.*—From 1978 through 2003, Texas Parks and Wildlife Department conducted annual spotlight surveys to monitor mesopredator abundance. Spotlight counts have been used previously as indices of relative mesopredator abundance (Ryberczyk et al. 1981; Ralls & Eberhardt 1997; Panek & Bresinski 2002; Ruelle et al. 2002). Surveys were originally designed to monitor these species because of their significance as furbearing animals. To the authors' knowledge, this survey provides the only long-term contin-

uous set of broad-scale carnivore abundance data in Texas. Surveys were conducted throughout the region during August-October in conjunction with annual white-tailed deer (*Odocoileus virginianus*) surveys.

Permanent survey routes were situated along public and occasionally private roads in rural areas of each county. Although confined to roads and not randomly distributed, transects typically were situated to give even coverage across individual counties. Most, but not all, routes were 24.1-km long (8.0-25.7 km,  $\bar{x} = 23.3$  km,  $SD = 2.6$  km). Length of individual lines remained constant throughout 1978-2003. From 1978 through 2003, 192 routes were established in the study area (Fig. 1). Most routes were not surveyed continuously during this period, with 24-101 ( $\bar{x} = 72$ ,  $SD = 21.4$ ) being surveyed each year. Routes were surveyed once per year.

Surveys were conducted beginning approximately 1 hour after sunset. Surveys were not conducted when weather conditions (e.g., rain) might impede visibility. Two observers were situated on elevated seats in the back of a pickup truck that was driven along a survey route at 16-24 km/hr. Observers continually scanned the area adjacent to each side of the roadway to the extent of their vision using spotlights (100,000 cp) and recorded the total number of individuals observed of the following species: raccoon, ringtail (*Bassariscus astutus*), Virginia opossum (hereafter opossum; *Didelphis virginiana*), skunk (striped and hog-nosed [*Conepatus mesoleucus*] skunks were not differentiated), gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), coyote (*Canis latrans*), bobcat (*Lynx rufus*), badger (*Taxidea taxus*), mink (*Mustela vison*), spotted skunk (*Spilogale gracilis* and *S. putorius*), and domestic cat (*Felis domesticus*). A total of 2130 survey nights was conducted, 1978-2003.

Red fox, coyote, bobcat, badger, mink, and spotted skunk comprised only 1.6% of the total animals identified. Because of

their infrequent occurrence and because domestic cats were not recorded prior to 2000, these species were excluded from the analysis.

*Regional abundance.*—Annual regional abundance ( $S_{Ri}$ ) calculated for raccoons, ringtails, opossums, skunks, and gray foxes as the number of individuals encountered per kilometer on each survey route and averaged across all survey routes in the region as

$$S_{Ri} = \frac{\sum_{j=1}^n \left( \frac{x_{ij}}{l_j} \right)}{n_R},$$

where  $x$  = the number of individuals of species  $i$  sighted on survey route  $j$ ,  $l$  = the length of the survey route, and  $n_R$  = the number of routes surveyed throughout the region. To identify trends in regional abundance, a simple linear regression (Ott & Longnecker 2001) was performed of  $S_{Ri}$  against year for each species after confirming the residuals were normally distributed using a Ryan-Joiner test.

*County-level abundance.*—Annual county-level abundance ( $S_{Ci}$ ) of each species for each county in the region was calculated as

$$S_{Ci} = \frac{\sum_{j=1}^n \left( \frac{x_{ij}}{l_j} \right)}{n_C},$$

where  $n_C$  = the number of routes surveyed in county  $C$ . Results of a Ryan-Joiner test indicated that the residuals for some county data were not normally distributed. Therefore, a Spearman rank correlation of  $S_{Ci}$  against year was used to test for abundance trends in each county over time.

*Local abundance.*—Local-level abundance trends were assessed by analyzing long-term abundance along individual survey routes. Not all survey routes were suitable for long-term analysis because many routes were surveyed for only a few years or were surveyed only during the early or late years of the survey period. Hence data from these routes would not have been representative of the entire survey period and might have biased estimates of abundance trends. To ensure that only those routes that represented both the early and late years of the survey period were examined, the survey period was arbitrarily divided into two phases: 1976-1990 and 1991-2003. Only those lines that had been surveyed for  $\geq 9$  years (approximately 2/3 of the phase) during each phase of the survey period were selected for analysis. Annual local abundance ( $S_{Li}$ ) of each species along each of the 53 resulting survey routes (hereafter, “long-term routes”) was calculated as

$$S_{Li} = \frac{x_{ij}}{l_j}.$$

As with the county data, residuals were not normally distributed for some routes, so Spearman rank correlation of  $S_{Li}$  against year was used to test for abundance trends. For all statistical tests, results were considered significant where  $P \leq 0.10$ .

## RESULTS

*Regional abundance.*—Abundance of raccoons and gray foxes increased ( $r^2 = 0.45$ ,  $P < 0.001$  and  $r^2 = 0.26$ ,  $P = 0.008$ , respectively) across central Texas during 1978-2003 (Fig. 2). No trends in abundance were detected for ringtail, skunk, or opossum ( $P = 0.170$ - $0.838$ ).

*County-level abundance.*—All species exhibited trends in abundance at the county level for at least one county. Raccoon abundance increased in 15 of the 38 counties studied, and did not decrease in any county (Fig. 3a). Ringtail abundance increased in

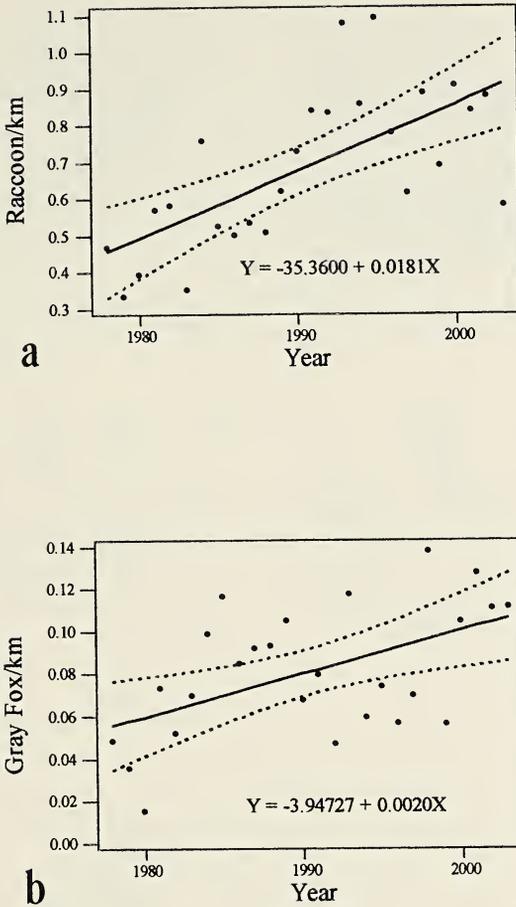


Figure 2. Raccoon (a) and gray fox (b) abundance throughout central Texas (Fig. 1), showing number of raccoons observed per kilometer on spotlight surveys, 1978–2003. Solid line is linear regression line and dashed lines indicate 95% confidence bands about the regression line.

seven counties but decreased in four others (Fig. 3b). Opossum abundance increased in four, but decreased in three counties (Fig. 3c). Skunk abundance showed the least variability among counties, increasing in two while decreasing in three counties (Fig. 3d). Finally, gray fox abundance increased in six counties, while decreasing in only one (Fig. 3d). In all, 27 counties (71% of counties studied) reported a significant change in abundance of at least one species.

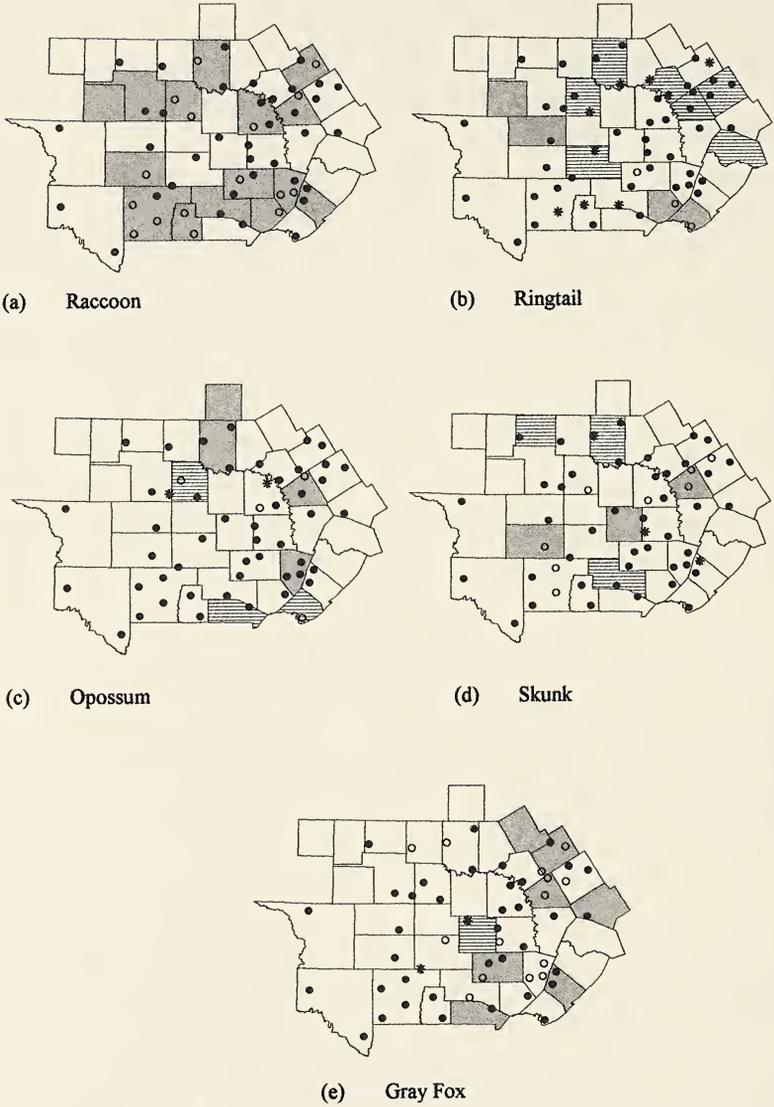


Figure 3. County- and local-level trends of raccoon (a), ringtail (b), opossum (c), skunk (d), and gray fox (e) abundance in central Texas, 1978–2003. Shaded areas indicate counties where abundance increased, cross-hatched areas indicate counties where abundance decreased, and white areas indicated counties where no trend was detected. Open circles indicate long-term survey routes where abundance increased, asterisks indicate routes where abundance decreased, and closed circles indicate routes where no trend was detected.

*Local abundance.*—At the survey-route level, raccoons again showed a consistent trend of increasing abundance. Raccoon abundance on 18 long-term routes significantly increased while decreasing on no routes (Fig. 3a). Three survey routes showed significant declines in ringtail abundance, but nine showed increases (Fig. 3b). Opossum abundance increased on four routes and decreased on two (Fig. 3c). Skunk abundance increased on eight routes, but decreased on three (Fig. 3d). Finally, for gray fox, 14 survey routes exhibited significant increases in abundance while two showed decreases (Fig. 3e).

## DISCUSSION

From 1978 though 2003, mesopredator populations in central Texas exhibited trends at the local and county level that often ran counter to those observed at the regional level. Most striking were the results for ringtail, opossum, and skunk, where county and local abundance trends were detected, but no trends were found at the regional level. In all, 23 counties (47%) exhibited abundance trends for at least one species that were undetected at the regional level. Moreover, 45% of long-term routes showed a trend for at least one species that was inconsistent with results for the region, whereas 45% exhibited a trend that was inconsistent with results for the county in which the route was located. This suggests that dynamics of mesopredator populations might be more complex than suggested by broad-scale trends alone, thus reinforcing the importance of addressing multiple spatial scales when investigating potential mechanisms driving carnivore population dynamics.

Inconsistency among spatial scales could lead to misunderstanding about the dynamics of mesopredator populations. As noted above, the public thought skunk abundance increased, whereas the analysis presented here showed no regional trend. Members of the general public, however, rarely are exposed to broad-scale population data. Instead, their observations occur at much smaller spatial scales that roughly correspond to the local-level analysis presented here. Trends in abundance were detected at

this spatial scale. Thus, controversies between the public and agency personnel could arise from what appear to be contradictory conclusions based on observation made at different spatial scales.

Silvy et al. (2000) analyzed furbearer spotlight data from the Edwards Plateau from 1980 through 1999, but failed to detect trends in mesopredator abundance. It is possible that the analyses presented herein were able to detect trends in raccoon and gray fox abundance because they (1) included data from the southern portion of the Cross Timbers as well as the Edwards Plateau, (2) were performed using a larger sample size (26 vs. 20 years), and (3) included several records not included in the summaries used by Silvy et al. (2000).

Studies conducted elsewhere in the United States also have reported broad-scale trends in mesopredator abundance. Using fur harvest data, Landholt and Genoways (2000) reported significant increases in raccoon abundance in Nebraska (1941-1997), while skunk numbers declined. Gehrt et al. (2002) used road-kill and spotlight-survey data to determine that raccoon abundance increased in Illinois (1975-1998).

Various authors have suggested explanations for long-term trends in mesopredator abundance. Rollins & Carroll (2001) hypothesized that declining demand for furs and the concomitant decline in furbearer harvest resulted in increased abundance of mesopredators, specifically raccoons. Conover (2001) also implicated low fur prices in an increase in raccoon population. These authors presented no evidence to support their claims.

Climate and weather have also been implicated as driving forces behind carnivore trends. For example, Dennis & Otten (2000) found that rainfall was a key variable for predicting San Joaquin kit fox (*Vulpes macrotis mutica*) abundance in California. Other carnivore species might be similarly impacted, probably due to

weather and climate effects on vegetation and, in turn, prey species such as rodents (Holmgren et al. 2001).

Landscape-scale habitat alteration also has been suggested as a causative mechanism for population changes in mesopredators (Rollins & Carroll 2001). Raccoons and gray fox are known to respond to landscape-scale phenomena (Pedlar et al. 1997; Dijak & Thompson 2000; Gehring & Swihart 2003), including habitat fragmentation (Oehler & Litvaitis 1996; Gehring & Swihart 2003), urbanization (Prange et al. 2003), rural residential development and human disturbance (Harrison 1997; Kuehl & Clark 2002), land-management practices (Chamberlain et al. 2002), and availability of free water (Gehrt & Fritzell 1998). These hypotheses offer fertile ground for future research.

Because mesopredator dynamics appear to be, in part, fine-scale phenomena, the effects of carnivore abundance on other components of the ecosystem also might manifest themselves at fine scales. For example, Rollins & Carroll (2001) suggested several possible explanations for the near continent-wide decline in northern bobwhite abundance (*Colinus virginianus*), including increased mesopredator numbers. Silvy et al. (2000) failed to find a correlation between mesopredator and northern bobwhite or scaled quail (*Callipepla squamata*) abundance in the Edwards Plateau of Texas. Because their analyses were conducted at only the regional scale, however, they were not able to address fine-scale relationships. It also is possible the bobwhite decline is the result of multiple factors operating at different scales in different physiographic regions.

Of course, other factors might operate to produce the patterns that were observed in this study. For example, sample error due to small sample size may have produced varying results across space and scales. Also, changes in vegetation both temporally and spatially may have altered the observability of mesopredators along survey routes, and perceived abundance patterns may have been an

artifact of this. These factors reinforce the necessity of critically evaluating abundance trends prior to using the data to justify management decisions.

These results also have implications for monitoring mesopredator populations. A standard Texas Parks and Wildlife Department procedure is to summarize carnivore data at the regional level. This analysis demonstrates that this practice masks small-scale effects, impeding efforts to identify trends in abundance at more local scales. Considerable information is lost when data are aggregated and analyzed at only the regional level. For this reason, it is recommended that natural resource agencies monitor mesopredator abundance and analyze data at multiple scales to better understand the dynamics of these populations.

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## CHARACTERISTICS OF A RINGTAIL (*BASSARISCUS ASTUTUS*) POPULATION IN TRANS PECOS, TEXAS.

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**Abstract.**—Despite the common occurrence of ringtails (*Bassariscus astutus*) few studies have been conducted to assess population characteristics. The objectives of this study were to determine (1) habitat selection, (2) home range, (3) denning characteristics, and (4) food habits of ringtails in the Trans Pecos region of west Texas. Seventeen ringtails were captured between November 1999 and January 2001 using Havahart live box traps. Second- and third-order habitat selection was determined for a ringtail population using range sites, slope, elevation, and vegetation communities. Diets were determined from volumetric scat analysis. The mean summer and winter range sizes (100% Minimum Convex Polygon [MCP]) for ringtails ( $n = 5$ ) were  $0.28 \pm 0.163 \text{ km}^2$  and  $0.63 \pm 0.219 \text{ km}^2$ , respectively. Overlap between ringtail ranges averaged 33.3%. Ringtails preferred catclaw (*Mimosa biuncifera*), persimmon (*Diospyros texana*), oak (*Quercus* sp.) bottom and catclaw/goldeneye (*Viguiera stenoloba*), sideoats (*Bouteloua curtipendula*) slope communities. Rock dens were used exclusively by ringtails, with 80.6% of dens found on slopes between 30-60%. Plant (seeds and miscellaneous vegetation) and animal material were found in 74.6 and 86.6% of scats, respectively. Findings suggest that ringtails in Trans Pecos, Texas, are an important component of the ecosystem and that management practices should conserve canyon habitats and adjacent slopes for ringtails.

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The ringtail is a common meso-carnivore in the southwestern United States and plays an important role in the ecosystem. Despite the common occurrence of this species, few studies have been conducted investigating its ecology. Many studies have focused on dietary information (Wood 1954; Toweill & Teer 1977; Trapp 1978; Alexander et al. 1994; Rodriguez-Estrella et al. 2000) and a few studies have focused on movements and activity patterns (Toweill 1976; Trapp 1978; Callas 1987; Yarchin 1994). However, little information exists on the ecology of ringtails in the Chihuahuan Desert. The purpose of this study was to describe ringtail ecology in the Trans Pecos eco-region of Texas. The

specific objectives of this study were to determine (1) habitat selection, (2) seasonal range size, (3) denning characteristics, and (4) food habits of ringtails in the Trans Pecos, Texas.

### STUDY AREA

This study was conducted on the Elephant Mountain Wildlife Management Area (WMA) located 41.9 km south of Alpine, Brewster County, Texas. Elephant Mountain WMA lies in the south-central portion of the Trans Pecos ecoregion. The topography of the study area consisted of a single igneous mountain (Elephant Mountain, 1,891 m elev.) with numerous canyons and washes. Average annual temperature from 1961-1990 for the Alpine, Texas region was 18.4°C. Average annual precipitation was 40.8 cm, with the majority of the precipitation occurring from June to September. Most precipitation was in the form of rain (USDA-NRCS 2000).

Elephant Mountain WMA lies between the Chihuahuan desert scrub and the desert grasslands, giving a mixture of vegetation types. Typical plants include creosotebush (*Larrea tridentata*), lechuguilla (*Agave lechuguilla*), sotol (*Dasyilirion* sp.), yucca (*Yucca* sp.), mariola (*Parthenium incanum*), and low native grasses. Ackerson (2001) lists a variety of fauna that can be found on Elephant Mountain WMA, including potential ringtail predators and competitors (e.g., coyote [*Canus latrans*], bobcat [*Lynx rufus*], gray fox [*Urocyon cinereoargenteus*], raccoon [*Procyon lotor*], striped skunk [*Mephitis mephitis*], and great-horned owl [*Bubo virginianus*]).

### MATERIALS AND METHODS

Trapping was conducted for 15 mo beginning in November 1999 by placing an average of eight Havahart live box traps (107 by 38 by 38 cm and 81 by 25 by 31 cm; Woodstream, Lititz, PA) 50 m apart in shaded areas where physical evidence suggested ringtail presence. Traps were baited with canned fish, set for six days, and

checked daily. Sampling effort was not equal for all months with most sampling occurring from November through March. No trapping was conducted from August through October 2000. Upon capture, ringtails were removed from traps and administered 0.07 cc tiletamine hydrochloride/zolazepam hydrochloride (Telazol®)/kg of body mass. Ringtails were observed until they recovered from sedation. Radiocollars with mortality sensors (Model 5902, Advanced Telemetry Systems, Isanti, MI) weighing 25 g were attached to ringtails. Sex and age class were determined for each individual. Then each individual was ear-tagged (Style 1005-4, National Band and Tag Co., Newport, KY), and various morphological measurements were taken. Ringtails were aged as juvenile or adult using crown-rump and ear and hindfoot length measurements (Richardson 1942; Toweill & Toweill 1978). All non-target species were released. All methods were approved by Sul Ross State University Animal Use and Care Committee.

*Habitat selection.*—A habitat map of Elephant Mountain WMA was produced using a digitized range site map (USDA-NRCS 1999) to determine second-order habitat selection (Samuel & Fuller 1996). Habitats around North Canyon and Double Windmill Canyon (core study area) of Elephant Mountain were further delineated using vegetation, elevation, and slope measurements (Ackerson 2001) for third-order habitat selection (Samuel & Fuller 1996). Vegetation characteristics were determined by delineating the communities using visual reconnaissance and a digital orthophoto quadrangle. Slope and elevation measurements were derived using a digital elevation model. A species list was then prepared for each community using the Braun-Blanquet method where each species within the community is assigned a rank (1-6) based on abundance and cover of the species (Smith 1996). Nomenclature of plants followed Hatch et al. (2001). Second- and third-order habitat selection was determined using simultaneous confidence intervals (Byers et al. 1984; Cherry 1998).

*Seasonal ranges.*—Ringtail locations were triangulated for radiocollared individuals at one randomly chosen time per day to determine seasonal ranges (Samuel & Fuller 1996). Coordinates of each ringtail location were determined and plotted on 7.5' USGS topographic maps. Seasonal ranges for each ringtail were determined using ArcView's Animal Movements Extension (Hooge & Eichenlaub 1997) using the minimum convex polygon estimator (100% Minimum Convex Polygon [MCP]); Mohr & Stumpf 1966) and adaptive kernel estimator (95%, 75%, and 50% ADK; Worton 1989). Data were separated into summer (April-September) and winter (January-March) seasons. Telemetry error was determined by triangulating and homing in on ringtail locations during daylight hours and using a global positioning system (GPS). The distance between the true and estimated locations was defined as the radial error.

*Den characteristics.*—Because ringtails are nocturnal, diurnal (day-use) sites were labeled den sites. Den sites were located by homing in on individuals during daylight hours (0800–1800 hrs) for four consecutive days to determine the number of consecutive days that dens were used. Dens were circled using telemetry equipment, flagged, and researchers then left the area to minimize disturbance. After ringtails left their den, measurements were taken including type of den, number of openings, size and direction of openings, internal size of den, vegetation, and slope. Den locations were marked using a GPS unit and imported into a GIS. Linear distance between consecutive den locations was measured in the GIS. Den use and characteristics were separated by seasons as defined prior. Rayleigh's test was used to determine if den openings were distributed uniformly around a circle (Zar 1999).

*Food habits.*—Ringtail scat was collected opportunistically, placed in plastic bags, marked with location and date, and frozen. Scat was determined to be ringtail based on size, smell, and location found (Elbroch 2003). In the lab, frozen scat was separated into categories (seed, arthropod, mammal, reptile, and other vegetation)

and the percent volume and frequency of each food category was determined. Scats were separated by the season they were collected. Reproductive season (15 March to 30 September) included breeding (March to April), parturition (May to June), and until the juveniles denned independently (September; Toweill 1976; Poglayen-Neuwall & Poglayen-Neuwall 1980). Non-reproductive season (1 October to 14 March) included the time when juveniles denned independently but remained in their maternal home ranges until the time of dispersal (Toweill 1976; Poglayen-Neuwall & Poglayen-Neuwall 1980). The difference in percent volume between seasons was determined using Mann-Whitney non-parametric test (Zar 1999).

*Population characteristics.*—Ringtail survival was determined using the Kaplan-Meier procedure with staggered entry (Pollock et al. 1989; White & Garrot 1990). Causes of mortality were determined by homing in on mortality signals. The immediate area was investigated for sign or cause of death and ringtails were necropsied to ascertain cause of death. A minimum population density was estimated using the minimum known number alive and applying that number to a buffer of 200 m on either side of the traplines (Ackerson 2001). Population size was estimated by extrapolating the number of animals based on canyon length and composition.

## RESULTS

Trapping occurred for a total of 983 trap nights (trap night = one trap open for one night) with 5.2% capture success (17 new captures and 34 recaptures). Ringtail sex ratio (male:female) was 1:0.96 and age ratio (adult:juvenile) was 1:0.23. Ringtail weights ranged from 0.7-1.75 kg ( $\bar{x} = 1.2 \pm 0.36$  kg,  $n = 17$ ). Non-target animals included gray fox, striped skunk, hog-nosed skunk (*Conepatus mesoleucus*), western spotted skunk (*Spilogale gracilis*), raccoon, and rock squirrel (*Spermophilus variegates*).

Table 1. Classification of slope and elevation for vegetation associations at Elephant Mountain WMA, Brewster County, Texas, 2000-2001.

Vegetation Community	Abbreviation	Slope (%)	Elevation (m)
Mesquite/black grama flat	MBGF	0-5	1,320-1,415
Catclaw/persimmon/black grama draw	CPBD	0-8	1,320-1,415
Catclaw/whitebrush flat	CWF	0-3	1,320-1,415
Catclaw/persimmon/oak bottom	CPOB	8-16	1,415-1,705
Catclaw/goldeneye/black grama slope	CGBS	3-16	1,320-1,415
Catclaw/whitebrush/sideoats slope	CWSS	16-30	1,415-1,705
Adolphia/whitebrush/sideoats slope	AWSS	16-30	1,415-1,512
Catclaw/goldeneye/sideoats slope	CGSS	≥ 16	1,512-1,705
Scrub oak/adolphia/sideoats slope	SOASS	≥ 16	1,415-1,801
Mountain laurel/sideoats slope	MLSS	≥ 16	1,512-1,705

*Habitat selection.*—There was 101 locations recorded for five ringtails in North Canyon and Double Windmill Canyon. The other 12 captured ringtails either died or slipped their collar before a substantial number of locations could be obtained. In this area of interest on Elephant Mountain, four range sites were found, Igneous Hill Mountain/Mixed Prairie, Igneous Hill Mountain/Desert Grassland, Gravelly/Mixed Prairie, and Foothill Slope/Desert Grassland. Ringtails preferred the Igneous Hill Mountain/Desert Grassland range site, with all 101 locations falling in this range site. Ten vegetative communities with associated slope and elevation were identified in North Canyon and Double Windmill Canyon (Table 1). Catclaw/persimmon/oak bottom and catclaw/goldeneye/sideoats slope communities were preferred by ringtails, and mesquite/black grama flat and scrub oak/adolphia/sideoats slope communities were avoided (Figure 1).

*Seasonal ranges.*—Sample sizes of ringtails in winter and summer were too small to compare seasonal ranges. However, winter ranges were larger than summer ranges using 100% MCP and 95%, 75%, and 50% ADK (Table 2). Using telemetry and den locations the mean summer range size at 100% MCP was  $0.28 \pm$

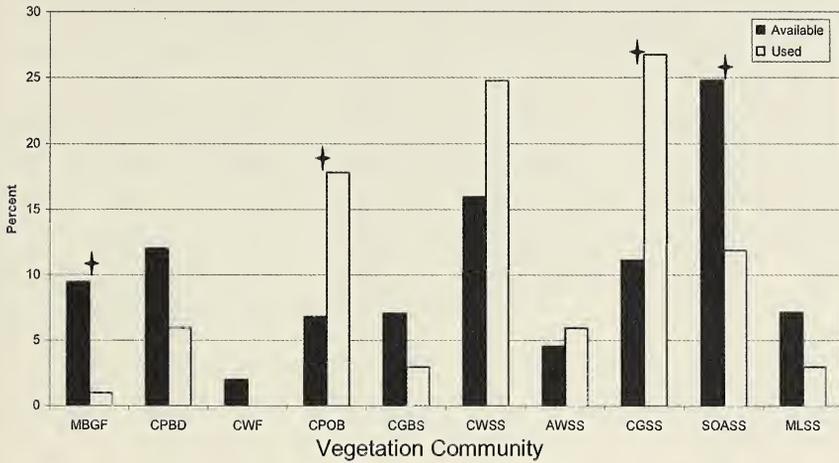


Fig. 1. Comparison between percent vegetation communities available and percent vegetation communities used within ringtail home ranges at Elephant Mountain WMA, Brewster County, Texas, 2000-2001. (+ Indicates significant difference between use and availability; MBGF = mesquite/black grama flat, CPBD = catclaw/persimmon draw, CWF = catclaw/whitebrush flat, CPOB = catclaw/persimmon/oak bottom, CGBS = catclaw/goldeneye/black grama slope, CWSS = catclaw/whitebrush/sideoats slope, AWSS = adolphia/whitebrush/sideoats slope, CGSS = catclaw/goldeneye/sideoats slope, SOASS = scrub oak/adolphia/sideoats slope, MLSS = mountain laurel/sideoats slope).

0.163 km<sup>2</sup>. The mean winter range size was  $0.63 \pm 0.219$  km<sup>2</sup>. Calculated telemetry error for 23 locations was  $178.2 \pm 129.22$  m.

All ringtail ranges in North Canyon overlapped to some extent (range = 17.5-46.6%) (Table 3). Only two pair of ringtails had overlapping ranges in the same season. All ringtails in North Canyon shared a common area of overlapping ranges (0.06 km<sup>2</sup>). Four vegetation communities (catclaw/persimmon/oak bottom, catclaw/whitebrush/sideoats slope, allthorn/whitebrush/sideoats slope, catclaw/goldeneye/sideoats slope), and two slope classes (16-30%, and 30-60%) occurred in this common area of overlap.

*Den characteristics.*—Measurements were taken from 40 different den sites that were located from April 2000-January 2001. Rock dens were the only type of den ringtails used at Elephant

Table 2. Seasonal range sizes (km<sup>2</sup>) calculated for ringtails at Elephant Mountain WMA, Brewster County, Texas, 2000-2001.

Individual <sup>a</sup>	<i>n</i>	Season	MCP <sup>b</sup>		ADK <sup>c</sup>	
			100%	95%	75%	50%
F02	24	Summer	0.400	0.221	0.054	0.263
F03	23	Summer	0.335	0.548	0.122	0.061
F18	12	Summer	0.091	0.143	0.076	0.036
F21	20	Winter	0.784	0.912	0.309	0.092
M22	17	Winter	0.474	0.493	0.117	0.050

<sup>a</sup>F = female, M = male<sup>b</sup>Minimum convex polygon<sup>c</sup>Adaptive kernel

Table 3. Percent overlap of ringtail home ranges in North Canyon, Elephant Mountain WMA, Brewster County, Texas, 2000-2001.

Individuals <sup>a</sup>	Overlap Area (km <sup>2</sup> )	Overlap (%)
F02.F03	0.171	46.56
F21-F02	0.187	31.51
F21-F03	0.233	41.58
M22.F02	0.096	21.96
M22-F03	0.113	17.53
M22-F21	0.249	39.65

<sup>a</sup>F = female, M = male

Mountain WMA. The mean length of stay in a den was  $2.2 \pm 4.62$  days (range = 1-27 days). The mean distance traveled between consecutive dens was  $186.4 \pm 199.61$  m (range = 0-580 m).

The mean area of den openings was  $290 \pm 245$  cm<sup>2</sup> (range = 120-1,023 cm<sup>2</sup>). The number of den openings per den ranged from 1-4 ( $\bar{x} = 2.1 \pm 0.75$ ). For dens with multiple openings, the distance between den openings ranged from 6.5-102 cm ( $\bar{x} = 79 \pm 27.8$  cm). Den openings were not distributed uniformly 360° ( $\bar{x} = 243 \pm 69.5^\circ$ ;  $r = 0.477$ ;  $P \leq 0.05$ ). Separating the den openings by canyon, both

North Canyon and Double Windmill Canyon dens were not distributed uniformly  $360^\circ$  ( $\bar{x} = 265 \pm 41^\circ$ ,  $r = 0.78$ ,  $P \leq 0.05$ , and  $\bar{x} = 155 \pm 62^\circ$ ,  $r = 0.56$ ,  $P \leq 0.05$ , respectively). The volume of ringtail dens ranged from 0.004-0.279  $m^3$  ( $\bar{x} = 0.074 \pm 0.069 m^3$ ). The slope surrounding dens averaged  $35.1 \pm 11.8\%$ , with 80.6% of ringtail dens occurring on slopes between 30-60%. Ringtail dens were located in six different vegetation communities, with dens primarily occurring in catclaw/persimmon/oak bottom and catclaw/goldeneye/sideoats slope communities.

*Food habits.*—Plant material in the form of seeds and miscellaneous vegetation was found in 50 of 67 scats, and animal material (mammals, arthropods, and reptiles) was found in 58 of 67 scats (Table 4). There was no difference in the percent volume of seed ( $P = 0.776$ ) or miscellaneous vegetation ( $P = 0.388$ ) in ringtail diets at Elephant Mountain WMA between seasons. Of the plants represented during the reproductive season, 52.2% of scats contained persimmon seeds. *Mahonia* sp., *Celtis* sp., *Juniperus* sp., *Ephedra* sp., *Sapindus* sp., and unidentified members of the Fabaceae and Cactaceae families were represented as seed in ringtail scats 47.3% of the time during the reproductive season. During the non-reproductive season all scats contained persimmon seeds, with only one other plant (a member of the Fabaceae family) occurring with a trace amount.

There was no difference in the percent volume of arthropods in ringtail diets at Elephant Mountain WMA between seasons ( $P = 0.589$ ). During the reproductive season, arthropods (Coleoptera, Orthoptera, Neuroptera, Scorpionida, Lepidoptera, Spirobolida, and Dermaptera) accounted for 69.0% of the animal matter. Arthropods were represented by four orders (Coleoptera, Orthoptera, Scorpionida, and Neuroptera) during the non-reproductive season, accounting for 63.9% of the animal matter. There was no difference in the percent volume of mammals present in ringtail diets at Elephant Mountain WMA between seasons ( $P = 0.862$ ). Sciuridae and Muridae families were represented as bones, hair, and other fragments in scats during the reproductive season accounting for

Table 4. Percent volume (V) and percent frequency of occurrence (F) of food categories found in 67 ringtail scats at Elephant Mountain WMA, Brewster County, Texas, 2000-2001.

Food Category	Season <sup>a</sup>					
	Reproductive ( <i>n</i> = 32)		Non-reproductive ( <i>n</i> = 12)		All seasons ( <i>n</i> = 67)	
	V	F	V	F	V	F
Vegetation	50.9	72.2	64.6	88.9	50.5	74.6
Seed	40.9	72.2	43.1	100	35.9	76.1
Misc. vegetation	9.9	42.6	21.5	66.7	14.6	46.3
Animal	49.2	87.0	35.4	77.8	49.4	86.6
Mammal	14.3	42.6	13.3	22.2	21.9	38.8
Arthropod	34.6	55.6	22.1	88.9	27.3	62.7
Reptile	0.3	5.6	0.0	0.0	0.2	4.5

<sup>a</sup> Reproductive season was 15 March – 30 September and non-reproductive season was 1 October – 14 March (Towell 1976).

37.9% of scats containing animal matter. Mammalian fragments were found in 30.0% of scats containing animal matter, with Muridae being the only family represented in mammals during the non-reproductive season. Reptiles (Eublepharidae and Iguanidae) were only found in the reproductive season in 10.4% of the scats.

*Population characteristics.*—Annual survival rate for ringtails at Elephant Mountain WMA was  $0.191 \pm 0.0990$  ( $n = 17$ ), with the majority of mortalities occurring in spring. Mortalities were recorded as avian ( $n = 3$ ), mammalian ( $n = 3$ ), and unknown ( $n = 1$ ). No mortalities were within two weeks of sedation, indicating no capture-related mortalities. The minimum population density for ringtails in North Canyon and Double Windmill Canyon from November 1999–November 2000 was 5.9 ringtails/km<sup>2</sup>. North Canyon had a higher density (6.25 ringtails/km<sup>2</sup>) than Double Windmill Canyon (3.42 ringtails/km<sup>2</sup>). Using density results and extrapolating to the canyon and draws of Elephant Mountain, the minimum population estimate on Elephant Mountain was 59 ringtails.

## DISCUSSION

Sex and age composition of ringtails in this study were similar to Trapp (1978), Toweill (1976), Callas (1987), and Yarchin (1994). The mean weight and morphological measurements were similar to previously recorded data from other studies in Texas (Toweill 1976; Toweill & Toweill 1978; Kortlucke 1984).

Differences in range size reported by Trapp (1978), Toweill & Teer (1982), Lacy (1983), Yarchin (1994) and this study were probably attributed to the method used, topography of study area, or resource abundance and distribution. Range size in this study using 100% MCP were larger ( $\bar{x} = 0.416 \text{ km}^2$ ) than reported by Toweill & Teer (1982) who relied on den locations only. In addition, central Texas has rolling hills, whereas Zion Canyon, Utah (Trapp 1978) or the canyons in west Texas have steep slopes that could create physical boundaries to ranges.

Both intersexual and intrasexual range overlap were found in this study, contrary to previous studies that found primarily intersexual range overlap (Toweill & Teer 1982; Lacy 1983). Even though ranges appear to overlap, some ringtail seasonal ranges did not overlap simultaneously. However, the two largest overlaps in ranges occurred when the ringtails were being monitored at the same time. One of these pair was a male and a female with overlapping ranges by 39.7%. Lacy (1983) found intersexual range overlap to be 51%. The largest overlap found in this study was between two females (46.6%). However, caution should be used when interpreting results from range overlap because of the small sample size in this study.

There was a common area of overlap that all radio-collared ringtails in North Canyon shared ( $0.06 \text{ km}^2$ ) dominated by catclaw/persimmon/oak bottom and the catclaw/goldeneye/sideoats slope communities with 16-60% slope. These two communities seem to be important for ringtails, as indicated by habitat selection results where catclaw/persimmon/oak bottom and catclaw/goldeneye/sideoats slope communities were preferred. Physical characteristics of the catclaw/persimmon/oak bottom community appear to be similar to Yarchin's (1994) and Toweill & Teer's (1982) riparian areas. The catclaw/

goldeneye/sideoats slope community appears to be similar to Trapp's (1978) blackbrush community because of the type of vegetation, but also because of the steep, boulder-strewn slopes of the community. Ringtails preferentially denned in the catclaw/goldeneye/sideoats slope community that has steep slopes. It is possible that ringtails forage on the slopes and canyon bottoms, but prefer to den on the slopes only. Ringtails have special adaptations that could allow them to escape predators and forage on these slopes better than other species. Slopes of the canyons may also provide additional food resources such as ephedra, *Opuntia* sp., and mast producing trees (i.e., Fabaceae).

Unlike previous studies, ringtails at Elephant Mountain WMA used only rock dens, with most dens occurring on the slopes of the canyon. Trapp (1978), Toweill (1976), Yarchin (1994), Lacy (1983), and Callas (1987) found ringtails use a variety of den types including rock dens, brushpiles, and tree dens. At Elephant Mountain WMA the riparian areas have trees and brushpiles. However, these areas have frequent flashfloods and potential competitors (foxes and skunks) were relatively abundant and able to traverse this terrain easily. Flashfloods may make these areas more dangerous and the competition from other species may make riparian areas less profitable to ringtails. The slopes, on the other hand, have little to no trees and brushpiles, but do provide cliffs, rock outcrops, and crevices that provide protection from predators. In addition, ringtails may use rock dens to maximize thermoregulation as suggested by Toweill (1976), Callas (1987), and Yarchin (1994).

Ringtails at Elephant Mountain WMA rarely used the same den consecutively, with a mean stay of 2.17 days. Ringtails in central Texas (Toweill 1976), California (Callas 1987), and Arizona (Yarchin 1994) seldom used dens consecutively. Toweill (1976) found ringtails in central Texas to have an average den stay of 1.58 days for males and 2.25 days for females, and Callas (1987) found that California ringtails never denned in the same den consecutively.

The mean distance between den sites reported for this study was smaller ( $\bar{x}$  = 186.36 m) than reported by Toweill (1976) ( $\bar{x}$  = 306 m).

This disparity could be due to the method used to calculate the distance. In this study, the distance was measured between consecutive dens using a straight route with GIS software and included dens that were used consecutively (i.e., distance = 0 m). With this method, less activity by females would be able to be detected. Dens may be more available at Elephant Mountain than in central Texas, where Toweill (1976) conducted his study. Greater availability of dens may decrease the distance required between dens.

Ringtail dens at Elephant Mountain WMA typically had openings facing down slope, which may be important in inclement weather (e.g., rain). North Canyon generally runs north-south with slopes facing east or west. Almost all dens were located on the west-facing slope and faced in a southwestern direction. Ringtails may have chosen this slope because of less human presence, climatic reasons, or habitat differences. The vegetation communities were different on the eastern and western slopes, where catclaw/goldeneye/sideoats slope community located on the western-facing slope of North Canyon was selected by ringtails. Ringtails in Double Windmill Canyon showed a preference for the north-facing slope, which contains the catclaw/goldeneye/sideoats slope community. Collectively, there were 18 den locations found in the catclaw/goldeneye/sideoats slope community.

Ringtails at Elephant Mountain WMA consumed more plant material in the non-breeding season and more animal material in the breeding season. Their foraging habits mirrored seasonal availability of food (Ackerson 2001). Similar trends in ringtail diets have been noted in previous studies (Wood 1954; Toweill & Teer 1977; Trapp 1978).

The survival estimates at Elephant Mountain WMA may have been lower than the actual survival due to the addition of radio-collars. However, no survival rates on ringtails have been previously published for comparison. Opossum (*Didelphis virginiana*) in Kansas (Kamler & Gipson 2004) had annual survival rates (0.02-0.21) lower than those found in the present study for ringtails. Compared to raccoons in Kansas (0.58-0.88; Kamler & Gipson 2004) and Texas (0.79-0.81; Gehrt & Fritzell 1999) annual survival rates for Trans Pecos

ringtails were much lower. Ringtails use dens with very small den openings. The battery of the radiocollar may have protruded from the neck and handicapped ringtails entering dens. In this study, there were a large number of ringtail mortalities in the spring, corresponding to dispersal and the breeding season. This may have increased a ringtail's exposure to predators. The primary predators in our study appear to be similar to those reported by Poglayen-Neuwall and Toweill (1988) and included great-horned owl, and to a lesser extent coyotes, raccoons, and bobcats.

In this study, the density of ringtails was greater than most previously reported (Toweill & Teer 1977; Trapp 1978; Yarchin 1994). Only Lacy (1983) reported densities greater than that reported for this study. Whereas, the ringtail density in Double Windmill Canyon is comparable to other studies, the density of ringtails in North Canyon was high. This may suggest a greater availability of resources in North Canyon than in Double Windmill Canyon.

Ringtails are important components of the Trans Pecos ecosystem. They provide food for larger predators, may impact arthropod and small mammal populations, and aid in seed dispersal. The canyons found in the Trans Pecos are an important area for ringtails where they use the canyon bottoms and the slopes for food and dens. Therefore, management practices should conserve the vegetation and structure of the slopes and bottoms of canyon habitats. Elephant Mountain WMA contains artificial water sources at several locations to provide water for wildlife. Although it would seem this management activity might benefit ring-tails, data from Black Gap WMA suggests that they do not frequent these water sources (only 4.7% of photographs from remote cameras contained ringtails) (Foster 2002). Instead ringtails may use pothole water and springs throughout much of the year and drink from artificial water sources only in low rainfall months. The results of this study suggest several possibilities for future research. First, the possible effects of radio-collars on ringtails and other species of similar size and behavior should be addressed. Second, additional data on survival rates of ringtail in a range of vegetation and habitats types are needed. Finally, ringtail food habits need to be related to productivity of an area.

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## GENERAL NOTES

DISCRIMINATION BETWEEN  
SMALL AND MEDIUM-SIZED SPECIES OF  
WEST TEXAS POCKET MICE (*PEROGNATHUS*, *CHAETODIPUS*)  
BY CHARACTERS OF THE LOWER DENTITION**Jennifer A. Craighead and Frederick B. Stangl, Jr.***Department of Biology, Midwestern State University,  
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Pocket mice (*Perognathus* and *Chaetodipus*) of the rodent family Heteromyidae are comprised of species generally adapted to arid and semi-arid regions of the western United States and Mexico. *Perognathus* are generally diminutive forms with silky pelages, while *Chaetodipus* are comparatively moderate to large in size with coarse pelages. Several species of pocket mice are known to occur in the Chihuahuan Desert region of Trans-Pecos Texas, and their ranges have been well documented in the state during the past few decades (e.g., Genoways et al. 1979; Wilkins & Schmidly 1977; Stangl et al. 1994; Yancey 1997; Yancey & Jones 2000; Schmidly 2004).

Pocket mouse remains are commonly represented in the regurgitated pellets of owls, which often litter the vicinity of roosting owls. However, the fragile skulls rapidly disintegrate by digestive processes, leaving the lower mandibles as the most readily recognizable skeletal remain. The hypsodont molars of the lower jaws often fall out of the alveoli, but the splayed roots of the p4 and the long arc of the incisor commonly permit those teeth of *Perognathus* and *Chaetodipus* to stay in place.

Disparity in lower jaw size permits easy separation into three size classes: the large *C. hispidus*, three medium-sized *Chaetodipus*, and two diminutive *Perognathus*. Large size prevents confusion of the hispid pocket mouse and any of its anatomical components with

smaller pocket mice. However, discrimination of comparable-sized species of *Perognathus* (*P. flavus*, *P. flavescens*) and *Chaetodipus* (*C. intermedius*, *C. eremicus*, *C. nelsoni*) can be difficult, especially when relying on the isolated jaws and disintegrated cranial remains in owl pellets. This study provides a means to distinguish these heteromyid taxa, which should prove useful both to those interested in the feeding habits of owls, and to those supplementing their own field collecting efforts with owl pellet analyses.

A total of 145 adult specimens of *Perognathus* and *Chaetodipus* from the collections of Midwestern State University (MWSU) and Texas Tech University (TTU) were examined and measured under a 10x jewelers' lens. Sufficient samples of each taxon were available from Trans-Pecos Texas, with the exception of *P. flavescens*, for which Texas Panhandle specimens were included in the analysis.

*Material examined.*—Following is a list of western Texas localities, sample size, and catalog numbers for specimens of pocket mice from the collections of Midwestern State University (MWSU) and Texas Tech University (TTU) used in this study.

*Perognathus flavescens* ( $n = 24$ ).—ANDREWS CO.: 1 mi SE of Frankel City, 1 (TTU 55327); 8.5 mi S of Andrews, 2 (TTU 56823, 56824); 9.5 mi S of Andrews, 1 (TTU 52865); 6 mi S of Andrews, 2 (TTU 52328, 52329); 4 mi N of Andrews, 2 (TTU 56821, 56822). WARD CO.: 2.4 mi S of Peyote, 1 (TTU 18308); 4.7 mi W of Wickett, 1 (TTU 18309). WINKLER CO.: 8.5 mi SE of Kermit, 3 (TTU 7055-7057); 6.5 mi SE of Kermit, 2 (TTU 655, 7839); 1 mi NW of Winkler, 1 (TTU 7838). WILBARGER CO.: 17 mi NW of Vernon, 1 (MWSU 3483). ROBERTS CO.: 6 mi N of Miami, 5 (MWSU 2457, 19613, 19640, 19641, 19650). HEMPHILL CO.: 7 mi NE of Miami, 2 (MWSU 7046, 19623).

*Perognathus flavus* ( $n = 21$ ).—CULBERSON CO.: 28 mi S of Pine Springs, 1 (MWSU 16909); 26 mi S of Pine Springs, 8 (MWSU 16908, 16910, 16911, 16934, 16961, 17251, 17254, 17255); 25 mi S

of Pine Springs, 2 (MWSU 13305, 16973); 22.1 mi S of Pine Springs, 4 (MWSU 16933, 16935, 17252, 17253); 7 mi N of Kent, 2 (MWSU 18081, 18082); 6 mi W of Kent, 1 (MWSU 13052); 3.5 mi S of jct. Hwys. 54 & 180, 1 (MWSU 17250); jct. Hwys. 2424 & 2184, 1 (MWSU 17249); 24.5 mi NE of Van Horn, 1 (MWSU 13420).

*Chaetodipus intermedius* ( $n = 15$ ).—CULBERSON CO.: 2 mi SSE of El Capitan, 7 (MWSU 13465, 13466, 14638, 17952, 18413, 18414, 18471); 21 mi S of Pine Springs, 1 (MWSU 16995); 25 mi S of Pine Springs, 3 (MWSU 16907, 16974, 17266); 25 mi NE of Van Horn, 3 (MWSU 12823-12825); 4.8 mi SW of jct. Hwys. 2185 and 2424, 1 (MWSU 13464).

*Chaetodipus nelsoni* ( $n = 30$ ).—BREWSTER CO.: Frog Canyon, Black Gap Wildlife Management Area, 10 (MWSU 19827-19829, 19887, 20346-20351); 26 mi SSE of Stillwell, 2 (MWSU 19260, 19261) Heath Canyon Ranch, Lalinda, 2 (MWSU 19649, 19650); Terlingua ghost town, 1 (MWSU 8318). CULBERSON CO.: 3.3 mi NNW of Van Horn, 4 (MWSU 18434, 18475, 18476, 18538); 3.5 mi S of jct. Hwys. 54 and 180, 1 (MWSU 17260). JEFF DAVIS CO.: 2 mi NW of Fort Davis, 1 (MWSU 8319); 16 mi NE of McDonald Observatory, 1 (MWSU 11557). PRESIDIO CO.: 13 mi E of Presidio, 1 (MWSU 8314); 30 mi of SSE Redford, 2 (MWSU 8315, 8317); Bandera Ranch, Bandera Mesa, 4 (MWSU 6028, 6903, 10877, 10878). VAL VERDE CO.: 9 mi N of Comstock, 1 (MWSU 8319).

*Chaetodipus eremicus* ( $n = 55$ ).—BREWSTER CO.: Big Bend National Park, 2 (MWSU 5085, 5317); Black Gap Wildlife Management Area, 2 (MWSU 19830, 20345). CULBERSON CO.: 6 mi E of Pine Springs, 2 (MWSU 16932, 16975); 26 mi S of Pine Springs, 1 (MWSU 16994); 28 mi S of Pine Springs, 5 (MWSU 17261-17265); 22 mi N of Van Horn, 2 (MWSU 17257, 17258); 16 mi SW of jct. Hwys. 2185 and 2184, 1 (MWSU 13467); 3.5 mi S of jct. Hwys. 54 and 180, 2 (MWSU 17256, 17259). EL PASO CO.: 15 mi E of Horizon City, 5 (MWSU 9536-9538, 9541, 9542).

HUDSPETH CO.: Fort Hancock, 4 (MWSU 9534, 9535, 9539, 9540). PRESIDIO CO.: San Jacinto Mountains, 1 (MWSU 10388); Bandera Ranch, Bandera Mesa, 1 (MWSU 6906); 1 mi E of Bandera Mesa, 11 (MWSU 8303-8308, 8311-8313, 8316, 8666); 30 mi SSE of Redford, 2 (MWSU 8302, 8320); 55 mi SSE of Marfa, 1 (MWSU 16678); 13 mi E of Presidio, 1 (MWSU 8309); 3 mi E of Presidio, 1 (MWSU 8310); Plata, 2 (MWSU 6904, 6905). WARD CO.: 10 mi W of Monahans, 8 (MWSU 11684, 17432, 17946-17951); 2.3 mi E of Peyote, 1 (MWSU 17444).

Adult status of each specimen was determined by the presence of a fully erupted p4. The p4 was measured as the greatest width of the crown. The lower incisor was taken as the greatest width at the point where the tooth emerged from the alveolus. Measurements were taken with digital calipers to the nearest 0.01 mm. Descriptive statistics and analyses were accomplished with the NCSS statistical package (Hintze 1990).

The lower incisor width is the single most useful character in discriminating between congeners, and p4 width is also useful in separating species of *Chaetodipus* (Table 1). Some degree of overlap characterizes the three species of *Chaetodipus*, although each taxon comprises a statistically significant subset for both premolar and incisor measurements. Among the three, *C. nelsoni* averages consistently smaller for each character.

Overlap of measurements among *Perognathus* is more extensive, although *P. flavus* averages smaller than *P. flavescens* for both dental characters. The extent of overlap of measurements of the two taxa, and larger coefficient of variation for the lower incisor of *P. flavus*, might reflect the inclusion of *P. merriami* (*sensu* Lee & Engstrom 1991; Schmidly 2004), or that the silky pocket mouse is simply a more variable species for dental characters examined in this study.

Table 1. Variation of mandibular tooth measurements (in mm) for two species of small pocket mice (*Perognathus*) and three species of medium-sized pocket mice (*Chaetodipus*) from western Texas. Descriptive statistics are sample size (*n*), mean, standard deviation (*SD*), range, 95% confidence interval (*C.I.*), and coefficient of variation (*C.V.*). Letters for Duncan's Multiple Means Test indicate statistically significant subsets at  $P \leq 0.05$ .

Species ( <i>n</i> )	Mean $\pm$ <i>SD</i>	Range	95% <i>C.I.</i>	<i>C.V.</i>	Duncan's
<i>Perognathus</i> incisor width*					
<i>P. flavescens</i> (24)	0.43 $\pm$ 0.04	0.37-0.53	0.42-0.45	9.30	A
<i>P. flavus</i> (21)	0.39 $\pm$ 0.07	0.25-0.47	0.36-0.43	17.95	B
<i>Perognathus</i> premolar width					
<i>P. flavescens</i> (24)	0.55 $\pm$ 0.06	0.42-0.70	0.53-0.57	10.91	A
<i>P. flavus</i> (21)	0.53 $\pm$ 0.06	0.44-0.62	0.50-0.56	11.32	A
<i>Chaetodipus</i> incisor width**					
<i>C. eremicus</i> (55)	0.52 $\pm$ 0.04	0.43-0.63	0.51-0.53	7.69	A
<i>C. intermedius</i> (15)	0.49 $\pm$ 0.03	0.42-0.52	0.48-0.51	6.12	B
<i>C. nelsoni</i> (30)	0.46 $\pm$ 0.03	0.41-0.54	0.45-0.47	6.52	C
<i>Chaetodipus</i> premolar width**					
<i>C. intermedius</i> (15)	0.84 $\pm$ 0.04	0.77-0.88	0.82-0.86	4.76	A
<i>C. eremicus</i> (55)	0.79 $\pm$ 0.03	0.70-0.85	0.78-0.79	3.80	B
<i>C. nelsoni</i> (30)	0.74 $\pm$ 0.06	0.62-0.85	0.72-0.76	8.11	C

One-way *MANOVA*: \*  $P \leq 0.05$ ; \*\*  $P \leq 0.001$ .

There are several excellent sources that aid in discrimination of superficially similar species of western Texas pocket mice (e.g., Schmidly 1977; Wilkins & Schmidly 1979). This study supports the conclusion that measurements of the p4 and lower incisor provide additional useful supplementary tools in discriminating among species of *Perognathus* and *Chaetodipus*, and especially where

remains are partial or fragmentary. Application to Pleistocene and Holocene materials might also prove informative.

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\* \* \* \* \*

REPORT OF A SECOND FEMALE SPECIMEN  
OF *PLENOCULUS GILLASPYI* KROMBEIN  
(HYMENOPTERA: CRABRONIDAE)

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While identifying digger wasps of the genus *Plenoculus* at the Brackenridge Field Laboratory Insect Collection at the University of Texas at Austin, a single female was noted that readily keyed to *Plenoculus gillaspyi* Krombein (Williams 1960). Krombein (1938) described this species from a single female collected in 1935 by James E. Gillaspy. Krombein (1938) gave the label information as “♀, Round River, Williamson Co., Texas, Oct. 10, 1935, (J. E. Gillaspy, on *Baccharis salicina*)”. Gillaspy provided this specimen with three labels: (1) state, date, and collector, (2) plant it was collected on (*Baccharis salicina*), and (3) a hand written label saying “Round R.”. Krombein (1938) interpreted “Round R.” as an abbreviation for “Round River”. However it actually represents Round Rock (Gillaspy, pers. comm.), a city located about 20 km north of the current city limits of Austin, Texas (also, there is not a “Round River” in Williamson County, nor anywhere in Texas).

The holotype of *P. gillaspyi* (loaned by David Furth, Collections Manager, Entomology, of the National Museum of Natural History (USNM), Smithsonian Institute) examined by the author, undoubtedly represents the second known collection of a female of this species. This female is smaller than the type (3.7 mm vs. 4.7 mm long) and its pygidium is more highly polished with shagreening limited to its edges, instead of the entire surface. This second female bears the following label information: TEXAS Val Verde County, Devils R. Dolan Falls, 15-26 March 1995, malaise trap, C. R. Nelson. Riley Nelson and the author ran malaise traps (1994-1996) as part of an insect survey for the Nature Conservancy

of Texas at their Dolan Falls Preserve, on the Devils River. The Dolan Falls Preserve is located at the junction of three biotic provinces (after Blair 1950), the Balconian, Chihuahuan, and the Tamaulipan, whereas Round Rock is roughly the northeastern boundary of the Balconian province. The two localities are separated by 334 km.

The Insect Collection of Texas A&M University was examined to determine if any sorted or unsorted specimens of this species could be located, but none were found. The male continues to remain unknown for this species. Given that the holotype has some damage, the present specimen has been deposited in the USMN where it resides with the holotype.

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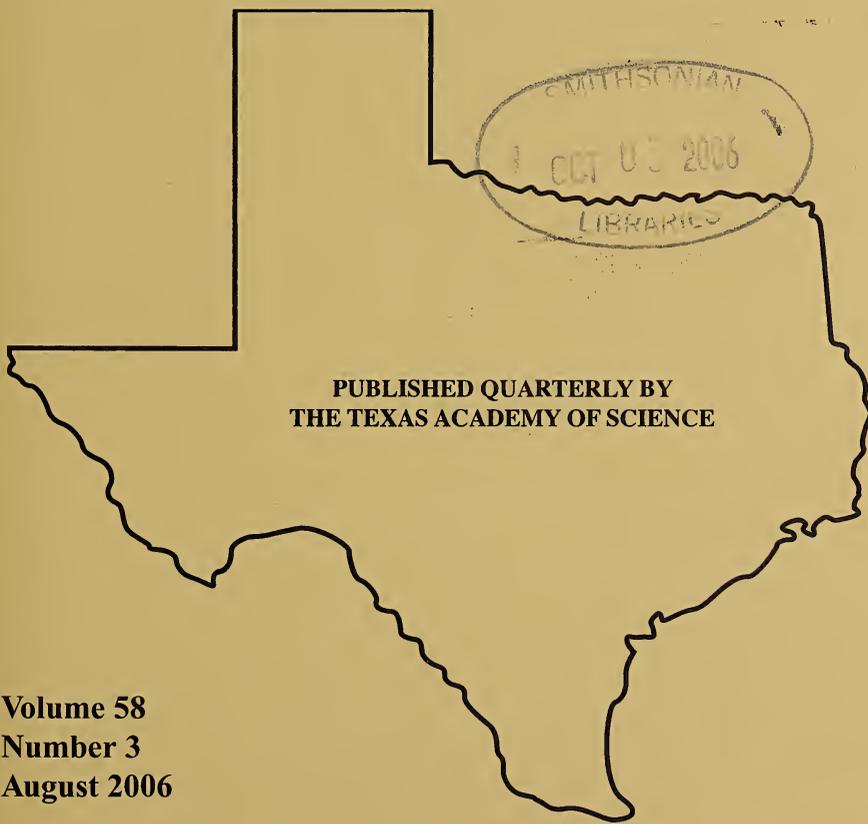
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## GROWTH AND MYCORRHIZAL INFECTION OF TWO ANNUAL SUNFLOWERS WITH ADDED NUTRIENTS, FUNGICIDE OR SALTS

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**Abstract.**—*Helianthus paradoxus* and *H. annuus*, two annual sunflowers, form symbiosis with arbuscular mycorrhizal fungi (AMF). This relationship and its influence on the growth and dry mass of the two sunflowers was examined. Time of growth, nutrient status, reduction of mycorrhizal fungi, and soil salinity were the experimental treatments. Their effects on the infection of the roots and the above and belowground dry mass of both species were the response variables. Plants were grown in a native Patrick series Mollisol and the plants were colonized by native AMF. *Helianthus annuus* and *H. paradoxus* mycorrhizal infection was relatively stable at  $49.7 \pm 1.9\%$  over ten weeks. When nutrients were increased in a separate experiment, the mean percent mycorrhizal infection of both species dropped from  $51.0 \pm 1.4\%$  to  $33.3 \pm 0.7\%$ , but dry mass increased. Total dry mass of *H. annuus* increased 12.3 times to  $18.35 \pm 2.61$  g/pot and for *H. paradoxus* it increased 3.1 times to  $4.15 \pm 0.51$  g/pot compared to no added nutrients. Reducing or eliminating the mycorrhizal fungi from the soil with heat sterilization or fungicide did not have a significant deleterious effect on growth in either species. Both species appear to be facultative mycotrophs. At increased levels of soil salinity, *H. paradoxus* mean percent mycorrhizal infection increased, but the infection in *H. annuus* decreased. *Helianthus paradoxus* showed greater survivorship at the highest levels of salinity. However, above and belowground dry mass of both species decreased with increased soil salinity.

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The relationship between plants and fungi is an ancient one. Many have suggested that the adaptation of plants to a terrestrial habitat took place at least in part because of this association (Pirozynski & Malloch 1975; Trappe 1987; Carlile 2001). The importance of this relationship can be realized by looking at its widespread occurrence, with 95% of all terrestrial plant families having species with mycorrhizal associations (Trappe 1987). These associations are usually described as symbiotic, and are typically mutualistic, but sometimes they can be parasitic (Allen 1991). The fungal partner induces the plant host to provide carbohydrates, and in turn provides nutrients to the plant, primarily in the form of

phosphorous and nitrogen absorbed via fungal hyphae in the soil (Carlile 2001).

The number and breadth of recent studies also indicates the importance of the mycorrhizal symbiosis (Rillig 2004). The fungal-plant association is pivotal in understanding plant mineral nutrition and thus puts this association at the interface between the abiotic and biotic environment. This is a critical point in understanding both natural and agricultural ecosystems, because soil resources limit primary productivity in most ecosystems (Chapin et al. 2002). In addition, mycorrhizal fungi appear to be responsive to many ecosystem level factors including global change phenomena, agricultural practices and various types of pollution including trace metal and nitrogen deposition (Rillig 2004). Some plants will grow very poorly unless the soil is inoculated with mycorrhizal fungi and many plants infected with mycorrhizal fungi show an increase in growth in phosphorous-poor soil (Rovira & Bowen 1966; Salisbury & Ross 1992; Carlile 2001). Some studies have indicated that mycorrhizal fungi may play a role in soil remediation, and affect the response of plants to different environmental factors, such as aridity and salinity (Hirrel & Gerdemann 1980; Allen 1982; Mathur & Vyas 2000).

If AMF (arbuscular mycorrhizal fungi) can confer resistance to the effects of saline soils in some plants, it could have tremendous importance for crop production. Salinity is the most widespread soil constraint for agricultural crops (Jindal et al. 1993), and understanding the role of mycorrhizal symbiosis in increasing crop yields could have real benefits. Understanding the role these symbioses play in non-agricultural species subject to stressful abiotic and biotic conditions may also prove important in understanding the biology and ecology of these plants.

The fungi that form these symbioses with plants are members of the class Glomeromycota (Schuessler et al. 2001). There are perhaps 200 species of fungi worldwide that form these associations and they are not highly species specific (Raven & Eichhorn 1999).

The fungal mycelium forms intracellular arbuscules and vesicles for nutrient exchange, and runner hyphae in the soil for absorbing nutrients (Bago 2000). It is assumed that the arbuscules serve as the location of nutrient exchange between the plant and the fungus (Allen 1991). The fungal partner apparently causes selective membrane leakage of hexose sugars, while phosphorous and other nutrients obtained from the soil pass from the arbuscules to the plant cell (Carlile 2001). In later stages of infection, vesicles form, for lipid and sometimes glycogen storage (Bowen 1987).

In addition to providing phosphorous, mycorrhizal fungi have been shown to increase the growth of some plant species in saline soils (Hirrel & Gerdemann 1980; Jindal et al. 1993; Pfeiffer & Bloss 1988). Amelioration of effects due to increased salinity may be simply due to improved phosphorous nutrition, or may involve mechanisms as yet unknown. Research in the area of salinity tolerance has focused mainly on wetland species and food crops. It is possible that AMF regulate osmotic effects by providing increased water flow-through (Allen 1982). Adding soluble phosphate to the soil has a similar effect to inoculating plants with AMF (Pfeiffer & Bloss 1988; Jindal et al. 1993). Whether it is solely increased nutrition that benefits plants or how the effect carries across species is unclear. Arbuscular mycorrhizae seem to confer a greater salt tolerance and promote growth more than phosphate alone, suggesting that some mechanism beyond phosphate nutrition might be involved. On the other hand, not all species of plants respond positively. For example there was little difference in dry mass between *Distichlis spicata* (saltgrass) plants infected with AMF and those without when grown at three different salt levels (Allen & Cunningham 1983). In addition, no interaction at all between the presence of AMF and salinity in citrus seedlings was found (Graham & Syvertsen 1989).

The current study focuses on two species of annual sunflowers, *Helianthus paradoxus* and *H. annuus*, both are tap-rooted member of the Asteraceae (Compositae or sunflower family, Correll & Johnston 1979). Sunflowers of the genus *Helianthus* are annual or

perennial herbaceous plants and they are found throughout the world (Heiser 1965; Heiser et al. 1969). They are found in disturbed habitats, early successional communities and various grasslands (Weaver 1965; Sims 1988). They are also found in savannas and woodlands, not usually under the canopy, but in openings or gaps. In forests they would be found in gaps or along the edges of openings. As such, they seem to be sun plants, requiring higher levels of solar radiation rather than the shade of the understory (Bohning & Burnside 1956; Fay & Knapp 1996). However, the authors have not found any studies of potential mycorrhizal associations with members of the genus *Helianthus*.

*Helianthus paradoxus* (Puzzle sunflower or Pecos sunflower) is a species of hybrid origin (Rieseberg et al. 2003) that grows in saline, wet or marshy soils in three locations in Texas and 22 in New Mexico (Van Auken & Bush 1998; McDonald 1999). All of these locations lie along the drainages of the Rio Grande and Pecos Rivers. *Helianthus annuus* is the common sunflower and one of the parent species of *H. paradoxus* (cf. Abbott 2003; Rieseberg et al. 1990; 2003), and varieties are used in cultivation around the world. It is found all across North America, typically on heavy, clay soils. *Helianthus paradoxus* is more tolerant of soil salts than *H. annuus* (cf. Mendez 2001; Welch & Rieseberg 2002) and can out-compete its parental species and other competitors in slightly salty soils (Bush & Van Auken 2004; Van Auken & Bush 2006).

The main purpose of this research was to examine the importance of the mycorrhizal fungal symbiotic relationship in two annual sunflowers, *H. annuus* and *H. paradoxus*. The consequence of the mycorrhizal symbiosis in the two species and how this symbiosis affects the growth of both species through time, with increased soil resources and at elevated salt levels was examined.

#### MATERIALS AND METHODS

Four experiments were carried out in a fiberglass greenhouse on the campus of the University of Texas San Antonio. Experiments

were conducted to examine the effects of time of growth, nutrient availability, salinity, and the presence of mycorrhizal fungi on the above ground dry mass, root ash-free dry mass and percent mycorrhizal infection of *H. annuus* and *H. paradoxus*. Daytime temperatures in the greenhouse were between 21°C and 32°C over the course of the experiments. Mid-day light levels were  $562 \pm 135 \mu\text{mol}\cdot\text{m}^2\cdot\text{s}^{-1}$  measured as photosynthetically active photon flux density (PPFD 400 to 700 nm) using a LI-COR<sup>®</sup> LI 190 SA integrating quantum sensor (LI-COR Inc., Lincoln, Nebraska). The PPFD was 36.4% of outdoor levels (Van Auken & Bush 1995).

Seeds of *H. annuus* were collected in northeastern Bexar County, Texas, during July and August of 1998. Seeds of *H. paradoxus* were collected at the Diamond Y Spring Preserve in Pecos County, Texas during October and November 1998. After collection, seeds were stored at 4°C until needed. Seeds were germinated in deionized water in paper-lined trays covered in clear plastic at approximately 25°C (Van Auken 2001). For each species, pots were initially planted with eight plants per pot. Plants were thinned after one week to five plants per pot but all plant material per pot was pooled at harvest.

The experiments utilized the top 20 cm of a Patrick series soil, a Mollisol classified as clayey over-sandy, carbonatic-thermic, typic calciustoll (Taylor et al. 1966; United States Department of Agriculture 2000). Soil was collected from a grassland in northern Bexar County, Texas, sifted through a 6.4-mm mesh screen, air-dried, mixed, and stored in plastic containers until used. The soil was friable, allowing for root recovery and supported the growth of both species. In addition, the soil was low in carbon (5-10 g/kg), nitrogen (1.0 mg/kg), phosphorus (12.0 mg/kg) and other nutrients (Van Auken et al. 1992). Plastic pots that were 15 cm in diameter by 15 cm deep, lined with 3.8 L plastic bags to prevent nutrient, salt and water loss were used in all experiments. Pots were filled with 1400 g of the air dried soil and watered thoroughly with deionized water at planting and then with approximately 150 mL as needed,

usually every other day. Plants were colonized by native AMF that were present in the Patrick series soil.

When harvested, plant above ground parts were clipped at the soil surface, placed in perforated aluminum foil and dried at 90°C to a constant mass. Roots were gently washed free of soil and then treated as above. The presence of mycorrhizae was detected by staining approximately 1.0 g of fresh root material, 0.3 g from the lower, middle, and upper part of the root (in cases where the root mass was less than 1.0 g, the entire root was used). Ash-free below ground dry mass was determined by drying the remaining roots to a constant mass at 90°C, weighing, ashing at 600°C for 3 h, reweighing, and subtracting the inorganic component (Böhm 1979).

Mycorrhizae were stained with Trypan Blue for visualization (Phillips & Hayman 1970). Root samples were washed and clipped to 1.0 cm lengths and then placed in 10% KOH at 90°C for 20 min for clearing. Samples were then decanted through a 1-mm fine mesh strainer and rinsed with 50 mL of distilled water. Root samples were next placed in 10% HCl for 10 min and then decanted through a strainer and stained for 10 min in 0.05% Trypan Blue at 90°C. Samples were de-stained in 85% lactic acid for 20 min at 90°C and then stored in 85% lactic acid until viewing.

The gridline-intercept technique was used to determine the percent root infection (Giovannetti & Mosse 1980). Stained root samples were placed in a Petri dish scored with 100 intersections (1.0 cm apart) and then evenly distributed. Each intersection was searched for the nearest root segment. The optical pointer in the eyepiece of the microscope was placed on the root segment, and the cell directly below the pointer was examined at 100X magnification. The microscope was focused through the entire root segment and if an arbuscule or vesicle was detected, the root segment at the intersection was scored as infected. The number of points with an arbuscule or vesicle in the cells examined was converted to percent infection.

The response variables, including above ground dry mass, ash-free root mass, and percent mycorrhizal infection, were analyzed separately as full-factorial analyses of variance (ANOVA, SAS Institute 1989). Percent data was arcsin transformed prior to analysis. The main effects were species (*H. annuus* and *H. paradoxus*) and time of growth or nutrient level or salinity or the presence of mycorrhizal fungi (soil treatment). The two-way interaction, between species and one of the other main effects was included in the model. Where a significant difference was detected the Scheffé multiple comparison test was used to determine significant differences among treatment groups. In cases where there was a significant interaction, a separate one-way analyses of variance was used for each species followed by the Scheffé multiple comparison test to determine significant differences among treatment groups ( $P < 0.05$ ).

Temporal changes in dry mass and percent mycorrhizal infection in low nutrient native soil was examined for both *H. paradoxus* and *H. annuus*. The experimental treatment variable was time of growth, with harvests from 2-10 weeks at two-week intervals. For each treatment or sampling time, there were five replicate pots. There were five plants per pot but all plant material per pot was harvested and pooled. There were a total of 50 pots used (5 treatment levels or times, five replicates and two species). Every two weeks, five pots with each species (total of 10) were chosen randomly (from those remaining) for harvest.

The influence of increasing soil nutrient levels on the dry mass and the percent mycorrhizal infection of *H. annuus* and *H. paradoxus* was examined next. The experimental treatment variable was an increase in the level of available soil nutrients. The general nutrient treatment (the 1.0 level) was 150 mg N as  $\text{NH}_4\text{NO}_3$ , 110 mg P as  $\text{Na}_2\text{HPO}_4$ , 80 mg K as KCl, and 30 mg S as  $\text{MgSO}_4$  per pot (Tiedemann & Klemmedson 1986). The amount added per pot was doubled in the 2.0 treatment, halved in the 0.5 treatment and no nutrients were added in the 0.0 treatment. Nutrients were added once at the beginning of the experiment.

There were five replicates for each level of nutrient treatment. Forty pots were used in the experiment (four nutrient treatments, five replicates per level, two species).

The next experiment tested the importance of the presence of soil mycorrhizal fungi on the dry mass and percent root infection of both *H. annuus* and *H. paradoxus*. There were four soil treatments including heat-sterilized soil, benomyl solution added directly to the soil, benomyl solution sprayed on the plants and no plant or soil manipulation (benomyl is a fungicide used to eliminate both pathological and non-pathological fungi from the soil, Hayman 1982; Anderson & Liberta 1992). Nutrients were added to each pot at the one level as in the previous experiment (Tiedemann & Klemmedson 1986). Forty pots were used (four soil treatment levels, five replicates per level, and two species). Pots were positioned in the greenhouse by treatment, with the spray treatment kept separate to prevent contamination. The control group had no heat treatment, soil additions or plant spray. The second treatment contained soil sterilized by dry heat at 160°C for 12 hours. Pots of the third treatment were filled with soil to which 33 mL of a 30 mg/L solution of benomyl was added once at the beginning of the experiment (Hetrick et al. 1989). For the fourth treatment, a spray mist of 33 mL of a 30 mg/L benomyl solution was applied directly to the plants using a plastic spray bottle. The spray treatment was applied three times (11 mL/time). The initial spray treatment was performed at planting. The second and third treatments were at two and four weeks, respectively.

The effect of increasing soil salinity was examined in the next experiment. This experiment was done to examine the changes in dry mass and mycorrhizal infection of *H. paradoxus* and *H. annuus* as soil salinity was increased. The salts used were similar to those in the soil at the Diamond Y Spring Preserve where *H. paradoxus* is found (Veni 1991; Boghici 1997). There were four soil treatments including no salt addition or 0.0 g/kg, 5 g/kg added salinity, 10 g/kg added salinity, and 20 g/kg added salinity. Salts were added directly to the soil as a dry mixture. For the 5 g/kg soil salinity treatment,

1.58 g  $\text{CaCl}_2$ , 2.19 g  $\text{MgCl}_2$  and 3.23 g  $\text{Na}_2\text{SO}_4$  were added per pot of 1400 g Patrick soil. For the 10 g/kg soil salinity treatment, twice as much of each salt was added per pot and for the 20 g/kg soil salinity treatment, four times as much salt was added per pot. Nutrients were added once at the beginning of the experiment to each pot at the one level as in the earlier experiments (Tiedemann & Klemmedson 1986). Forty pots were used in the experiment (four salinity treatment levels, five replicates per level, two species).

## RESULTS

The time of growth was significant, affecting the percent mycorrhizal infection and dry mass of *H. annuus* and *H. paradoxus*. For mean percent infection, results of the two-way *ANOVA* indicated the overall model was significant and there was a significant difference due to the time of growth. However, there was no significant difference among species, and no significant interaction (Fig. 1a). The Scheffé Multiple Comparison Test showed there were significant differences between the mean percent infection values, but the differences were not large. The mean overall percent infection for the experiment was 49.7%.

The results of the two-way *ANOVA* for above ground dry mass indicated the overall model was significant, as was the time of treatment, species and the two-way interaction ( $P < 0.0001$  for all, Fig. 1b). Mean above ground dry mass increased over time, and was generally greater for *H. annuus* ( $0.76 \pm 0.73$  g/pot) than *H. paradoxus* ( $0.39 \pm 0.34$  g/pot, Fig. 1b). Because there was a significant interaction between the two main factors, the effect of time of treatment was analyzed separately as a one-way *ANOVA* for each species. Both *H. annuus* and *H. paradoxus* above ground dry mass was significantly different among weeks (one-way *ANOVA*,  $P < 0.0001$  for both). The Scheffé Multiple Comparison Test showed which treatments were significantly different. Mean above ground dry mass of *H. annuus* was  $1.78 \pm 0.45$  g/pot at the end of the 10 weeks and for *H. paradoxus* it was  $0.87 \pm 0.15$  g/pot.

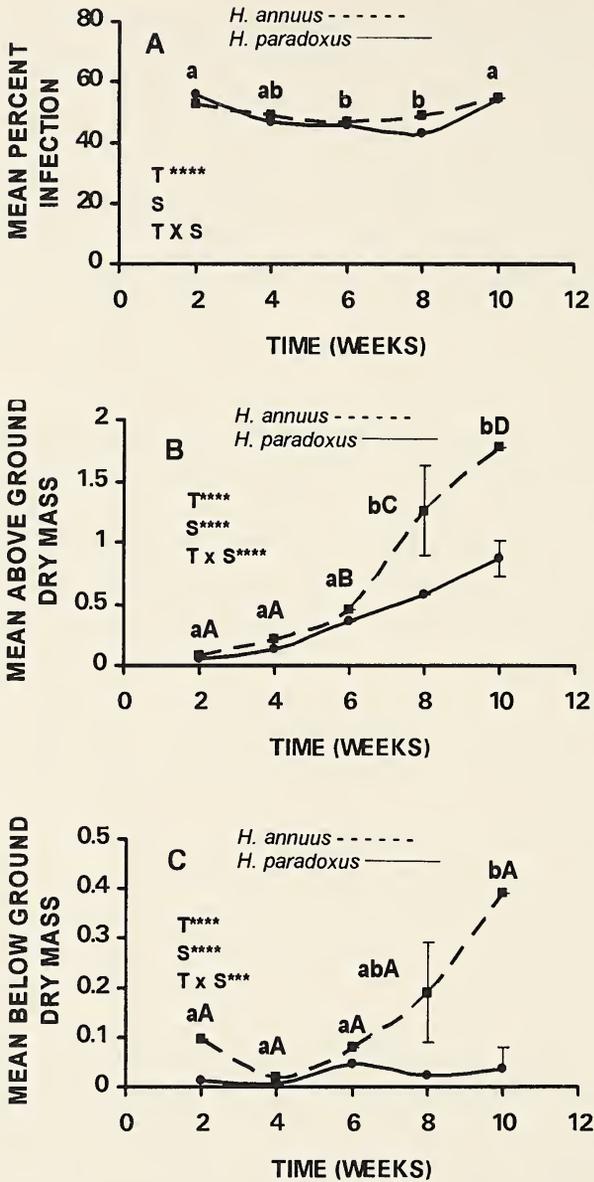


Fig. 1. Mycorrhizal (a) percent infection, (b) above ground and (c) below ground dry mass per pot for both species in time. ANOVA results:  $*=P<0.05$ ,  $**=P<0.01$ ,  $***=P<0.001$ ,  $****=P<0.0001$ . If there are species differences, lower case letters indicate differences for *H. annuus*. Error bars =  $\pm$  one SD. T = time, S = species and T x S = interaction.

The results of the two-way *ANOVA* for mean ash-free root mass indicated the overall model was significant ( $P < 0.0001$ ), as was the time of growth ( $P < 0.0001$ ), species ( $P < 0.0001$ ) and the two-way interaction ( $P = 0.0002$ , Fig. 1c). Mean ash-free root mass increased over time, but was generally greater overall for *H. annuus* ( $0.16 \pm 0.15$  g/pot) than *H. paradoxus* ( $0.03 \pm 0.02$  g/pot, Fig. 1c). Since the interaction was significant, the effect of time of treatment was analyzed separately for each species as a one-way *ANOVA*. Both *H. annuus* and *H. paradoxus* mean ash-free root mass were significantly different among weeks or time of growth (one-way *ANOVA*,  $P = 0.0005$ , and  $P = 0.0223$ , respectively). The Scheffé Multiple Comparison Test showed which treatments were significantly different. Despite finding a significant difference among weeks with a one-way *ANOVA*, the Scheffé Multiple Comparison Test found no significant differences between weeks for *H. paradoxus*. Mean ash-free dry mass of *H. annuus* at the end of the experiment was  $0.39 \pm 0.19$  g/pot and for *H. paradoxus* it was  $0.04 \pm 0.04$  g/pot.

The influence of soil nutrient level on mycorrhizal infection and dry mass of *H. annuus* and *H. paradoxus* was examined. The results of the two-way *ANOVA* for mean percent mycorrhizal infection indicated the overall model was significant, as was the nutrient level ( $P < 0.0001$  for both, Fig. 2a). However, species and the two-way interaction was not significant ( $P > 0.05$  for both). Mean percent infection decreased as the amount of added nutrients increased but was the same for both species. The Scheffé Multiple Comparison Test showed that the mean percent mycorrhizal infection for the zero added nutrient level was significantly different than all other nutrient levels ( $51.0 \pm 1.4\%$ ). The mean mycorrhizal infection was  $33.3 \pm 0.7\%$  at the highest level of nutrients added to the soil.

For the two-way *ANOVA* of above ground dry mass, the overall model, the nutrient level, species and the interaction were significant ( $P < 0.0001$  for all, Fig. 2b). Mean above ground dry

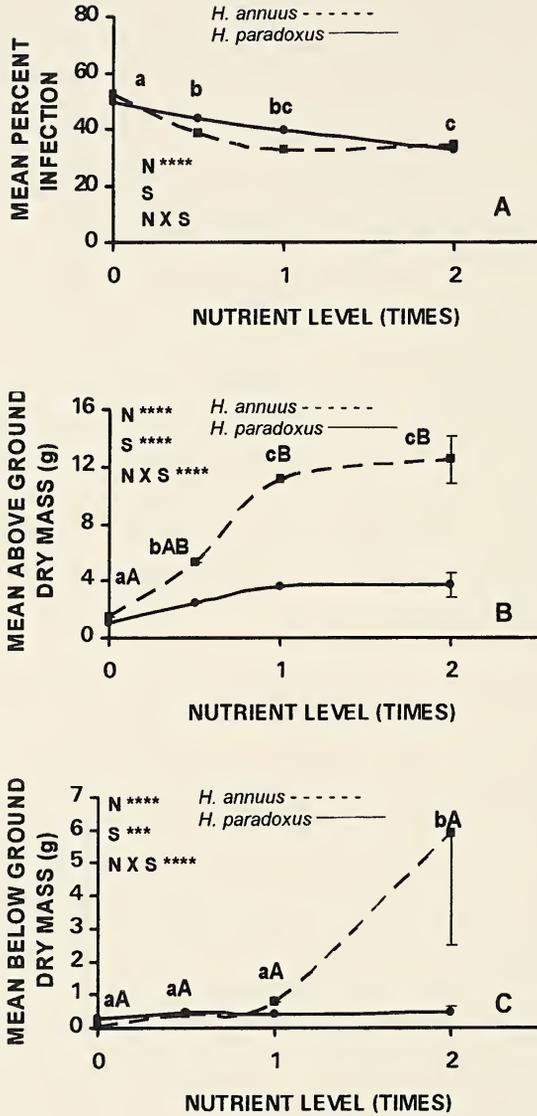


Fig. 2. Mycorrhizal (a) percent infection, (b) above ground and (c) below ground dry mass as a function of added nutrient levels. ANOVA results:  $*=P<0.05$ ,  $**=P<0.01$ ,  $***=P<0.001$ ,  $****=P<0.0001$ . If there are species differences, lower case letters indicate differences for *H. annuus*. Error bars = ± one SD. N = nutrients, S = species and N x S = interaction.

mass was generally greater over all for *H. annuus* ( $7.59 \pm 5.14$  g/pot) than *H. paradoxus* ( $2.72 \pm 1.23$  g/pot). In addition, mean above ground dry mass increased with added nutrient level. The interaction was significant therefore the effect of nutrient levels was analyzed separately for each species as a one-way ANOVA. Both *H. annuus* and *H. paradoxus* above ground dry mass was significantly different among treatments (one-way ANOVA,  $P < 0.0001$  for both). In both species the mean above ground dry mass increased as nutrient levels increased, but the effect was larger for *H. annuus* than for *H. paradoxus*. The Scheffé Multiple Comparison Test showed which treatments were significantly different (Fig. 2b). The above ground dry mass of *H. annuus* increased to  $12.46 \pm 1.64$  g/pot at the end of the experiment and *H. paradoxus* increased to  $3.71 \pm 0.85$  g/pot.

The results of the two-way ANOVA for mean ash-free root mass indicated the overall model was significant ( $P < 0.0001$ ), as was the nutrient level ( $P < 0.0001$ ), species ( $P < 0.001$ ), and the two-way interaction ( $P < 0.0001$ , Fig. 2c). Mean ash-free root mass was greater overall for *H. annuus* ( $1.78 \pm 2.76$  g/pot) than it was for *H. paradoxus* ( $0.39 \pm 0.09$  g/pot). The effect of nutrient level was analyzed separately for each species as a one-way ANOVA. *Helianthus annuus* mean ash-free root dry mass was significantly different among nutrient levels ( $P < 0.0001$ ), but this was not the case for *H. paradoxus* ( $P < 0.05$ ). Ash-free root dry mass increased as nutrient levels increased for *H. annuus*, but the 2.0 level was the only treatment significantly different from the other levels that were not different from each other.

The influence of various sterilization treatments on the mycorrhizal infection and dry mass of *H. annuus* and *H. paradoxus* was examined. When mean percent mycorrhizal infection was examined, a two-way ANOVA demonstrated the model was significant ( $P < 0.0001$ ), as was the soil treatment ( $P < 0.0001$ ), and species ( $P = 0.0069$ ), but the interaction between species and soil treatment was not significant ( $P > 0.05$ , Fig. 3a). The mean percent

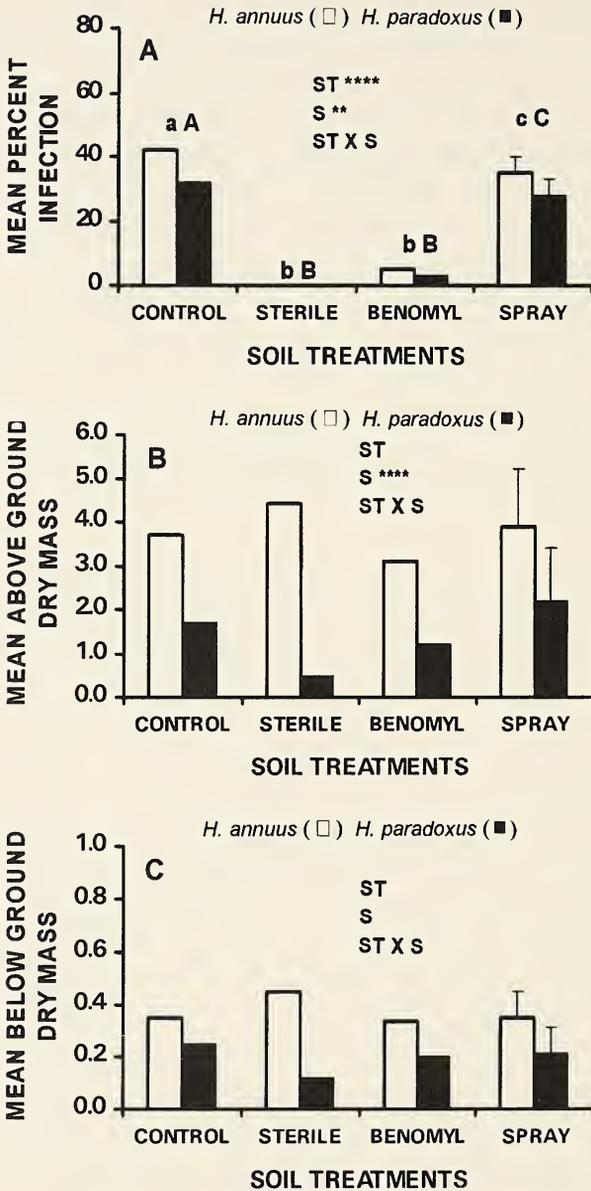


Fig. 3. Mycorrhizal (a) percent infection, (b) above ground and (c) below ground dry mass per pot in various soil treatments. ANOVA results:  $*=P<0.05$ ,  $**=P<0.01$ ,  $***=P<0.001$ ,  $****=P<0.0001$ . Lower case letters indicate differences for *H. annuus*. Error bars =  $\pm$  one SD. ST = sterilization treatment, S = species and ST x S = interaction.

infection of all treatments was significantly different than the control and all other treatments had smaller percent infections. There was no significant difference between the heat sterilized soil treatment and the soil application of the Benomyl for either species, but both were significantly different than the control treatment and the Benomyl spray treatment. In general, *H. annuus* had a greater overall mean percent infection than *H. paradoxus* ( $19.7 \pm 20.1\%$  vs.  $15.0 \pm 16.0\%$ , respectively).

The two-way *ANOVA* for mean above ground dry mass revealed the overall model was significant ( $P < 0.001$ ), there was no significant difference between soil treatments ( $P > 0.05$ ), species was significant ( $P < 0.0001$ ), but the interaction was not significant ( $P > 0.05$ , Fig. 3b). *Helianthus annuus* overall mean above ground dry mass was greater than *H. paradoxus* ( $3.82 \pm 0.58$  g/pot versus  $1.37 \pm 0.75$  g/pot).

There was no significance in the overall model for the two-way *ANOVA* for mean ash-free root mass (Fig. 3c). However, *H. annuus* mean ash-free root mass was greater than the *H. paradoxus* ash-free root mass ( $0.40 \pm 0.04$  g/pot versus  $0.22 \pm 0.05$  g/pot).

The effect of added soil salinity on the percent mycorrhizal infection and dry mass of *H. annuus* and *H. paradoxus* was examined. The results of the two-way *ANOVA* of percent infection demonstrated the model, soil salinity, species and the interaction were significant ( $P < 0.0001$  for all, Fig. 4a). *Helianthus paradoxus* had a greater mean percent mycorrhizal infection overall compared to *H. annuus* ( $41.1 \pm 8.0\%$  versus  $28.1 \pm 29.0\%$ ). The interaction between soil salinity level and species was significant and the effect of salinity was analyzed separately for each species (one-way *ANOVA*). Both *H. annuus* and *H. paradoxus* mean percent mycorrhizal infection was significantly different among salinity treatments (one-way *ANOVA*,  $P < 0.0001$  for both). The interaction plot shows that *H. annuus* and *H. paradoxus* mean percent infection responded differently to increasing levels of soil salinity. The Scheffé

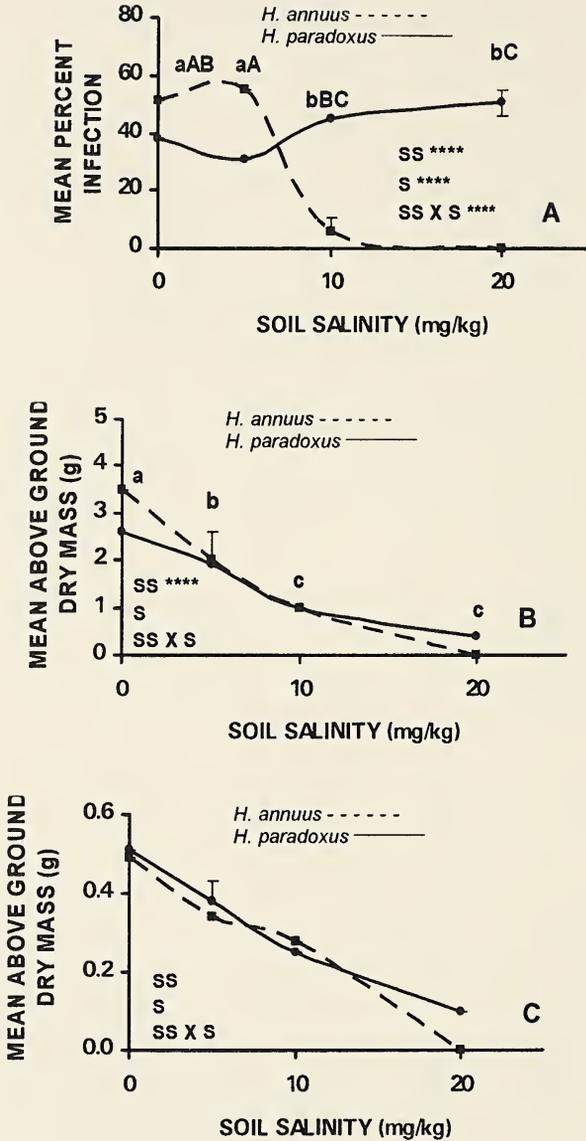


Fig. 4. Mycorrhizal (a) percent infection, (b) above ground and (c) below ground dry mass per pot as a function of soil salinity levels. ANOVA results: \*= $P < 0.05$ , \*\*= $P < 0.01$ , \*\*\*= $P < 0.001$ , \*\*\*\*= $P < 0.0001$ . If there are species differences, lower case letters indicate differences for *H. annuus*. Error bars = ± one SD. SS = soil salinity, S = species and SS x S = interaction.

Multiple Comparison Test showed which treatments were significantly different. It should be noted that all *H. annuus* plants in the 20 g/kg soil salinity treatment died before the end of the experiment. For *H. paradoxus* the mycorrhizal infection decreased from zero to 5 g/kg, and then increased as salinity levels increased. For *H. annuus*, infection decreased in the 10 g/kg treatment.

The effects of salinity on the above ground dry mass of *H. annuus* and *H. paradoxus* was examined with a two-way *ANOVA*. The model and soil salinity were significant ( $P < 0.0001$  for both, Fig. 4b). Species and the interaction were not significant ( $P > 0.05$ ). Mean above ground dry mass for the zero added soil salinity level was significantly different from all other levels. In general, above ground dry mass decreased as salinity levels increased for both species. There was no significant difference in the model for the two-way *ANOVA* of mean ash-free root mass, although ash-free root mass did decline for both species with increased soil salinity (Fig. 4c).

## DISCUSSION

As plant communities change during succession, both the types and species of AMF (arbuscular mycorrhizal fungi) change as well (Allen 1991). It is difficult if not impossible to partition the influence of the observed changes in AMF and plant community properties, probably because they change concomitantly and are really interdependent (Rillig 2004). Greater proportions of the plant species present in early successional communities are either non-mycotrophic or facultative mycotrophic while most of the later successional species are either obligate or facultative (Allen 1991).

Both species studied here, *Helianthus annuus* and *H. paradoxus*, were infected early in growth experiments and the infection remained fairly constant over the course of the experiment. Both species responded similarly to added nutrients, the mycorrhizal infection decreased with increased levels of soil nutrients but the dry mass of both species increased, more for *H. annuus*. This

response has been reported for many species (Buwalda et al. 1982; Hayman 1982; Johnson et al. 1997; Bryla & Koide 1998; Bago 2000).

Heat sterilization of the soil or the application of benomyl, a fungicide commonly used in this type of fumigation experiment, reduced the root infection to zero or very low levels, but the dry mass of both species was not changed significantly. These results suggest that these members of the genus *Helianthus* are facultative mycotrophs (Allen 1991). They do not require the fungal infection although their growth was modified when they were infected.

The importance of the AMF to either species of *Helianthus* examined in the current studies in terms of dry mass production could be transitory. In addition, high variation in the experiments carried out may have prevented detection of significant differences. *Helianthus annuus* above ground dry mass increased by 19% with heat sterilization of the soil (Fig. 3). This suggests that the presence of the mycorrhizal fungi has a metabolic cost (carbon-drain on the plant to satisfy fungal metabolism) and uses 19% of the dry mass produced by *H. annuus* and this cost reduces plant growth by that amount. For *H. paradoxus*, above ground dry mass decreased by 76% with heat sterilization and the change was in the opposite direction compared to *H. annuus*. This suggests that the fungi were important to *H. paradoxus* and their loss caused a reduction in growth or dry mass production by 76%.

It is possible that the advantage or benefit of the fungal infection to the two sunflowers is only important or can only be detected in low nutrient soils, especially low phosphate soils (Rovira & Bowen 1966; Salisbury & Ross 1992; Carlile 2001). If phosphate was added to the soil, as in the present studies, the fungal infection may not be important to the plant because sufficient phosphate is available in the soil or the infection may be important to some other non-measured plant function.

There are some major differences in habitat preference or requirements of the two sunflowers examined in the current study. *Helianthus annuus*, one of the parent species of *H. paradoxus* (cf. Abbott 2003; Rieseberg et al. 2003), is found all across North America, typically on heavy, clay soils. The other species, *H. paradoxus* is of hybrid origin and grows in saline, wet or marshy soils in a few locations in west Texas and New Mexico (McDonald 1999). *Helianthus paradoxus* is more tolerant of soil salts than *H. annuus* (cf. Mendez 2001; Welch & Rieseberg 2002) and can out-compete its parental species in slightly salty soils but the outcome is reversed in non-saline soils (Bush & Van Auken 2004). It out-competed ecosystem competitors at all salt levels (Van Auken & Bush 2006).

The slight differences in dry mass seen in these two species in sterile soil, and the difference in direction of their response to the symbiosis, might be a reflection of their differences in habitat requirements (Bush & Van Auken 1997). *Helianthus annuus* appears to be an early successional species, it is found in open habitats, usually grasslands or disturbed sites, and may require vegetation gaps for establishment. If it is truly an early successional species, it is probably a facultative mycotrophic species, that is, a species that can survive and grow with or without the symbiosis (Allen 1991). In addition, the change in dry mass with or without the fungus was small. On the other hand, *H. paradoxus* has only been found in saline, wet or marshy soils (McDonald 1999). It may be an obligate mycotrophic species, a species that requires the symbiosis (Allen 1991). This is suggested by the 76 % reduction in dry mass in the plant with the loss of the fungal infection.

In a saline environment the loss of the fungus may not be alleviated or compensated by the addition of nutrients. The presence of AMF in the roots of salt marsh plants was noted quite early (Mason 1928). In some respects this is surprising, since many researchers have found that increasing levels of salinity and the presence of flooding generally reduce the presence of mycorrhizal

fungi in plant roots (Brown & Bledsoe 1996; Copeman et al. 1996). A number of studies have shown that plants with AMF exhibit increased tolerance to salinity and to drought when compared to non-AMF plants, or plants treated with fungicide or grown in sterilized soil (Allen 1982; Allen & Cunningham 1983; Pfeiffer & Bloss 1988; Jindal et al. 1993; Copeman et al. 1996; Mathur & Vyas 2000). Growth of guayule (*Parthenium argentatum*) in saline soil was equally enhanced by either the addition of phosphorous or inoculation with AMF (Pfeiffer & Bloss 1988). Similar results were found for bell pepper plants grown under different soil salinity levels (Hirrel & Gerdemann 1980). These findings have led to speculation that mycorrhizal fungi can increase salt tolerance through improved phosphate nutrition. However, findings are not universal. There was no improvement in salt tolerance in citrus seedlings with or without AMF (Graham & Syvertsen 1989). Thus, there may be some unidentified species-specific effects.

*Helianthus paradoxus* and *H. annuus* both showed reduced growth as soil salinity increased in the present study (Fig. 4). *Helianthus paradoxus* survived at the highest levels of salinity, while *H. annuus* did not survive. It should be noted that although there were no significant differences in shoot or root mass between species in the salinity experiment, relatively speaking, the overall reduction in growth with increased salinity was less in *H. paradoxus* compared to *H. annuus*. These results are in accordance with previous studies that showed *H. paradoxus* was somewhat more salt tolerant than *H. annuus* (Mendez 2001; Welch & Rieseberg 2002).

While both *H. annuus* and *H. paradoxus* plants showed a reduction in growth, the effect of salinity on mycorrhizal infection was different for each species (Fig. 4). *Helianthus annuus* had an overall reduction in mean percent infection, while *H. paradoxus* had an overall increase. In general, plants show a decrease in mycorrhizal infection as salinity increases (Brown & Bledsoe 1996), but in some species infection increases (Copeman et al.

1996). There does not seem to be an obvious and consistent relationship between increases or decreases in infection and increases or decreases in salinity tolerance. It is possible that *H. paradoxus* has multiple mechanisms for dealing with high levels of salinity; mycorrhizal fungi may provide additional protection in the form of increased plant nutrient status.

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## HISTORIC VEGETATION OF CAMP BULLIS AND CAMP STANLEY, SOUTHEASTERN EDWARDS PLATEAU, TEXAS

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**Abstract.**—Historic land survey data were used to test a previously proposed nineteenth century landscape model of southeastern Edwards Plateau (Balcones Canyonlands or Texas Hill Country) counties; the model (47% wooded pre-1860) was supported by the results of this study. Woodland and savanna were common, open grassland was uncommon, and forest was rare on the study area. Five tree species were recorded; Plateau oak (*Quercus fusiformis*) was the most common species, post oak (*Quercus stellata*) was the largest species, and Texas oak (*Quercus buckleyi*) was the rarest and smallest species. Mean tree density varied from 6.8-307.8 per ha across savanna, woodland, and forest. There was no association between generalized vegetation types (grass-dominated vs. wooded) and range sites/site groups.

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Proper planning of ecological restoration requires that the nature of historic landscapes be known to the greatest degree possible. The Edwards Plateau of central Texas has been and continues to be a major focus of study for plant ecologists and range managers (Amos & Gehlbach 1988; Taylor 1997) interested in understanding and managing this region for biodiversity and economic benefits to society. However, little quantitative treatment of the subject of its original condition has been published (Weniger 1984; 1988; Wills 2005). Narrative accounts, though historically valuable as qualitative statements about a particular locality, or (in aggregate) a region, are too often substituted for quantitative analyses of the early composition and structure of regional plant communities.

In 1839, surveyors began delineating tracts of land along Salado Creek in northern Bexar County granted by the Republic of Texas. John Leonard Riddell (Breedon 1994:58-59) described the contemporaneous landscape seen by surveyors of this area in the following manner: “The musquit tree now disappears and is replaced by live oak, post oak etc.... Land sparsely timbered, but no uninterrupted large prairies. Real thickets occur only in the *cañadas* or ravines of

water courses.” Spaight (1882:25) provides additional detail, stating that the “timber in the northern portion [of Bexar County] is live oak on the hills and high plateaus, post oak on the flats, and elm, walnut, pecan, and hackberry along the streams.”

Based on study of historic land surveys, an assessment of the amount of woodland in the southeastern edge of the Edwards Plateau region (Comal County and Kendall County data) occurring during the middle of the nineteenth century found that 47.0% (range 46.1-47.9%) of the landscape was wooded; historic vegetation in the Balcones Canyonlands as a whole was also 47.0% wooded (Weniger 1988). The purpose of this paper is to use site-specific historic land survey data, comprising the tree component of the vegetation, to test the results of Weniger’s analysis, and to describe the relationship of wooded versus grass-dominated vegetation to range site types.

#### MATERIALS AND METHODS

*Study area.*—Camp Bullis (subpost of Fort Sam Houston) and Camp Stanley (part of the Red River Arsenal) are contiguous sites located in northern Bexar and western Comal counties approximately 29 km NNW of San Antonio, Texas (29°41’N, 98°34’W). These two sites, together formerly known as the Leon Springs Military Reservation, cover approximately 12,870.8 ha within the Balcones Canyonlands subregion of the Edwards Plateau. The topography is hilly, with numerous intermittent streams draining to the east and south. The most prominent of these are Cibolo Creek and Salado Creek. Mean annual precipitation is 74 cm; mean annual temperature is 19.8°C. Elevation ranges from 306-462 m. Many of the hills exhibit a terraced or “stair step” appearance due to alternating harder and softer strata. Most (74%) of the rock outcropping on Camp Bullis is Upper Glen Rose limestone. Lower Glen Rose limestone is exposed over 14% of the northern portion of Camp Bullis, while 12% (mainly in the southeastern corner of the site) is Kainer limestone of the Edwards group (Anonymous 1990). Soils belong primarily to the Tarrant-Brackett association; these are

shallow to very shallow soils over limestone parent material. Specific soils within the study area include Bexar, Brackett, Crawford, Krum, Lewisville, Patrick, Tarrant, Frio, and Venus. These fall into nine range sites: Clay Loam (Lewisville, Venus), Loamy Bottomland (Frio), Low Stony Hill (Tarrant), Redland (Bexar, Crawford), Rocky Upland (Tarrant), Shallow (Patrick), Steep Adobe (Brackett), Steep Rocky (Tarrant), and Valley (Krum) (Anonymous 1990; Taylor et al. 1991).

Recently, the vegetation of Camp Bullis was estimated to be 18.3% grassland, 11.9% savanna, 62.7% woodland (including forest), and 7.1% other (sotol [*Dasyilirion texanum*], yuccas [*Yucca* spp.], rock outcrop, other) (Hudler 2000). Grassland is more frequent at higher elevations, whereas oak savanna is more common at lower elevations (Hudler 2000). Riskind & Diamond (1986, 1988) described the region as comprising evergreen woodland, deciduous woodland, and floodplain forest (riparian woodland), and these types are all represented at Camp Bullis. American sycamore (*Platanus occidentalis*) is the principal riparian species, although pecan (*Carya illinoensis*) is also present.

*Data collection and analysis.*—Witness tree data were obtained from field notes of 64 original land surveys (Whitney & DeCant 2001) of the study area conducted during the years 1839-1862. These survey reports are held in the Archives and Records Division, General Land Office of Texas, Austin; a list is available from the author. Data collected included survey number, year, tree species, tree diameter, bearing of tree from survey corner, and distance of tree from survey corner. All survey corners were inside, or within 750 m of, the Camp Bullis/Camp Stanley boundary. Abbreviations for tree species, as used by surveyors, were interpreted as follows: L.O. = Plateau (live) oak (*Quercus fusiformis*), P.O. = post oak (*Quercus stellata*), and Sp.O. = Texas (Spanish) oak (*Quercus buckleyi*). Other species included blackjack [oak] (*Quercus marilandica*) and [cedar] elm (*Ulmus crassifolia*). Diameters, recorded in whole inches, were converted to meters. Distances,

recorded by surveyors in fractional varas, were converted to meters by multiplying by 0.84667 (Reasonover 1946), and the tree radius (in meters) was added to the converted distance. The Texas General Land Office apparently did not specify how far surveyors should go from a survey corner to record witness (bearing) trees. Maximum tree distance reported for the study area was 85.6 m. Most survey corners with reported trees had two witness trees, but a few indicated one or three. Only those with two or three witness trees could be used in computing mean tree distance at a corner. Corners with a single tree were assigned to a coarser vegetation type (grass-dominated). Corners with no reported trees were scored as open grassland. One hundred and seventy-two corners were included in the analyses. Most surveys having shared corners agreed with respect to presence/absence of trees, tree species, diameters, bearings, and distances at a given corner. In the few cases where any of these data differed, information from the oldest survey was given priority to eliminate changes due to settlement (e.g., tree cutting) and possible field note transcription errors.

Trees per hectare were calculated according to the following formula:  $10,000/d^2$  where  $d$  is the mean tree distance in meters (Smeins & Slack 1982). Weniger's (1988) distance criteria were adopted to determine if a given survey corner (point) represented savanna (>21 m), woodland (7-21 m), or forest (<7 m). For some analyses, corners were grouped into wooded (woodland and forest) and grass-dominated (savanna, grassland, and single trees) categories. The only differences between the methods used in this paper and those of Weniger (1988) are that data on plant communities are reported by five categories herein instead of two, and that one of these five categories (single trees) was eliminated from consideration by Weniger.

Using ArcView 9.02 GIS, a digital Texas General Land Office original land survey map (RRC 2000, slightly modified *sensu* Boggs & Giles 1932) was superimposed on a digital NRCS range site map to provide a basis for locating survey corners and their

witness trees within range site types on the study area. Some range sites were combined into larger groups if they had similar potential vegetation and/or similar topographic positions (Taylor et al. 1991). The following range sites were combined into range site groups (percent of study area in parentheses): Steep Adobe and Steep Rocky (35.9%), Clay Loam and Shallow (1.6%), and Rocky Upland and Low Stony Hill (27.3%). This process reduced the number of range sites or range site groups from nine to six, including Loamy Bottomland (4.7%), Redland (15.7%) and Valley (14.9%). Chi-square analysis was used to compare the results of this study with those of Weniger (1988). A similar analysis comparing range site groups with respect to wooded and grass-dominated vegetation was performed (Bruning & Kintz 1968). The sample sizes for Clay Loam/Shallow and Loamy Bottomland were too limited; these sites were excluded from the analysis.

## RESULTS

Surveyors found five species of trees on the study area, including four oak species and cedar elm (*Ulmus crassifolia*). Almost 99% of the 294 trees reported were oaks. Riparian trees were absent from the survey notes, as was Ashe juniper (*Juniperus ashei*), the most abundant species in the contemporary woodland (Van Auken 1988). Post oak (*Quercus stellata*) was the largest species (mean diameter 38 cm), Plateau oak (*Quercus fusiformis*) was intermediate in size (mean diameter 27 cm), and blackjack oak (*Quercus marilandica*), Texas oak (*Quercus buckleyi*), and cedar elm were the smallest species (mean diameters 20, 18, and 22 cm, respectively). Plateau oak was the dominant tree (ca. 85% of all trees), post oak and blackjack oak (between 5-8% each) were uncommon, and Texas oak and cedar elm (<2% each) were rare (Table 1).

In decreasing order of abundance, woodland, savanna, grassland, and forest plant communities were found to occur in the historic landscape (Table 2). Woodland and savanna were common and together accounted for 77.3% of the total landscape. Grassland was

Table 1. Witness trees (1839-1862) and their diameters (nearest cm) on the study area.

Species	<i>n</i> (Percent)	Range (cm)	Mean (cm)
<i>Quercus marilandica</i>	16 (5.4%)	13-30	20
<i>Quercus fusiformis</i>	249*(84.7%)	8-61	27
<i>Quercus stellata</i>	23 (7.8%)	23-71	38
<i>Quercus buckleyi</i>	2 (0.7%)	10-25	18
<i>Ulmus crassifolia</i>	4 (1.4%)	13-30	22

\*no diameter available on one tree

Table 2. Plant communities at survey corners on the study area, 1839-1862. Distance units are meters.

Community	<i>n</i>	Percent	Mean Distance (range)	Mean Distance (grand mean)	Mean Density (trees/ha)
Grassland	23	13.4	—	—	—
Savanna	59	34.3	21.7-85.6	38.3	6.8
Woodland	74	43.0	7.4-20.5	13.6	54.1
Forest	7	4.1	2.6- 6.9	5.7	307.8
Single Tree	9	5.2	—	—	—

uncommon and appeared to be concentrated in the southern portion of the study area. Forest was rare and located mostly in the western part. Points having a single tree (ca. 5% of the total points) were not assigned to any plant community as no average tree distance could be calculated. Grass-dominated communities (grassland, savanna, and unclassified [single trees]) occurred at 91 points (52.9%), while wooded communities (woodland and forest) occurred at 81 points (47.1%). Comparable values reported by Weniger (1988) were 563 grass-dominated points (53.0%) and 500 wooded points (47.0%). There was no difference between the Weniger model and the data in this study ( $X^2 < 0.01$ ,  $df = 1$ , n.s.). Grass-dominated and wooded communities were apparently equally abundant in the landscape of the study area during the historic period ( $Z = 0.76$ , n.s.).

Range sites and site groups appeared to vary in the extent to which they were wooded. Clay Loam/Shallow ( $n = 4$ , 3 wooded), Loamy Bottomland ( $n = 6$ , 4 wooded), and Redland ( $n = 32$ , 19

wooded) sites had more wooded points than grass-dominated points. Low Stony Hill/Rocky Upland ( $n = 38$ , 22 grass-dominated), Steep Adobe/Steep Rocky ( $n = 63$ , 36 grass-dominated), and Valley ( $n = 29$ , 17 grass-dominated) sites had more grass-dominated points than wooded points. However, differences in these two general categories of vegetative cover among range sites/site groups were insignificant ( $X^2 = 3.05$ ,  $df = 3$ , n.s.). Much of the grassland (56.5% of all grassland points) was within the Steep Adobe/Steep Rocky site group. Woodland was the predominant community type in Redland (53.1% of its points) and Steep Adobe/Steep Rocky (41.3% of its points). The most common community type in the Valley site was savanna (55.2% of Valley points). Formation of mottes (small stands of trees in a grassy matrix) appears to have been uncommon in savanna. Only four of 59 savanna points (6.8%) had two Plateau oaks with similar distances and bearings from the survey corner stake, suggesting they were part of a tree cluster. These four points were all in the general vicinity of the confluence of Salado and Lewis Valley creeks.

Direct evidence of landscape disturbance is rare in the survey field notes, but there is some pertinent data. By 1860, three Plateau oaks in the vicinity of Salado Creek and the Pinta Trail were reported to have been killed. One 46-cm diameter tree had been reduced to a stump. Two others (diameters 13 and 25 cm) had been destroyed, one of them (13 cm) by fire.

## DISCUSSION

The species composition of historic plant communities on the study area varied from that reported by Weniger (1984) for the Edwards Plateau as a whole. He found that 54% of the trees were oaks, about 7% were elms, 5% were baldcypress (*Taxodium distichum*), <1% were pecan, and about 33% other (the percentage of Ashe juniper was not given). Of the oak species, he reported that 40% were Plateau (live) oak, 33% were post oak, 9% were blackjack oak, 6% were Texas (Spanish) oak, and 12% other.

While the rank order of these oaks was found to be the same in this paper, their relative abundances are quite different. These differences can be attributed partly to dissimilarities in methodology. Many of the surveys used by Weniger had two of their corners on a permanent stream, thus accounting for the presence of riparian species such as baldcypress and pecan. The high relative abundance of Plateau oak and low numbers of post oak, blackjack oak, and Texas oak on the study area is striking. It seems clear that Plateau oak dominated many Edwards Plateau uplands, and the high proportions of other oaks reported by Weniger are likely a reflection both of the larger scope of his study (13 counties) and differences in methods. Most of the surveys used in his study were concentrated along valleys where post oak and blackjack oak were more common (Weniger 1984). Points used in the present study are fairly well distributed between valley and upland areas, and apparently none fall in riparian corridors. On the other hand, the plant community results of this study are in close agreement with Weniger (1988), who found that there were historically about equal proportions of grass-dominated landscapes and wooded landscapes in the Edwards Plateau as a whole and in the Balcones Canyonlands subregion that includes Camp Bullis/Camp Stanley.

The apparent absence of Ashe juniper in the historic landscape has four possible explanations: (1) Surveyors considered Ashe juniper to be a poor witness tree and ignored it, (2) Ashe juniper was completely missing from the region, (3) Ashe juniper was too small to make a good witness tree, or (4) Ashe juniper was fairly rare. The first two reasons can probably be rejected; this species has been recorded in survey notes in other parts of the Edwards Plateau and it was the second most common species on a site in Kerr County (Wills 2005). Whitney & DeCant (2001:155) believe “it is unlikely that [surveyor bias] obscured real differences in the relative abundances of the species.” The third alternative appears somewhat more likely (Inglis 1964). However, small junipers are very sensitive to hot fires and would have been consumed quickly unless they occupied refuges, including rocky outcrops and

canyons. The fourth alternative, which appears to agree with the narrative description of Riddell (Breedon 1994), might be the best one. At the time of the surveys, this fire-sensitive species apparently had not expanded from those refugia due to recurrent fire at intervals of 13-25 yr (Frost 1998). Thus it covered a relatively small proportion of the landscape. Juniper fence post harvest and charcoal burning became major industries only after 1878 (Toepperwein 1950; Cartwright 1966), and their effect on the abundance of Ashe juniper was probably minimal prior to that time. The geographic proximity of disturbance to a travel corridor and places of early settlement suggests causality. However, considering the brief period of settlement ( $\leq 15$  yr) and what appear to be fairly low numbers of livestock, it seems improbable that major changes in tree cover had occurred before 1862. Such changes likely began around the early 1880s with the advent of barbed wire fencing (Wills 2005).

Comparison of contemporary vegetative cover with the historic prevalence of plant communities suggests that juniper woodland (including forest) has increased (Van Auken 1993) on the study area over time at the expense of savanna (woodland/forest: 47.1% increasing to 62.7%; savanna: 34.3% decreasing to 11.9%). The true extent of this increase is somewhat speculative due to the different methodologies employed (land survey versus remote sensing). Land use change, primarily decreased fire frequency after 1947 and possibly lower levels of juniper harvest, is the likely cause of juniper population expansion. Climatic change is a possible mechanism for juniper increase (Smeins & Fuhlendorf 1997), but this has not been conclusively demonstrated. The 1950s drought of record caused 90% mortality in some Edwards Plateau populations of mature Ashe juniper and a 56% reduction in total woody canopy cover, including a loss of 30% of scalybark oak (*Quercus sinuata*) and 54% of Plateau oak on some sites. Junipers <2 m tall survived this bottleneck and might have been able to occupy the space vacated by the dead oaks. However, there was little change in community composition evident at the end of the

drought (Young 1956; Merrill & Young 1959). Some features of historic vegetation patterns on the study area have apparently persisted over time, including savanna in the valleys and grassland on steep hills. The amount of grassland (including single tree points) does not appear to have decreased from 1862-2000. Indeed, a modest increase in the grassland percentage during that period is suggested when rosette plant patches are considered part of the grassland category. Expansion of grassland is likely due to clearing for agriculture, for flood control structures, and for military use.

Weniger (1988:22) argued that the “historic [Edwards] Plateau was thus a blend zone [(ecotone)]....” As such, it constituted an inviting region for nineteenth century settlers. There were woods for construction, fuel, posts, and shade, and abundant pasturage for grazing animals. Historical ecology and restoration ecology in the southeastern Edwards Plateau can benefit by taking into consideration that the region was not mainly grassland or savanna during the mid-nineteenth century settlement period, though these communities collectively accounted for  $\geq 50\%$  of the landscape.

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DETECTION OF ARBUSCULAR MYCORRHIZAL FUNGI  
IN AN EAST TEXAS FOREST BY ANALYSIS  
OF SSU rRNA GENE SEQUENCE

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**Abstract.**—PCR amplification of a region of the small subunit ribosomal RNA gene sequence with Glomeromycota-specific primers was used to detect arbuscular mycorrhizal fungi (AMF) in the roots of *Chasmanthium sessiliforum* (Poir.) Yates (longleaf woodoats) from the east Texas Pineywoods. Nineteen amplified sequences along with sequences from forty other AMF species reported from previous studies outside Texas with >97% pairwise sequence identity were used to build a phylogenetic tree. At least four AMF types, several of which do not appear to be closely related to previously described taxa or sequences, were identified from east Texas clones.

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Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota, Helgason et al. 2003) form mutualistic symbiotic associations with the roots of 80-90% of all terrestrial plant species, acting as an extension of the plant root system, increasing the uptake of nutrients, improving soil stability and protecting plants against soil pathogens (Jakobsen & Nielsen 1983; Newsham et al. 1995a; & 1995b; Budi et al. 1999).

Because of their significance to plants, knowledge of AMF could be considered a prerequisite for sustainable agriculture, effective forest management (Smith & Read 1997), or conservation. While AMF communities have been described for British croplands and forests (Daniel et al. 2001), Panama tropical forest (Husband et al. 2002), Danish croplands (Kjoller & Rosendahl 2001), Minnesota experimental fields (Burrows & Pflieger 2002), various Southern Ontario and Quebec fields (Klironomos 2002), and others, they have not been described for east Texas forests. This study attempts to isolate and identify AMF from a natural forest in the Pineywoods region (Hatch et al. 1990) of east Texas with the goal of sampling local AMF diversity by isolating AMF gene sequences from roots

of a widespread host growing in a typical east Texas forest habitat. The results provide preliminary baseline data that can then be compared to other habitats, hosts, and seasons.

Although AMF associated with plant roots are extremely difficult to identify using traditional morphological methods, recent molecular techniques (Simon et al. 1992; Daniel et al. 2001) show promise in detecting and identifying AMF (Hahn et al. 1993; Gadkar et al. 1997; Longato & Bonfante 1997). The small subunit ribosomal RNA (SSU rRNA) gene was chosen for PCR because it has variable regions that have been proven to provide phylogenetic information (Cedergren et al. 1988; Hamby & Zimmer 1988; Amann et al. 1991; Simon et al. 1992; 1993; Gehrig et al. 1996; Simon 1996; Redeker et al. 2000; Schüßler et al. 2001), while also possessing highly conserved regions that enable the use of universal primers to amplify DNA (Bousquet et al. 1990; White et al. 1990; Clapp et al. 1995). While the phenomenon of variable nuclear ribosomal repeats even within single individuals of Glomeromycota may obscure differences between taxa, the region of rRNA used in this study is fairly conserved and was successfully used by De Souza et al. (2004).

Designed by Helgason et al. (1998), the reverse primer AM1 excludes both plant and non-Glomeromycota fungal SSU sequences while amplifying SSU rRNA genes from the three traditional Glomeromycota families of Acaulosporaceae, Gigasporaceae, and Glomaceae. While it also excludes several AMF types, particularly the newly-discovered ancestral lineages (Morton & Redecker 2001; Schwarzott & Schüßler 2001), AM1 in combination with forward primer NS31 appears to detect a large portion of the AMF community and has been used successfully in several ecological studies including those of Helgason et al. (1998), Helgason et al. (1999), and Daniell et al. (2001).

#### MATERIAL AND METHODS

*Study area and host plant.*—The study site was a moderately-steep south-facing slope adjacent to the floodplain of the Angelina

River located in the Stephen F. Austin Experimental Forest in Nacogdoches County, Texas. The topsoil was a brown (7.5YR 5/4) sandy loam. Vegetated with common native species including *Pinus taeda* L., *Pinus echinata* p. Mill, *Liquidambar styraciflua* L., *Toxicodendron radicans* (L.) Kuntze, *Quercus alba* L., *Dichanthelium comutatum* (J.A. Schultes) Gould, *Smilax bonanox* L., *Callicarpa Americana* L., *Cornus florida* L., *Vitis rotundifolia* Michx., *Quercus nigra*, L. *Parthenocissus quinquefolia* (L.) Planch, *Arisaema triphyllum*, (L.) Schott, *Elephantopus tomentosus* L., *Ulmus alata* Michx., *Ilex opaca* Ait., and *Sanicula Canadensis* L., the site represents a widespread east Texas forest community type and is classified as a white oak-loblolly pine/*Callicarpa* loamy mesic slope under the US Forest Service ecological classification system (Turner et al. 1999).

*Chasmanthium sessiliflorum* (Poir.) Yates, (Poaceae), a native forest understory grass, was selected as the mycorrhizal host for the study. It is a tufted, summer-blooming, shade-tolerant perennial up to 1 m tall which bears nearly sessile, laterally-compressed, wedge-shaped spikelets arranged in a slender, erect, spike-like inflorescence (Correll & Johnston 1979). It is present across a wide range of east Texas forest habitat types ranging from wet-mesic stream bottoms to dry-mesic uplands on various soils. A significant component of most any east Texas community in which it is found, it is characteristic of the pine-broadleaf deciduous forests that cover much of eastern Texas and in many such stands is a dominant ground layer plant (Turner et al. 1999).

*Field sampling and sample processing.*—Three random sample points were located along a transect established parallel to the direction of the slope, one from the upper third of the transect and one each from the middle and lower thirds. During July 2003, root samples were obtained from the three colonies of *C. sessiliflorum* found nearest to each sample point. Samples were transported to the laboratory at 4°C, washed free of soil, dried on tissue paper and kept at -70°C until analysis. Sub samples of each root sample were

stained with ink (Vierheilig et al. 1998) to verify AMF infection prior to DNA analysis. Roots were sonicated two times for five minutes each, first in 10mM sodium pyrophosphate and then in distilled water (De Rooij Van Der Goes 1995) to remove root surface fungi.

*DNA isolation & amplification.*—DNA was isolated from a 20 mg portion of ground root material from three replicates of each of the nine original root samples by the modified potassium ethyl xanthate (PEX) extraction method of Edwards et al. (1997). Fragments of SSU DNA ( $\approx$ 520bp) were amplified by PCR with Pfu DNA polymerase (Stratagene Inc.) using the universal eukaryotic primer NS31 (Simon et al. 1992) in combination with the primer AM1 (Helgason et al. 1998), which is specific to many AM species but excludes plant and non-glomalian fungal species. The conditions described by Helgason et al. (1999) were used for PCR.

*DNA sequencing.*—The PCR products were cloned into pCR-Script Amp SK(+) (Stratagene Inc.) and transformed into *Escherichia coli* (XL1-blue MRF', Stratagene Inc.) following Helgason et al. (1999). Colonies were screened using the blue–white screening technique. Ten putative positive transformants from each of the three slope positions were selected for sequencing of plasmid inserts. Sequencing was performed by Amplicon Express Inc. (Pullman, Washington).

*Sequence analysis of 18S rRNA sequenced from the clones.*—Forward and reverse sequences were aligned and assembled using the CAP–Contig Assembly Program (Huang 1992). To verify glomalean origin and identify sequences with a high degree of similarity, BLAST search (Altschul et al. 1990) was used with the sequences obtained to search the GenBank at NCBI (<http://www.ncbi.nih.gov>). Multiple sequence alignment was performed using ClustalX (Thompson et al. 1994). The alignment is available from the corresponding author upon request.

All phylogenetic trees were computed with MEGA version 3.0 (Kumar et al. 2004) using 422 sites certain to be in alignment. Two separate trees were generated: the first using maximum parsimony analysis with the initial tree generated by random addition, and the second using neighbor-joining analysis (Saitou & Nei 1987) with the Kimura two-parameter model (Kimura 1980). The robustness of the inferred trees was evaluated after 1000 bootstrap resampling. Only values >70% are shown on the trees. Sequences were deposited in the NCBI database and GenBank accession numbers were received.

## RESULTS

Staining revealed the presence of AMF infection in all root samples (Figure 1). Three clones were not able to be sequenced and eight clones showed low similarity to AM fungal sequences and were removed from the analysis. These may have resulted from contaminating organisms, nonspecific binding, or PCR errors. The 19 remaining sequences, 528-548 bp in length, as expected, were undoubtedly of glomalean origin.

Results from a BLAST search yielded 35 sequences from the NCBI nucleotide data bank with at least 97% pairwise sequence identity with at least one of the east Texas clones. Each belonged to the genus *Glomus*. Moreover, those sequences in the search results which were included in Schüßler et al. (2001) belonged to the group "GIGrA" (*Glomus* Group A) described in that publication. A preliminary neighbor-joining tree (not shown) constructed using only the east Texas sequences and those of Schüßler et al. (2001) also showed that the Texas sequences occurred within a monophyletic group of the genus *Glomus* and belonged to Schüßler's group "GIGrA". Accordingly, neighbor-joining and maximum parsimony trees were constructed using the best-matching NCBI sequences (>97% sequence identity), the east Texas clones, and five sequences from groups "GIGrB" (*Glomus* Group B) and "GIGrC" (*Glomus* Group C) of Schüßler et al. (2001) which were used as an outgroup (Figure 2).



Figure 1. Light microphotograph of arbuscular mycorrhizal fungi on an east Texas *Chasmanthium sessiliflorum* root sample.

Both trees resulted in similar topologies. Definitions of groups were based on tree topology and pairwise distance. Pairwise distance between groups was  $>0.03$ . The 19 east Texas sequences

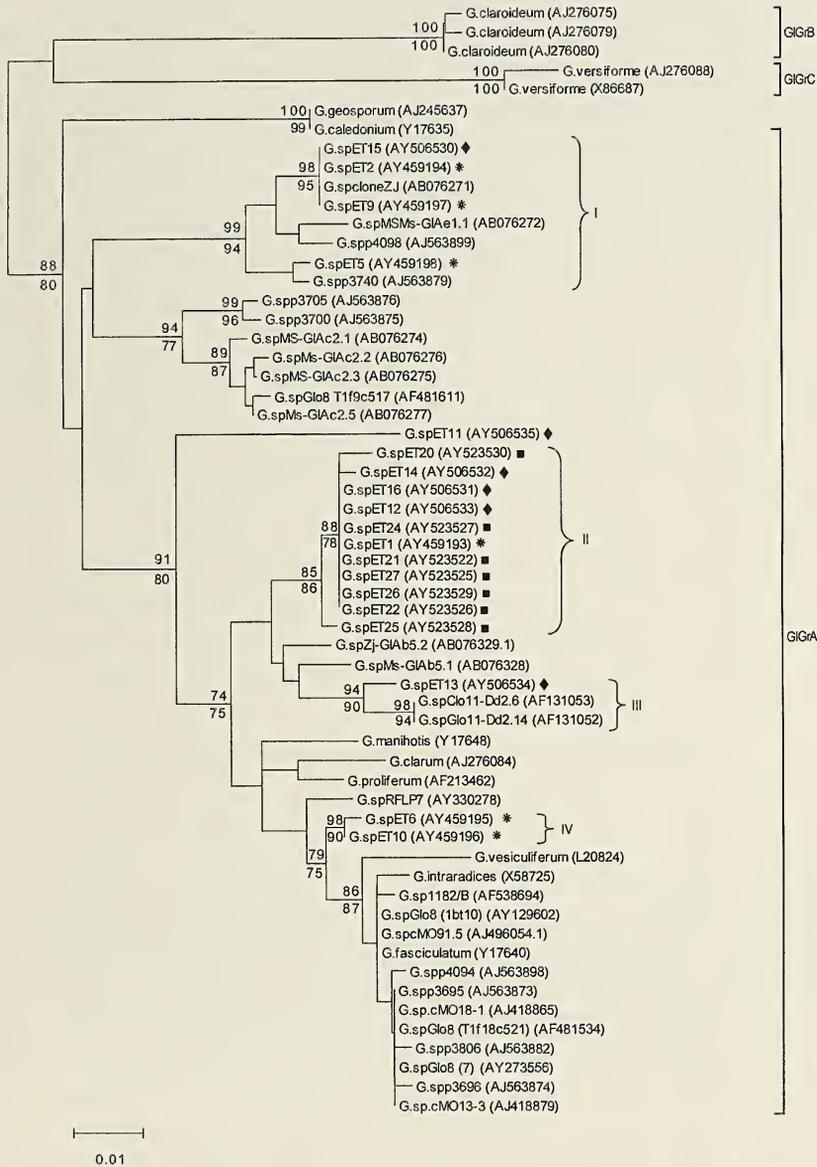


Figure 2. Neighbor joining tree of 59 partial 18S rRNA sequences. Labelling for new sequences are: diamond (◆) = lower slope, asterisk (\*) = midslope, and square (■) = upper slope. Bootstrap values from neighbor joining and parsimony analysis are shown above and below the branches, respectively. Groups I, II, III and IV are based on topology and pairwise distance (within subgroup, <0.03).

fell within four well-supported groups with bootstrap values of at least 70% (Figure 2). The first group received 99% (neighbor joining analysis) and 94% (Parsimony analysis) bootstrap support and included the sequences ET2, ET9, ET15 and ET5 whose closest neighbors on the tree included *Glomus* sp. ZJ (GenBank accession number AB076271) and uncultured *Glomus* clone p3740 (GenBank accession number AJ563879, Figure 2). The second group consisted of 11 east Texas clones (Figure 2) and received 85%/86% bootstrap support. The third group, which had 94%/90% bootstrap support, included the east Texas clone (ET13) along with two additional *Glomus* species clones reported from the GenBank database: *Glomus* sp. Glo11 isolate Dd2.14 (AF131052) and *Glomus* sp. Glo11 isolate Dd2.6 (AF131053). The fourth group, ET6 and ET10 belonged to a branch with 98%/90% bootstrap support that included *G. fasciculatum*, *G. interadices*, and *G. vesiculiferum*, but was not closely related to them. A single east Texas clone, ET11, appeared to form an additional separate lineage but was not well supported statistically. No formally named species closely matched any of the four groups of east Texas clones.

The AMF types detected were not uniformly distributed across the sample site. Group III was found in a sample from the lower portion of the hill slope. Group IV was restricted to midslope samples while Group I occurred in both lower slope and midslope samples. Group II was found on all three landscape positions; however, the majority of sequences (7/11) were isolated from the upper slope. Moreover, several AMF types (Groups I, II, and III for the lower slope and groups I, II, and IV for the mid-slope) were isolated from the root samples taken from the lower and middle slopes, while only Group II types were found in upper slope samples.

## DISCUSSION

While it is not possible to directly infer numbers of species from this limited study, it is clear that at least four AMF taxa were detected - including several not reported elsewhere. By compari-

son, Daniell et al. (2001) and Husband et al. (2002) in far more extensive studies involving multiple hosts and sites isolated eight and 30 fungal types from British cropland and tropical forest respectively. The results of this current study support the emerging consensus that there are many undiscovered species of AM fungi and that there is significant localized diversity (Husband et al. 2002; Helgason et al. 2002). Future data from different hosts, habitats, and seasons will enable us to further understand AMF diversity in east Texas ecosystems.

Small sample size does not permit a definitive statement as to whether the uneven distribution of AMF types is the result of habitat differences associated with the hill slope gradient or the result of chance distributions of fungal taxa across the landscape. However, it is evident that different combinations of local AMF species may occur on the same species of host plant in the natural environment and that these differences can occur across a relatively small spatial scale (<150m in the case of the current study). This suggests that future ecological studies relating differences in east Texas AMF composition to local differences in habitat or vascular plant species composition will be a fruitful area of inquiry.

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A SURVEY OF FRESHWATER MUSSELS (UNIONIDAE) OF  
THE OLD SABINE WILDLIFE MANAGEMENT AREA,  
SMITH COUNTY, TEXAS

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**Abstract.**—This study examined diversity and abundance of freshwater mussels (Family Unionidae) in the Sabine River and an old channel of the river that occur within the boundaries of the Old Sabine Bottom Wildlife Management Area (OSBWMA) of the Texas Parks and Wildlife Department (TPWD). Nine sites on the river and nine sites on the Old Channel each 40m in length were surveyed by hand searches for buried mussels. A total of 477 individuals of 17 species were found and three additional species were recorded during other studies within the OSBWMA. Species diversity indices were not different but some species were restricted to either the river or Old Channel. Nearly 60% of the species that could potentially occur in these waters were found but their numbers at all sites were low compared to other studies and a large percentage of specimens from the river samples were dead. Even though over 90% of the OSBWMA is forested erosion of the riverbank was evident and some beds of dead mussels were covered in sediment.

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Riverine mussels have historically dominated aquatic systems of the southeastern United States and their biomass in undisturbed rivers can exceed all other benthos by an order of magnitude (Strayer et al. 1994). They often occur in dense multispecies beds and perform significant functional roles such as removing suspended organic material, moving sediments and providing habitat for other invertebrates (Strayer et al. 1999; Vaughn & Hakenkamp 2001). The mussels of the family Unionidae of the United States are highly speciose with over 300 species known (Neves 1993). However, North American mussel populations have been declining for over a century with 35 species now presumed extinct and more than 40% imperiled to some degree (Neves et al. 1997; Vaughn 1997; Brown & Banks 2001). For many states, the extent of declines for specific species is simply not known (Bogan 1993; Layzer et al. 1993; Neves 1993). Over harvesting, pollution, reservoirs and other human activities have been implicated in

mussel losses elsewhere and are certainly issues for this fauna in Texas (Shannon et al. 1993; Howells et al. 1996; Howells 1997).

Texas has over fifty species of unionid mussels in multiple river basins that often have isolated drainage into the Gulf of Mexico (Howells et al. 1996). Consequently, species composition in eastern Texas differs from that of central and western areas (Neck 1982). Water pollution has long impacted the freshwater fauna of east Texas (Shira 1913; Howells 1997). Further, 31 reservoirs have been constructed on its rivers over the years and the change dams cause to water flow downstream is known to have major impacts on mussel diversity and abundance (Vaughn & Taylor 1999; Howells et al. 2000). In addition, erosion from agricultural land and commercial harvesting of mussels have also impacted the freshwater mussels of this area (Neck 1986; Howells 1997). Yet almost no studies on the mussels of the river basins in eastern Texas have been conducted (Howells 1997; Bordelon & Harrel 2004). For example, the upper stretches of the Sabine River have been intensely fished by commercial musselers (Howells 1993; Howells et al. 1996) but the only published surveys of the mussels of this drainage are for Lake Tawakoni (Neck 1986), a Master's thesis written in 1940 (Bechtel 1940) and in-house Texas Parks and Wildlife Department (TPWD) reports summarized in Howells (1997).

#### STUDY AREA

The Sabine River arises in the eastern part of north-central Texas (Hunt, Collin, and Rockwall counties), flows southeastward through eastern Texas for approximately 890 km and terminates at Sabine Lake, an estuary of the Gulf of Mexico. The TPWD's Old Sabine Bottom Wildlife Management Area (OSBWMA) is located 109 km south of Lake Tawakoni in Smith County, Texas and receives flow from both the Sabine River and Lake Fork Creek. The OSBWMA is 2318 hectares of mostly bottomland, hardwood forest along 38 km of the southern bank of the Sabine River. Eighteen km of an old channel of the Sabine River forms much of

the southern boundary of the area. The Sabine River at this site has a fall of 0.2 m per km, an average depth of less than 1 m and a flow averaging less than 5 CMS in the summer and 2 m and less than 115 CMS in the winter. Bank erosion exists in the area even though the river along this stretch is forested by the management area on the south and The Little Sandy Hunting Club, an extremely natural bottomland that is in a U. S. Conservation easement, located on its northeastern end.

The "Old Channel" is a smaller cut-off section of the Sabine River running somewhat parallel for 22 km. It meanders through the southern boundary of the OSBWMA for 18 km with a total fall of 0.2 m per km. It is structurally complex with numerous oxbows that may be up to 2.5 m in depth and narrow riffles that can be very shallow. The river feeds the Old Channel when the river level reaches 3.6 m and flow is approximately 28 CMS at the Mineola gauging station. It is also fed by 5 large creeks and several springs so that it flows even during lower water levels. The average summer flow of the Old Channel where it crosses the main road into the OSBWMA is 0.7 CMS and in winter it averages 2.3 CMS.

The substrate in the river consists of sand and clay with large areas of silt and detritus. The Old Channel has less silt but organic debris trapped by fallen trees is extensive in some areas. Although the banks of both the river and channel are lined with typical bottomland trees the waterways should receive some additional improvement over time as a 230 hectare pasture upstream acquired by TPWD in 2003 returns to native vegetation. This project provides a preliminary survey of the mussel fauna of the OSBWMA for future monitoring of species diversity and relative abundances.

## METHODS

*Sampling techniques.*—Nine sites on the river and nine sites on the Old Channel were surveyed between June and September 2005. The waterway was first explored for shells and stream characteristics that were appropriate for mussels and when a site

was located a timed hand search was initiated (Vaughn et al. 1997). A 40-m stretch was divided into 10 m sections and two persons searched the substrate for mussels for 15 to 20 minutes in each section. Both live and dead mussels were collected, identified and measured (shell length, width and height in mm), with one voucher of each species sent to the Stephen F. Austin State University invertebrate collection. Any questionable specimens were sent to Robert Howells of TPWD for identification.

*Data analysis.*—The general recommendations of Krebs (1998) were followed for measuring aspects of diversity. A Shannon-Wiener species diversity ( $H'$  base e) and evenness ( $J'$ ) were calculated for each waterway and the rarefaction method (James & Rathbun 1981) was used to calculate the expected number of species at comparable sample sizes. A Jaccard's coefficient of community was used to compare species similarity between watercourses (Brower et al. 1997).

## RESULTS AND DISCUSSION

A total of 477 individuals of 17 species was found during approximately 40 person-hours of sampling of the 18 sites (Table 1). Measurements of these 17 species of Unionids are given in Table 2. Three additional species were found at the OSBWMA during other surveys. A sampling during a mark-recapture study at site 5 in the Old Channel recorded one tapered pondhorn, *Unio declivis* and one pond mussel, *Ligumia subrostrata*. A large number of pond mussels, was also found in one oxbow pool off the Old Channel. Valves of two flat floaters, *Anodonta suborbiculata* were found on the river but not at a sample site. The diversity indices for the Sabine River and the Old Channel were not significantly different (Old Channel richness adjusted to the sample size of the river = 13.15; 95% confidence limits of 12-14). However, differences in the species composing the assemblages were evident ( $CC_J = 58.8\%$ ) and abundances for species in common were often different (Fig. 1). The river had half the total individuals found in the Old Channel and over 75% of those recorded were

dead. Over half of those in the Old Channel were collected alive. The most obvious environmental difference between the river and the Old Channel was the erosion of the steep riverbanks. The banks of the Old Channel were less elevated and often were forested up to the water's edge. Although some silting was evident, erosion was restricted to shorter stretches of the waterway.

Abundances of all mussels were much lower than in comparable surveys in other east Texas rivers (Howells et al. 2000; Bordelon & Harrel 2004). The major substrates of the waterways of the OSBWMA are sand and clay, neither of which are excellent habitats for unionids. However, it is useful to discuss the abundances of each species relative to others in the two habitats with suggestions as to factors that may be involved in the numbers recorded.

*Abundant species.*—Four species were found in at least five of the samples in the Sabine River (Table 1). Yellow sandshell, *Lampsilis teres*, was present at eight of the nine river sites. However, it was even more common in the Old Channel (all nine samples, Fig. 1). Interestingly, a large percentage of the individuals in the Old Channel were recently dead. This species is susceptible to drought conditions (Howells et al. 1996) but even individuals found underwater were often dead. The valves were nearly always of large adults so possibly the high number was the result of natural senescence in this fast growing species (Howells et al. 1996). Washboard, *Megalonaias nervosa*, was also abundant in the river but never found alive. Erosion of the riverbanks was common in all sites and often the valves of this species were found buried. Some live individuals have been found in nearby sections of the Sabine River, but our data suggests the species may be extirpated in the river bordering the OSBWMA. Fragile papershell, *Leptodea fragilis*, was found in most sites in both the river and the Old Channel and this may be because it is a species that can tolerate silting (Buchanan 1980). Bleufer, *Potamilus purpuratus*, was found in five sites on the river and most of these were live specimens.



Table 1. Cont.

Species	Old Channel Sites										Totals	No. sites present
	1	2	3	4	5	6	7	8	9			
<i>Amblyema plicata</i>	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
<i>Arcidens confragosus</i>	1/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/0	0/0	1/2	3
<i>Lampsilis hydiana</i>	3/4	0/2	3/0	0/0	1/0	4/0	1/0	0/0	0/0	0/0	12/6	6
<i>Lampsilis teres</i>	4/15	0/2	0/4	23/5	7/8	5/1	5/27	5/14	5/5	5/8	54/81	9
<i>Leptodea fragilis</i>	0/2	0/0	0/1	0/3	1/3	2/0	0/1	1/1	0/2	0/2	4/13	8
<i>Megalonaia nervosa</i>	0/0	0/0	0/0	0/0	0/0	0/0	0/2	0/0	0/0	0/0	0/2	1
<i>Obliquaria reflexa</i>	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
<i>Plectomerus dombeyanus</i>	10/3	0/2	11/1	4/0	0/1	0/0	7/2	0/0	2/0	0/0	34/9	7
<i>Potamilus purpuratus</i>	0/0	0/0	0/1	9/3	3/1	0/0	1/1	1/1	0/0	0/0	14/7	5
<i>Pyganodon grandis</i>	0/0	0/0	0/0	0/0	0/1	0/1	0/2	0/1	0/5	0/10	0/10	5
<i>Quadrula apiculata</i>	6/0	1/0	4/0	4/0	1/0	0/0	4/0	1/0	0/0	21/0	21/0	7
<i>Quadrula mortoni</i>	1/0	0/0	2/0	0/0	0/2	0/0	0/2	0/0	0/1	3/5	3/5	5
<i>Quadrula verrucosa</i>	3/0	2/2	3/0	6/2	0/0	0/0	0/1	1/0	0/0	15/5	15/5	6
<i>Toxolasma texasensis</i>	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/1	0/1	1
<i>Truncilla truncata</i>	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
<i>Unio merus tetralasmus</i>	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/2	1/2	1/2	1
<i>Villosa lienosa</i>	0/0	0/2	0/0	0/2	0/1	0/0	0/0	0/0	0/0	0/5	0/5	3
Total abundance (live/dead)	159/148											
Species richness	14											
Shannon diversity index	1.92											
Evenness	0.73											

Table 2. Measurements of Unionid mussels collected at the Old Sabine Wildlife Management Area. Mean  $\pm$  SD (minimum-maximum).

Species	Length (mm)	Width (mm)	Height (mm)
<i>Amblema plicata</i>	76.3 $\pm$ 9.2 (63-83)	59.5 $\pm$ 63.5 (51-66)	38.3 $\pm$ 58.0 (32-46)
<i>Arcidens confragosus</i>	93.0 $\pm$ 34.1 (57-139)	69.3 $\pm$ 20.9 (45-96)	59.8 $\pm$ 53.4 (22-139)
<i>Lampsilis hydiana</i>	63.7 $\pm$ 15.3 (34-87)	40.2 $\pm$ 9.8 (21-54)	29.3 $\pm$ 9.2 (12-42)
<i>Lampsilis teres</i>	94.1 $\pm$ 20.1 (15-124)	44.4 $\pm$ 9.2 (10-63)	31.0 $\pm$ 8.3 (2.0-47)
<i>Leptodea fragilis</i>	85.4 $\pm$ 17.6 (47-122)	53.0 $\pm$ 10.9 (27-72)	31.1 $\pm$ 6.5 (18-43)
<i>Megaloniais nervosa</i>	160.5 $\pm$ 43 (68-220)	106.3 $\pm$ 29.5 (44-158)	69.7 $\pm$ 23.3 (27-101)
<i>Obliquaria reflexa</i>	37.9 $\pm$ 6.5 (28-45)	30.4 $\pm$ 7.0 (20-39)	16.3 $\pm$ 3.6 (10-21)
<i>Plectomerus dombeyanus</i>	91.6 $\pm$ 14.9 (47-120)	60.9 $\pm$ 11.8 (27-84)	35.5 $\pm$ 16.0 (13-101)
<i>Potamilus purpuratus</i>	90.4 $\pm$ 36.0 (45-166)	58.3 $\pm$ 23.2 (27-100)	39.7 $\pm$ 18.5 (16-75)
<i>Pyganodon grandis</i>	89.3 $\pm$ 15.9 (566-122)	53.7 $\pm$ 9.1 (39-68)	41.4 $\pm$ 7.0 (25-45)
<i>Quadrula apiculata</i>	54.3 $\pm$ 8.3 (37-71)	45.7 $\pm$ 7.4 (28-56)	28.6 $\pm$ 5.8 (19-48)
<i>Quadrula mortoni</i>	52.6 $\pm$ 9.8 (22-64)	43.9 $\pm$ 7.7 (19-52)	32.1 $\pm$ 7.1 (11-39)
<i>Quadrula (=Tritogonia) verrucosa</i>	109.2 $\pm$ 25.5 (50-142)	62.9 $\pm$ 12.5 (28-97)	34.7 $\pm$ 10.2 (12-32)
<i>Toxolasma texasensis</i>	33.0	1.7	1.2
<i>Truncilla truncata</i>	35.6 $\pm$ 12.6 (20-49)	29.6 $\pm$ 7.7 (18-38)	19.4 $\pm$ 4.6 (12-24)
<i>Unio merus tetralasmus</i>	91.0 $\pm$ 4.6 (87-96)	46.0 $\pm$ 1.7 (45-48)	29.3 $\pm$ 2.3 (28-32)
<i>Villosa lienosa</i>	55.8 $\pm$ 10.7 (42-66)	31.8 $\pm$ 6.2 (24-40)	19.0 $\pm$ 5.2 (12-24)

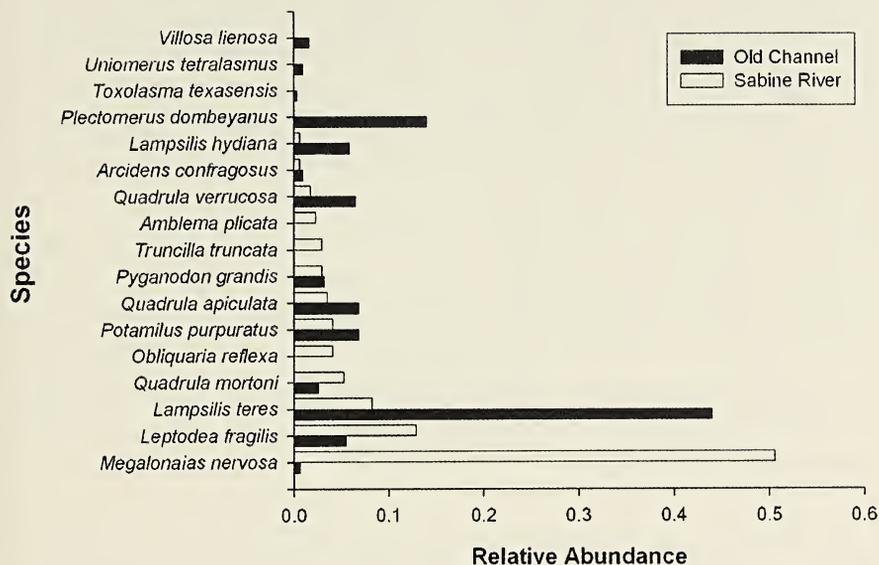


Fig. 1. Relative abundances of Unionid mussels from Texas Parks and Wildlife Department's Old Sabine Wildlife Management Area (Smith County, Texas).

This species was also relatively abundant in the Old Channel with 14 live specimens recorded. Several other species were found in at least five sites in the Old Channel including Louisiana fatmucket, *Lampsilis hydiana*, and bankclimber, *Plectomerus dombeyanus*, which were rare in the river (Fig. 1). Additionally, giant floater, *Pyganodon grandis*; southern mapleleaf, *Quadrula apiculata*; western pimpleback, *Q. mortoni*; and pistolgrip, *Q. (=Tritogonia) verrucosa*, were each found in two or three sites in the river (Table 1).

*Uncommon species.*—Eight species were found in less than five sites in both habitats. This includes threeridge, *Amblema plicata*, which was only found dead in three sites on the river. This species was once abundant enough in the Sabine River to support a commercial harvest (Howells et al. 1996), and overharvesting may have caused its apparent decline. Rock-pocketbook, *Arcidens*

*confragosus*, was found in small numbers in both the river and the channel. This species is not rare in eastern Texas but is generally found in limited numbers where it occurs (Howells et al. 1996). Threehorn wartyback, *Obliquaria reflexa*, was only found in two sites in the river of which one had six individuals and deertoe, *Truncilla truncata*, was only found in three sites in the river. These two species were abundant in other nearby sites on the river. Texas lilliput, *Toxolasma texasensis*: pondhorn, *Uniomerus tetralasmus*: and little spectaclecase, *Villosa lienosa*, were only found in the Old Channel. Little spectaclecase was found in three sites while only one Texas lilliput and three pondhorns at one site were found. The latter species is known to occur in large numbers in ponds (Howells et al. 1996) and a pool isolated off the Old Channel did have a large number of pondhorns.

In this study, 58% of the species of mussels previously recorded in the Sabine River were found. This suggests the waters bordering and within the OSBWMA support a diverse bivalve fauna. However, some other studies of mussels in east Texas rivers have samples much higher than in this report and other sites on the Sabine River outside the OSBWMA had much higher densities of unionids. None of the species recorded in either of these habitats appeared to be abundant and the lack of optimal substrate is likely the main cause. However, anthropogenic impacts that are known to affect freshwater mussels are evident in the area, such as sand and silt deposition from upstream, and erosion of banks. In addition, during this very dry summer, damage to the banks and substrate of the channel from wild hogs was evident. The waterways of the OSBWMA go through bottomland forest habitat and the impacts of agriculture should be limited. It would be useful to monitor recruitment of young in the unionid fauna at the OSBWMA to determine if individual species are recovering or declining.

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REPRODUCTIVE CYCLE OF THE SPOTTED SAND LIZARD,  
*MEROLES SUBORBITALIS* (SQUAMATA: LACERTIDAE)  
FROM SOUTH AFRICA

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**Abstract.**—The reproductive cycle of the spotted sand lizard, *Meroles suborbitalis* was studied from a histological examination of gonads. The reproductive cycle is prolonged with males undergoing spermiogenesis in all months examined. Females with reproductively active ovaries were found in all months except July. Histological evidence is presented (corpora lutea and yolk deposition in the same ovary) that more than one clutch can be produced in the same reproductive period. Females undergo a quiescent period between clutches. Regression analysis revealed a significant correlation between female body size and clutch size. Mean clutch size for 55 gravid females was  $3.98 \pm 1.01$  SD, range 1-6. One and two eggs are new minimum clutch sizes for *M. suborbitalis*. The smallest reproductively active male measured 45 mm SVL; the smallest reproductively active female measured 48 mm SVL. The prolonged reproductive cycle of *M. suborbitalis* resembles that of *Meroles anchietae*.

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The spotted sand lizard, *Meroles suborbitalis*, lives in arid savannah to desert from the Central Karoo to Little Namaqualand and Southern Namibia, extending to the central parts of the Namib Desert (Branch 1998). It is a sit and wait predator (Pianka 1971; Auerbach 1987). Brief information on its reproduction appeared in Fitzsimons (1943), Auerbach (1987) and Branch (1998). Branch (1998) reported breeding appeared to be continuous in the central Namib Desert with females producing eggs throughout the year. The purpose of this paper is to examine the reproductive cycle of *M. suborbitalis* from the Republic of South Africa and to compare it with reproduction in other south African lacertid lizards.

#### MATERIALS AND METHODS

One-hundred eighty-nine male (mean snout-vent length, SVL =  $53.8$  mm  $\pm$   $3.5$  SD, range = 45-63 mm), 130 female (mean SVL =  $55.6$  mm  $\pm$   $3.3$  SD, range = 48-65 mm) and 8 neonates (mean SVL =  $27.0$  mm  $\pm$   $1.6$  SD, range = 24-29 mm) were examined from the herpetology collection of the Natural History Museum of Los

Angeles County, Los Angeles, CA. Lizards were collected during 1969-1970. The left testis and epididymides were removed from males and the left ovary was removed from females for histological examination. Enlarged follicles ( $> 4$  mm length) were counted but not examined histologically. Oviductal eggs were previously removed for an ecological study (Pianka 1986). Tissues were embedded in paraffin, sectioned at  $5 \mu\text{m}$  and stained with Harris' hematoxylin followed by eosin counterstain. Testes slides were examined to determine the stage of the spermatogenic cycle and epididymides were examined for the presence of sperm. Ovary slides were examined for the presence of yolk deposition or corpora lutea. The relationship between body size (SVL) and clutch size was examined by linear regression analysis. *Meroles suborbitalis* male versus female mean body sizes as well as body sizes of males with testes in recrudescence versus males with testes in spermiogenesis were compared using an unpaired  $t$  test. Statistical tests were performed using InStat, vers 3.0b (Graphpad Software, San Diego, CA).

*Material examined.*—Specimens of *Meroles suborbitalis* from the Republic of South Africa (Northern Cape Province) examined from the herpetology collection of the Natural History Museum of Los Angeles County, Los Angeles (LACM).

53 km E, 43 km N Upington ( $28^{\circ}01'S$ ,  $21^{\circ}50'E$ ) LACM 82369.

18 km S, 22 km E Witkoms ( $27^{\circ}58'S$ ,  $21^{\circ}32'E$ ) LACM 82372, 82373, 82375, 82381, 82384, 84096.

66 km N, 35 km W Upington ( $27^{\circ}51'S$ ,  $20^{\circ}53'E$ ) LACM 82393.

Kalahari-Gemsbok National Park, 1 km W. Kameelsleep ( $25^{\circ}45'S$ ,  $20^{\circ}44'E$ ) LACM 82366-82368.

29 km S, 40 km E Rietfontein ( $27^{\circ}00'S$ ,  $20^{\circ}27'E$ ) LACM 82005, 82006, 82008, 82011-82014, 82018-82020, 82023, 82025.

121 km N, 16 km E. Upington ( $27^{\circ}22'S$ ,  $27^{\circ}22'E$ ) LACM 82276, 82278, 82284, 82287, 82288, 82291-82293, 82295, 82296, 82298-82301, 82303, 82305, 82306, 82308-82311, 82313-82316, 82318-82320, 82322-82327, 82329, 82330, 82332, 82333, 82336-82346, 82348-82350, 82352, 82353, 82355-82360, 82362, 82363, 82365.

31 km N, 100 km E. Upington (28°13'S, 22°16'E) LACM 82026, 82060, 82061, 82063-82067, 82069, 82070, 82077, 82087, 82099, 82102, 82104, 82106-82128, 82130-82133.

129 km N, 65 km W Upington (27°17'S, 21°54'E) LACM 82036, 82037, 82039-82041, 82044-82058.

24 km N, 83 km E Upington (28°17'S, 22°05'E) LACM 82139, 82140, 82142, 82145-82153, 82155-82160, 82162, 82163, 82166, 82167, 82170, 82172, 82175-82185, 82187, 82190-82192, 82194, 82196, 82198, 82200-82204, 82206, 82208-82210, 82212, 82214-82220, 82222, 82223, 82225, 82226, 82228-82232, 82234-82236, 82238, 82239, 82241-82249, 82251-82254, 82256, 82257, 82260, 82264-82271, 82273.

120 km N, 54 km W Upington (27°22'S, 20°43'E) LACM 81848, 81857, 81858, 81872, 81875, 81878, 81882, 81883, 81886, 81889-81891, 81897, 81899, 81900, 81902, 81905, 81909-81913, 81916, 81918, 81920, 81921, 81924, 81926-81929, 81931, 81932, 81934, 81935, 81938, 81939, 81941, 81943-81950, 81952-81962, 81964-81972, 81975, 81977, 81979, 81982-81990, 81992-81996.

## RESULTS

Seasonal changes in the testicular cycle are presented in Table 1. Only two stages were present: (1) recrudescence in which there is a renewal of germinal epithelium for the next period of sperm formation; seminiferous tubules contain primary, secondary spermatocytes and varying amounts of spermatids and (2) spermiogenesis in which clusters of spermatozoa line the lumina of the seminiferous tubules and several rows of metamorphosing spermatids are present. No males were recorded with regressed "inactive testes" in which seminiferous tubules are greatly reduced in size and contain mainly spermatogonia and interspersed Sertoli cells. Testes undergoing spermiogenesis were found in all months examined (June and November samples were not available). All epididymides from males undergoing spermiogenesis contained sperm. Males with testes in recrudescence were found in January through March. Males undergoing recrudescence ( $n = 8$ , mean SVL =  $47.6 \pm 2.1$  SD) were significantly smaller ( $t = 5.5$ ,  $df = 187$ ,  $P$

Table 1. Monthly distribution of reproductive conditions in seasonal testicular cycle of 189 *Meroles suborbitalis* from South Africa. Values are the number of males exhibiting each of the two conditions.

Month	N	Recrudescence	Spermiogenesis
January	9	2	7
February	11	5	6
March	3	1	2
April	63	0	63
May	27	0	27
July	10	0	10
August	24	0	24
September	16	0	16
October	20	0	20
December	6	0	6

<0.0001) than males undergoing spermiogenesis ( $n = 181$ , mean SVL =  $54.0 \pm 3.3SD$ ) suggesting smaller males enter the breeding population later in the year. The smallest reproductively active males measured 45 mm SVL and were from April (LACM 81926, 81934).

The mean of the female *M. suborbitalis* sample was significantly larger than that of the male sample ( $t = 4.6$ ,  $df = 317$ ,  $P < 0.0001$ ). Data on the seasonal ovarian cycle is presented in Table 2. There was ovarian activity in all months examined except for July; no sample was available from June. One female from October (LACM 82273) contained corpora lutea from a previous clutch and yolk deposition for a subsequent clutch indicating *M. suborbitalis* may produce more than one clutch in the same breeding cycle. Only one female of fourteen with corpora lutea (7.1%) had initiated yolk deposition for the next clutch suggesting females may spend time recovering their resources before commencing vitellogenesis for the next clutch. The smallest reproductively active female (three enlarged ovarian eggs > 4 mm length, LACM 82051, measured 48 mm SVL). Mean clutch size for 55 sets of enlarging ovarian follicles (> 4 mm diameter) from *M. suborbitalis* females from South Africa was  $3.98 \pm 1.01$ , range: 1-6. Linear regression analysis for 55 gravid females revealed a significant correlation

Table 2. Monthly distribution of reproductive conditions in seasonal ovarian cycle of 130 *Meroles suborbitalis* from South Africa. Values are the numbers of females exhibiting each of the five conditions.

Month	<i>n</i>	No yolk deposition	Early yolk deposition	Eggs > 4mm length	Corpus luteum	Corpus luteum and yolk deposition
January	4	3	0	1	0	0
February	4	3	0	1	0	0
March	2	1	0	0	1	0
April	35	18	0	16	1	0
May	20	3	4	7	6	0
July	7	7	0	0	0	0
August	17	10	0	6	1	0
September	17	3	5	8	1	0
October	20	3	1	12	3	1
November	2	0	0	2	0	0
December	2	1	0	1	0	0

between female body size (SVL) and clutch size:  $Y = -2.08 + 0.11X$ ,  $r = 0.33$ ,  $P = 0.0142$  (Fig. 1). The clutches of one from LACM 82339 collected 24 September 1970 and two each from LACM 82359 (27 Nov 1969), LACM 82306 (21 May 1970), LACM 82125 (24 April 1970) are new minimum clutch sizes for *M. suborbitalis*.

Neonates (SVL between 24-29 mm) were collected in December and January.

## DISCUSSION

*Meroles suborbitalis* has a testicular cycle in which some males were undergoing spermiogenesis in all months examined (no November sample was available). It thus differs from males of other African lacertid lizards in which the peak of spermiogenesis is in summer: (*Pedioplanis burchelli* Nkosi et al. 2004; *Pedioplanis lineocellata*, Goldberg 2006a; *Pedioplanis namaquensis*, Goldberg 2006b). Males of *Meroles cuneirostris* differed in having a long period of spermiogenesis from May to December a short regression in March followed by recovery in April (Goldberg & Robinson 1979). *Meroles anchietae* males followed a testicular cycle some-

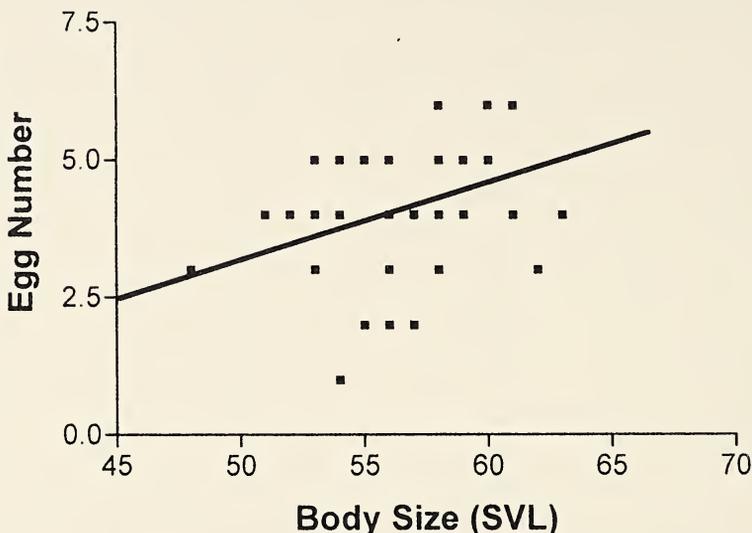


Fig. 1. Linear regression of body size, SVL (mm) on egg number for 55 *Meroles suborbitalis* females from the Republic of South Africa. Some values (same SVL and number of eggs) occurred more than once.

what similar to *M. suborbitalis* as some males in spermiogenesis were found in each month, however, regressed males were found in February-March and males in recrudescence were found in April and May (Goldberg & Robinson 1979).

*Meroles suborbitalis* males with testes in recrudescence were all from January-March and were significantly smaller than males undergoing spermiogenesis. This may suggest they were coming into breeding condition for the first time and that sub-adult males enter the breeding population in late summer. Subsequent study to determine the time of first breeding for *M. suborbitalis* is required.

The ovarian cycle of *M. suborbitalis* was prolonged with reproductive activity exhibited in all months except July. *Aporosaura anchietae* also exhibited a long period of ovarian activity with vitellogenic to oviductal eggs recorded in all months (Goldberg & Robinson 1979). It was postulated that the long

period of egg production in *A. anchietae* might, in part, result from the utilization of seeds as part of the diet when insects were not abundant (Robinson & Cunningham 1978; Goldberg & Robinson 1979). *Meroles suborbitalis* is insectivorous (Branch 1998) and thus may be subject to periodic seasonal food shortages. However, the apparent quiescent period between clutches of *M. suborbitalis* females might allow sequestration of sufficient energy resources to produce clutches through most of the year. In contrast, the ovarian cycles of *M. cuneirostris* (cf. Goldberg & Robinson 1979); *P. namaquensis* (cf. Goldberg 2006a); *P. lineoocellata* (cf. Goldberg 2006b) were seasonal with egg production mainly restricted to summer.

Fitzsimons (1943) and Auerbach (1987) reported *M. suborbitalis* laid about 6 eggs. Branch (1998) reported clutches of 3-8 eggs were laid. Pianka (1986) reported a value of  $4.2 \pm 0.87$  for 108 *M. suborbitalis*. In the central Namib *M. suborbitalis* may produce clutches throughout the year; in the Kalahari females may produce two clutches (Branch 1998). The results of this study support the statement of Branch (1998) that reproduction in *M. suborbitalis* appears to be continuous. Subsequent studies will be required to elucidate the diversity of reproductive strategies of South African lacertid lizards.

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MINIMUM SIZE AT MATURATION IN THE  
MUD SNAKE, *FARANCIA ABACURA* (SERPENTES: COLUBRIDAE)  
FROM THE SOUTHEASTERN UNITED STATES

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**Abstract** - Museum specimens of 129 female mud snakes (*Farancia abacura*) were examined for body size and reproductive condition. Specimens from Texas, Oklahoma, Louisiana, Mississippi, Florida, Tennessee and South Carolina provided data on the minimal size at maturation. A small (42.0 cm snout vent length) reproductive female *F. abacura* with Class I follicles was observed in this investigation and is reported here with an analysis for the relationship between body size and mean follicle length. The theoretical significance of early reproduction with respect to increased fecundity and fitness is presented and may provide future researches with additional information for investigating the evolutionary ecology of this secretive snake species.

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The age and size at which a species becomes reproductive is one of its most important natural history traits. This trait is often more influential to a species' fitness than any other natural history trait (Sterns 1992). Species that become reproductive earlier in life have a greater probability of survival to reproductive age and the potential for greater fecundity. Although there are costs associated with this life history strategy, for many species the benefits of early reproduction are much greater than the potential costs.

Constraints on the age at maturation include phylogeny and body size. In squamates, researchers have demonstrated a positive correlation between adult body size and age at maturation (e.g., Dunham & Miles 1985). Although there may be exceptions, this relationship is conserved among most reptiles.

There is limited information on the reproductive life history of mud snakes, *Farancia abacura* (see Fitch 1970; Mitchell 1982). Quantifying the reproductive traits in this species is difficult due to its secretive habits. There are several reports of oviposition and subsequent hatching (Meade 1935; 1937; 1940a; 1940b; Goldstein 1941; Reynolds & Solberg 1942; Meade 1945), but little is known about the minimum body size at which this species becomes reproductive.

More recently, Robinette & Trauth (1992) investigated both female ( $n = 22$ ) and male ( $n = 22$ ) reproductive cycles from *F. abacura* collected throughout Arkansas. The smallest female with follicles was approximately 56.0 cm snout-vent length (SVL). This female is only 29.0 to 40.1 cm larger than the reported 15.9 to 27.0 cm hatching size of *F. abacura* (Conant & Collins 1998).

#### METHODS AND MATERIALS

A total of 129 female *F. abacura* museum specimens from throughout their range were examined to determine the minimum body size at maturation. Seventy-six of the 129 specimens provided reproductive data that could be used in the analyses; 53 female specimens had damaged follicles (e.g., road killed specimens) or were dissected previously. Specimens were sampled primarily from Louisiana and Texas, with some adults from Florida, Mississippi, Oklahoma, South Carolina, and Tennessee. Ovarian follicles and oviductal tissue samples were removed through a ventral incision and stored in 70% ethanol. Follicles were measured with a Fowler dial caliper and classified based on length according to Betz's (1963) system (Class I = 0.1 – 5.0 mm; Class II = 5.1 – 10.0 mm; Class III = 10.1 – 20.0 mm; and Class IV = 20.1 – 46.0 mm). This system allows for grouping follicles into one of four distinct size classes and is commonly used in studies investigating follicle size and development (e.g., Kofron 1979; Kofron 1983; Holycross & Goldberg 2001; Goldberg 2002; Rosen & Goldberg 2002).

*Material examined.*— Specimens of *Farancia abacura* examined. Standard museum symbolic codes for institutional resource collections follow Leviton et al. (1985).

**Florida:** DADE CO.: TNHC 50103, LAKE CO.: OMNH 18993, PUTNAM CO.: OMNH 34414.

**Louisiana:** AVOYELLES CO.: LSUMZ 2724, LSUMZ 75894, LSUMZ 75914, BEAUREGARD CO.: LSUMZ 22552, BOSSIER CO.: LSUMZ 24247, CADDO CO.: LSUMZ 4838, CAMERON CO.: TCWC 17417, EAST BATON ROUGE CO.: LSUMZ 2723, LSUMZ 5959, LSUMZ 11895, LSUMZ 20331, LSUMZ 20332, LSUMZ 24248, LSUMZ 24249, LSUMZ 31246, LSUMZ 38093, LSUMZ 38959, LSUMZ 39191, LSUMZ 44913, LSUMZ 65908, LSUMZ 83190, LSUMZ 83386, LSUMZ 83390, LSUMZ 84521, EAST FELICIANA CO.: LSUMZ 2725, LSUMZ 6109,

LSUMZ 9121, LSUMZ 18296, LSUMZ 34306, EVANGELINE CO.: LSUMZ 29097, LSUMZ 58466, LSUMZ 58467, LSUMZ 74846, LSUMZ 75895, FRANKLIN CO.: LSUMZ 43537, GRANT CO.: LSUMZ 74849, IBERVILLE CO.: LSUMZ 18770, LSUMZ 46868, LSUMZ 75888, LSUMZ 75889, JEFFERSON CO.: LSUMZ 9163, LSUMZ 9164, LSUMZ 18282, LSUMZ 58389, LSUMZ 58454, LSUMZ 58455, JEFFERSON DAVIS CO.: LSUMZ 59063, LAFAYETTE CO.: LSUMZ 74848, LSUMZ 75909, LAFOUCHE CO.: LSUMZ 19178, LIVINGSTON CO.: LSUMZ 12884, LSUMZ 13008, LSUMZ 55927, LSUMZ 79283, LSUMZ 80501, LSUMZ 80503, NATCHITOCHEES CO.: LSUMZ 75896, LSUMZ 83451, LSUMZ 83485, LSUMZ 83504, LSUMZ 84597, LSUMZ 84674, ORLEANS CO.: LSUMZ 9162, LSUMZ 14154, PLAQUEMINES CO.: LSUMZ 75900, POINTE COUPEE CO.: LSUMZ 4149, LSUMZ 18295, RAPIDES CO.: LSUMZ 74853, LSUMZ 75869, LSUMZ 75893, RICHLAND CO.: LSUMZ 42524, ST. LANDRY CO.: LSUMZ 20330, LSUMZ 74850, LSUMZ 75862, LSUMZ 75868, TCWC 38241, ST. JOHN THE BAPTIST CO.: LSUMZ 39805, LSUMZ 58438, LSUMZ 59624, LSUMZ 80948, ST. MARTIN CO.: LSUMZ 74843, LSUMZ 74844, LSUMZ 74845, LSUMZ 74847, LSUMZ 74851, LSUMZ 75866, LSUMZ 75891, LSUMZ 75892, LSUMZ 75897, LSUMZ 75898, LSUMZ 75907, LSUMZ 75913, LSUMZ 75915, LSUMZ 75916, LSUMZ 75917, LSUMZ 75910, LSUMZ 75911, LSUMZ 75912, LSUMZ 79053, ST. MARY CO.: LSUMZ 75890, LSUMZ 75899, ST. TAMMANY CO.: LSUMZ 24098, LSUMZ 24099, LSUMZ 28816, LSUMZ 58518, LSUMZ 80255, LSUMZ 80898, LSUMZ 81207, TANGIPAHOA CO.: LSUMZ 17674, LSUMZ 23175, LSUMZ 47458, LSUMZ 57956, LSUMZ 57959, LSUMZ 57960, LSUMZ 80507, LSUMZ 80508, LSUMZ 80509, LSUMZ 80510, LSUMZ 80511, TERREBORNE CO.: TCWC 74150, TERREBORNE CO.: TCWC 71458, VERNON CO.: LSUMZ 20174, WASHINGTON CO.: LSUMZ 21026, WEST CARROLL CO.: LSUMZ 20333.

**Mississippi:** ATTALA CO.: LSUMZ 75989, HANCOCK CO.: LSUMZ 41368, LSUMZ 19176, JACKSON CO.: LSUMZ 57957, SHARKEY CO.: LSUMZ 47883.

**Oklahoma:** MCCURTAIN CO.: OMNH 30111, OMNH 38351, OMNH 24380, OMNH 30706.

**South Carolina:** CHARLESTON CO.: LSUMZ 36919, JASPER CO.: LSUMZ 74432.

**Tennessee:** LAKE CO.: LSUMZ 74856.

**Texas:** ANDERSON CO.: TCWC 64992, TCWC 81207, ANGELINA CO.: SFA 654, ARANSAS CO.: TCWC 81205, AUSTIN CO.: TCWC

4583, TCWC 6453, BURLESON CO.: TCWC 18279, BRAZORIA CO.: TCWC 53155, BRAZOS CO.: TCWC 5164, TCWC 13838, TCWC 45620, CHAMBERS CO.: TCWC 60707, COLORADO CO.: TCWC 64322, DEWITT CO.: TCWC 82477, FORT BEND CO.: TCWC 81641, GALVESTON CO.: TCWC 27368, GRIMES CO.: TCWC 64991, TNHC 36319, HARDIN CO.: TNHC 4534, TNHC 19800, TNHC 21940, TNHC 28728, HARRIS CO.: TCWC 183, TCWC 8711, TCWC 18278, HARRISON CO.: TCWC 79273, HOUSTON CO.: TCWC 67299, JACKSON CO.: TCWC 29467, JASPER CO.: SFA 2896, TCWC 48425, TCWC 78732, JEFFERSON CO.: TCWC 8710, TCWC 16178, LEON CO.: TCWC 2614, TCWC 5158, TCWC 5159, TCWC 5160, TCWC 5161, TCWC 5162, TCWC 5163, TCWC 5177, TCWC 8709, TCWC 8712, LIBERTY CO.: TNHC 21846, MADISON CO.: TCWC 17389, TCWC 49322, MORRIS CO.: TCWC 78731, MONTGOMERY CO.: TCWC 57916, TCWC 68233, TCWC 68237, TCWC 81209, TCWC 82476, NACODOCHES CO.: SFA, SFA 1216, SFA 1233, SFA 2033, SFA 2291, SFA 2309, NEWTON CO.: TCWC 48426, ORANGE CO.: TCWC 33646, TNHC 21963, REFUGIO CO.: TNHC 20583, TNHC 32202, SAN JACINTO CO.: LSUMZ 34289, TYLER CO.: TCWC 78730, TCWC 81204, VICTORIA CO.: TCWC 70080, WALKER CO.: TCWC 67234, TCWC 82818, WHARTON CO.: TCWC 4757, TCWC 81206.

**Unknown Locality:** SFA, SFA, TCWC 31956.

## RESULTS AND DISCUSSION

Snout-vent length (SVL) of the 76 females examined ranged from 42.0–191.0 cm ( $\bar{x} = 92.4$ ,  $SE = 3.53$ ,  $n = 75$ ); six individuals (TCWC-81206 = 42.0 cm; LSUMZ-75890 = 43.0 cm; LSUMZ-75913 = 43.0 cm; LSUMZ-75891 = 44.0 cm; TCWC-82477 = 53.5 cm; LSUMZ-17674 = 55.0 cm) had a smaller SVL than the 56.0 cm SVL reported by Robinette & Trauth (1992). All six of these individuals had either Class I or II follicles. The smallest individual (TCWC-81206 = 42.0 cm) was 14.0 cm smaller than the female reported by Robinette & Trauth (1992) and had Class I follicles (4.08 mm). This individual represents a significant finding for this species' minimum size at maturation. All other reproductive females examined fell within the range of adult body size reported by Conant & Collins (1998). A frequency distribution of body size for 75 of the 76 specimens examined showed a normal distribution (Kolmogorov-Smirnov Distribution = 0.078,  $P > 0.20$ ) for female reproductive size (Fig. 1).

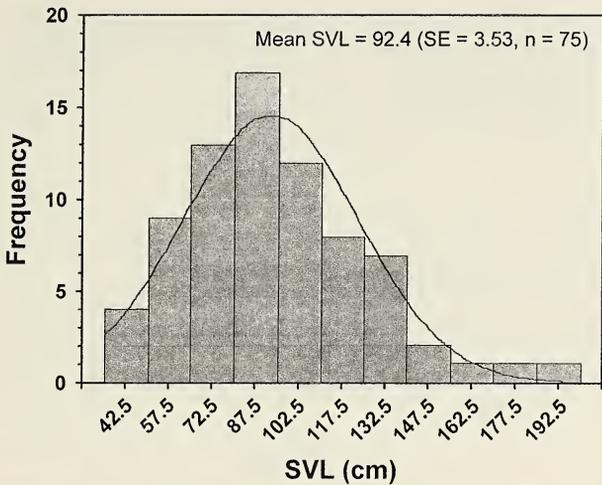


Fig. 1. A frequency distribution of snout-vent-length (SVL) for 75 of the 76 snakes examined for reproductive condition. Snout-vent length in one female specimen was not measured due to its poor condition.

No relationship was found between female body size (SVL) and mean follicle length (Fig. 2). This is because follicular development is mostly dependent on time of year and not body size (Lutterschmidt et al. 2005). Snout-vent length explained only 2% ( $r^2 = 0.0203$ ) of the variation in the mean follicle size ( $F = 1.51$ ;  $df = 1, 74$ ;  $P = 0.223$ ). However, what may be of interest is the sequential increase in variation among Class I ( $SE = 0.149$ ,  $n = 17$ ), Class II ( $SE = 0.302$ ,  $n = 44$ ), and Class III ( $SE = 0.636$ ,  $n = 14$ ) follicle lengths (Fig. 2). These increases in variation among classes may be due to differences among females and their rates of follicular development through the reproductive season.

This report provides important information regarding minimum body size at maturation in a Colubrid that typically reaches 137 cm as an adult with hatchlings ranging from 15.9 to 27.0 cm (Conant & Collins 1998). As discussed by Sterns (1992), there are potential reproductive advantages in having a moderately large body size at hatching. The 42.0 cm female with Class I follicles (TCWC-81206) observed in this study was only 15 cm larger than the reported 27 cm maximum hatchling size. Unfortunately, there appears to be no information regarding growth rate of *Farancia* under field conditions and how long it may take an individual female to reach reproductive

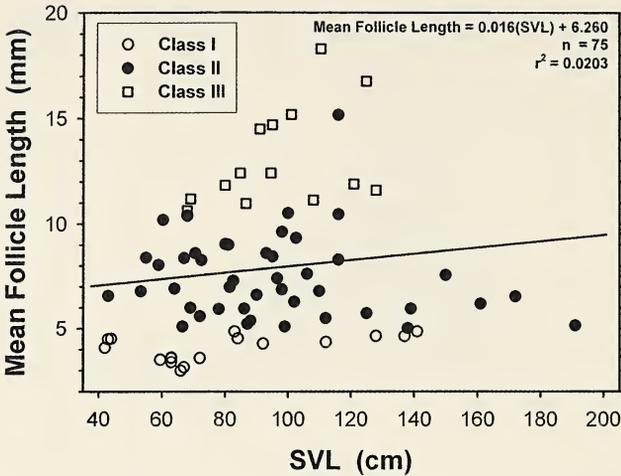


Fig. 2. Regression analysis of mean follicle length (mm) versus snout-vent length (SVL).

size.

Growth rates for *Crotalus* (a large-bodied snake in the family Viperidae) have been documented under field conditions. Prival et al (2002) reported that *Crotalus pricei* may grow 0.726 cm/shed or 0.0063 cm/day (i.e. 2.3 cm/year). Conversely, growth rates for immature female *Crotalus viridis* are considerably faster and have been reported as 10cm/year (see Fig. 1 in Diller & Wallace 2002). Himes et al. (2002) studied a large-bodied Colubrid (*Pituophis ruthveni*) from Louisiana and Texas using radio telemetry and reported a growth rate of 12.0 cm/19.4 months (i.e., 7.4 cm/year). Gibbons & Dorcus (2004) also report growth rates within species accounts of North American watersnakes (Colubridae: *Nerodia*). For example, Trauth (1990) in Gibbons & Dorcus (2004) estimated the growth rate in an Arkansas population of *N. cyclopion* (10.2 – 26.5 cm/year,  $n = 72$ ) from size classes representing presumed age. Faster growth rates were reported for *N. erythrogaster* (0.77 – 1.63 mm/day or 28.1-59.5 cm/year) from three recaptures (Preston 1970 in Gibbons & Dorcus 2004). However, a slower rate of growth (9.5 cm/year) was observed for 20 newborn snakes raised in captivity (Conant & Downs 1940 in Gibbons & Dorcus 2004). Because *Farancia* is also a Colubrid snake and may experience similar lengths of seasonal

activity as *P. ruthveni*, *N. cyclopion*, and *N. erythrogaster* due to similar geographic distributions, *F. abacura* may also demonstrate similar growth rates. Thus, a female could conservatively grow 10-20 cm in SVL and reach reproductive size within its second activity season.

Sterns (1992) discusses the evolutionary advantage of early reproduction and its most important limiting factors, minimum adult body size at maturation. However, as for other larger-bodied snakes, large hatchling size of *F. abacura* may allow this species to demonstrate early reproduction resulting in an overall increase in fitness.

### CONCLUSIONS

This information on the natural history of *Farancia* may help future researchers studying the reproductive strategy and evolutionary ecology of this fully aquatic and secretive snake species.

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## GENERAL NOTES

NOTES ON INVASIVE PLANTS IN THE RIO GRANDE DELTA  
OF CAMERON COUNTY, TEXAS**Robert I. Lonard and Frank W. Judd***Department of Biology, University of Texas-Pan American  
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Agricultural practices in the Rio Grande Delta from the 1930s to the 1980s resulted in the removal of 74,000 ha of the native woodland vegetation in Cameron County, Texas (Tremblay et al. 2005). Subsequently, this area has been prone to the invasion of exotic tropical and subtropical species. These introduced species have contributed to altered ecosystem functions.

Resource managers at the 420 ha Lennox Foundation Southmost Preserve (LFSP) are attempting to restore sites dominated by introduced species. Therefore, the purpose of this note is to report species richness of invasive species and the locations of these species in the preserve.

The LFSP is located along the Rio Grande about 15 km east of Brownsville. The tract is part of the Boscaje de la Palma region of the Lower Rio Grande Wildlife Corridor. An endangered Texas palmetto (*Sabal mexicana*) forest (Diamond et al. 1987) occupies a small area. Commercial operations include row crop farming and citrus production. Discontinued palm, cycad, and lacebark elm nurseries occupy extensive areas, and roads and paths provide additional disturbances to the mesic landscape. Wetland sites include resacas (old ox-bows of the Rio Grande) and a narrow riparian zone adjacent to the Rio Grande.

Vegetation sampling was conducted by randomly establishing 100-m line transects (Canfield 1941) at each of 11 sites (Table 1). Transects were divided into 10 m intervals and readings were taken along the total length of each interval. Frequency, relative frequency, foliage cover, relative cover, and an importance value

Table 1. Study sites and sampling dates at the Lennox Foundation Southmost Preserve, Cameron County, Texas.

Study site	Location	Sampling date
Re-vegetated nursery	25°51'05.31"N 97°23'55.08"W	4 September 2003
Riparian woodland	25°51'01.89"N 97°23'53.95"W	4 September 2003
Weedy roadside	25°50'56.98"N 97°23'46.90"W	25 September 2003
Giant reed/weedy riparian	25°50'39.66"N 97°24'01.33"W	2 October 2003
Old sorghum field	25°50'56.81"N 97°23'42.27"W	16 October 2003
Mediterranean fan palm nursery	25°50'23.55"N 97°23'31.56"W	20 November 2003
Resaca margin	25°51'03.29"N 97°23'50.58"W	12 January 2004
Washington fan palm nursery	25°50'35.24"N 97°23'50.04"W	12 January 2004
Levee margin/re-vegetated	25°51'17.77"N 97°23'25.61"W	12 January 2004
Lacebark elm nursery	25°50'21.89"N 97°24'13.64"W	18 February 2004
Texas palmetto forest	25°50'32.64"N 97°22'55.01"W	4 March 2004

that indicates dominance were recorded for each species (Lonard & Judd 2002). The line intercepts were supplemented with nine driving and walking surveys. Nomenclature follows Jones & Wipff (2003) for scientific names and the Subcommittee on Standardization of Common and Botanical Names of Weeds (1966) for common names.

*Dicots*.—Four introduced dicots were present on the transects (Table 2). *Leucaena leucocephala* (white popinac) was the most extensively established woody dicot. Thickets of this aggressive

invasive species are established in the lacebark elm (*Ulmus parviflora*) nursery. Seedlings are abundant in the ground layer, and the shrub and tree layers are dominated by this species. *Ricinus communis* (castorbean), first reported by land developers in 1869 (Dougherty 1869), was present in the Mediterranean fan palm (*Chamaerops humilis*) nursery and was fairly common on the banks of the Rio Grande.

*Chenopodium murale* (nettleleaf goosefoot) was present only along a weedy roadside adjacent to a citrus grove, and *U. parviflora* was planted in rows in the lacebark elm nursery. Neither of these species shows evidence of spreading to other sites.

During reconnaissance of the preserve, small populations of *Melia azedarach* (Chinaberry) and *Tamarix aphylla* (athel tamarisk) were noted in the Washington fan palm nursery and on the banks of the Rio Grande, respectively. Drupes of *M. azedarach* are dispersed by perching birds, and propagules of *T. aphylla* probably have been carried down stream to the preserve.

*Leucaena leucocephala*, and to a lesser extent, *M. azedarach* pose threats to biodiversity. The former species is quickly invading sites near the river. Large colonies are developing with several focal point trees serving as a seed source for the development of a monotypic stand. A management effort directed at mechanical removal is an option before this species invades other sites. Legumes should be removed by hand, bagged, and burned. Girdling beetles prune branches, but this promotes adventitious growth in intact stems. The same procedure could be followed for removal of *M. azedarach* plants. It is impractical to remove drupes from the ground layer.

*Monocots.*—Eighteen introduced monocots including 15 species of grasses were recorded on transects at LFSP. *Urochloa maxima* (Guineagrass) has had the greatest impact on the native vegetation

Table 2. Introduced species on transects at the Lennox Foundation Southmost Preserve. 1 = re-vegetated nursery, 2 = riparian woodland, 3 = weedy roadside, 4 = giant reed/weedy riparian, 5 = old sorghum field, 6 = Mediterranean fan palm nursery, 7 = resaca margin, 8 = Washington fan palm nursery, 9 = levee margin/re-vegetated, 10 = lace-bark elm nursery and 11 = Texas palmetto forest.

Species	1	2	3	4	5	6	7	8	9	10	11
<b>Magnoliopsida</b>											
<i>Chenopodium murale</i>			X								
<i>Leucaena leucocephala</i>										X	
<i>Ricinus communis</i>						X					
<i>Ulmus parviflora</i>										X	
<b>Liliopsida</b>											
<i>Chamerops humilis</i>						X					
<i>Washingtonia robusta</i>								X		X	
<i>Arundo donax</i>				X							
<i>Cynodon dactylon</i>			X			X					
<i>Dactyloctenium aegyptium</i>			X								
<i>Dicanthium annulatum</i>				X		X		X	X		
<i>Dicanthium aristatum</i>	X					X			X	X	
<i>Digitaria bicornis</i>			X								
<i>Eleusine indica</i>			X								
<i>Eragrostis barrelieri</i>			X								
<i>Oplismenus hirtellus</i>											X
<i>Pennisetum ciliare</i>			X					X	X		
<i>Sorghum bicolor</i>					X						
<i>Sorghum halepense</i>	X					X			X		
<i>Urochloa maxima</i>	X	X	X	X		X		X	X	X	X
<i>Urochloa mutica</i>				X			X				
<i>Urochloa panicoides</i>			X		X						
<i>Eichornia crassipes</i>				X							

at the preserve. It was present on nine transects, and it had high importance values at eight sampling sites. It was the dominant or co-dominant at six sites. *Urochloa maxima* has supplanted native species at all sites except the Texas palmetto forest, the old sorghum field, and the resaca margin. However, it is abundant in grassy areas adjacent to these sites. *Urochloa maxima* is replaced by a monotypic stand of *Urochloa mutica* (paragrass) about 15 m wide that forms a belt around the shallow water resaca.

At sites not dominated by *U. maxima* other aggressive perennial grasses including *Pennisetum ciliare* (buffelgrass), *Dichanthium annulatum* (Kleberg bluestem), *Dichanthium aristatum* (Angleton bluestem), *Cynodon dactylon* (Bermudagrass), and *Sorghum halepense* (Johnsongrass) have replaced native species. Farming and road maintenance operations have favored the spread of the seasonally abundant federally listed noxious weed *Urochloa panicoides* (panic liverseed grass). This annual roots at the nodes, forms dense prostrate mats, and produces abundant caryopses.

Large, asexually reproducing stands of *Arundo donax* (giant reed) co-occurs with dense populations of the native *Phragmites australis* (common reed) on the margins of the Rio Grande. Dense stands of *A. donax* dominate large segments of the Rio Grande including the riparian zone at Del Rio, Laredo, and Presidio (Everitt et al. 2004; Everitt et al. 2005). The world's most invasive aquatic weed, *Eichornia crassipes* (waterhyacinth) rafts on the slow moving currents of the Rio Grande and lodges in the giant reed/common reed zone. However, it was not seen in the shallow water resaca.

Other introduced grasses including *Dactyloctenium aegyptium* (crowfootgrass), *Digitaria bicornis* (Asian crabgrass), *Eleusine indica* (goosegrass), and *Eragrostis barrelieri* (Mediterranean lovegrass) were present only on weedy roadsides. Due to their low stature or prostrate growth forms, they do not compete with taller invasive grasses. *Sorghum bicolor* (sorghum) was present only in the old sorghum field. Adventitious shoots were growing from basal culms of harvested plants. The pan tropical *Oplismenus hirtellus* subsp. *setarius* (shortleaf basket grass) was found only on the transect in the Texas palmetto forest. It may have escaped from former commercial nursery operations at the preserve.

The introduced palms, *Chamaerops humilis* (Mediterranean fan palm) and *Washingtonia robusta* (Washington fan palm) were present only in discontinued nurseries. Adventitious growth of *C.*

*humilis* produced impenetrable thickets in some sections of the nursery, but no plants had escaped to other locations.

*Urochloa maxima* and *P. ciliare*, introduced from Africa and Eurasia, respectively, have supplanted the herbaceous ground layer at most sites in the riparian zone of the lower Rio Grande (Lonard & Judd 2002). However, *U. maxima* dominates the landscape at LFSP. It is impractical, if not impossible, to remove *U. maxima* and other invasive grasses from the preserve. Fire only serves to promote vegetative growth of *U. maxima* and *P. ciliare*, and plowing and disking serve to transport rhizomes of *S. halepense*. Selective herbicides are a poor option because a viable seed bank of native herbaceous dicots and grasses may no longer be available to fill the void.

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TWO NEW TROPICAL COLUBRID SNAKE HOSTS FOR  
THE PENTASTOMID WORM, *KIRICEPHALUS COARCTATUS*  
(PENTASTOMIDA: POROCEPHALIDAE) FROM PANAMA

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*Kiricephalus coarctatus* (Pentastomida, tongue worm) is known to utilize snakes as both second intermediate and definitive hosts throughout Central and North America (Riley & Self 1980). Eggs of *K. coarctatus* are apparently non-infectious to snakes; however, the typical life cycle includes infection of the first intermediate host (mammals, amphibians, and lizards) through consumption of eggs (Guidry & Dronen 1980; Riley & Self 1980). Infective nymphs are then transmitted to a second intermediate or definitive host through ingestion of the first intermediate host (Riley & Self 1980). In snakes serving as definitive hosts, adult worms are typically found in the lungs and body cavity (Detterline et al. 1984; Foster et al. 2000). This study reports the presence of *K. coarctatus* from the large-scaled black treesnake (*Chironius grandisquamis*) and parrot snake (*Leptophis ahaetulla*) which were collected within the Omar Torrijos National Park in Cocle Province of Panama.

At 2200 hours on 10 September 2005 a single adult female specimen (82.7 cm SVL; 64.6 cm TL; 147.1 g) of *Chironius grandisquamis* was collected from an arboreal habitat approximately 2.0 meters above the ground. This species ranges from northern Honduras to northern Colombia (Köhler 2003) and feeds primarily on frogs, but is known to feed on other amphibians, lizards, birds, and mice (Savage 2002). The specimen was collected, returned to the lab for processing, and left in the collecting bag overnight. The next morning the snake had expired and a single parasite was visible within the collecting bag and a second specimen was in the process of exiting the host through a nostril. Dissection of the host specimen recovered an additional 10 parasites (12 total; 7M:5F) from the lungs, trachea, and attached to the body cavity. Some specimens had burrowed through the lung lining and attached themselves to the body wall. A total of 10 specimens were collected, preserved in 70% ethanol, and later identified as mature adult *Kiricephalus coarctatus*. The preserved *C. grandisquamis* was deposited in the Museo de Vertebrados de la Universidad de Panama (MVUP-1864) and voucher parasite specimens were deposited in the United States National Parasite Collection, USNPC, Beltsville, Maryland (USNPC 97485).

At 2130 hours on 4 October 2005 an adult male specimen of *Leptophis ahaetulla* (71.0 cm SVL; 50.3 cm TL; 54.3 g) was collected from an arboreal habitat approximately 2.1 m above the ground. This species ranges from southern Mexico to southern Brazil (Solorzano 2004); its diet consists mainly of frogs, although lizards, insects, birds, and bird eggs have been reported (Savage 2002). The specimen was collected, brought back to the lab for processing, and left overnight in the collecting bag. The following morning a single parasite was observed exiting the live snake through the nostril. The snake was euthanized and dissected; four additional parasites (five total; 1M:4F) were removed from the lungs and body cavity of the snake. Two of these specimens were collected, preserved as before, and later identified as mature adult *K. coarctatus*. The preserved *L. ahaetulla* was deposited in the

Museo de Vertebrados de la Universidad de Panama (MVUP-1873) and voucher specimens of *K. coarctatus* were deposited in the United States National Parasite Collection (USNPC 97486).

*Kiricephalus coarctatus* exhibits a prominent sexual dimorphism: males 30-35 mm in length, females 81-114 mm in length (Riley & Self 1980); males 13-47 mm lengths, females 55-76 mm lengths (Detterline et al. 1984). Measurements observed in this study, 24-29 mm length ( $n = 6$ ) for males and 65-89 mm length ( $n = 6$ ) for females, are within the combined range of values of Riley & Self (1980) and Detterline et al. (1984). Riley & Self (1980) suggested that males may be absent from mature infections of *K. coarctatus*. Observations of a 7M:5F and 1M:4F infection in this study combined with the 8M:3F ratio reported by Detterline et al. (1984) indicate that males are often present; however, males die shortly after mating, resulting in a decreased longevity relative to females (Riley & Self 1980). Therefore, observations reported in this study may be of more recent infections relative to those reported by Riley & Self (1980).

The observed escape behavior of *K. coarctatus* exiting through the nostrils of a stressed or dead host has been previously reported for the infective nymph stage, but not for the mature stage (Riley & Self 1980). This behavior was thought to be a response by the nymphs to move out of the intermediate host into the gut of the definitive host, initiated by rough handling or death of the host (Riley & Self 1980). The response in adult worms observed here may be a similar response to move into the gut of a different definitive host, but may also simply be a response to move out of the potentially inhospitable environment of a dead or dying host.

Nymphs of *K. coarctatus* have been previously reported from the false coral snake (*Erythrolamprus bizona*) collected in Panama (Riley & Self 1980); but, to the authors' knowledge, this is the first report of adults specimens in Panamanian snakes. Other Central and North American snakes harboring adults of *K. coarctatus* include *Chironius carinatus*, *Coluber constrictor*, *Drymarchon*

*corais*, *Elaphe obsoleta*, *Lampropeltis getulus*, *Masticophis flagellum*, *Mastigodryas bifossatus*, *Nerodia cylopion*, *N. erythrogaster*, *N. fasciata*, *N. rhombifera*, *N. sipedon*, and *Thamnophis sirtalis*, (cf. Riley & Self 1980; Detterline et al. 1984; Foster et al. 2000). *Leptophis ahaetulla* and *Chironius grandisquamis* represent new definitive host records for *K. coarctatus*.

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\* \* \* \* \*

*BAYLISASCARIS PROCYONIS* (NEMATODA: ASCARIDOIDEA)  
IN RACCOONS (*PROCYON LOTOR*) FROM  
DUVAL COUNTY, TEXAS

**David B. Long, Tyler A. Campbell, and Scott E. Henke\***  
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*Baylisascaris procyonis*, or the raccoon roundworm, primarily utilizes the raccoon as its definitive host, and has been found in >90 species of North American animal intermediate hosts (mostly birds, lagomorphs, and rodents) (Kazacos 2001). In non-raccoon hosts, including humans, larvae of this parasitic nematode can cause severe neurological disease (cerebrospinal nematodiasis) and often damage visceral and ocular tissues (Kazacos 2001). Formerly, *B. procyonis* was not thought to occur in Texas (Chandler 1942; Schaffer et al. 1981). However, it was recently discovered in raccoons occurring in moist environments of coastal areas (Kerr et al. 1997) and in eastern portions of the state where infection rates of 70% and 23%, respectively, were reported. It is believed that *B. procyonis* is not common in semi-arid, hot environments and is probably limited by soil types and low raccoon densities (Amundson & Marquenske 1986). The purpose of this note is to report *B. procyonis* presence in raccoons from a previously unstudied and semi-arid region of Texas.

The study was conducted in April 2005 on a private ranch near Conception in Duval County, Texas (27°22'N, 98°18'W). The 1,721 ha ranch was purchased in 1996 and managed for recreational uses, including the maintenance of white-tailed deer (*Odocoileus virginianus*) feeders at an approximate density of 1.2 feeders/km<sup>2</sup>. The ranch is located within the Rio Grande Plains ecoregion (Gould 1975). Climate for the area is considered semi-arid, with mean annual rainfall of 67.9 cm (National Climatic Data Center, <http://www.ncdc.noaa.gov>), although yearly rainfall varies greatly (Norwine & Bingham 1986). Soils commonly found in the area are

well drained fine-sandy loams, belonging to the Runge-Delfina-Delmita soil associations (Fair 1995).

Raccoons were captured using 20 Tomahawk<sup>®</sup> live traps (23 by 23 by 66 cm; Tomahawk Live Trap Co., Tomahawk, WI,) baited with canned meat (i.e., Vienna sausages) and/or whole kernel corn. Upon capture, raccoons were sexed, weighed, aged by body weight to age class (juvenile and adult) (Kaufmann 1982), and euthanized (AVMA 2001). Viscera were stored in labeled 3.8 liter bags on an individual raccoon basis and frozen until examination. All capture and handling procedures were approved by the Institutional Animal Care and Use Committee (Permit No. QA-1310). Gastrointestinal tracts were thawed, separated from other viscera and opened longitudinally; then, mucosa were scraped and contents were washed using a washing and sedimentation process in conical glasses as outlined in Wallace & Pence (1986). The washed sediment was examined with a dissecting microscope. Helminths were collected, identified, and quantified. Nematodes were fixed in glacial acetic acid and stored in 70% ethanol with 8% glycerin. Nematodes were identified in alcohol-glycerin wet mounts. Identification followed the taxonomic keys of Yamaguti (1961) and Sprent (1958). Representative specimens were deposited in the United States National Parasite Collection (Beltsville, MD, accession numbers USNPC 97475 [*Baylisascaris procyonis*] and USNPC 97474 [*Toxascaris procyonis*]). Prevalence and abundance of raccoon helminths were determined.

A total of 19 raccoons were captured over 180 trap-nights. Of these, 14 were male, five were female, 15 were adult, and four were juvenile. *Baylisascaris procyonis* was found in three (16%) individuals (one adult male, one adult female, and one juvenile male). Two raccoons (adult male and juvenile male) had four *B. procyonis* present and one raccoon (adult female) had one *B. procyonis* present. In total, eight species of helminths (five nematodes, one acanthocephalan, and two cestodes) were found, and only one raccoon, an adult female, was free of intestinal

parasites (Table 1). The most common parasite found was *Macrocanthorhynchus ingens*, which occurred in 11 of 19 raccoons and the most abundant was *Atriotenia procyonis* ( $n = 63$ ).

Feeding supplements to white-tailed deer is a common practice in southern Texas intended to promote population health and visibility (Synatzske 1981). However, much of this feed is consumed by raccoons and other non-target wildlife (Lambert & Demarais 2001). Because the occurrence of *B. procyonis* often is correlated with raccoon abundance (Amundson & Marquenske 1986), landowners who provide supplemental feed to deer may inadvertently be encouraging *B. procyonis* expansion into areas where it might not normally occur (e.g., hot and dry climates). Raccoons often defecate in specified latrine areas, which accumulate *B. procyonis* eggs and are sites for infection (Page et al. 1998; Page et al. 1999). Undigested feedstuff from deer feeders (e.g., corn) in raccoon feces is often consumed by other wildlife. For example, Page et al. (1999) documented 15 species of birds and 16 species of mammals that visited raccoon latrines to forage. It is likely that the presence of *B. procyonis* in this study from a semi-arid region is due to elevated raccoon densities, maintained through feed supplements. Furthermore, other wildlife that forage at raccoon latrines may be at risk of *B. procyonis* infection.

Average parasite intensity of *B. procyonis* is reported between 12-22, with documented parasite loads as high as 1321 ascarids/raccoon (Kazacos 2001). It is believed that raccoons can self-cure, or eliminate some or all *B. procyonis*, when loads become excessive. This usually occurs in the winter and new infections are reported to be recruited in the late spring (Kidder et al. 1989). The present study was conducted during the late spring, which may explain why *B. procyonis* intensity was comparatively low.

Due to the high reproductive and dispersal rates of raccoons, it is difficult to control populations (Conover 2002), especially those that have access to supplemental feed. To reduce the risk of *B. procyonis* range expansion and transmission to other species

Table 1. Scientific name of parasite (total number of specimens recovered), infection rate (% infected),  $\bar{x}$  (mean number of parasites per infected *Procyon lotor*) and range (low to high number of parasites per infected *P. lotor*) found in raccoons ( $n = 19$ ) from Duval County, Texas during April 2005.

Parasite	Infection rate (%)	$\bar{x}$	Range
<b>Nematodes</b>			
<i>Physaloptera rara</i> ( $n = 22$ )	8/19 (42%)	2.75	(1-5)
<i>Placoconus lotoris</i> ( $n = 3$ )	3/19 (16%)	1.00	(1)
<i>Molineus barbatus</i> ( $n = 2$ )	2/19 (10%)	1.00	(1)
<i>Baylisascaris procyonis</i> ( $n = 9$ )	3/19 (16%)	3.00	(1-4)
<i>Toxascaris procyonis</i> ( $n = 11$ )	2/19 (10%)	1.00	(1)
<b>Acanthocephalans</b>			
<i>Macrocanthorhynchus ingens</i> ( $n = 39$ )	11/19 (58%)	3.55	(1-17)
<b>Cestodes</b>			
<i>Atriotaenia procyonis</i> ( $n = 63$ )	6/19 (32%)	10.50	(1-43)
<i>Mesocostoides lineatus</i> ( $n = 1$ )	1/19 (5%)	1.00	(1)

(including humans) in semi-arid regions of Texas, it may be necessary to limit supplemental feeding activities. However, before definitive recommendations can be made, additional experimental study is needed to determine the dynamics of *B. procyonis* occurrence and transmission at supplemental feeder locations in this region.

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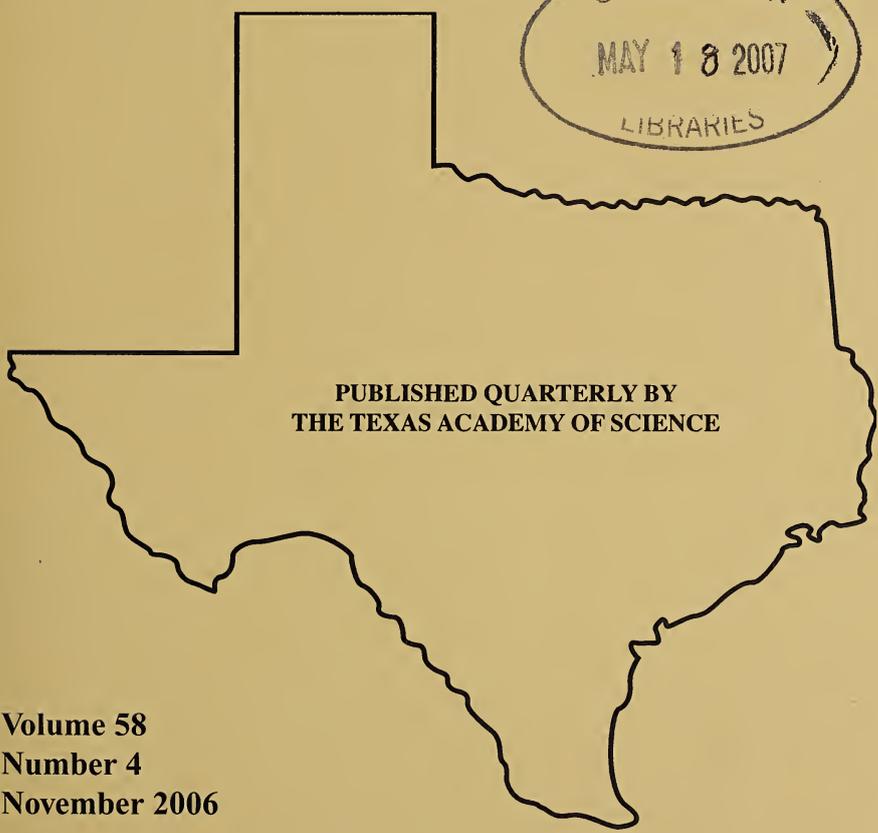
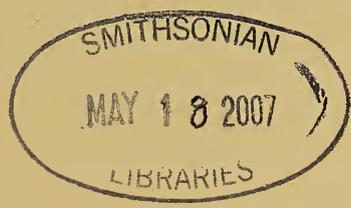


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REPRODUCTIVE CYCLE OF THE KALAHARI TREE SKINK,  
*TRACHYLEPIS SPILOGASTER* (SQUAMATA: SCINCIDAE)  
FROM SOUTHERN AFRICA

Stephen R. Goldberg

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**Abstract.**—The reproductive cycle of the viviparous Kalahari tree skink, *Trachylepis spilogaster*, was studied from a histological examination of gonadal material. Males follow a seasonal testicular cycle in which spermiogenesis occurred in spring and early summer. Regression followed by recrudescence took place during summer and autumn. Females began to deposit yolk the previous summer from which young were born. Not all females reproduce annually. Female litter size was positively correlated with body size. Mean litter size for 29 *T. spilogaster* females was  $4.93 \pm 1.6$  SD; range 2-9. Litter sizes of 2 and 9 are new minimum and maximum sizes for *T. spilogaster*.

---

The Kalahari tree skink, *Trachylepis spilogaster*, (formerly *Mabuya spilogaster*), is an arboreal species that frequents *Acacia* trees along dry river beds in arid savannah from Kimberley and the Lower Orange River in Northern Cape Province, Republic of South Africa through Botswana and Namibia to southern Angola (Branch 1998). There is little information on its reproduction. Branch (1998) reported *T. spilogaster* gave birth to 3-5 offspring. Pianka (1986) reported a mean litter size of  $4.4 \pm 1.3$  SD for 74 females. The purpose of this paper is to supplement known information on *T. spilogaster* reproduction from a histological examination of museum specimens.

#### MATERIALS AND METHODS

One-hundred twenty-five female (mean snout-vent length, SVL =  $66.1$  mm  $\pm 6.5$  SD, range = 53-85 mm), 102 male (mean SVL =  $63.5$  mm  $\pm 7.0$  SD, range = 47-76 mm) and seven neonates (mean SVL =  $25.3$  mm  $\pm 2.3$  SD, range = 23-28 mm) *T. spilogaster* were examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, CA. Lizards were collected during 1969-1970, 1972. The left testis and

epididymis were removed from males and the left ovary was removed from females for histological examination. Enlarged follicles ( $> 4$  mm length) were counted but not examined histologically. Oviductal eggs or embryos were previously removed (Pianka, 1986). Tissues were embedded in paraffin, sectioned at  $5\ \mu\text{m}$  and stained with hematoxylin followed by eosin counterstain. Testicular slides were examined to determine the stage of the spermatogenic cycle, and epididymides were examined for the presence of sperm. Ovary slides were examined for the presence of yolk deposition (secondary vitellogenesis *sensu* Aldridge 1979) or corpora lutea. *Trachylepis spilogaster* adult male versus adult female mean body sizes were compared using an unpaired *t* test. The relationship between body size (snout-vent length, SVL), and clutch size was examined by linear regression analysis. Statistical tests were performed using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

The following specimens of *Trachylepis spilogaster* from Botswana (Kgalagadi Province), the Republic of South Africa (Northern Cape Province) and Namibia were examined from the herpetology collection of the LACM.

#### BOTSWANA

59 km N Tsabong ( $25^{\circ}32'S$ ,  $22^{\circ}18'E$ ) LACM 81245; 131 km N Tsabong ( $24^{\circ}55'S$ ,  $22^{\circ}05'E$ ) LACM 81247, 81249, 81251, 81253-81257; 14 km W Middleputs ( $26^{\circ}51'S$ ,  $21^{\circ}38'E$ ) 81230-81239; 1 km W Tsabong ( $26^{\circ}03'S$ ,  $22^{\circ}25'E$ ) 81240-81242; 59 km N Tsabong ( $25^{\circ}32'S$ ,  $22^{\circ}18'E$ ) 81244, 81246; 31 km S Tsabong ( $26^{\circ}20'S$ ,  $22^{\circ}27'E$ ) LACM 81000; 11 km S Tsabong ( $26^{\circ}08'S$ ,  $22^{\circ}28'E$ ) LACM 81002-81005, 81009-81013, 81017, 81019, 81021, 81023, 81024, 81026, 81029, 81031, 81034, 81035, 81039-81042, 81047, 81050, 81051, 81053, 81057, 81058, 81066-81068, 81073, 81074, 81076, 81077, 81081, 81082, 81085, 81091, 81104, 81105, 81120, 81122, 81124, 81129, 81130, 81132-81134, 81136, 81140-81142, 81144-81146, 81151, 81155, 81156, 81158, 81163-81167, 81172, 81175, 81176, 81178, 81186, 81187, 81190, 81192, 81195-81201, 81203-81205, 81210, 81212, 81214-81216, 81219-81228.

## REPUBLIC OF SOUTH AFRICA

129 km N, 65 km W Uington LACM 80868; 4 mi SE Aansluit, Kurumar River (26°45'S, 22°32'E) LACM 80990-80995, 80998; 3 mi W, 4 mi S. Vanzylsrus (27°04'S, 21°48'E) LACM 80973-80976, 80978, 80980-80982, 80986, 80988; 8 mi E, 1 mi N Vanzylsrus (26°55'S, 21°52'E) LACM 80986, 80987; Kalahari Gemsbok National Park (27°17'S, 21°54'E) LACM 80870-80872, 80874-80877, 80879-80881, 80883-80890, 80892-80894, 80896-80904, 80906, 80908, 80909, 80912, 80915, 80920-80924, 80926, 80928-80930, 80934, 80937, 80939-80944, 80947-80949, 80953-80957, 80960, 80964-80967, 80970, 80979, 139057.

## NAMIBIA

Karas Region, 89 km ENE Koes (26°00'S, 19°15'E) LACM 77243; Karas Region, 25 km WNW Helmeringhausen (24°80'S, 15°05'E) LACM 77012, 77013, 77015; Karas Region, 77 km W Helmeringhausen (25°88'S, 16°81'E) LACM 77078-77081; Erongo Region, 47 km S Wilhelmstal (22°21'S, 16°21'E) LACM 77618-77622; Khomas Region, 110 km E. Windhoek (22°41'S, 18°08'E) LACM 77419-77421, 77423-77425, 77427-77429.

## RESULTS

Males followed a seasonal testicular cycle (Table 1). In the regressed testis, the germinal epithelium is exhausted and the predominant cells are Sertoli cells and spermatogonia. In testes undergoing recrudescence, there is renewal of the germinal epithelium for the next period of spermiogenesis. Primary and secondary spermatocytes are the predominant cells; some spermatids, but no spermatozoa may be present. During spermiogenesis the seminiferous tubules are lined by clusters of spermatozoa and metamorphosing spermatids and the epididymides are packed with sperm.

The period of spermiogenesis (Table 1) encompassed July through January (winter-summer); epididymides contained sperm. Since 98% (65/66) males were undergoing spermiogenesis during this time, breeding most likely occurs during this period. There was a period of regression in February-March (summer) followed by recrudescence which occurred in summer and autumn. The

Table 1. Monthly distribution of reproductive conditions in the seasonal testicular cycle of 102 *Trachylepis spilogaster*. Sequence of months begin with austral spring. Values are the numbers of males exhibiting each of the conditions.

Month	<i>n</i>	Regressed	Recrudescence	Spermiogenesis
September	15	0	0	15
October	11	0	0	11
November	5	0	0	5
December	12	0	0	12
January	5	0	0	5
February	14	1	5	8
March	3	1	2	0
April	2	0	2	0
May	12	1	6	5
June	5	1	2	2
July	10	0	0	10
August	8	0	1	7

smallest reproductively active male (spermiogenesis) measured 47 mm SVL (LACM 81176) and was from August.

The mean body size of the female *T. spilogaster* sample was significantly larger than that of the male sample ( $t = 2.84$ ,  $df = 225$ ,  $P = 0.01$ ). Monthly changes in the ovarian cycle are in Table 2. Oviductal eggs and/or embryos were previously removed and their mean value appears in Pianka (1986). In a few cases they were not removed or lizards were collected by other individuals; these data are in Table 2. Females with enlarged follicles ( $> 4$  mm length), oviductal eggs, embryos or corpora lutea only were present September to April (spring-summer). There was no evidence (corpora lutea and early yolk deposition) in the same female to suggest more than one brood is produced during the same reproductive season. The presence of 40/96 (42%) of mature females with inactive ovaries during the period when other females were reproductively active (Table 2) suggests that not all females in the population produce young each year. Four females (Table 2) were undergoing early yolk deposition in January-February (summer) when it would not have been possible to complete it during the current reproductive season. Assuming the follicles continued development

Table 2. Monthly distribution of reproductive conditions in the seasonal ovarian cycle of 125 *Trachylepis spilogaster* from southern Africa. Sequence of months begin with austral spring. Values shown are the numbers of females exhibiting each condition.

Month	<i>n</i>	Inactive	Early yolk deposition	Enlarged follicles (> 4 mm length)	Oviductal eggs	Embryos	Corpora lutea
September	17	10	0	3	1	0	3
October	20	6	0	2	4*	7	1
November	9	6	0	0	2	1	0
December	21	7	1	7	1	0	5
January	7	2	3	0	1	0	1
February	14	4	1	1	0	1	7
March	3	1	0	0	1*	0	1
April	5	4	0	0	0	0	1
May	6	6	0	0	0	0	0
June	9	9	0	0	0	0	0
July	10	10	0	0	0	0	0
August	4	4	0	0	0	0	0

\*One female contained squashed oviductal eggs that could not be counted.

without undergoing atresia, they would have resulted in embryos which would have completed development during the next reproductive season. The smallest reproductively active female (corpus luteum present) measured 53 mm SVL (LACM 80998) and was from December. Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch = litter size for 29 *T. spilogaster* females (Fig. 1):  $Y = -5.26 + 0.15X$ ,  $r = 0.61$ ,  $P = 0.0004$ . Mean clutch = litter size was  $4.93 \pm 1.6$  SD, range: 2-9. Two eggs is a new minimum litter size and nine is a new maximum litter size for *T. spilogaster*. Young < 30 mm SVL (presumably neonates) were collected (November,  $n = 1$ ; February,  $n = 4$ ; April,  $n = 2$ ).

## DISCUSSION

The sequence of events in the testicular cycle of *T. spilogaster* is similar to that which occurs in the African lacertids *Meroles cuneirostris* (cf. Goldberg & Robinson 1979), *Pedioplanis namaquensis* (cf. Goldberg 2006a) and *Pedioplanis lineoocellata* (cf. Goldberg 2006b). All undergo spring spermiogenesis followed

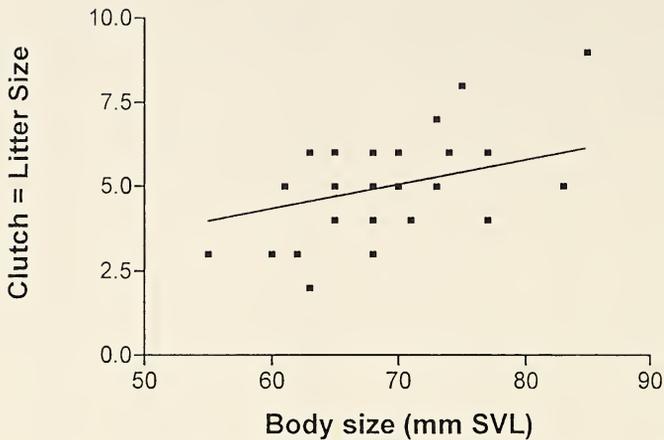


Figure 1. Linear regression of body size (mm SVL) on clutch = litter size for 29 *Trachylepis spilogaster* females from southern Africa. Some values (same SVL and clutch = litter size) occurred more than once.

by summer regression and subsequent recrudescence. Males of the scincid lizard, *Mabuya capensis*, from South Africa followed a testicular cycle similar to *T. spilogaster* as peak spermiogenesis occurred during late winter to early summer followed by summer regression (Flemming 1994). The timing of the testicular cycle of *T. spilogaster* with spring spermiogenesis followed by summer regression is similar to that of the North American skinks, *Eumeces skiltonianus* (cf. Goldberg 2005); *Eumeces anthracinus* and *Eumeces fasciatus* (cf. Trauth 1994). In contrast, two other African skinks, *Mabuya quinquetaeniata* and *Mabuya striata* from Zambia exhibited cycles in which spermiogenesis was continuous (Simbotwe 1980).

The timing of the *T. spilogaster* female reproductive cycle was similar to that of the African skink *Mabuya capensis* reported by Flemming (1994) as ovulation occurred during mid-spring to mid-summer and gravid females were collected from October to February. It was indicated by Van Wyk (1991) that *Cordylus giganteus* females exhibited a biennial reproductive strategy with females reproducing once every two years. This may be the case for *T. spilogaster* females as almost half of the female population

was not reproductively active during the reproductive season and there were no regressing corpora lutea in these lizards to indicate recent reproduction. Goldberg & Bezy (1974) similarly reported that only about half of the population of live-bearing *Xantusia riversiana* females from California were reproductively active in a given year. It appears that some *T. spilogaster* females start yolk deposition during summer for next year's litter.

As was the case for *M. capensis* (cf. Flemming 1994), there was no evidence that *T. spilogaster* females produce more than one litter per year. In contrast, Patterson (1990) reported the viviparous *Mabuya striata* might be multi-clutched. *Trachylepis spilogaster* females apparently mated at different times during the reproductive period as evidenced by the eight month span (September to April) in which females were found in different stages of the ovarian cycle (oviductal eggs, embryos or corpora lutea).

As occurred in *M. capensis* (cf. Flemming 1994), female body sizes and clutch sizes in *T. spilogaster* were positively correlated. Male and female reproductive cycles of *T. spilogaster* appear synchronized as males undergo spermiogenesis during spring at which time females are close to ovulation. The appearance of *T. spilogaster* neonates during November, February and April is consistent with the period during which females were reproductively active. Pianka (1986) reported a mean clutch/litter size of  $4.4 \pm 1.3$  SD for *T. spilogaster* which is close to the value of  $4.9 \pm 1.6$  SD reported herein.

Skinks are the second most diverse group of lizards in southern Africa with 69 species in 11 genera (Branch 1998). Subsequent studies on the reproductive cycles of additional scincid species from southern Africa are needed to ascertain whether the reproductive pattern exhibited by *T. spilogaster* is common to other members of the Scincidae from this region.

## ACKNOWLEDGMENTS

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ADDITIONAL DISTRIBUTIONAL RECORDS FOR  
SCOLOPENDROMORPH CENTIPEDES (CHILOPODA) FROM  
ARKANSAS, KANSAS, LOUISIANA, NEW MEXICO, OKLAHOMA,  
AND TEXAS, WITH THE FIRST REPORT OF  
*THEATOPS SPINICAUDUS* (WOOD) (CRYPTOPIDAE) FROM TEXAS

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**Abstract.**—This study reports 44 new county records and one state record for 10 scolopendromorph centipede species collected between October 2003 and December 2006 from six states in the southern United States west of the Mississippi River (Arkansas, Kansas, Louisiana, New Mexico, Oklahoma, and Texas). Detailed distributional information is included. The following species were collected: *Scolopendra heros*, *S. polymorpha*, *S. viridis*, *Hemiscolopendra marginata*, *Arthrorhabdus pygmaeus*, *Scolopocryptops rubiginosus*, *S. sexspinosus*, *Theatops posticus*, *T. spinicaudus*, and *Cryptops leucopodus*. In addition, *T. spinicaudus* is reported from Texas for the first time, and *S. rubiginosus* from Arkansas for the second time.

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Shelley (2002) provided the most comprehensive summation to date on the scolopendromorph centipedes of North America. More recently, McAllister et al. (2003) reported 43 new geographic distribution records for scolopendromorphs in Arkansas, Oklahoma, and Texas. In addition, McAllister et al. (2004) provided new distributional records for *Theatops posticus* from Oklahoma. As part of the continuing effort to document distributions in the southcentral region, this study provides 44 new records for species in Arkansas, Louisiana, Kansas, Oklahoma, New Mexico, and Texas. *Theatops spinicaudus* is reported from Texas for the first time, and *Scolopocryptops rubiginosus* is cited from significant counties in Arkansas and Oklahoma.

#### MATERIALS AND METHODS

Between October 2003 and December 2006, scolopendromorph centipedes were collected at sites throughout much of Arkansas, southcentral Kansas, northwestern Louisiana, southeastern New

Mexico, west-central and eastern Oklahoma, and southwestern and eastern Texas. When specimens were found, they were generally taken by forceps from damp areas off trails in pine and hardwood forests or from sites supporting mesquite, live oak, prickly-pear, and cedar by overturning decaying logs, leaf litter, and rocks with potato rakes. At each locale, centipedes were placed in individual vials containing 70% ethanol and taken to the laboratory for preliminary processing and identification. Specimens were later shipped to the second author (RMS) for confirmation of preliminary identification and deposited in the North Carolina State Museum of Natural Sciences (NCSM), Raleigh, North Carolina, U.S.A.

## RESULTS AND DISCUSSION

Ten scolopendromorph centipedes, representing six genera and three families, were encountered during this survey. As expected, the most common was the widespread *Scolopocryptops sex-spinosus*, reported herein from 12 Arkansas counties and one parish or county each in Kansas, Louisiana, Oklahoma, and Texas. Of 44 new county records documented, 11 (25%) are for *Hemiscolopendra marginata*; every centipede represented at least one new county record. Taxa recovered are presented below along with distributional (state, county/parish, specific locality) information.

## ANNOTATED LIST OF SPECIES

### SCOLOPENDRIDAE

*Scolopendra heros* Girard.—ARKANSAS: Pulaski County, 2.2 km S of Mayflower, 28 August 2004. OKLAHOMA: Okmulgee County, Okmulgee State Park, 10 September 2004 (new county record). Pushmataha County, off Indian Nations Turnpike, 15.4 km NW of Antlers, 9 September 2004 (new county record). TEXAS: Edwards County, 45.1 km SW of Rocksprings off Ranch Road 674, 7 March 2004. Terrell County, 37.0 km SE of Sheffield at Independence Creek Preserve, 7 October 2005. The largest North American centipede, *S. heros* has now been reported from 22

Oklahoma counties (Shelley 2002). In Texas, the species is expected statewide, but vouchers are available from only 58 of 254 (23%) counties (see Shelley 2002).

*Scolopendra polymorpha* Wood.—KANSAS: Barber County, 10.3 km W of Medicine Lodge, off River Road, 4 May 2005. TEXAS: Jeff Davis County, McDonald Observatory off St. Hwy 118, 10 March 2004. Kimble County, 8.0 km SW of Junction off US 377 at Bailey Creek, 21 February 2004 (new county record). Reagan County, 13.7 km NW of Barnhart off US 67 on Owen Road, 11 November 2005 (new county record). Tom Green County, 8.0 km SE of Christoval at Head of the River Ranch, 17 September 2005 (new county record). This centipede has been previously reported from 59 counties of central and west Texas, including Jeff Davis County (Shelley 2002). Except for historical records from Franklin and Polk counties (Shelley 2002), *S. polymorpha* is unknown from the eastern periphery of Texas. Individuals noted herein from Barber County, Kansas, and Kimble County, Texas, are anatomically intermediate between *S. polymorpha* and *S. viridis*. These two species are distinct in Utah and Arizona and are clearly reproductively isolated; however the distinctions become blurred in the western Plains, where they both appear to be capable of hybridizing (see Shelley 2002).

*Scolopendra viridis* Say.—NEW MEXICO: Doña Ana County, Dripping Springs Natural Area, 11 March 2004. TEXAS: Brewster County, 33.8 km S of Alpine off St. Hwy 118, 9 March 2004. Edwards County, 3.2 km SSW of Telegraph off US 377, 21 February 2004, and 45.1 km SW of Rocksprings off Ranch Road 674, 7 March 2004 (new county record). Irion County, 9.7 km SSE of Mertzon off Ranch Rd. 915, 18 November 2005 (new county record). Jeff Davis County, Fort Davis State Park, 8 March 2004. Reagan County, 13.7 km NW of Barnhart off US 67 on Owen Road, 11 November 2005 (new county record). Terrell County, 4.8 km SE of Dryden off St. Hwy 90, 8 March 2004. Uvalde County, Garner State Park, 6 March 2004. Val Verde County, 8.0 km NW of Del Rio off St. Hwy 90, Amistad Reservoir, 8 March 2004, and

Seminole Canyon State Park, 8 March 2004. *Scolopendra viridis* is expected statewide in Texas except for the northeastern corner and has been reported previously from 45 counties (Shelley 2002).

*Hemiscolopendra marginata* (Say).—ARKANSAS: Cleveland County, 4.0 km N of Rison off US 79, 11 November 2005 (new county record). Columbia County, 1.6 km E University Road off St. Hwy 79 in Magnolia, 2 March 2004. Hempstead County, Fulton, 22 October 2004 (new county record). Johnson County, Clarksville, 25 March 2006 (new county record). Lafayette County, Conway Cemetery State Park, 18 November 2004 (new county record). Nevada County, White Oak Lake State Park, 7 January 2004 (new county record). Ouachita County, Poison Springs State Park, 7 January 2004. Polk County, Ouachita Mountains Biological Station, 9 June 2004. Scott County, Waldron City Lake, 26 January 2006 (new county record). LOUISIANA: Caddo Parish, Ida off US 71 and Munnerlyn Chapel Road, 24 October 2003. OKLAHOMA: LeFlore County, Choctaw Nation Historic Site off St. Hwy 1, 16 April 2004 (new county record). McCurtain County, 4.8 km W OK/AR State Line, near Tom off St. Hwy 3, 22 April 2004 (new county record). TEXAS: Bandera County, Lost Maples State Park, 6 March 2004 (new county record). Bowie County, Lake Wright Patman, 17 October 2004 (new county record). Grayson County, 6.6 km N of Gordonville off US 377, 21 April 2006 (new county record). Titus County, Argo off FM 1993, vicinity Snake Creek, 10 November 2003 (new county record). The range of *H. marginata* is extensive and covers parts of 17 states, including all of Louisiana and all of five states east of the Mississippi River (Hoffman & Shelley 1996; Shelley 2002:Fig. 71). In Oklahoma, the centipede is expected in the southeast and the Red River Valley; however, the only previous record is from Latimer County (Shelley 2002), to which this study adds two neighboring counties.

*Arthrorhabdus pygmaeus* (Pocock).—TEXAS: Brewster County, 33.8 km S Alpine off St. Hwy 118, 9 March 2004. Culberson County, Van Horn city limits off I-10, 10 March 2004. El Paso

County, Franklin Mountains State Park off Loop 375, 11 March 2004 (new county record). Presidio County, 8.0 km S Shafter off US 67, 9 March 2004, and Big Bend Ranch State Park off FM 170, 9 March 2004. Schleicher County, 25.7 km SE Christoval off FM 2235, 29 April 2005 (new county record). Terrell County, 37 km SE of Sheffield at Independence Creek Preserve, 7 October 2005. In Texas, *A. pygmaeus* has been reported previously from 16 counties (Shelley 2002; Shelley & Chagas 2004) and is projected to span the entire southern border of the state, with potential occurrence in the Panhandle *per se* plus southwestern Oklahoma. Shelley & Chagas (2004) reported the centipede from Tucson, Pima County, Arizona, which spans New Mexico and corroborates Crabill's statement (1960) of occurrence in this state. The collection of *A. pygmaeus* in El Paso County, the westernmost in Texas, further implies occurrence in New Mexico, particularly in adjacent Doña Ana and Otero counties.

#### SCOLOPOCRYPTOPIDAE

*Scolopocryptops rubiginosus* L. Koch.—ARKANSAS: Drew County, 13.0 km S Monticello off St. Hwy 81, 11 November 2005 (new county record). OKLAHOMA: Caddo County, Red Rock Canyon State Park off St. Hwy 8, 8 November 2003 (new county record). Carter County, Lake Murray State Park off St. Hwy 77S, 6 November 2004 (new county record). Cleveland County, Lake Thunderbird State Park, 7 November 2003 (new county record). Murray County, Turner Falls off US 77, 6 November 2004 and 6 November 2005, and Chickasaw National Recreation Area off St. Hwy 177, near Sulphur, 6 November 2004 (new county record). Okmulgee County, Okmulgee State Park, 10 September 2004 (new county record). In Oklahoma, *S. rubiginosus* was previously known from only six counties of the state (see McAllister et al. 2003:Fig. 1), and the former record is just outside the range as depicted in Shelley (2002:Fig. 108). As such, five new county records are added for *S. rubiginosus* in Oklahoma (Fig. 1), bringing to 11 the known counties supporting this centipede in the state. In addition, the Arkansas record provided herein represents only the

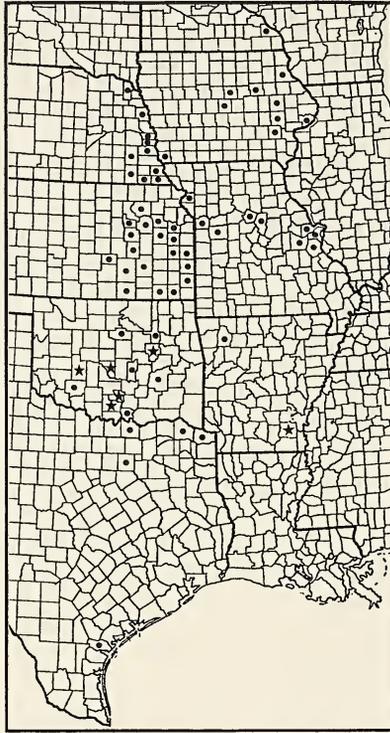


Figure 1. Geographic distribution of *Scolopocryptops rubiginosus* west of the Mississippi River showing county distributions in Arkansas, Kansas, Iowa, Minnesota, Missouri, Nebraska, Oklahoma, and Texas (dots = previous records; stars = new records).

second time this centipede has been reported from the state; it is also the southeasternmost locality reported for *S. rubiginosus* (Fig. 1).

*Scolopocryptops sexspinosus* (Say).—ARKANSAS: Ashley County, 6.4 km S Crossett off St. Hwy 133, 11 November 2005 (new county record). Columbia County, 1.6 km E University Road off St. Hwy 79 in Magnolia, 2 March 2004, and Logoly State Park, 8 December 2004. Desha County, 0.4 km N of Masonville off St. Hwy 159, 12 November 2005 (new county record). Hot Spring County, DeRoche off St. Hwy 128, 24 October 2004. Independence County, Salado Creek Rest Stop off US 167, 30 December

2004 (new county record). Nevada County, White Oak Lake State Park, 7 January 2004 (new county record). Ouachita County, Poison Springs State Park, 7 January 2004 (new county record). Pike County, Daisy State Park, 12 June 2004. Polk County, Pipistrelle Mine, 6 June 2004. Saline County, Shannon Hills, 26 December 2006 (new county record). Scott County, Waldron at Brush Creek, 17 January 2006 (new county record). Sevier County, Jefferson Ridge Park near Dierks, 16 November 2003 (new county record). KANSAS: Cherokee County, 1.6 km S of Galena at Schermerhorn Park off St. Hwy 26, 4 May 2005. LOUISIANA: Caddo Parish, Ida off US 71 and Munnerlyn Chapel Road, 24 October 2003. OKLAHOMA: McCurtain County, 4.8 km W OK/AR State Line, off St. Hwy 3 near Tom, 22 April 2004. TEXAS: Hardin County, Village Creek State Park, Lumberton, 5 June 2004 (new county record). *Scolopocryptops sexspinosus* is commonly encountered in a host of native and urban environments in eastern North America. The projected distribution includes all of Arkansas, the eastern half of Oklahoma, and eastern and east-central Texas (Shelley 2002), and the foregoing records lie within this area.

## CRYPTOPIDAE

*Theatops posticus* (Say).—ARKANSAS: Columbia County, 1.6 km E of University Road off St. Hwy 79, 3 February 2004, and Logoly State Park, 8 December 2004. Lincoln County, 5.6 km NE Cornersville off St. Hwy 11, 11 November 2005 (new county record). OKLAHOMA: Pittsburg County, 3.2 km SE Canadian, Arrowhead State Park, 11 September 2004. *Theatops posticus* is another scolopendromorph with projected statewide occurrence in Arkansas. However, voucher specimens are available for only seven counties of this state and 10 of Oklahoma (Shelley 1990; 1997; McAllister et al. 2003; 2004).

*Theatops spinicaudus* (Wood).—ARKANSAS: Independence County, Blowing/Cushman Cave, 28 June 2004, and Salado Creek Rest Stop off US 167, 30 December 2004. Johnson County, Clarksville, 11 November 2005 (new county record). Perry

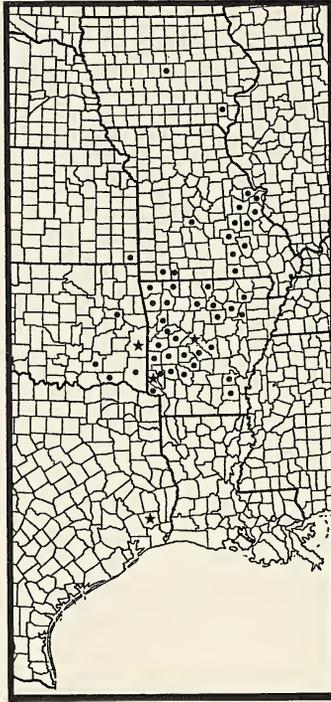


Figure 2. Geographic distribution of *Theatops spinicaudus* west of the Mississippi River showing county distributions in Arkansas, Kansas, Iowa, Missouri, Oklahoma, and Texas (dots = previous records; stars = new records).

County, 6.4 km S Hollis, 26 November 2005 (new county record). Saline County, Shannon Hills, 26 December 2006. Sevier County, Jefferson Ridge Park at Dierks Lake, 16 November 2003 (new county record). Scott County, Blue Moon and Waldron, 26 January 2006. OKLAHOMA: LeFlore County, Heavener-Runestone State Park, 16 April 2004 (new county record). TEXAS: Hardin County, Village Creek State Park, Lumberton, 15 June 2004 (new state record). *Theatops spinicaudus* comprises two allopatric populations, an eastern one that extends from southwestern Virginia and western North Carolina to east-central Alabama and eastern Tennessee, and a western one extending from central Iowa and northern Illinois to southern Arkansas and eastern Oklahoma (Shelley 2002:Fig. 155). McAllister et al. (2003) provided four additional records for the western population in parts of Arkansas

and Oklahoma and projected occurrence for northeastern Texas. The Hardin County locality lies approximately 435 km (270 mi) southeast of the nearest site in Oklahoma (Fig. 2); occurrence in Louisiana has not been documented, but the Texas record suggests that *T. spinicaudus* may occupy adjacent Calcasieu Parish and potentially may be discovered in the northwestern corner of the state, in Caddo Parish. In addition, the centipede is now known from five counties of Oklahoma (McAllister et al. 2003).

*Cryptops leucopodus* (Rafinesque).—ARKANSAS: Columbia County, 1.6 km E of University Road off St. Hwy 79, 3 February 2004 (new county record). With this citation, *C. leucopodus* is known definitely from only 10 of 75 (13%) Arkansas counties (McAllister et al. 2003), though occurrence is still expected statewide (Shelley 2002).

#### ACKNOWLEDGMENTS

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PESTICIDE USAGE ON THE SOUTHERN HIGH PLAINS AND  
ACUTE TOXICITY OF FOUR CHEMICALS TO THE FAIRY SHRIMP  
*THAMNOCEPHALUS PLATYURUS* (CRUSTACEA: ANOSTRACA)

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**Abstract**—Extensive pesticide use on crops grown on the Southern High Plains (SHP) represents a considerable anthropogenic stressor to ephemeral aquatic ecosystems. These short-lived aquatic ecosystems, known in the southwest as playas, are epicenters of biodiversity on the High Plains. Cotton is the major agricultural crop grown on the SHP, accounting for over half of all cotton produced in Texas. Currently there are 67 different chemicals used to control cotton pests, and when other crops such as grain sorghum are included the number approaches 100. *Thamnocephalus platyurus* is a fairy shrimp indigenous to the Southern High Plains that is also available commercially. In addition it is used as an invertebrate model for water quality and toxicity testing. Acute toxicity of four agricultural pesticides widely used on the SHP (Methyl Parathion 4E, Tempo® SC Ultra [active ingredient cyfluthrin], Roundup® [glyphosate], and Karmex® DF [diuron]) was determined using laboratory-derived *T. platyurus*. Twenty-four hour old nauplii experienced mortality (48 hour LC50) at concentrations ranging from 10.99 µg/L for Tempo® SC Ultra and 1.248 mg/L for Roundup®. These results suggest that the current pesticide application rates have the potential to endanger the native playa invertebrate *T. platyurus*.

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The Southern High Plains (SHP) is an 8.2 million hectare, 27 county region that starts at the Canadian River in west Texas and extends south past Lubbock, Texas, and west into eastern New Mexico (Fig. 1; Smith 2003). The major land use throughout this region is agriculture, with cotton being the most commonly grown crop species contributing 64% of Texas' crop (Crop Profile for Cotton in Texas 1999). Grain sorghum, soybean, corn, alfalfa, and sunflowers are relatively minor crops and although they may be important in local ecosystems, their limited areas of growth make them "islands" in a sea of cotton (Agricultural Statistics Board 2001). Therefore, the primary focus of this study is with the chemicals currently associated with the production of cotton in the SHP.

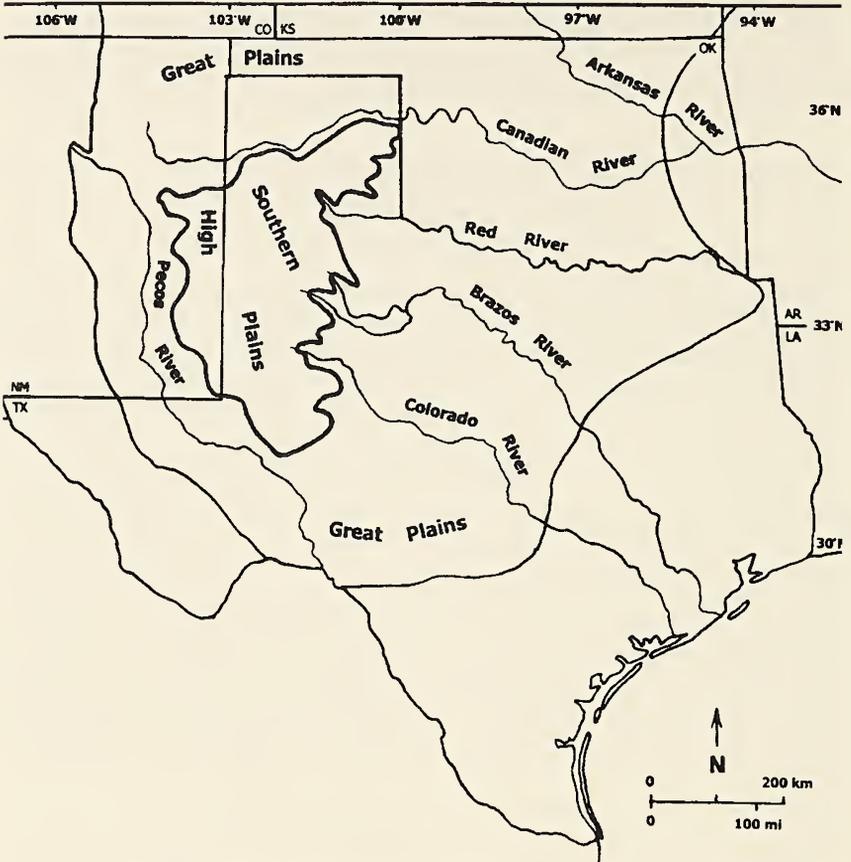


Figure 1. Location of the Southern High Plains in Texas and New Mexico and in relation to the Great Plains. (Adapted from Smith 2003, *Playas of the Great Plains*, University of Texas Press).

Cotton is grown throughout eleven different states in the southern United States, and geographic variation in agricultural practices results from differences in weed and insect pressures. This, in turn, results in major variation in the types and usage of pesticides (Gianessi & Puffer 1990).

Cotton typically is planted between May 5 and May 25 in Lubbock and the surrounding counties. Farther to the south, the planting window is slightly longer, lasting through early June.

Blooms usually appear in mid July with heat unit accumulation ending around 5 October. Harvest begins around 15 October and lasts through November (Ritchie et al. 1994).

Cotton farming requires three to five times more kilograms of chemicals per hectare (ha) than corn or soybeans, and as much as 7 kg/ha of herbicides and 5 kg/ha of insecticides are applied annually to cotton fields throughout the United States. Cotton also requires more applications per year; 4.7 chemical applications as opposed to 1.2 applications for corn (Coupe et al. 1998).

Herbicides are one of the major chemical classes used in the SHP to control cotton weeds. Twenty-four different active ingredients in herbicide formulations are used on the SHP. Herbicides are applied at ten different times throughout the year starting with winter treatments and ending in preharvest applications. The most commonly used herbicide on the SHP was Roundup® and its other brand names used as a post-emergent herbicide, which can be applied at five different times throughout the growing season. It is used in high concentrations, with up to 4.5 kg active ingredient/hectare (a.i./ha) as the recommended application rate. Roundup® is very water soluble, therefore it can infiltrate into playa ecosystems through runoff or spray drift. The LC50 value for glyphosate (the active ingredient in Roundup®) (Relyea 2005) is relatively high (4,000 µg/L for *Daphnia magna*), indicating that it poses minimal risk to aquatic invertebrates. However, the surfactant used in most Roundup formulations, polyethoxylated tallowamine [POEA], is exceedingly toxic to aquatic species (Relyea 2005). Another common herbicide used is Karmex®, with the active ingredient diuron. It is primarily used on both oranges and cotton as a pre-emergent herbicide. The recommended usage of diuron is as much as 1.36 kg a.i./ha/yr. Diuron has a long half life of 90 days in aquatic environments and a high solubility of 42 mg/L, allowing diuron to infiltrate into playas as well as remain for extended periods of time. Diuron is one of the more toxic

herbicides with 48 h LC50 values of 1,400  $\mu\text{g/L}$  for *D. magna* (PAN Pesticides Database 2005, Muschal & Warne 2003).

A second major class of chemicals used on cotton in the SHP is insecticides. The major pest species to cotton on the SHP is the boll weevil (*Anthonomus grandis*). Thirty-six different active ingredients are used as insecticides on crops in the SHP. The organophosphate Methyl Parathion 4E was determined to be the most widely used insecticide in this region, and third in the US, with over 3.3 million pounds of active ingredient applied annually (Thurman et al. 1998). It is used extensively on cotton and other crops in the SHP for control of fourteen pests, including the most common pest, boll weevil (Baugh et al. 2004). Based on crop consultant recommendations, Methyl Parathion 4E can be used at the highest application rate on the SHP with up to 2.25 kg a.i./ha (Baugh et al. 2004). Methyl parathion is one of the more toxic active ingredients used on the SHP, with a 48 hour LC50 value of 0.14  $\mu\text{g/L}$  for the aquatic invertebrate *D. magna* (Hazardous Substance Data Base 2005).

Cyfluthrin, the active ingredient in Baythroid® and Tempo®, also is extremely toxic to aquatic invertebrates with a similar LC50 value of 0.14  $\mu\text{g/L}$  for *D. magna*. It is not used as heavily as Methyl Parathion 4E with only 28-56 g a.i./ha used (www.pesticideinfo.org 2005). However, it was deposited more often than Methyl Parathion 4E with over 40 million applications a year in the United States (Kiely et al. 2004). It was used on six cotton pests and many other sorghum and corn pests. Tempo® SC Ultra is composed of the active ingredient cyfluthrin as well as crystalline silica and the solvents, xylene, ethyl benzene, and trimethyl benzene which do not appear to affect acute toxicity (Cox 1994).

Harvest aids are used on the SHP but to a much lower extent than herbicides and insecticides. Also, their time of application, October through November, makes them irrelevant to this study.

In the cotton growing regions of the SHP there are over 20,000 playa lakes, which are small, shallow wetland depressions (Gustavson et al. 1994). These low-lying areas are fed solely by precipitation, thus playas are watersheds throughout the SHP. Playas are vital to maintaining the biodiversity and they harbor many species that could not otherwise survive in the arid environment of the SHP (Smith 2003). The actual number of species living in each playa is highly variable based on size of playa and relation to other playas, however all playas increase the diversity of species in the surrounding areas (Smith 2003). In addition to increasing biodiversity, playas in the SHP are important as the only recharge points for the Ogallala Aquifer, which underlies much of this region, inputting between 13 to 82 ml of water per year (Nativ & Riggio 1989, Stone 1990, Zartman et al. 1994).

Fairy shrimp are macroinvertebrates that are commonly found in playas of the SHP, as well as temporary water bodies throughout the world. *Thamnocephalus platyurus* (Crustacea: Anostraca) is the largest fairy shrimp species found in the SHP and can also be found throughout the southern United States living in ephemeral water bodies between 17°C and 32°C (Belk 1977). They typically emerge between May and October, which coincides with cotton growing season in the SHP and the application of most agricultural chemicals. Fairy shrimp are a major source of metabolizable energy to many vertebrates (Munuswamy & Subramoniam 1986) including mammals, amphibians, and waterfowl (Anderson & Hsu 1990, Mizutani et al. 1991, Fischer et al. 1982). *Thamnocephalus platyurus* are non-discriminate filter feeders that help maintain water quality in playa ecosystems (Eriksen & Belk 1999). *Thamnocephalus platyurus* eggs, known as cysts, are available commercially, therefore they are frequently used as a test species for water quality and toxicity testing (Thamnotoxkit F [Creasel Ltd., Belgium] and *Thamnocephalus platyurus* Toxkit [Vickers Laboratories Ltd., United Kingdom]).

The pesticide registration process requires acute toxicity testing to nontarget organisms such as birds, mammals, fish, plants, and terrestrial and aquatic invertebrates. However, toxicity tests used in the pesticide registration process often are conducted on species that are not present in the ecosystems impacted by pesticide application. Generally, aquatic invertebrate toxicity testing is done on five species, *Daphnia magna*, *D. pulex*, *Gammarus fasciatus*, *G. pseudolimnaeus*, and *G. lacustris* (cf. Walker 1995). Currently, the EPA only requires that the active ingredient be tested for toxicity, not the pesticide formulation (EPA document 40 CFR 158.145, Touart 1995). Pesticide formulations consist of both active ingredients and inert ingredients. The active ingredient destroys or mitigates the pest while inert ingredients, or other ingredients, are not intended to affect the target pest. Inert ingredients consist of surfactants, wetting agents, dispersing agents, emulsifiers, solubilizers, and bioenhancers (Tominack 2000). The US Environmental Protection Agency currently acknowledges almost 1,700 inert ingredients being used on crops in the United States. Twelve-hundred of these are classified as unknown toxicity and of the 500 tested, only eight are classified as non-toxic (United States Environmental Protection Agency 1987). This study was conducted to determine if pesticide application of formulations used on the SHP would be injurious of a native macroinvertebrate (*T. platyurus*).

This study evaluated the acute toxicity (48 hour LC50) of four commonly used pesticides: Methyl Parathion 4E, Tempo® SC Ultra, Roundup®, and Karmex® DF in order to determine their potential effects on *T. platyurus*. Chemicals were selected based on their potential to occur in playas in the SHP due to the amount used, number of applications per year, and the time when they are applied.

#### METHODS AND MATERIALS

Chemicals were purchased from a local pesticide distributor. The chemicals purchased were Methyl Parathion 4E [methyl parathion; O,O-dimethyl O-4-nitrophenyl phosphorothioate, CAS 298-00-0] (Cheminova Inc., Wayne, NJ), Tempo® SC Ultra [cy-

fluthrin; cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate, CAS 68359-37-5] (Bayer Corporation, Kansas City, MO), Roundup® [glyphosate; N-(phosphonomethyl) glycine, CAS 1071-83-6] (Monsanto Company, St. Louis, MO), and Karmex® DF [diuron; N'-(3,4-dichlorophenyl)-N,N-dimethylurea, CAS 330-54-1] (Griffin Chemical, Valdosta, GA).

Laboratory derived *T. platyurus* cysts were obtained from Thamnotoxkit F and hatched in moderately hard synthetic freshwater as defined by EPA document EPA/600/4-90/027F. The water contained 60 mg/L magnesium sulfate, 96 mg/L sodium bicarbonate, 4 mg/L potassium chloride, and 60 mg/L calcium sulfate dehydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) (pH = 7.53, alkalinity = 92  $\text{CaCO}_3/\text{L}$ , and hardness = 66  $\text{CaCO}_3/\text{L}$ ) (United States Environmental Protection Agency 1993). Cysts were placed in 250 ml beakers and hatched in a Plant Growth Chamber (Altair Refrigeration, Stafford, Texas) on a 14:10 light:dark schedule with light intensity of 1,575 lux and temperature regulated at 25°C. Twenty-four hours after cysts were placed in water, free-swimming nauplii were randomly selected for toxicity testing. Toxicity tests were conducted in 250 ml beakers using twenty, 24-hr-old nauplii. Each experiment was carried out using seven different chemical concentrations (eight for Karmex® DF) and one control group. Each treatment group was replicated three times. All beakers were filled with 175 ml of EPA water plus chemical. Each concentration was made using a serial dilution with final concentrations being 0.01, 0.1, 4, 50, 500, 10,000, 20,000  $\mu\text{g}/\text{L}$  for Methyl Parathion 4E, 0.008, 0.08, 2.2, 10.1, 84.7, 8,474, 84,745 for Tempo® SC Ultra, 19.9, 199, 747, 1,595, 4,175, 19,920, 199,200  $\mu\text{g}/\text{L}$  for Roundup®, and 0.006, 0.06, 6, 37.5, 187.5, 625, 6,250, 12,500  $\mu\text{g}/\text{L}$  for Karmex® DF as nominal concentrations. At 4, 8, 12, 24, and 48 h after addition of the chemical, dead nauplii were counted and recorded with total mortality at 48 hours used to generate a dose response curve. Death was defined by lack of phyllopod movement.

Table 1. Acute toxicity values of four common pesticides on *Thamnocephalus platyurus* (this study) and *Daphnia magna* (literature) in the Southern High Plains.

Pesticide	Water Quality Parameters			Acute Toxicity	Literature Value
	Temperature	pH	Dissolved Oxygen	( $\mu\text{g/L}$ ) Mean $\pm$ SE	( $\mu\text{g/L}$ ) <i>Daphnia magna</i>
Methyl Parathion 4E	24.6 $\pm$ 0.2°C	7.67 $\pm$ 0.05	8.32 $\pm$ 0.02	31.30 $\pm$ 7.52	0.14
Tempo SC Ultra	24.6 $\pm$ 0.2°C	7.26 $\pm$ 0.05	8.19 $\pm$ 0.01	10.99 $\pm$ 1.24	0.14
Roundup Super Concentrate	24.6 $\pm$ 0.2°C	5.05 $\pm$ 0.05	8.27 $\pm$ 0.04	1243.38 $\pm$ 47.75	4000
Karmex DF	24.6 $\pm$ 0.2°C	7.70 $\pm$ 0.05	8.30 $\pm$ 0.05	75.97 $\pm$ 7.41	1400

Water was not changed during the testing period because water replacement would have added an additional external stressor and possibly confounded results of toxicity tests. Water quality parameters were measured three times throughout each test for the highest concentration.

Mortality data was modeled using logit analysis. The concentration that killed 50% of test organisms (i.e. LC50) was determined using the statistical program R version 2.0.1 (R Development Core Team, Boston, MA, USA) and recorded.

## RESULTS AND DISCUSSION

Tempo® SC Ultra was the most lethal of the compounds tested (Table 1) with an LC50 value for *T. platyurus* of 10.99  $\pm$  1.24  $\mu\text{g/L}$  (mean  $\pm$  SE). Methyl Parathion 4E had a 48 hour static LC50 value of 31.30  $\pm$  7.52  $\mu\text{g/L}$ . Interestingly, the herbicides were much more toxic than expected based on previous studies on the active ingredient alone. The herbicide Karmex® DF had LC50 values of 75.97  $\pm$  7.41  $\mu\text{g/L}$ . Roundup® had an LC50 of 1,243.38  $\pm$  47.75  $\mu\text{g/L}$  (Table 5). Acute lethality curves are given for Methyl Parathion 4E (Fig. 2), Tempo® SC Ultra (Fig. 3), Roundup® (Fig. 4), and Karmex® DF (Fig. 5).

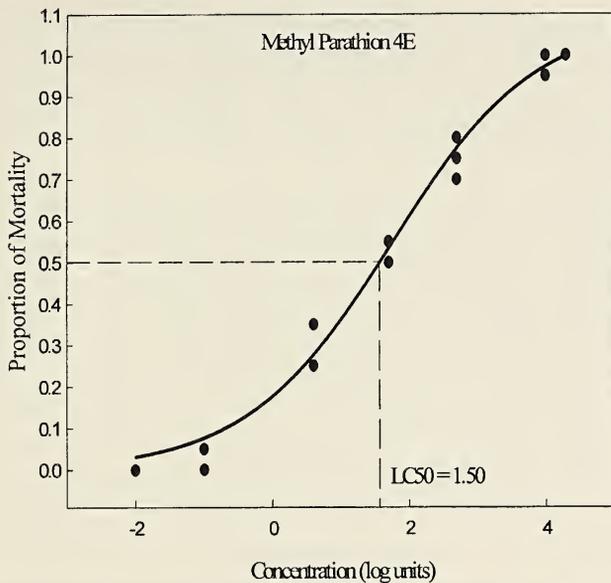


Figure 2. Mortality percentage versus chemical concentration for Methyl Parathion 4E used on the SHP. The superimposed line denotes line of best fit. Value of LC50 is in log units.

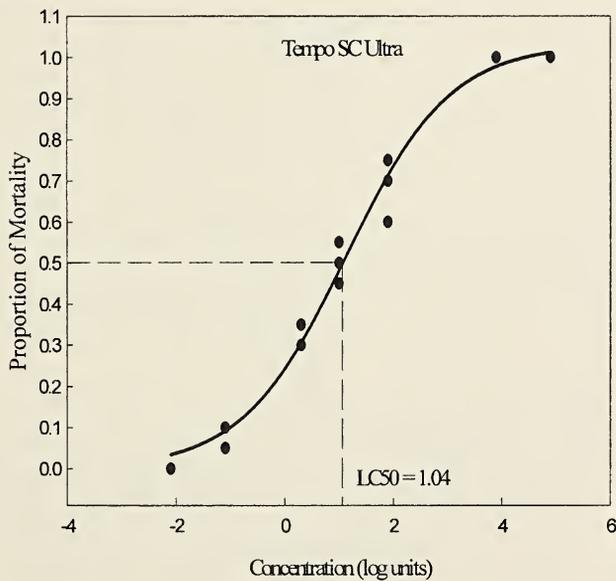


Figure 3. Mortality percentage versus chemical concentration for Tempo<sup>®</sup> SC Ultra used on the SHP. The superimposed line denotes line of best fit. Value of LC50 is in log units.

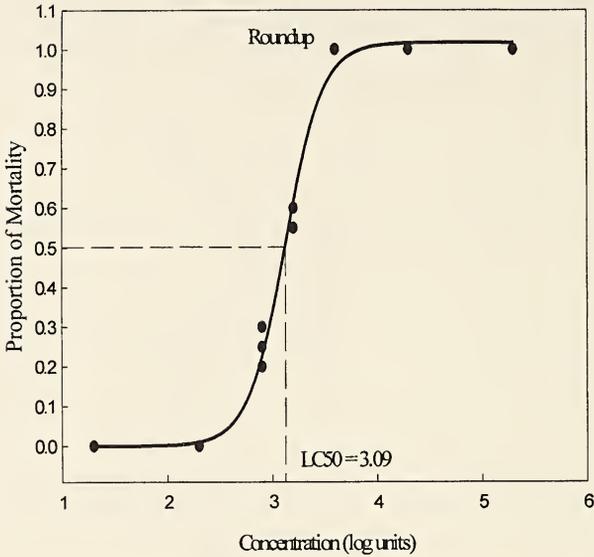


Figure 4. Mortality percentage versus chemical concentration for Roundup<sup>®</sup> used on the SHP. The superimposed line denotes line of best fit. Value of LC50 is in log units.

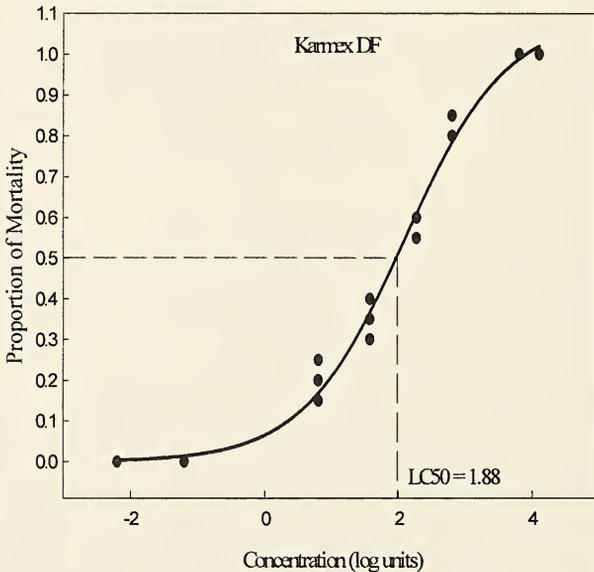


Figure 5. Mortality percentage versus chemical concentration for Karmex<sup>®</sup> DF used on the SHP. The superimposed line denotes line of best fit. Value of LC50 is in log units.

Overall, the insecticides were more toxic to *T. platyurus* than the herbicides, as expected. This study found that *T. platyurus* is more tolerant of Methyl Parathion 4E and Tempo® SC Ultra than *Daphnia magna*, but less tolerant of Karmex® DF and Roundup®.

The use of pesticide formulations rather than active ingredient alone can substantially alter the toxicity of pesticides. In this study, a 4-fold higher toxicity (as compared to *D. magna*) was determined for Roundup® compared to glyphosate alone. In three frog species (*Rana sylvatica*, *Rana pipiens*, *Hyla versicolor*), the addition of POEA surfactant can lower the acute lethal concentration (48 h LC50) by 10 fold (3.9-15.5 mg/L with POEA versus 108-161 mg/L for technical grade glyphosate) (Relyea, 2005). POEA is an ethoxylated long-chain alkyl amine that is derived from animal fatty acids and functions by enhancing uptake of glyphosate by decreasing water surface tension and breakdown of the cuticle (Giesy et al, 2000). It is hypothesized that POEA also can affect the structural integrity and function of the exoskeleton of aquatic invertebrates. In this study, a 20-fold increase in toxicity was seen for Karmex® DF (tested on *T. platyurus*) over diuron (*D. magna*) alone. It's suspected that this is also a result of inert ingredients found in Karmex® DF although no research exists on this. Also, *T. platyurus* could potentially be more sensitive to Karmex® DF than other previously studied tested species as a result of interspecies variation. This result for herbicides opposes the finding of Centenu et al. (1995) suggesting that acute toxicity values are typically higher for *T. platyurus* than for *D. magna*.

Typically, herbicides as a whole are only minimally toxic to aquatic invertebrates, and are virtually nontoxic at ecologically relevant concentrations (Thurman et al. 1998). However, alterations in metabolism causing decreased fecundity and retarded growth are possible when macroinvertebrates are exposed to herbicides (Sibly & Calow 1989, Thurman et al. 1998). *T. platyurus* becomes sexually mature based on size (Ali & Dumont 1995). If growth is significantly retarded, reproduction will not

occur before the playa dries eventually resulting in the elimination of *T. platyurus* in the playa. However, from this study it is the consensus of the authors that there is a need to reevaluate the toxicity of herbicides based on the pesticide formulations, not the active ingredient alone due to the increased toxicity seen in this study.

The insecticide with the greatest potential for exposure among the aquatic invertebrates in the SHP based on usage and time of application was determined to be Methyl Parathion 4E. However, Methyl Parathion 4E is relatively insoluble in water, thus potentially limiting exposure among aquatic species (Thurman et al. 1998). Other insecticides which have the potential to infiltrate into playas include Baythroid® and Tempo® (cyfluthrin), Asana® XL (esfenvalerate), Decis® 1.5E (deltamethrin), and Scout® X-tra 0.9E (tralomethrin), based on high concentrations used and high toxicity values. Of the top five insecticides used on the SHP, four are synthetic pyrethroids.

The pesticides least likely to affect playa ecosystems are insect growth regulators due to extremely low concentrations used and limited numbers of applications. Insect growth regulators counteract Juvenile hormone in insects and therefore do not allow the larva to morph into a pupa and inhibit activation of ovarian follicles and development of accessory sex glands in adults (Muegge et al. 2003). Each of the insect growth regulators are specific to the group of insects it is designed to eliminate. All of these insecticides are used in low concentrations and have very high LC50 values for aquatic invertebrates.

The LC50 value for methyl parathion for *D. magna* is 0.14 µg/L whereas the formulation found in Methyl Parathion 4E had a 48 hour toxicity value of 31 µg/L in fairy shrimp. Similarly for cyfluthrin the LC50 for *D. magna* is 0.14 µg/L and found a value of 1.1 µg/L for Tempo® SC Ultra. These results coincide with the findings of Centenu et al. (1995).

The results of this study demonstrate that pesticide formulations can potentially be much more toxic than what is typically reported for the active ingredient alone. This is especially true for herbicides where many inert ingredients are added. Both herbicides tested in this study, Roundup® and Karmex® DF, were toxic to *T. platyurus* in concentrations less than what was seen for the active ingredient in the test species *D. magna*. This indicates that these herbicides may have the potential to cause mortality and metabolic alterations of a native playa species like fairy shrimp. Methyl Parathion 4E and Tempo® SC Ultra were less toxic to *T. platyurus* than in tests using their active ingredients seen in the test species *D. magna*. However, results of this study show added ingredients used in herbicides to increase weed killing capabilities can also increase toxicity of the herbicide to aquatic invertebrates possibly through common mechanisms. Therefore, herbicide toxicity needs to be reevaluated for herbicide formulations when surfactants and other inert ingredients are incorporated into the mixture.

Pesticides are not typically found individually in the environment, but are usually found as mixtures, thus altering toxicity, which needs to be considered further. Also, the actual concentration of chemicals in playas needs to be examined for a wide range of time periods to determine the extent of which chemicals get into the playa ecosystem. Future work needs to be done in order to determine non-lethal effects at lower concentrations as well as determine the acute toxic concentrations of native shrimp and other species living in the playas of the SHP.

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STATUS OF THE COMMON SNOOK  
(*CENTROPOMUS UNDECIMALIS*)  
IN TEXAS

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**Abstract.**—Catch data are summarized for common snook, (*Centropomus undecimalis*) from 1975 through 2004 from the lower Laguna Madre, the only area along the Texas coast where common snook are routinely captured. Catch rates of common snook were low (<1 common snook per gill net set) and varied among years, as did size structure. Based on the catch rate and size structure data, the adult common snook population is characterized by low abundance and erratic recruitment (i.e., missing or extremely weak year-classes are common). Additional comments on the status of common snook in Texas are provided.

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The common snook, *Centropomus undecimalis*, is a tropical fish whose range extends into sub-tropical waters. In the western Atlantic Ocean, common snook occur from  $\approx 34^{\circ}\text{N}$  to  $\approx 25^{\circ}\text{S}$  latitude (North Carolina to Rio de Janeiro, Brazil), with common snook frequently captured in waters off Galveston and the southern tip of Texas (Robins & Ray 1986; Rivas 1986). Although common snook reproduction is limited to salt water, juvenile habitat is usually characterized by low salinity waters whereas adult habitat includes rivers, estuaries, coastal lagoons, and outer shores of barrier islands (Marshall 1958; McMichael et al. 1989; Shafland & Koehl 1979). Common snook distribution is restricted primarily by cold weather and freeze events (Storey & Gudger 1936). These fish have been observed as far north as New York (Shaefer 1972), but their sensitivity to cold weather prevents establishment of a permanent population further north than the  $14^{\circ}\text{C}$  isotherm. The lower Laguna

Madre and its associated estuaries appear to be the northern-most range of the Texas-Mexico population.

The common snook is valued recreationally in Texas and commercially in Mexico. During the early part of the 20th century, common snook populations supported commercial fisheries in Florida, Texas, and the Caribbean (Marshall 1958; Alvarez-Lajonchere et al. 1982; Matlock & Osburn 1987). Annual commercial landings in Texas were greater than 45,360 kg in the 1930s. These landings declined through the 1940s and 1950s until 1961, after which no landings were reported (Matlock & Osburn 1987). The sale of common snook in Texas was prohibited in 1987. From 1978 to 1983, very few common snook were recorded in either Texas Parks and Wildlife Department fishery independent sampling or sport angler surveys; as a result, stocking was recommended as a means to revitalize the fishery (Matlock & Osburn 1987). Efforts to collect brood fish eligible for strip-spawning and attempts to mature snook in ponds and cages were unsuccessful (Colura & Matlock 1989). This study summarizes catch data for common snook from 1975 through 2004 from the lower Laguna Madre, which is the only area along the Texas coast where common snook are routinely captured, and comment on the status of common snook in Texas.

## METHODS

Total length (mm) was measured on common snook collected coastwide between 1975 and 2004 by Texas Parks and Wildlife Department personnel during routine gill-net sampling. Forty-five gill nets were set at random locations during 10-wk periods in the fall and spring of each year in each of eight bay systems. Gill nets were 183 m long and 1.2 m deep with 45.7-m sections of 76-, 102-, 127- and 152-mm stretched monofilament meshes. Gill nets were set perpendicular to the shoreline with the smallest mesh size adjacent to the shore. The nets were set within 1 hr of sunset and picked up within 4 hr after sunrise. Due to lower catch rates in the spring and to minimize difficulties with assessment of seasonal

catches (Pope & Willis 1996), this study restricted the assessment to fall (September through November) catch data. Spectral analysis (Chatfield 1989) was used to determine whether cyclical patterns were evident in the catch of adult common snook.

From 1992-97, juvenile fish were collected using otter trawls and bag seines in the Rio Grande from its confluence with the Gulf of Mexico to 48.3 river-km upstream. Ten trawls and six bag seines were collected monthly at randomly selected sampling stations. Otter trawls were 5.7 m wide at the headrope with 38-mm stretched nylon multifilament mesh. Trawl tows were made in alternating directions (upstream and downstream) in the center of the channel. Bag seines were 9.1 m long with 19-mm stretched mesh in wings and 13-mm stretched mesh in bag. Bag seines were pulled parallel to the riverbank. To minimize seasonal biases in catches (Pope & Willis 1996) and ensure that age 0+ common snook had recruited to these gears, this study restricted assessment to winter (December through January) catch data.

## RESULTS AND DISCUSSION

Most (83 percent) common snook caught in gill nets came from the lower Laguna Madre and no common snook were caught north of Matagorda Bay. A total of 209 common snook was captured in the lower Laguna Madre during fall sampling. Annual variation in the catch rate of these common snook was evident (Fig. 1). Spectral analysis revealed statistically significant 3-, 5- and 10-year cycles in the catch rate of common snook (Fisher-Kappa test,  $P = 0.019$ ), with the 10-year cycle as the strongest. Although there is no linear trend in the 30-year time series of catch rates, there is a significant recurring pattern of years with higher and lower catches of common snook. In all years, catch rates were low (<1 common snook per gill net set). There were ten years in which enough common snook ( $n \geq 10$ ) were captured to examine size structure, which also varied among years (Fig. 2). Based on the catch rate and size structure data, the adult common snook population is

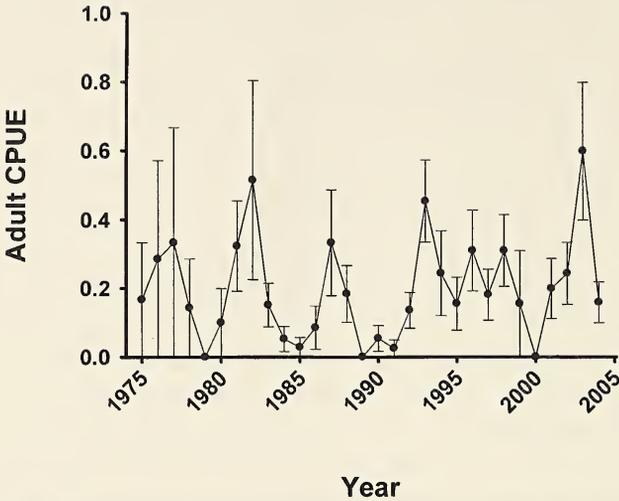


Figure 1. Mean  $\pm$  SE catch per unit effort (CPUE; number per gill net set) for adult common snook captured in the Lower Laguna Madre, Texas from 1975 to 2004.

characterized by low abundance and erratic recruitment (to 300-mm TL); namely, missing or extremely weak year-classes are common.

The common snook is a protrandric hermaphrodite (Peters et al. 1998; Taylor et al. 2000). In Florida populations of common snook, 50 percent of the young males are believed to transform into females by the age of 5-7 years (Taylor et al. 2000). Thus, adequate growth and some protection of younger males are necessary for the production of females. The current recreational harvest regulation (a reverse slot, which allows the harvest of fish between a minimum and maximum length and requires the release of fish shorter than the minimum length or longer than the maximum length) is designed to provide protection to males, while allowing some harvest. Given the low numbers of common snook captured during routine monitoring, it is unlikely that density-dependent mechanisms are hindering growth rates of these fish. Even so, very few large (>750 mm) common snook were captured during fall gill-net sets. It is possible that production of common snook year classes in Texas is egg-limited because few mature females exist. This may be an important key for future conservation efforts with common snook in Texas.

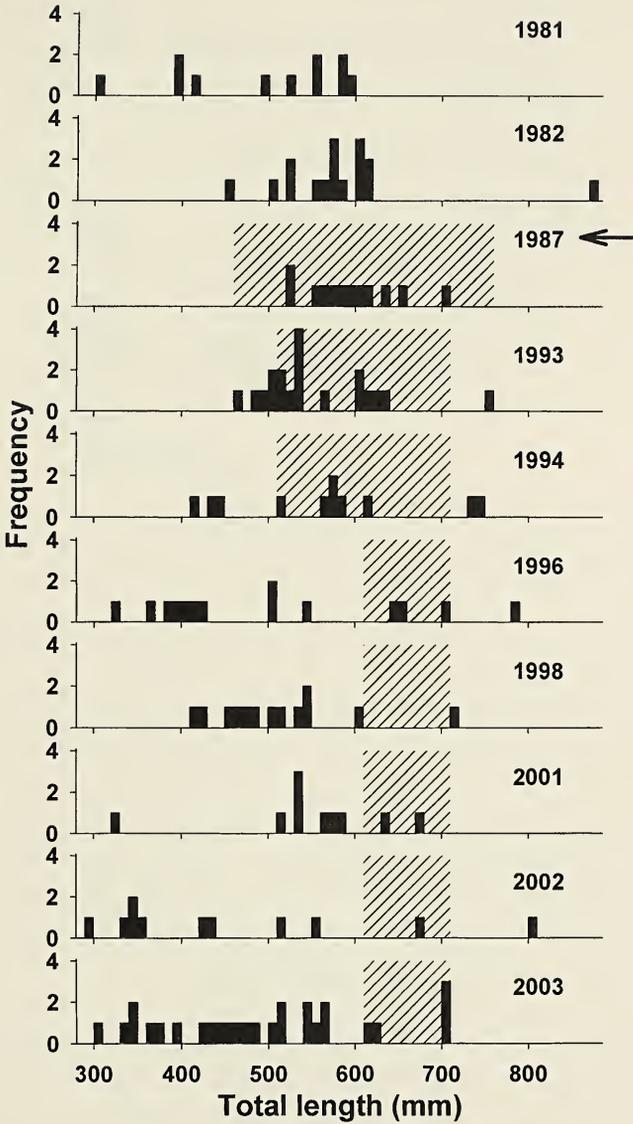


Figure 2. Size structure for adult common snook captured with gill nets in the Lower Laguna Madre, Texas from 1981 to 2004. The arrow indicates when the sale of common snook was prohibited and size limits were approved for recreational harvest of common snook. The legal harvestable size range for common snook is represented by the hatched region.

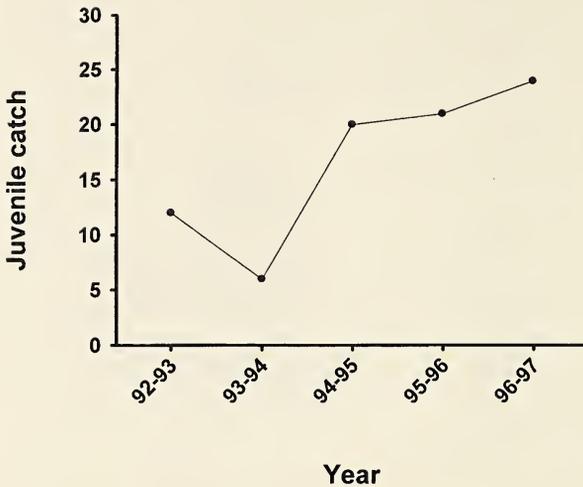


Figure 3. Number of age 0+ common snook caught during winter (December-January; fish arbitrarily became age 1 on January 1) using bag seines and otter trawls in the lower 48.3 river-km of the Rio Grande, Texas from 1992 to 1997.

During the years assessed in this study, juvenile common snook were captured each year in the Rio Grande (Fig. 3), although catches and size structure varied among years (Fig. 4). Thus, it appears that common snook successfully spawn most years in south Texas. Given the size structure of the adult population, these young fish may not successfully recruit each year to the adult population, a common trait of longer-lived fishes. While this trait may not be a concern for common snook, research on the factors that influence recruitment of common snook may provide the knowledge necessary for rebuilding the populations.

At present, the common snook population in Texas is small in size and appears to persist at a relatively steady state. Several factors may limit the growth of this population including over fishing, sudden winter freezes, and loss or degradation of habitat including environmental contamination. For example, the blockage of the mouth of the Rio Grande that occurred in 2001 and 2002 prevented larval common snook from reaching their nursery habitat upriver, at least during the period of blockage. The difficulty of

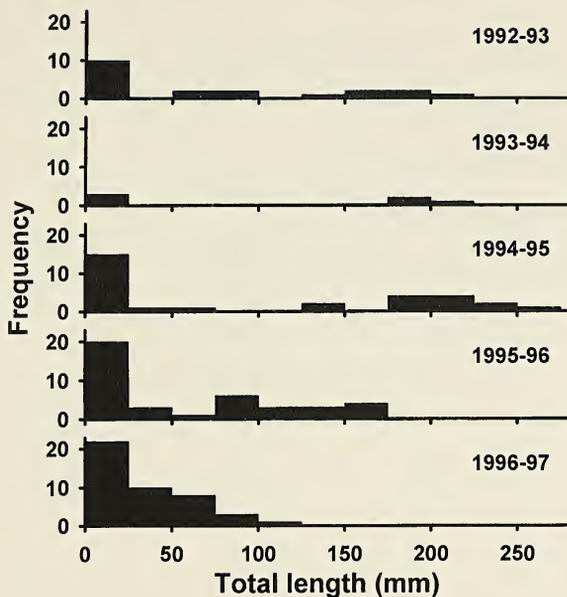


Figure 4. Size structure for age 0+ common snook during winter (December-January; fish arbitrarily became age 1 on January 1) that were captured in the lower 48.3 river-km of the Rio Grande, Texas from 1992 to 1997.

managing these factors and their interactions constrain the return of the common snook population in Texas to historic levels.

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A COMPARISON OF THE BIRD COMMUNITY OF CREOSOTE BUSH  
SCRUB DURING TWO CONSECUTIVE SUMMERS IN THE  
MUNICIPALITY OF GARCÍA, NUEVO LEÓN, MEXICO

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**Abstract.**—This study examined the avifauna of a creosote bush (*Larrea tridentata*) community in the municipality of García, Nuevo León, during the summers of 1995 and 1996. Based on foraging strategies, the 37 species (1995=29; 1996=31) of insectivorous species observed were grouped into seven functional groups (or guilds), which were subdivided into 12 subgroups. Nine functional subgroups were observed during the summer of 1995 and 12 subgroups observed during 1996. While this diversity of insectivorous foraging strategies was found to be high in this area of the Chihuahuan Desert, the exact nature of the differences observed between 1995 and 1996 remains unclear. It appears to be dependent upon variation in the productivity of the desert ecosystem.

**Resumen.**—El presente trabajo estudia la avifauna de una comunidad de gobernadora (*Larrea tridentata*) en el municipio de García, Nuevo León, durante los veranos de 1995-1996. Basado en las estrategias de forrajeo, se registraron 37 especies (1995=29; 1996=31) incluidas en siete grupos funcionales subdivididos en 12 subgrupos. Nueve subgrupos funcionales fueron observados durante el verano de 1995 y doce subgrupos observados durante 1996. La diversidad de estrategias de forrajeo fue encontrada alta en esta área de Nuevo León, y es comparada con otras localidades del Desierto Chihuahuense. Esta área representa un sitio importante del Desierto Chihuahuense, tanto por su riqueza específica, como por el número de subgrupos funcionales presentes.

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Both the diversity and feeding habits of those avian species inhabiting the Chihuahuan Desert of northern Mexico have been examined in several different regions. Babb-Stanley & Verhulst-R. (1992) recorded 92 avian species at a site in Durango, at the southern limit of the Chihuahuan Desert and these authors reported insectivorous birds as the most important guild with 27 species. Contreras-Balderas et al. (1997) studied the ecological and seasonal composition of the avifauna of the valley of Cuatro Ciénegas,

Coahuila. In this same valley, García-Salas et al. (1997) analyzed the trophic structure of the bird community that inhabits the creosote bush scrub. Recently, Contreras-Balderas et al. (2000), also determined the trophic dynamics of the bird community associated with the creosote bush in the same locality (García, Nuevo Leon) studied here. The purpose of this study was to investigate the similarity of the insectivorous bird communities during two consecutive summers in this region of the Chihuahuan Desert of Nuevo Leon in northern Mexico.

### Study Site

The municipality of García (25°4'48" N and 100°54'18" W) is located approximately 35km WNW of Monterrey in the state of Nuevo Leon in northern Mexico. It is located near the east central edge of the Chihuahuan Desert at an elevation of approximately 705m. Annually, the rainfall varies between 200 and 400 mm, whereas the annual average temperature is between 18 and 20°C. The vegetation is dominated mainly by creosote bush scrub (*Larrea tridentata*) along with other plant species such as: anacahuite (*Cordia boisieri*), black brush (*Acacia rigidula*), ocotillo (*Fouquieria splendens*), coyotillo (*Karwinskia humoldtiana*), bear-rass (*Yucca* sp.), lechugilla (*Agave lecheguilla*), leather stem (*Jatropha dioica*), *Opuntia microdesys*, tree cholla (*Opuntia imbricata*), desert christmas cactus (*Opuntia leptocaulis*), candelilla (*Euphorbia antisiphylitica*), hedgehog cactus (*Echinocereus* sp.) and other cactus (*Opuntia* spp.).

### METHODS

The study site was visited six times in 1995 (late June to early August) and five times in 1996 (late June and July), according to Skirvin's (1981) census method, from sunrise until approximately 4 h after sunrise. Five 1 ha plots were sampled three times a day and the observed species were categorized into functional groups and resident status following Howell & Web (1995) and Contreras-Balderas et al. (2000). The same site of creosote bush scrub vegetation was sampled fortnightly. Species names follow the

systematic nomenclature of the A.O.U. (1998). The assignment of species to foraging strategies was based on Ehrlich et al. (1988). Several ecological indexes that describe the dynamics and structure of the community (Brower et al., 1997) were computed for each season of summer: Shannon's species diversity, Margalef's species richness, and Jaccard's species similarity. In order to compare if the species abundances between both summers were correlated, Pearson's correlation test was applied.

### RESULTS AND CONCLUSIONS

A total of 37 species (Table 1) were found during both summers (29 in 1995 and 31 in 1996) for the study area. The most important functional group for both summers combined was the insectivorous guild with 26 species, which represented 70% of the total bird community. There was considerable variation in the absolute and relative abundances of the species for each summer, including the presence of seven groups and 12 subgroups (Table 1). Similarly, the calculated species diversity and similarity indices also varied for each summer (Table 1). In the summer of 1995, five species were the most abundant taxa in the bird community: *Amphispiza bilineata* (25.23%), *Cardinalis sinuatus* (12.30%), *Thryomanes bewickii* (8.0%), *Chordeiles acutipennis* (8.30%) and *Polioptila melanura* (7.07%). In 1996, the most abundant were *A. bilineata* (28.24%), *C. sinuatus* (9.51%), *P. melanura* (8.93%), *Campylorhynchus brunneicapillus* (5.47%), and *Auriparus flaviceps* (5.18%). The species abundance for both summers was significantly correlated ( $r=0.941$ ,  $df=34$ ,  $P<0.001$ , Fig. 1) and the abundance of individuals of each species followed a logarithmic distribution during each of the years (Fig.2). Also, both summer bird communities exhibited a similarity of 0.6216 (62.16%). Species diversity for summer 1995 and summer 1996 were 2.61 and 2.71, respectively (Table 1).

If the guild of insectivorous birds (the richest guild in species) is considered exclusively in the comparative analysis between seasons (Table 2), the same tendency of abundances is still observed ( $r=0.959$ ,  $df=24$ ,  $P<0.001$ ). The specific diversity for summer 1995 and summer 1996 was 1.9921 and 2.2061 respectively; species

Table 1. Species account of all birds on creosote bush scrub *Larrea tridentata* community at García, Nuevo León, México, during summers of 1995-1996. Shown are the number of individuals and proportion (in parenthesis) for each species, functional group (FUG) and resident status (RES). The values of different ecological indexes in these communities are presented in the bottom of the table.

Species	1995	1996	FUG	RES
<i>1-Nycticorax violaceus</i>	---	1(0.28)	Insectivorous, ground glean	Transient
<i>2-Cathartes aura</i>	8(2.46)	10(2.88)	Carrion, high patrol	Permanent Resident
<i>3-Buteo jamaicensis</i>	2(0.61)	1(0.28)	Prey, high patrol	Permanent Resident
<i>4-Falco sparverius</i>	---	2(0.57)	Insectivorous, swoops	Winter Resident
<i>5-Callipepla squamata</i>	15(4.61)	4(1.15)	Granivorous, ground glean	Permanent Resident
<i>6-Zenaida macroura</i>	23(7.07)	16(4.61)	Granivorous, ground glean	Permanent Resident
<i>7-Bubo virginianus</i>	1(0.30)	---	Prey, swoops	Permanent Resident
<i>8-Chordeiles acutipennis</i>	26(8.00)	17(4.89)	Insectivorous, aerial	Summer Resident
<i>9-Archilochus alexandri</i>	3(0.92)	3(0.82)	Nectivorous, hover glean	Winter Resident
<i>10-Picoides scalaris</i>	3(0.92)	11(3.17)	Insectivorous, bark glean	Permanent Resident
<i>11-Empidonax minimus</i>	---	2(0.57)	Insectivorous, aerial	Winter Resident
<i>12-E. sp. Indet.</i>	1(0.30)	1(0.28)	Insectivorous, aerial	Winter Resident
<i>13-Myiarchus cinerascens</i>	3(0.92)	---	Insectivorous, aerial	Summer Resident
<i>14-M. tyrannulus</i>	---	1(0.28)	Insectivorous, aerial	Winter Resident
<i>15-Tyrannus tyrannus</i>	1(0.30)	---	Insectivorous, aerial	Transient
<i>16-Hirundo rustica</i>	2(0.61)	8(2.30)	Insectivorous, aerial	Summer Resident
<i>17-Auriparus flaviceps</i>	20(6.15)	18(5.18)	Insectivorous, bark glean	Permanent Resident
<i>18-Corvus corax</i>	12(3.69)	7(2.01)	Omnivorous, ground glean	Permanent Resident
<i>19-Campylorhynchus brunneicapillus</i>	5(1.53)	19(5.47)	Insectivorous, ground glean	Permanent Resident
<i>20-Salpinctes obsoletus</i>	---	3(0.86)	Insectivorous, ground glean	Permanent Resident
<i>21-Thryomanes bewickii</i>	27(8.30)	16(4.61)	Insectivorous, ground glean	Permanent Resident
<i>22-Poliptila melanura</i>	23(7.07)	31(8.93)	Insectivorous, foliage glean	Permanent Resident
<i>23-P. caerulea</i>	5(1.53)	2(0.57)	Insectivorous, foliage glean	Winter Resident
<i>24-Mimus polyglottos</i>	1(0.30)	5(1.44)	Insectivorous, ground glean	Permanent Resident
<i>25-Toxostoma curvirostre</i>	6(1.84)	12(3.45)	Frugivorous, ground glean	Permanent Resident
<i>26-Lanius ludovicianus</i>	2(0.61)	3(0.86)	Insectivorous, swoopPrey, swoops	Winter Resident
<i>27-Vireo bellii</i>	1(0.30)	5(1.44)	Insectivorous, foliage glean	Summer Resident
<i>28-Dendroica coronata</i>	---	3(0.86)	Insectivorous, foliage glean	Transient
<i>29-D. townsendi</i>	1(0.30)	---	Insectivorous, foliage glean	Transient
<i>30-Wilsonia pusilla</i>	1(0.30)	---	Insectivorous, foliage glean	Transient
<i>31-Arremonops rufivirgatus</i>	---	1(0.28)	Insectivorous, ground glean	Permanent Resident
<i>32-Amphispiza bilineata</i>	82(25.23)	98(28.24)	Insectivorous, ground glean	Permanent Resident
<i>33-Cardinalis sinuatus</i>	40(12.30)	33(9.51)	Granivorous, ground glean	Permanent Resident
<i>34-Guiraca caerulea</i>	1(0.30)	---	Insectivorous, ground glean	Summer Resident
<i>35-Molothrus ater</i>	7(2.15)	5(1.44)	Granivorous, ground glean	Summer Resident
<i>36-Icterus parisorum</i>	3(0.92)	5(1.44)	Insectivorous, foliage glean	Summer Resident
<i>37-Carpodacus mexicanus</i>	---	4(1.15)	Granivorous, ground glean	Permanent Resident
N Species (N Individuals)	29(325)	31(347)		
Similarity		0.62		
Species Diversity (Shannon)	2.61	2.72		
Species Diversity (Margalef)	4.84	5.13		

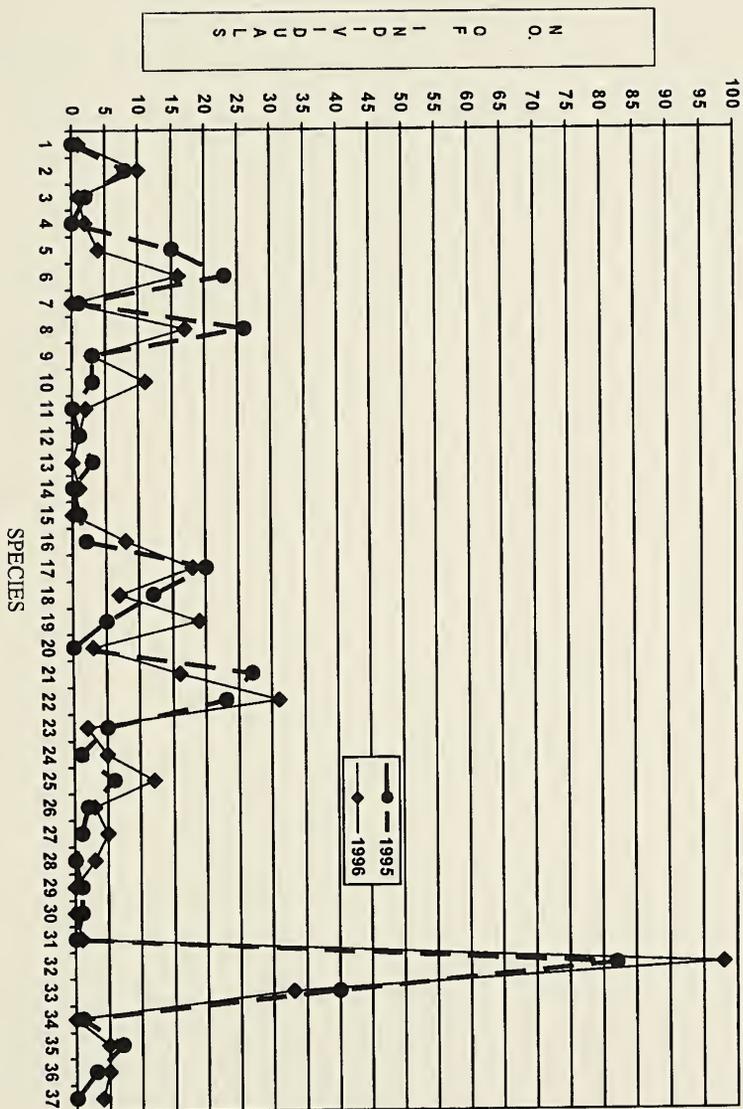


Figure 1. Relation graphical of bird species and individuals on Creosotebush scrub at a locality of García, Nuevo León, México, showing a same tendency in both summers of 1995 and 1996.

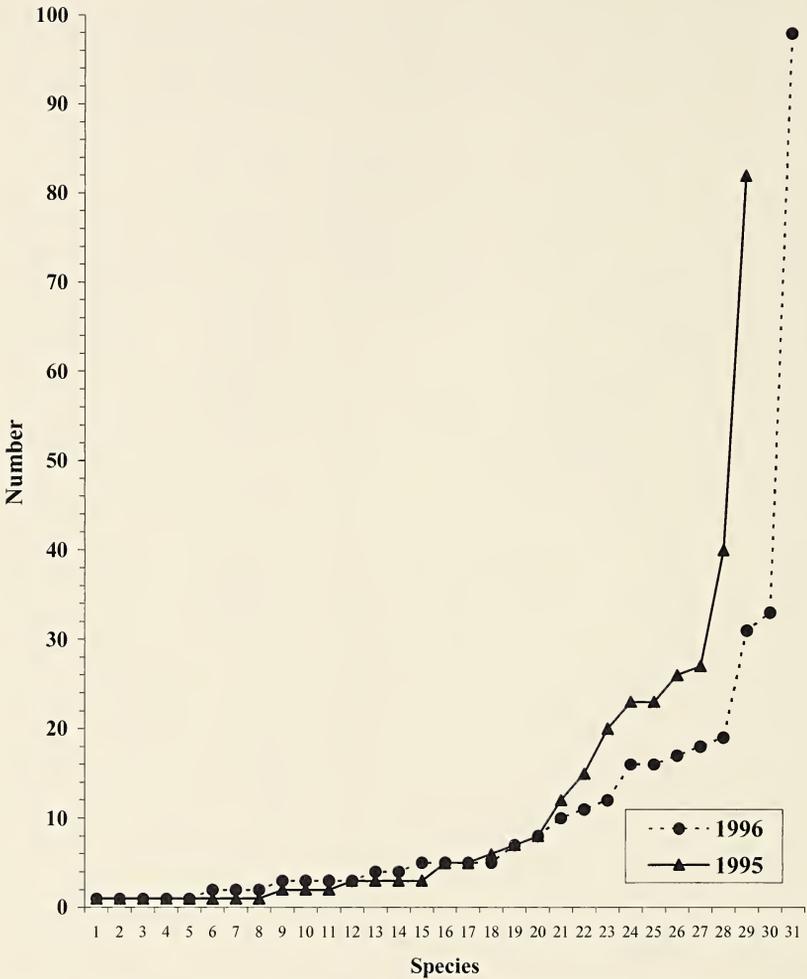


Figure 2. Model of logarithmic distribution for the abundance of the species in the bird community of creosote bush in García, Nuevo León, México, during the summers of 1995 and 1996.

richness was 3.3723 (summer 1995) and 3.6170 (summer 1996). The species similarity of this insectivorous bird guild between both summers was 0.60 (60.0%) (Table 2). In terms of functional groups represented in the bird community (Table 3), the values of the different ecological indexes were also very similar for both summers ( $r = 0.939$ ,  $df = 7$ ,  $P > 0.001$ ).

Table 2. Species account of insectivorous birds on creosote bush scrub *Larrea tridentata* community at García, Nuevo León, México, during summers of 1995-1996. Shown are the number of individuals and proportion (in parenthesis) for each species, functional group (FUG) and resident status (RES). The values of different ecological indexes in these communities are presented in the bottom of the table.

Species	1995	1996	FUG	RES
<i>1-Nycticorax violaceus</i>	----	1(0.28)	Insectivorous, ground glean	Transient
<i>2-Falco sparverius</i>	----	2(0.57)	Insectivorous, aerial	Winter Resident
<i>3-Chordeiles acutipennis</i>	26(8.00)	17(4.89)	Insectivorous, aerial	Summer Resident
<i>4-Picoides scalaris</i>	3(0.92)	11(3.17)	Insectivorous, bark glean	Permanent Resident
<i>5-Empidonax minimus</i>	----	2(0.57)	Insectivorous, aerial	Winter Resident
<i>6-E. sp. Indet.</i>	1(0.30)	1(0.28)	Insectivorous, aerial	Winter Resident
<i>7-Myiarchus cinerascens</i>	3(0.92)	----	Insectivorous, aerial	Summer Resident
<i>8-M. tyrannulus</i>	----	1(0.28)	Insectivorous, aerial	Winter Resident
<i>9-Tyrannus tyrannus</i>	1(0.30)	----	Insectivorous, aerial	Transient
<i>10-Hirundo rustica</i>	2(0.61)	8(2.30)	Insectivorous, aerial	Summer Resident
<i>11-Auriparus flaviceps</i>	20(6.15)	18(5.18)	Insectivorous, bark glean	Permanent Resident
<i>12-Campylorhynchus brunneicapillus</i>	5(1.53)	19(5.47)	Insectivorous, ground glean	Permanent Resident
<i>13-Salpinctes obsoletus</i>	----	3(0.86)	Insectivorous, ground glean	Permanent Resident
<i>14-Thryomanes bewickii</i>	27(8.30)	16(4.61)	Insectivorous, ground glean	Permanent Resident
<i>15-Poliptila melanura</i>	23(7.07)	31(8.93)	Insectivorous, foliage glean	Permanent Resident
<i>16-P. caerulea</i>	5(1.53)	2(0.57)	Insectivorous, foliage glean	Winter Resident
<i>17-Mimus polyglottos</i>	1(0.30)	5(1.44)	Insectivorous, ground glean	Permanent Resident
<i>18-Lanius ludovicianus</i>	2(0.61)	3(0.86)	Insectivorous, swoops+D49	Winter Resident
<i>19-Vireo bellii</i>	1(0.30)	5(1.44)	Insectivorous, foliage glean	Summer Resident
<i>20-Dendroica coronata</i>	----	3(0.86)	Insectivorous, foliage glean	Transient
<i>21-D. townsendi</i>	1(0.30)	----	Insectivorous, foliage glean	Transient
<i>22-Wilsonia pusilla</i>	1(0.30)	----	Insectivorous, foliage glean	Transient
<i>23-Arremonops rufivirgatus</i>	----	1(0.28)	Insectivorous, ground glean	Permanent Resident
<i>24-Amphispiza bilineata</i>	82(25.23)	98(28.24)	Insectivorous, ground glean	Permanent Resident
<i>25-Guiraca caerulea</i>	1(0.30)	----	Insectivorous, ground glean	Summer Resident
<i>26-Icterus parisorum</i>	3(0.92)	5(1.44)	Insectivorous, foliage glean	Summer Resident
N Species (N Individuals)	19(208)	21(252)		
Similarity		0.6		
Species Diversity (Shannon)	1.99	2.21		
Species Diversity (Margalef)	3.37	3.62		

These results differ in the number of functional groups or subgroups of foraging strategies when compared to other studies conducted in different regions of the Chihuahuan Desert. Tomoff (1974) reported 3/7, Thiollay (1979) 5/10, Babb-Stanley & Verhulst-R. (1992) 6/0 and Garcia-Salas et al. (1997) 5/0, respectively. In this study at García in Nuevo León, seven functional groups (or guilds) with 12 subgroups were recognized. In comparison, Blake (1984) reported 47 species of birds (four groups/seven

Table 3. Species account of different functional groups of birds on creosote bush scrub *Larrea tridentata* community at García, Nuevo León, México, during summers of 1995-1996. Shown are the number of individuals and proportion (in parenthesis) for each species, functional group (FUG) and resident status (RES). The values of different ecological indexes in these communities are presented in the bottom of the table.

Species	1995	1996	FUG	RES
<i>1-Cathartes aura</i>	8(2.46)	10(2.88)	Carrion, high patrol	Permanent Resident
<i>2-Buteo jamaicensis</i>	2(0.61)	1(0.28)	Prey, high patrol	Permanent Resident
<i>3-Callipepla squamata</i>	15(4.61)	4(1.15)	Granivorous, ground glean	Permanent Resident
<i>4-Zenaidura macroura</i>	23(7.07)	16(4.61)	Granivorous, ground glean	Permanent Resident
<i>5-Bubo virginianus</i>	1(0.30)	----	Prey, swoops	Permanent Resident
<i>6-Archilochus alexandri</i>	3(0.92)	3(0.82)	Nectivorous, hover glean	Winter Resident
<i>7-Corvus corax</i>	12(3.69)	7(2.01)	Omnivorous, ground glean	Permanent Resident
<i>8-Cardinalis sinuatus</i>	40(12.30)	33(9.51)	Granivorous, ground glean	Permanent Resident
<i>9-Carpodacus mexicanus</i>	----	4(1.15)	Granivorous, ground glean	Permanent Resident
N Species (N Individuals)	8(104)	8(78)		
Similarity		0.78		
Species Diversity (Shannon)	1.65	1.65		
Species Diversity (Margalef)	1.51	1.61		

subgroups) from a creosote bush community in a desert site of Nevada (Christmas Tree Pass region of the Newberry Mountains, Clark Co.). This increase in the overall number of foraging strategies observed at García is important for understanding the role of the creosote bush scrub community in providing this diversity of foraging strategies.

Considering that the creosotebush represents a uniformly stable plant community, it would appear unlikely that this area would exhibit such an unusually high variation in its production of insects. It would also appear that the increased variation of foraging strategies of insectivorous birds is dependent upon the variation in the productivity of the desert ecosystem rather than being specifically dependent upon the creosote bush, or the presence of other species besides *Larrea*. In this current study, nine functional subgroups were observed in 1995 and 12 subgroups observed in 1996. While the exact nature of the basis for this increased diversity of insectivorous foraging strategies remains unclear, it appears to be dependent upon variation in productivity of the desert ecosystem rather than upon the creosote bush.

The presence of species categorized as winter resident or transient in the study area is because they were present in August to the end of the summer. It is important to document the year-to-year changes in the structure and composition of the bird community in terms of species richness and abundance as well as in number of guilds based on foraging strategies. It is the intent of the authors that this study will increase the interest of the ecology of the birds of creosote bush scrub community in northern México, especially with respect to the dynamic nature of avian species richness, abundance and seasonal status.

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## LEAD CONTAMINATION IN IMPORTED CANDIES AND THEIR WRAPPERS

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**Abstract.**—The dangers of ingesting lead, especially in childhood, are well documented. Some studies, recently reported in the media, have found a correlation between Mexican candies and cases of childhood lead poisoning. A few researchers have found lead in some brands of imported Mexican candies sold in the United States; it has not been conclusively determined whether the lead contamination originates in the candy itself or the wrapper. This ongoing project utilizes atomic absorption spectrophotometry to test several brands of candies, as well as their packaging material, for lead content.

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During the last few decades, researchers and medical practitioners have begun to appreciate the clear dangers associated with lead poisoning, especially in children. This increased awareness has raised childhood lead poisoning to the position of a public health threat that needs to be addressed and monitored. Lead is now known to be detrimental to nearly every organ system. It has been documented to cause problems with the kidneys, the blood, liver, reproductive organs, and hearing (USEPA 1985). Its ingestion has been linked to problems with neurological, and cognitive development. Children living with the effects of lead ingestion suffer from a reduced IQ, behavioral problems, and a slowed development (Lidsky & Schneider 2006). For several reasons, children are at a greater risk for lead poisoning than adults. First, a child's stomach readily absorbs lead when ingested. Also, children are more likely to place non-food items in their mouths. And finally, children are in a vulnerable stage of development that places their bodies at a greater risk of damage from lead poisoning (USEPA 1985).

Traditionally, childhood lead poisoning has widely been associated with ingesting lead paint (Jones & Moore 1999). During the 1970's, the United States government, along with some industries, began a concerted effort to combat childhood lead poisoning. The pillars of these programs were the restriction of lead paint, the banning of leaded gasoline, and an increased control and surveillance for lead in

food and consumer products. The implementation of these programs greatly reduced the incidences of lead exposure in children, along with the general population. However, with the globalization of the United States economy, non-traditional vectors for exposure have been noted (CDC 1998).

Several state health departments, along with the Center for Disease Control, have issued warnings outlining the risks associated with ingesting imported lead contaminated candies and their wrappers (CDC 2002). Several cases of children having an elevated blood lead level have been traced to certain Mexican candies (CDC 2002). These reported poisonings have been followed up by several research studies. One particular study from the University of Oklahoma reported that more than 50% of the Mexican lollipops tested exceeded the threshold of lead toxicity tolerated for this type of product (Lynch et al. 2000).

A method was designed and initiated in order to ascertain whether lead contamination does exist in locally available Mexican candies. Also, the method was used to determine if a correlation exists between any lead contamination noted in the candies and the presence of lead tainted wrappers.

#### METHODS AND MATERIALS.

Samples of seventy-one imported candies were randomly collected from various sites throughout Texas. These products represented a wide variety and type of imported products. The criteria imposed on the sample collection were that they must be purchased in Texas, and that they must have been manufactured in Mexico.

The samples were separated from their wrappers and prepared for analysis. The wrappers and candies were weighed and digested using 10% nitric acid, under heat, allowing a gentle reflux for two hours. A few of the positive wrapper samples were also digested with glacial acetic acid for 24 hours to determine if any leachable amount of lead was detectable. After digestion, any suspended organic materials were removed from the samples by filtration (USEPA 1998). All of the prepared samples were analyzed using a Buck Scientific flame atomic absorption spectrophotometer. The detection limit for this spectrophotometer lies between 1 and 3 parts per billion using this

method of lead analysis. Background correction was employed (USEPA 1998).

### RESULTS.

One wrapper, sample 23, was digested with 4% acetic acid for 24 hours to ascertain if any leaching occurred. The test for leaching is a milder digestion than one performed with nitric acid.

Three of the wrappers had detectable amounts of lead. These levels ranged from 3700 ppm to 6800 ppm. Also, another wrapper from the same lot number and purchased at the same time as product #23 was tested for lead by utilizing leaching rather than oxidation. This test indicated a lead level of 5100 ppm. Four of the candies had detectable lead levels. Nearly ten percent (7/71) of the products tested had either lead contamination in their wrapper or in the candy itself. This did not include the retest of product #23.

### DISCUSSION

The wrappers that had detectable lead levels could pose a lead hazard to any person placing them in their mouth. The wrapper that was subjected to acetic acid for a 24-hour period clearly indicated that the lead in the ink had the ability to leach out. This is of concern since children have a greater tendency to place and suck on non-food items. Since these wrappers are being utilized for candy, it is clear that they may pose a significant health threat to children.

Recently, the state of California enacted legislation effectively banning lead in candy wrappers (USFDA 2005). In the past, the Federal government has tolerated up to 500 ppm of lead on any painted surface (42 CFR §1303.1-5 1977). All three of the wrappers with detectable lead levels are well over the traditional Federal guideline. However, recently the Consumer Product Safety Commission has written an open letter to Mexican candy manufacturers informing them that using lead pigments in the ink of candy wrappers and packaging would not be allowed (CDC 2002).

The letter calls attention to the candy manufactures article #15 United States Code under the Federal Hazardous Substance Act: "household substances that expose children to hazardous quantities of lead under reasonably foreseeable conditions of handling or use are

Table 1. Concentration of lead in samples.

Product #	Wrappers and Candy	Concentration
1 (Chamoy Miguelito)	Outer wrapper, plastic bag	5400 ppm
23 (Rabanaditas)	Cellophane wrapper	6800 ppm
37 (Ta-Rico)	Cellophane wrapper	3700 ppm
23-a (retest for leaching)	Cellophane wrapper	5100 ppm
15 (Pica Limon)	Salt/lemon powder	1.92 ppm
16 (Limon 7)	Salt/lemon powder	4.67 ppm
20 (Limonazado)	Salt/lemon powder	4.91 ppm
22 (Super Rabanaditas)	Chili coated lollipop	1.32 ppm

hazardous substances. Any substance intended for use by children, which contains a hazardous amount of lead that is accessible for children to ingest, is a banned hazardous substance (Schoem 2004)". The letter also directs importers and manufactures to stop exporting candies to the United States that contain lead based ink (Schoem 2004).

The lead levels detected in the candies clearly constitute a health risk for anyone ingesting them. These candies had levels ranging from 1.32 ppm to 4.91 ppm. At the time of testing, the Federal government had placed the threshold of lead in candy at 0.5 ppm (USFDA. 2005). All of these candies are well over the Federal guideline. Within the last few months, the Food and Drug Administration has indicated that in 2006 it will lower the lead level allowed in candy to 0.1 ppm (USFDA 2005). The products that had detectable lead levels in the candy did not have detectable levels of lead in their wrappers. This indicates that the wrappers are most likely not a source of contamination of the candy in this study. However, the finding of lead contamination in ten percent of the products tested indicates a possible systematic problem in the industry. The products that had detectable lead levels are dangerous and should be deemed a potential public health threat.

The lead detected in the candies is a contaminant. Its presence is inconsistent and will greatly vary from sample to sample. However, the source of the lead detected in the wrappers can probably be traced to the use of lead based pigments in the ink. The lead is not a contaminant but an actual element of the ink. Lead based inks and

pigments are still utilized in food wrappers even though ingesting lead is a known health danger (Fuortes & Bauer 2000). This is completely avoidable and should not be tolerated. The colors in the ink can be safely and easily duplicated without the use of leaded pigments. This study's findings clearly point to a need for further testing, along with increased surveillance and government regulation of the industry.

With the globalization associated with free trade and increased travel, lead contamination of foreign candies and food products can no longer be considered just a regional issue. Products are produced all over the world with the intention of global distribution. Some localities lag behind current acceptable standards of food safety, thus affecting the world consumer. Many of these areas do not have effective government oversight, making research and surveillance of the industry even more critical. These products are being manufactured overseas; United States regulators have not been able to completely stop the importation and sale of lead contaminated products (Lynch et al. 2000). The problem first being noted in the 1990s still continues today.

Monitoring in this field is very important, especially since the products are traditionally targeted to children. Children are more susceptible to damage from ingesting toxins because of their continued developmental processes. The damage caused by lead poisoning usually lasts a lifetime. Many children who have been poisoned by lead ingestion require long term treatment well into their adult lives.

The tragedy is that childhood lead poisoning is nearly completely preventable. Education and awareness are the cornerstones of any prevention program. Identifying the sources of contamination in these products would be invaluable knowledge when combating this problem. Also, health practitioners need to explore the possibility of unconventional sources of lead exposure when confronted with cases of childhood lead poisoning that seem not to have an obvious cause (Woolf & Woolf 2005). In order to be effective, information about known contamination must be shared with the community at large. It is clearly a responsibility of the scientific and medical community to share research and information with the general population that could possibly prevent lead poisoning incidents in children.

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SAMPLING REQUIREMENTS FOR ESTIMATION OF  
THE SOIL SEED BANK OF A WEST TEXAS SALT MARSH

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**Abstract**—The purpose of this study was to determine the sample incubation time, surface area, depth of the soil sample and number of soil samples required to adequately estimate the seed bank of the *Distichlis spicata* (saltgrass) community, the most widespread of the salt marsh communities, in the Diamond Y Spring Preserve, north of Fort Stockton, Texas. The locally abundant but geographically rare and threatened puzzle or Pecos sunflower (*Helianthus paradoxus*) is found in this salt marsh community. Soil samples should be incubated for 6 to 8 weeks after cold stratification, but there were no significant differences in the total number of seeds germinated between 5 and 13 weeks (260–323 seeds germinated). A sample size with an area of 0.1 m<sup>2</sup> and 6 cm in depth was sufficient to sample the soil seed bank in this inland salt marsh community. No viable seeds of any species were found in the 10 cm deep samples and 99.4% were less than 6 cm deep. Additionally, 15 soil samples seemed adequate to sample the area, although the variance was high ( $\bar{x}=92.4$ ,  $SD=85$  seeds/m<sup>2</sup>).

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The soil seed bank is especially important for the re-establishment of populations of annual species after disturbances such as drought, fire or in areas with periods of unfavorable growing conditions including high or low temperatures (Leck et al. 1989; Zammit & Zedler 1994). In addition, seed banks could serve as reservoirs for seeds and increase the likelihood of species survival. Seed banks may be important for the conservation of rare species because many seem to use seed banks to overcome genetic challenges to their long-term survival (Falk & Holsinger 1991; Ellstrand & Elam 1993). These challenges may be related to small population size because a small number of individuals increases the susceptibility to genetic drift (Barrett & Kohn 1991) and will increase the likelihood of inbreeding and inbreeding depression (Ellstrand 1992). Stochastic events, such as fire or drought, could further reduce a population, producing genetic bottlenecks. However, a seed bank could act to mediate the genetic consequences of rarity, increasing the likelihood of persistence and

maintaining the evolutionary potential of a species (Levin 1990; Kalisz & McPeck 1993; McCue & Holtsford 1998).

The soil seed bank of a species includes all the detached viable seeds both above and below the soil surface at a specific time (Thompson & Grime 1979). A seed bank consists of two components. A transient seed bank contains seeds that are not dormant or become non-dormant and germinate the first season when conditions are adequate for plant growth (Harper 1979; Baskin & Baskin 1998; Fenner & Thompson 2005). The persistent seed bank contains dormant seeds and non-dormant seeds that do not germinate during the first season (Baskin & Baskin 1998; Fenner & Thompson 2005). The seed bank is determined by the seed rain that is composed in part of seeds produced by plants growing in the immediate area in the soil and partly by seeds blown in or otherwise transported into an area (Harper 1979; Simpson et al. 1989). Typically within a community, the seed rain and the species in the seed bank are dominated by local species (Harper 1979).

The importance of seed banks to wetland communities in general has been documented by numerous studies (see Leck et al. 1989). Seed banks have been shown to be particularly important in maintaining populations of annual halophytes in some inland salt marshes (Ungar 1991; Badger & Ungar 1994). The apparent reason for the importance of a seed bank in wetlands and inland salt marshes is the variability of the wet and dry cycle in these communities, resulting in high annual or inter-annual mortality. However, none of the published studies that were reviewed examined all of the factors that seem important to adequately sample the soil seed bank of these communities. These factors include sample incubation time, surface area, depth, or the number of soil samples required. Some studies examined at least one factor and showed high variance while attempting to adequately estimate the soil seed bank (Gross 1990; Bonir & Lepart 1994; Brock et al. 1994; Baskin & Baskin 1998; Hanlen & Williams 1998; Fenner & Thompson 2005).

There are a number of salt marshes in western Texas, New Mexico and northern Mexico (Hendrickson 1977), but their ecology and population biology have been largely ignored. Some of these inland salt marshes have species that are little studied, rare, threatened or endangered (McDonald 1999), such as *Helianthus paradoxus*, the puzzle or Pecos sunflower. This rare, threatened annual species of sunflower (Compositae, Correll & Johnston 1979), is a stabilized hybrid species with *H. annuus* (common sunflower) and *H. petiolaris* (plains sunflower) being the supposed parental species (Abbott 2003; Rieseberg et al. 2003).

*Helianthus paradoxus* is presently found in 22 sites in New Mexico and three sites in Texas (Fig. 1). Two of the Texas sites are in the vicinity of Fort Stockton in Pecos County, both are on the Leon Creek drainage (Seiler et al. 1981). The third site is in the vicinity of Balmorhea in Reeves County (Karges 1998). These three Texas populations are in the Pecos River drainage. One population is on the Diamond Y Spring Preserve, a 6.1 km<sup>2</sup> nature preserve, owned by the Nature Conservancy of Texas (Fig. 1). The Diamond Y Spring is the last major spring still flowing in Pecos County, Texas (Veni 1991). This preserve protects six federally endangered or threatened species including the Puzzle Sunflower (*H. paradoxus*), Leon Springs Pupfish (*Cyprinodon bovinus*), Pecos Gambusia (*Gambusia nobilus*), Diamond Y Spring Snail (*Tryonia adamantia*), Gonzalez Spring Snail (*Tryonia stocktonensis*), and Pecos Assiminea Snail (*Assiminea pecos*) (McDonald 1999).

In addition to the lack of studies on soil seed banks of the inland salt marshes of west Texas, there are no soil seed bank studies for *H. paradoxus*. Thus one objective of this study was to investigate the soil seed bank in the zone of the salt marsh where *H. paradoxus* grows. The community examined was the *Distichlis spicata* (saltgrass) community of the Diamond Y Spring Preserve, which is where the majority of the *H. paradoxus* plants in the preserve are found. Additionally, there is an absence of any published studies



Figure 1. The black dots represent the counties in New Mexico and Texas where known populations of *H. paradoxus* are found. The star represents the location of the population of *H. paradoxus* studied within the Diamond Y Spring Preserve.

that specified the incubation time, surface area, depth, or the number of samples required for adequately sampling inland salt marsh communities. Thus the objectives of the study were to determine (1) the time required for the seeds in the soil seed bank to germinate, (2) the surface area required, (3) the depth required and (4) the number of soil samples required to adequately estimate the soil seed bank of this inland salt marsh.

#### MATERIALS AND METHODS

*Study site.*—The study was conducted at the 607 ha Diamond Y Spring Preserve of the Nature Conservancy of Texas, approximately 15 km north of Fort Stockton, Texas, in Pecos county (31° 00.54'N, 102° 55.49'W). The study site, an inland salt marsh, is unlike typical marine wetlands where the total salinity is

approximately 35 mg/kg with chloride as the major anion. It is spring fed with water containing sulfate as the major anion followed secondarily by chloride (Veni 1991; Boghici 1997). The major cations in descending order are Na, Ca, Mg and K (Veni 1991; Boghici 1997). The soil salt concentrations are highly variable depending on the distance from the springs or the drainage and the time of year, but are usually 10-40 mg/kg (Van Auken & Bush 1998).

There are several plant communities on The Diamond Y Spring Preserve that are apparently associated with distinct levels of soil water and soil salinity (Poole 1992; Bush 2006a; Bush 2006b; Van Auken et al. 2007). The plant communities also seem to be related to distance and elevation from the watercourse. At the lowest elevation in the salt marsh is found the intermittently flowing water of the springs or the drainage of Leon Creek. Immediately adjacent to the water-course in areas that are usually flooded, *Schoenoplectus americanus* (bulrush) forms a narrow, discontinuous, nearly monoculture community. Moving away from the watercourse and gaining slightly in elevation, are more extensive floodplains that lack permanent surface water. *Distichlis spicata* (saltgrass) grasslands are the dominant communities on these floodplains. Associated with *D. spicata* are populations of the federally threatened annual sunflower *H. paradoxus*. *Helianthus paradoxus* appears to be restricted to areas like this with soil surface salt levels of approximately 10 mg/kg (Poole 1992; Poole & Diamond 1993; Sivinski 1995; Bush & Van Auken 2004; Bush 2006a; Bush 2006b). On more elevated secondary floodplains is the *Sporobolus airoides* (alkali sacaton) grassland with occasional *Limonium limbatum* (sea lavender) plants (Poole 1992; Van Auken & Bush 1998; Van Auken et al. 2007). Upslope soils become shallower and dryer and dominated by *Prosopis glandulosa* (mesquite) woodlands which give way to various *Larrea tridentata* (creosote bush) communities more typical of dry shallow soils of the Chihuahuan Desert (Powell 1988; Hart 2001).

Soil in the marsh study area belongs to the Balmorhea association, an alluvial gray to black silty clay-loam containing fine concretions of calcium carbonate and is moderately alkaline (pH 7.9 to 8.4, Rives 1980). These soils are deep (>152 cm) moderately saline and poorly drained often occupying lower elevations in and around spring fed marshes where they are usually anaerobic due to continuous saturation (Rives 1980; Lavelle & Spain 2001; Bush 2002; Grunstra 2003; Bush 2006b). Balmorhea soils are Mollisols and are classified as fine-silty, thermic Cumulic Haplaquolls (United States Department of Agriculture 2000). The types of soil and water chemistry, as well as the plant communities present are all indicators that the low elevation area at the Diamond Y Spring Preserve is a salt marsh (Chapman 1974; TPWD 2003). The two main springs, the Diamond Y Spring and Leon Spring, issue from a deep hole in Comanchean limestone (Brune 1981). Smaller unnamed springs found within the Diamond Y Spring Preserve also flow into the main drainage. The water from the springs flows through the study site and apparently continues underground into the Pecos River by way of Leon Creek.

*Sampling procedure.*—Soil was collected from a *D. spicata* community with a population of *Helianthus paradoxus* plants in the Diamond Y Spring Preserve. All aboveground biomass, both living and dead, was carefully removed prior to soil collection. A hand trowel was used to collect the surface area soil samples to a depth of one cm. The soil samples were located haphazardly within a *D. spicata* community. Soil surface samples from 1 cm<sup>2</sup>, 10 cm<sup>2</sup> (2 x 5 cm), 100 cm<sup>2</sup> (10 x 10 cm), 1,000 cm<sup>2</sup> (10 x 100 cm) and 2,000 cm<sup>2</sup> (20 x 100 cm) were collected and treated as a block ( $n = 5$  for each surface area sampled). All seed emergences from each complete set of soil samples (block) were pooled weekly to examine the time required to adequately sample the soil seed bank from the salt marsh. In addition, the number of seedlings emerging from each of the different size surface soil samples was noted and recorded separately. This information was used to evaluate the surface area

of the soil necessary to adequately sample the soil seed bank from the salt marsh.

To determine the depth of the soil seed bank, separate soil cores with a diameter of 20 cm (314 cm<sup>2</sup>) were collected to a depth of 45 cm. Each core was extracted and one cm soil horizons were sliced at depths corresponding to 0-1 cm, 5-6 cm, 10-11 cm, 20-21 cm, 30-31 cm and 40-41 cm. There were five replicates of each soil horizon. The remaining soil was returned to the excavated holes to minimize damage to the marsh. All samples for this phase of the study were collected on 11 and 12 April 1998. This time was chosen because it was after most, if not all, of the *H. paradoxus* seeds germinated for the current cohort of seedlings and before any current year seeds were produced. Thus, collecting the soil samples at this time would allow sampling the persistent seed bank, rather than the transient seed bank (Harper 1979).

Soil samples were placed in plastic bags, sealed and then placed in dark storage at 5°C for 90 days. This cold stratification time appeared to be sufficient for subsequent germination of fall maturing seeds (Baskin et al. 1998). After cold stratification, the soil samples were sieved using a 1 cm-wire screen to remove detritus and rhizomes and were evenly spread over a 2 cm deep layer of sharp sand in 23x33x6 cm deep trays (Haukos & Smith 1994). The trays were placed in a fiberglass greenhouse with a daytime temperature range of 26° to 38°C and light levels of 562 ± 135 μmol m<sup>-2</sup> s<sup>-1</sup> (Van Auken & Bush 1997). The soil was kept moist with deionized water during the study. The germinated seedlings, an estimate of the number of germinable buried seeds, were counted by species at weekly intervals from 20 July through 12 October 1998. Once a seedling was identified and counted it was removed from the tray. When seedling identification was difficult, the seedling was transferred to a pot and grown to maturity for positive identification.

Once the necessary time of incubation, surface area and depth were determined, 25 additional soil samples were collected from

another area within the *H. paradoxus* population in the *D. spicata* community. This sampling was done on 17 and 18 October 1999. To avoid any local patchiness that could occur (Bigwood & Inouye 1988; Brock et al. 1994), a series of smaller cores were taken. Each soil sample was an aggregate of 13 smaller cores of 10 cm diameter (Brock et al. 1994) that were aligned side by side in two rows measuring approximately 20 cm by 70 cm. The surface area sampled was 1,021 cm<sup>2</sup> for each of the samples collected. The soil cores were extracted to a depth of 6 cm for a total volume of 6,126 cm<sup>3</sup> for each soil sample. The soil samples were collected before the autumn dispersal of mature seeds of *H. paradoxus*. Soil samples were handled and stored as previously indicated. The germinated seedlings were counted by species initially at weekly then at biweekly intervals from 8 February through 12 July 1999, and then summed over the total time period. The total number of seeds germinated over the course of the incubation time for the experiment for each independent sample was used in the analysis.

*Analysis.*—In evaluating surface area needed for accurate sampling, seedling counts were normalized to 1 m<sup>2</sup> and log transformed ( $n + 1$ ) to attempt to stabilize the variances. Since the variances could not be stabilized for the surface measurements, Friedman's non-parametric *ANOVA*, SAS statistical software (SAS Institute 1991), was used with area (five levels: 1 cm<sup>2</sup>, 10 cm<sup>2</sup>, 100 cm<sup>2</sup>, 1,000 cm<sup>2</sup> and 2,000 cm<sup>2</sup>) as the main effect. Additionally, a one-way analysis of variance of seeds/m<sup>2</sup> for the 1,000 cm<sup>2</sup> and 2,000 cm<sup>2</sup> was performed.

To evaluate the depth needed to accurately determine the depth of the seed bank, a one-way *ANOVA* using the number of emergences and depth as the main effect was used (six levels: 1 cm, 5 cm, 10 cm, 20 cm, 30 cm and 40 cm). When significance was detected with the *ANOVA* it was followed by Bonferroni's multiple comparison *t*-test for pair wise comparisons among means (Milliken & Johnston 1992).

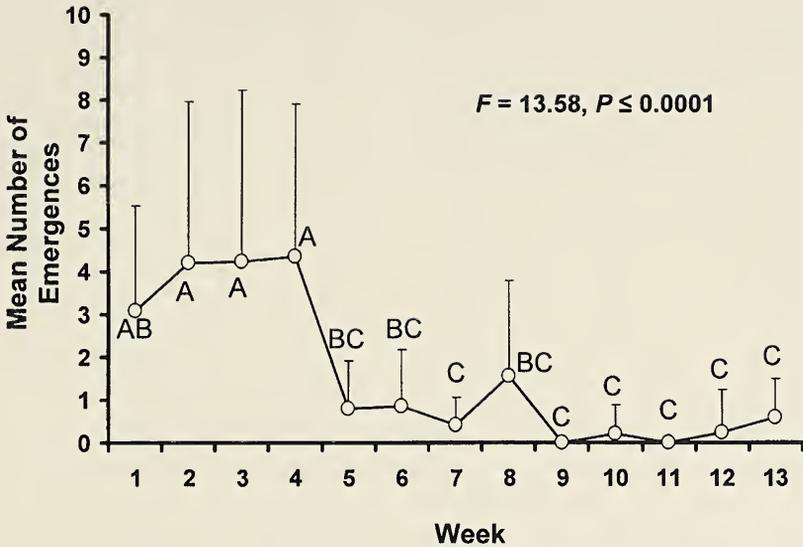


Figure 2. Mean number of newly emerged seedlings plus one standard deviation is plotted. The area of each sample was 3,111 cm<sup>2</sup> and the number of samples was five. One-way ANOVAs showed there were significant differences between the mean number of new seedling emergences ( $F$  values and  $P$  values are on the figure). Different letters indicate significant differences between means (Bonferroni's  $t$ -test).

In addition, the germinations per soil volume measurements were analyzed using the running mean number of germinations and variance of cumulative replicate samples ( $n = 2$  to 25). Further analysis of the data by the Bootstrap method (Kannan pers. corr.; Krebs 1999) using the mean number of germinations provided standard deviations and 95 percent confidence limits for the increasing replicate samples. Regression analyses were also conducted with the surface area, soil depth and sample adequacy data (Mason et al. 1989). Regressions included linear as well as several nonlinear transformations.

## RESULTS

In the temporal study there were significant differences in the mean number of seedlings emerging per week (ANOVA,  $F=13.58$ ,  $P \le 0.0001$ , Fig. 2). The mean number of seedlings emerging

Table 1. Mean number of seedling emergences and mean number of seedling emergences normalized to one square meter from each of the areas sampled with standard deviations during the 13 weeks of the study.

Area	Mean Number of Seedling Emergences	Mean(m <sup>2</sup> ) ± S.D. Number of Seedling Emergences
1 cm <sup>2</sup>	0.4	4,000 ± 8,944
10 cm <sup>2</sup>	1.4	1,400 ± 1,342
100 cm <sup>2</sup>	11.0	1,100 ± 1,030
1,000 cm <sup>2</sup>	19.8	198 ± 106
2,000 cm <sup>2</sup>	32.0	160 ± 30

increased to  $4.5 \pm 3.1$  per week at the end of 4 weeks and then declined to about  $0.5 \pm 1.1$  seedlings per week at the end of the experiment. The mean number of emergences per week for weeks 1-4 was significantly different from the number emerging in weeks 7, 9-13 (Bonferroni *t*-test). The weekly number of newly emerging seedlings for the 5 weeks between week 9 through week 13 were not significantly different from each other, and the temporal seedling germination experiment was discontinued. A total of 323 seedlings emerged during the temporal study of the soil samples.

The surface area study demonstrated large differences in the mean number of emerged seedlings per unit area (Table 1). These mean values were associated with large standard deviations. The variance could not be stabilized with various transformations. Consequently, standard parametric statistics could not be used. Further analysis using Friedman's non-parametric tests did not show significant differences between means in spite of overall large differences. When the three smallest size samples (1 cm<sup>2</sup>, 10 cm<sup>2</sup> and 100 cm<sup>2</sup>) are removed from the analysis because they appear to be statistical outliers (Milliken & Johnson, 1992) and a one-way ANOVA is performed between the emergences in the 1,000 cm<sup>2</sup> and 2,000 cm<sup>2</sup> area samples there is no significant difference between the two area sample values ( $F=1.63$ ,  $P=0.4794$ ). Thus, the 1,000 cm<sup>2</sup> surface soil sample should be adequate to sample the salt marsh community.

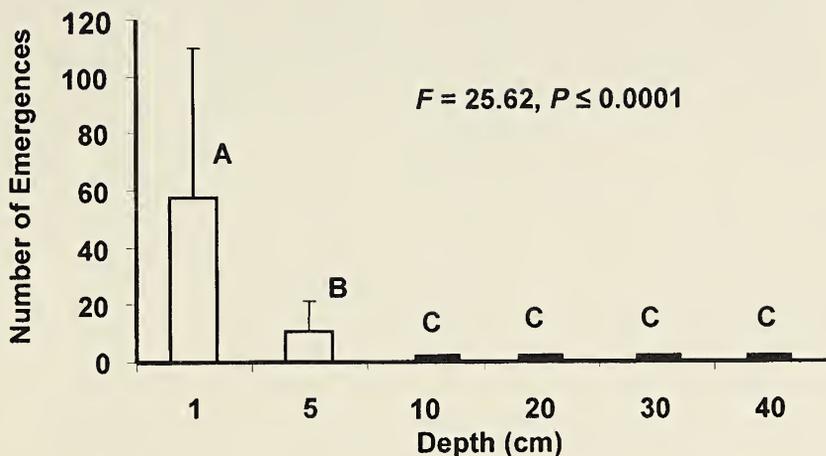


Figure 3. Mean number of seedling emergences at each soil depth sampled during the study. Horizon depths are for surface to 1cm (1), 5cm to 6cm (5), 10cm to 11cm (10), 20cm to 21cm (20), 30cm to 31cm (30), 40cm to 41cm (40). No seedlings emerged at horizons 10, 20, 30 or 40cm. Different letters indicated significant differences (*ANOVA*, Bonferroni's *t*-test) in mean seedling emergence between horizon depths. Lines above the bars represent one standard deviation.

A total of 340 seedlings emerged during the depth study. The greatest number of seedlings emerged from the surface samples (Fig. 3). No seedlings emerged from the 10-cm-deep horizon or below. There was a significant difference in the number of emergences from the different depths (one-way *ANOVA*,  $F=25.62$ ,  $P \leq 0.0001$ ). The mean number of seedlings in the surface sample was significantly different from the 5-cm-depth sample, and both were significantly different from all of the other samples (Bonferroni *t*-test). The distribution was best described by a second order quadratic regression, but it was not a strong relationship ( $y = 0.72x^2 - 12.98x + 57.4$ ,  $R^2 = 0.45$ ,  $P=0.0291$ ). The number of germinable seeds expected to be found between the surface and a depth of 6 cm is estimated at 99.4 % of all seeds in the seed bank with 0.6 % remaining between the 6 cm and 10 cm depth (calculated).

Table 2. Total number of seedlings that emerged and mean number of seedlings emergences standardized to 10,000 cm<sup>3</sup> (actual soil volume was 6126 cm<sup>3</sup>: 1021 cm<sup>2</sup> by 6 cm) ( $n = 25$ )  $\pm$  one standard deviation for each species found from a *Distichlis spicata* community after 23 weeks of incubation. Samples were collected on 17-18 Oct 1998; the emergence study ran from 1 Feb 1999 through 12 July 1999.

Species	Total Number of Seedling Emergences	Mean $\pm$ S.D. Number of Seedling Emergences
<i>Distichlis spicata</i>	2,211	144.3 $\pm$ 108.2
<i>Peganum harmala</i>	78	5.1 $\pm$ 6.7
<i>Helianthus paradoxus</i>	61	3.9 $\pm$ 3.9
<i>Sporobolus airoides</i>	9	0.7 $\pm$ 1.5
<i>Heliotropium curassavicum</i>	5	0.3 $\pm$ 0.8
<i>Prosopis glandulosa</i>	1	0.07 $\pm$ 0.3
Total	2,365	154.3 $\pm$ 121.5

Twenty-five independent soil samples were collected within the *D. spicata* salt marsh community. A total of 2,365 seedlings were counted during the study and six species were identified: *Distichlis spicata*, *Peganum harmala*, *Helianthus paradoxus*, *Sporobolus airoides*, *Heliotropium curassavicum* (seaside heliotrope) and *Prosopis glandulosa* (Table 2). The running mean and running variance were plotted (Fig. 4). The means of the number of seedlings emerging and the corresponding standard deviations were calculated and presented based on a sample size of two through 25. The mean and one standard deviation of the first two samples was 197.5  $\pm$  142. As the sample size increased both the mean and standard deviation decreased and at a sample size of 15 they were 92.4  $\pm$  85. From sample 15 to sample 25 the mean number of germinations changed by 1%. The standard deviation decreased by 29% at the same time. Results suggest that 15 or 16 soil samples yielded a reasonable estimate of the soil seed bank in the *D. spicata* community of the salt marsh.

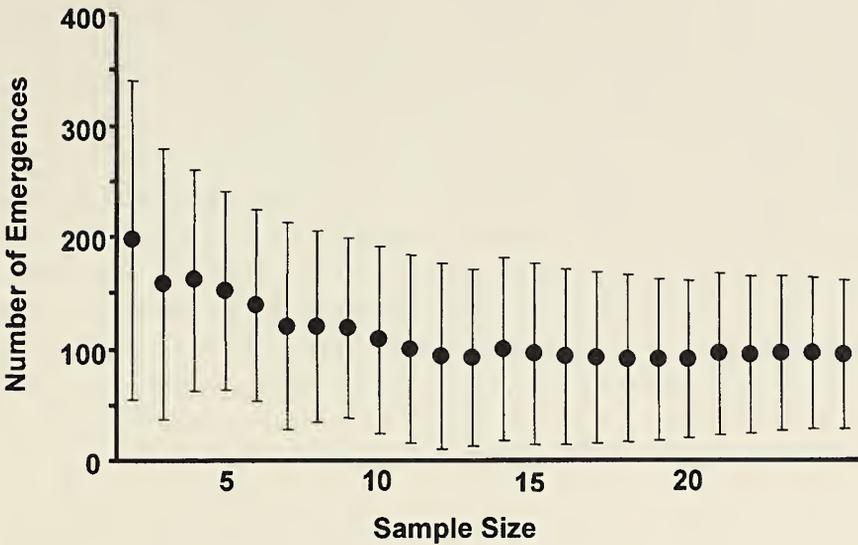


Figure 4. Running mean number of seedling emergences as a function of increasing number of samples. There was no significant difference between the number of emergences (*ANOVA*,  $F = 0.66$ ,  $P = 0.8806$ ). Means and standard deviations appear to stabilize after sample 15. Bars represent  $\pm$  one standard deviation.

## DISCUSSION

Difficulties associated with adequately estimating the soil seed bank reported here have been demonstrated by others (see Baskin & Baskin 1998; and Fenner & Thompson 2005). Factors including incubation time, surface area, depth to sample, as well as the number of samples required must be addressed, probably in each study, if a reasonable estimate of the soil seed bank is to be found. Consequently, care must be taken when examining seed bank estimates that have been reported and in conceptualizing and performing new studies.

Two techniques are available to determine seed bank densities including direct counting and seedling emergence (Kropac 1966; Brock et al. 1994). Direct counting uses flotation or sieving techniques to determine the number of seeds present, but gives no information about the species present or whether the seeds are

viable and is time and labor intensive. In contrast, the seedling emergence method solves two major problems that are inherent with direct counts. It eliminates the need to determine if the seeds are viable and if the seeds would live until the time of the normal germination season in the habitat sampled (Baskin & Baskin 1998; Fenner & Thompson 2005). The seedling emergence method provides an estimate of viable seeds in the soil based on emergence of seeds maintained under conditions favorable to germination. However, since conditions required for all species may not be met, emergence may be underestimated (Leck et al. 1989). Despite this limitation, it has been shown that the seedling emergence method is suitable for most studies (Poiani & Johnson 1988; Brock et al. 1994; Haukos & Smith 1994).

In addition to the above, many seeds dispersed in autumn lose viability in the soil during winter (Forcella 1992). These seeds are part of the transient seed bank, not the persistent seed bank. Consequently, the timing of soil collection is critical if the persistent seed bank is to be correctly predicted. It has been estimated that 54% of 171 seed bank studies may have contained a mixture of both transient and persistent seed or only transient seed (Baskin & Baskin 1998; Fenner & Thompson 2005). In some of these studies, soil samples were collected after the species had dispersed their seeds, but before the first germination season was complete (Dessaint et al. 1991; Warr et al. 1994). Therefore, the samples most likely contained seeds of the transient bank, and failed to correctly estimate the persistent seed bank. Depending on the habitat and species, there may be a definite germination season, one that maximizes a seedling's chance of survival. Most of the species found in the present study seems to have a definite germination season that coincides with or is a direct result of low winter temperatures and the high water table at that time of year (Bush & Van Auken 1997). Seed dispersal for *H. paradoxus* occurs during late October and early November (Bush & Van Auken 1997). Thus if the persistent soil seed bank of *Helianthus paradoxus* is to be accurately estimated, sampling of the soil should

occur after the germination season and before dispersal of *H. paradoxus* seeds. The present study used soil samples collected in April or early October after the current germination season occurred and before *H. paradoxus* dispersed its seeds for the next growing season. However, the number of viable seeds estimated to be in the seed bank may be overestimated because of possible inclusion of part of the transient seed banks for certain species. For example, *S. airoides* and *D. spicata* seeds probably germinate in late spring and early summer and then flower and set seed in late summer and early fall (Correll & Johnston 1979) prior to or during the time the October soil samples were collected in the present study. This difficulty could not be avoided because of the asynchronous flowering and seed set of the different species, i.e. flowering and seed dispersal in late summer and early fall for *S. airoides* and *D. spicata* and late fall for *H. paradoxus*.

An additional difficulty was to estimate the length of the required soil incubation time. Cold stratification was included as suggested for soil samples with fall maturing seeds (Gross 1990; Baskin & Baskin 1998). In the temporal study, it was shown that this experiment could be stopped when the number of new seedling emergences fell to a low level and remained at a low level. There were no significant differences among weeks for the last 9 weeks of the study (Fig. 2). The experiment was terminated after 13 weeks. An additional temporal study was carried out for 23 weeks with no significant differences in germinations between the third and 23<sup>rd</sup> week (Coteff 2000). These time periods are within the range of 12 to 28 weeks used by others (Coffin & Lauenroth 1989; Kinucan & Smeins 1992; Brock et al. 1994; Haukos & Smith 1994; Hanlon & Williams 1998).

The surface area to sample in any soil seed bank study is difficult to estimate because of the variance between samples. Others report large standard deviations (see Baskin & Baskin 1998; Fenner & Thompson 2005). The reason for this is the dispersal of seeds in any given habitat is not even, but clustered around the

parents that are irregularly distributed (Harper 1979; Thompson 1986; Bigwood & Inouye 1988; Chauvel et al. 1989, Matlack & Good 1990; Dessaint et al. 1991). In addition, the location of annual plants can change from year to year. New species-area curves for an Australian pasture and wetland showed that new species did not increase significantly after 800-1,000 cm<sup>2</sup> was sampled (Forcella 1984; Brock et al. 1994) which is what was found in the *D. spicata* community at the Diamond Y Spring Preserve (Coteff 2000). In addition, as the area sampled increased the number of seedlings per unit area decreased. The smaller areas sampled all had high variance attributed to the clumped distribution of the parent plants and thus typical parametric procedures could not be used. Furthermore, non-parametric procedures did not detect significant differences. Variance was reduced dramatically in the 1,000 and 2,000 cm<sup>2</sup> areas and met the conditions for analysis of variation. Since there was no significant difference between the 1,000 cm<sup>2</sup> and 2,000 cm<sup>2</sup> area, the smaller sample (1,000 cm<sup>2</sup>) could be used without jeopardizing the validity of the estimates.

In the present study, viable seeds of all species had a pronounced vertical distribution. Eighty-four percent of the emerging seedlings were found at the soil surface. The top 6 cm of soil contained an estimated 99.4 % of the total viable seeds in the soil seed bank. At the 10 cm depth the number of emerged seedlings decreased to zero and remained at zero down to the 40 cm sampling depth. Sampling the soil to a depth of 6 cm would capture 99.4 % of the soil seed bank of the Diamond Y Spring Preserve. The vertical structure of the seed bank is similar to that found in many types of ecosystems including two temporary marshes with a seed bank depth of 2 cm (Bonis & Lepart 1994), a shoreline seed bank with a 5 cm deep seed bank (Nicholson & Keddy 1983), and two studies of nontidal salt marshes with 4 and 10 cm deep seed banks (Ungar & Riehl 1980; Kadlec & Smith 1984,).

Previous soil seed bank studies collected a wide variety of samples, but few considered the adequacy of the sample (Brock et

al. 1994). Studies of salt marsh communities in South Wales and Ohio were based on 20 soil samples each (Badger & Ungar 1994; Ungar & Woodell 1996), while one in the Carolinas Bays used six samples from each area investigated (Poiani & Dixon 1995). The soil seed bank from floodplain swamps or marshes was estimated from five or 10 samples collected at each sites (Titus 1991; Bonis & Lepart 1994). In the few studies that did determine the adequacy of the number of samples needed, the number seemed to depend on the habitat investigated. For example, in a cultivated field in Ontario, 60 samples were required to adequately quantify the seed bank for *Chenopodium* spp. (Benoit et al. 1989). In Michigan, 15 - 20 samples were required to determine the number of species in the soil seed bank (Gross 1990). In the present study, the sample mean could only be reduced by an additional 1% if the sample size was increased from 15 to 25. Standard deviations could be reduced by an additional 29% at the same time, but because the standard deviation was so high no significant differences were found when the data was analyzed by one-way *ANOVA*.

These above mentioned difficulties unfortunately are common in soil seed bank analyses. Based upon these analyses, to adequately sample the soil seed bank of the dominant *D. spicata* communities found within the Diamond Y Spring Preserve, it was necessary to collect a minimum of 15 soil cores, each with a surface area of 0.1 m<sup>2</sup> and a depth of 6 cm. These samples would have to be incubated for 5-13 weeks. However, using the 15 samples shown to be adequate to estimate the soil seed bank, rare species may not be adequately represented. The soil seed bank at the Diamond Y Spring Preserve was very limited in the number of species present and is thus similar in terms of simplicity to the above ground community (Van Auken & Bush 1998).

This investigation demonstrated that there is a viable soil seed bank containing the threatened *H. paradoxus* and other species found in the Diamond Y Spring Preserve. This soil seed bank may be very critical to the survival of *H. paradoxus* and the other

species especially during extended periods of environmental stress. However, it is not clear that the seed bank is the only factor that is important to the overall survival of *H. paradoxus* and the other species present in this marsh habitat.

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## GENERAL NOTE

HELMINTH PARASITES (TREMATODA, NEMATODA)  
OF THE WESTERN SLIMY SALAMANDER,  
*PLETHODON ALBAGULA* (CAUDATA: PLETHODONTIDAE),  
FROM CENTRAL TEXAS**Chris T. McAllister***Department of Physical & Life Sciences, Chadron State College  
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The western slimy salamander, *Plethodon albagula*, is one of at least 13 species in the *P. glutinosus* complex (see Highton 1989). This medium to large salamander ranges from central Missouri southwestward through the Interior Highlands of Arkansas, with disjunct populations in eastern Texas (Upshur and Walker counties) and several counties of the Edwards Plateau (Conant & Collins 1998; Dixon 2000; LaDuc & Infante 2001; Trauth et al. 2004; Hibbitts 2006).

Although information is available on parasites of this salamander from Arkansas (Winter et al. 1986; McAllister et al. 1993; Upton et al. 1993), nothing, to the author's knowledge, has been published on helminth parasites of *P. albagula* from Texas. Herein is presented data on some helminth parasites of a small sample of *P. albagula* from near the westernmost extreme of its range.

On 6 March 2004, 12 juvenile and adult salamanders (six males, six females, mean  $\pm$  1 SD snout-vent length [SVL] =  $52.3 \pm 15.0$ , range 27-70 mm) were collected by hand in Bandera County, 10.1 km (6.3 mi) N Vanderpool, off FM 187, vicinity of Lost Maples State Natural Area (29° 45.3'N, 99° 33.5'W, elevation = 495.6 m). Salamanders were euthanized with a concentrated Chloretone (chlorobutanol) solution, fixed in 10% neutral buffered formalin for 48 hr, and later transferred to 70% ethanol until necropsy. The integument and underlying tissues were examined for chiggers and the entire gastrointestinal tract (including the liver and gall blad-

der), coelomic cavity, kidneys, urinary bladder, and reproductive organs were examined for endoparasites. Trematodes were stained with acetocarmine and mounted in Canada balsam. Nematodes were placed in a drop of glycerol on microscope slides and identifications were made from these temporary mounts.

Helminth voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, as follows: *Brachycoelium salamandrae* (HWML 48201), *Batracholandros magnavulvaris* (USNPC 48202). Host voucher specimens were deposited in the Angelo State Natural History Collection (ASNHC 14120-14131), San Angelo, Texas.

Five of 12 (42%) *P. albagula* harbored parasites, including four (33%, two males, two females,  $64.5 \pm 7.2$ , 54-70 mm SVL) with six (mean intensity =  $1.5 \pm 1.0$ , range 1-3) *Brachycoelium salamandrae* in the small intestine; a single salamander (8%, female, SVL = 54 mm) harbored three *Batracholandros magnavulvaris* in the rectum. None of the salamanders were infested with chiggers or harbored cestodes. A summary of all known parasites of *P. albagula* is provided in Table 1.

The plagiorchid trematode, *B. salamandrae*, is a common endoparasite of amphibians and some reptiles from various sites in Europe, Brazil, Mexico, Canada, and the United States. This parasite has been previously reported in members of the *P. glutinosus* complex from across the eastern U.S. (see Rabalais 1970; Dyer & Brandon 1973; Brooks 1979; and others). In Texas, *B. salamandrae* (variously reported as the synonyms *B. daviesi*, *B. hospitale*, and *B. meridionale*) has been reported from green treefrogs (*Hyla cinerea*), western chorus frogs (*Pseudacris triseriata*), southern leopard frogs (*Rana sphenocephala utricularia*), smallmouth salamanders (*Ambystoma texanum*), Texas black-spotted newts (*Notophthalmus meridionalis meridionalis*), ground skinks (*Scincella lateralis*), and brown snakes (*Storeria dekayi*) (Harwood 1932). This is the first time this helminth has been reported from *P. albagula*.

Table 1. Summary of parasites reported from *P. albagula* in Arkansas and Texas.

Parasite	Locality	Prevalence <sup>1</sup>	Reference
Protista			
<i>Cytamoeba baptifera</i>	Arkansas	3/37 (8%)	McAllister et al. (1993)
<i>Cepedietta michiganensis</i>	Arkansas	1/5 (20%)	Winter et al. (1986)
	Arkansas	2/37 (5%)	McAllister et al. (1993)
Unknown isosporan <sup>2</sup>	Arkansas	4/37 (11%)	McAllister et al. (1993)
<i>Isospora hightoni</i>	Arkansas	8/46 (17%)	Upton et al. (1993)
Trematoda			
<i>Brachycoelium salamandrae</i>	Texas	4/12 (33%)	This report
Cestoidea			
<i>Cylindrotaenia americana</i>	Arkansas	10/37 (27%)	McAllister et al. (1993)
Nematoda			
Unknown oxyuroids <sup>3</sup>	Arkansas	1/5 (20%)	Winter et al. (1986)
<i>Batracholandros magnavulvaris</i>	Texas	1/12 (8%)	This report
<i>B. salamandrae</i>	Arkansas	4/37 (11%)	McAllister et al. (1993)
Acanthocephala			
unknown cystacanth	Arkansas	1/37 (3%)	McAllister et al. (1993)

<sup>1</sup> Prevalence = number infected/number examined (percent).

<sup>2</sup> Subsequently described as *Isospora hightoni* (see Upton et al. 1993).

<sup>3</sup> Most likely *Batracholandros salamandrae*.

The oxyurid nematode, *B. magnavulvaris* is a ubiquitous parasite of numerous salamanders (Joy & Tucker 2001). Their host list, along with more recent surveys include other members of the genus *Plethodon*, namely the Caddo salamander (*P. caddoensis*), Fourche Mountain salamander (*P. fourchensis*), Rich Mountain salamander (*P. ouachitae*), Sequoyah slimy salamander (*P. sequoyah*), and southern redback salamander (*P. serratus*), and Ouachita dusky salamander (*Desmognathus brimleyorum*) from the surrounding states of Arkansas and Oklahoma (Winter et al. 1986; McAllister et al. 1995; 2002; McAllister & Bursey 2004). Interestingly, McAllister et al. (1993) reported the related species, *B. salamandrae* from *P. albagula* from Arkansas. The discovery of *B. magnavulvaris* in *P. albagula* represents a new host and locality for this nematode parasite.

In summary, two new host and a geographic record is documented for helminth parasites of *P. albagula*. Future surveys

on *P. albagula* from Texas should include a more complete examination of host components, including blood, feces, and gall bladder contents for protozoan parasites.

#### ACKNOWLEDGMENTS

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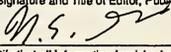
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