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MYCOSPORINE-LIKE AMINO ACIDS AND ANTIOXIDANT CAPACITY IN GRACILARIA DOMINGENSIS: CHEMICAL INVESTIGATION PROVIDING VALUABLE INSIGHTS

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Abstract: The Chihuahua Desert is the largest hot desert (BWh) in North America. Orthopterans are an integral part of desert ecosystems. They include grasshoppers, katydids and crickets. A large section of the Northern Chihuahua Desert is in Luna County, New Mexico. There is a dearth of information on the Orthopterans in this area. Between May and October of 2020, sixty adult grasshoppers, two katydids and one camel cricket were captured from a 5-hectare (ha) area at base of the Florida Mountains, which is the extreme southern portion of Luna County. Luna County was in a severe drought during 2020. The insects were identified using several taxonomic keys (Cigliano, Braun, Eades & Otte, 2018; Guala & Doring, 2019; Triplehorn & Johnson, 2005; Richman, Lightfoot, Sutherland & Fergurson, 1993, Otte, 1984, 1981; Tinkham, 1944). A previous New Mexico State University (NMSU) survey from 1993 had only documented grasshoppers in the Acrididae and Romaleidae families. The objective of this continuing study is to identify and document all species of Orthopterans found in Luna County, and correlate the populations with changing weather patterns. In this portion of the study, the majority of Orthopterans captured were *Leprus wheeleri* (Thomas), a previously documented specie. However, seven undocumented species were also captured. Given the ecological importance of these insects in the Chihuahua Desert, this research is crucial because of the scarcity of information on the Orthopterans in Luna County.

Keywords: Chihuahua Desert, *Aulocara elliotti* (Thomas), *Ateloplus hesperus* (Heband), *Ceuthophilus maculatus* (Harris), *Hesperotettix speciosus* (Scudder), *Insara covilleae* (Rehn & Hebard), *Leprus wheeleri* (Thomas) *Schistocerca alutacea albolineata* (Harris), and *Trimerotropis californica* (Bruner).

INTRODUCTION

Ten percent of the Chihuahua Desert is in New Mexico (National Park Service, 2018). Luna County, located entirely in the Chihuahua Desert, is rich in wild, arid dwelling florae and faunae including Orthopterans. Orthopterans include grasshoppers, katydids, and crickets. They are essential members of the faunae in an arid ecosystem (Richman, Lightfoot, Sutherland & Ferguson, 1993). Orthopteran specimens were collected from a 5-ha area (~32.17°N,

107.63°W) at an elevation of ~1,400m from the bajada on the north side of the Florida Mountains, approximately 25 kilometers (km) to the southeast of the city of Deming. The Florida Mountains are an inactive fault-block mountain range comprised predominately of Paleozoic limestone and dolomite rocks that extends north-south for approximately 38.6 km (Clemons, 1998). The range is surrounded by an extensive bajada formed from ephemeral streams transporting sediments during occasional rainfall events (Rech, 2021). The study area receives an average of 23.37 centimeters per year precipitation, predominately during winter.

The average high temperature is 35.5 ℃, and an average low temperature is 18.3 ℃ (US Weather Service, 2021). Soils across the alluvial fan surfaces are well developed and contain petrocalcic soil horizons that are sometimes exposed on the surface. In the drainages, however, most alluvial fill terraces are non-calcareous (Rech, 2021). The florae include a high percentage of creosote bushes, *Larrea tridentate* (Coville), interspaced with Joshua trees, *Yucca brevifolia* (Engelm), a few leguminous mesquite trees, *Prosopis glandulosa,* wild grasses, and several species of the genus *Opuntia*, prickly pear cacti (Dodson, 2012). The presence of leguminous mesquite trees indicates the presence of wild rhizobia, which fix atmospheric nitrogen (Salisbury and Ross, 1992).

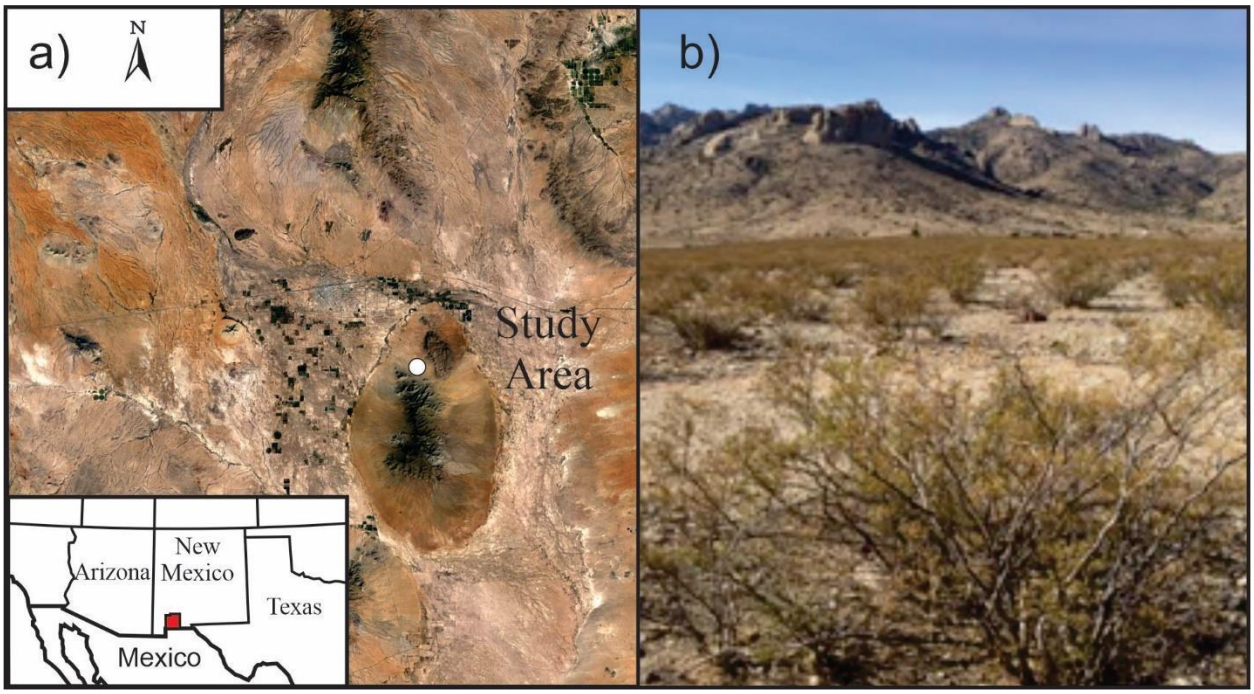

Figure 1. (a) Downloaded satellite image from Google Earth, (b) photograph of the study area.

Creosote bushes are now a major component of the northern Chihuahua Desert comprising of approximately 95% of the vegetation, and are an important part of the changing ecosystem (Lightfoot & Whitford, 1989). These bushes have been replacing the grasslands of the northern Chihuahua Desert for the last 100-150 years (He, D'Odorico & DeWekker, 2015).

The change in vegetation has altered the faunae inhabiting the area. Creosote bushes consist of autopolyploid races. Diploid bushes only exist in the Chihuahua Desert. Tetraploid populations occur in the Sonoran Desert, and hexaploid populations exist in the Mojave Desert (Laport, Minckley & Ramsey, 2012). Creosote bushes have high concentrations of foliar nitrogen and foliar resin. Orthopterans require a diet high in nitrogen (Wiesenborn, 2011). While foliar nitrogen is advantageous to phytophagous insects, foliar resin is not

(Lightfoot & Whitford, 1989). Resin is high in nordihydroguaiaretic acid (NDGA) which serves as a defense mechanism against insects (Rhoades, 1977). Orthopterans tend to inhabit bushes whose leaves have low concentrations of NDGA (Alcock, 1989). The leaves of the creosote bushes have low moisture content as well as other anti-feeding phytochemicals besides NDGA (Plagens, 2008). Soil concentrations of nitrogen are highest when located under creosote bushes (Lightfoot & Whitford, 1989).

Orthopterans are an indispensable part of desert ecosystems. They recycle nutrients by increasing the degradation of cellulose and breaking down plant material into smaller pieces which can be attacked by soil florae and faunae (Capinera, Scott & Walker, 2004). They also stimulate plant growth by combining acids from their crop and midgut with auxin, a plant hormone (Latchininsky, Sword, Sergeev, Cigliano & Lecoq, 2011). Orthopterans have also been shown to increase translocation of nutrients, facilitate the leaching of foliar nutrients in chewed leaves and to stimulate nitrification (Schowalter, 1981). Plus, Orthopterans, mainly grasshoppers, serve as nutrition for many species including humans (Capinera, Scott & Walker, 2004). Orthopterans of New Mexico are under researched; however, in 1993 New Mexico State University (NMSU) did survey grasshoppers in the Acrididae and Romaleidae families in the state, and documented 160 species (Richman, Lightfoot, Sutherland, & Ferguson, 1993). *Leprus wheeleri* (Thomas) was the only specie captured in Luna County for both studies. The seven additional undocumented species captured are: *Aulocara elliotti* (Thomas), *Ateloplus hesperus* (Hebard), *Insara covilleae (*Rehn & Hebard), *Hesperotettix speciosus* (Scudder), *Ceuthopolus maculatus* (Harris), *Schistocerca alutacea albolineata* (Harris), and *Trimerotropis californica* (Bruner).

Five of the Orthopteran species identified belong to the Acrididae family, which includes true grasshoppers and locust. They are commonly called "short-horned" grasshoppers due to the short antenna (Capinera, Scott, & Walker, 2004). Two specimens are in the Tettigoniidae family, which are katydids. And one specimen belongs to the Rhaphidophoridae, the cave or camel crickets (Triplehorn & Johnson, 2005). There is a scarcity of information, other than taxonomic keys, on these Orthopterans. Most of the published research found focused on three species, *L. wheeleri*, *I. covilleae* and *A. elliotti*.

The five Acrididae species are *A. elliotti, H. speciosus, L. wheeleri, S. alutacea albolineata,* and *T. california*. *Leprus wheeleri* (Thomas), commonly known as the "blue-winged grasshopper" inhabits the central and northern regions of the Chihuahua Desert. It is sometimes confused with *Leprus intermedius* (Saussure). However, *L. wheeleri* and *L. intermedius* are distinguished by the base *L.wheeleri's* hind wing being greenish-yellow and the hind tibia being pale blue or grayish (Capinera, Scott & Walker, 2004). Also, *L. wheeleri* has a yellow-wing dimorphism that exists in the Chihuahua Desert. Possible reasons for the yellow-wing dimorphism in *L. wheeleri* are thermoregulation and homochromy. Blue pigmentation absorbs more energy than yellow pigmentation. In a desert it is logical that absorption of less energy would be beneficial. Likewise, yellow coloration blends with the yellow-tan desert soil color (Valverde & Schielzeth, 2015).

Aulocara elliotti (Thomas), the big-headed grasshopper, mainly inhabits mid-western arid grasslands. The species only feeds grasses. Its distribution range is from Lower Canada to Mexico (Capinera, Scott & Walker, 2004). *A. elliotti* has not been previously documented in Luna County, but specimens have been captured in other parts of the state (Richman, Lightfoot, Sutherland & Ferguson, 1993). Populations can be abundant at times, but varies depending primarily on climate changes. Population density has been decreasing since the mid-twentieth century. It is hypothesized that decreasing moisture and vegetation are the causes for the drop in population density (Pfadt, 1949).

Schistocerca alutacea albolineata (Harris), the white-lined bird grasshopper, lives mostly in mountains, and usually feeds on plants other than grasses. Distribution is from the east coast to the mid-west and down into Mexico. In New Mexico, it has only been documented in the eastern part of the state (Richman, Lightfoot, Sutherland, & Ferguson, 1993).

Hesperotettix speciosus (Scudder) is commonly called the western grasshopper. In New Mexico it was previously documented in the northeastern part of the state (Richman, Lightfoot, Sutherland, & Ferguson, 1993). It is generally found in the Great Plains, and feeds on broadleaf plants. The grasshopper is identified by its green coloration with slight red and purple markings (Capinera, Scott, & Walker, 2004).

Trimerotropis californica (Bruner), the California band winged grasshopper, is quite easy to identify due to the large bands on the forewings, red or orange hind tibia, plus a tooth like structure on the pronotum. They inhabit rocky, arid areas and feed on grasses. *T. californica* can found throughout the southwest (Capinera, Scott, & Walker, 2004). It has previously been documented in several eastern and northern counties in New Mexico, but not in Luna County (Richman, Lightfoot, Sutherland, & Ferguson, 1993).

Ensifera, a suborder of Orthoptera, includes both Tettigoniidae and Rhaphidophoridae families among others. Tettigoniidae is the family of katydids and Rhaphidophoridae comprises camel crickets. Katydids tend to be strikingly beautiful and prone to singing. Camel crickets are usually nocturnal, inhabit dark- moist places, and are easily identified by their humpback (Capinera, Scott, & Walker, 2004). *Insara covilleae* and *Atetoplus hesperus* are katydids in the Tettigoniidae family. *Ceuthophilus maculatus* is in the Rhaphidophoridae family.

Insara covilleae, the creosote bush katydid, evolved via adaptive radiation about 11,000 years ago from *Insara elegans*. Evolution was prompted by the introduction of the creosote bush from Argentina (Plagens, 2008). The katydid is perfectly camouflaged in the creosote bush due to "top-down" pressures. It selects bushes with highest water and protein content, regardless of its resin levels (Schultz & Floyd, 1999).

Ateloplus hesperus, commonly known as the shield back katydid, was identified in 1934 and is the smallest katydid in the genus. Very little information was found on the biology of this insect. The host plant is Sage *Artemisia tridentate*, which is used as an ornamental plant in many arid locations. This katydid tends to be nocturnal. Stridulating can be heard at night as a soft buzzing (Tinkham, 1944).

Ceuthophilus maculatus, is an easily identified as a camel cricket due to its humpback appearance. There are 150 species of camel crickets in North America. Common features are long antennae that touch or nearly touch at the base, the tarsi are laterally compressed and the femora extend beyond the abdomen (Capinera, Scott, & Walker, 2004). They inhabit dark, damp places such as caves, hollow trees, and under logs or stones. Most are brownish in color.

They are distributed throughout North America (Triplehorn & Johnson, 2005).

METHODS

Daily specimen collection sweeps were made between 10:00 am and 2:00 pm in a 5-ha area (~32.17°N, 107.63°W) the base of the Florida Mountain during the six-month period between May and October 2020. Volunteers were equipped with insect nets and centrifuge tubes. Upon capture, the insects were placed in 50 mL centrifuge tubes. The tubes were identified with the date, location, and specimen number. Specimens were then frozen for further testing. There were no safety measures in place other than to watch out for rattlesnakes.

RESULTS

Sixty grasshoppers, two katydids and one camel cricket were caught during the sixmonth period in 2020 between May and October. *Leprus wheeleri* were the most abundant specie, comprising 83% of the insects captured. Only a minimal number of specimens were captured from the other species.

Table 1. Number of specimens caught from each specie.

Species	Number of Specimens Caught
Leprus wheeleri	52
Hesperotettix specious	4
Aulocara elliotti	1
Insara covilleae	1
Trimerotropis californica	2
Ceuthophilus maculatus	1
Schistocerca alutacea albolineata	1
Ateloplus hesperus	1

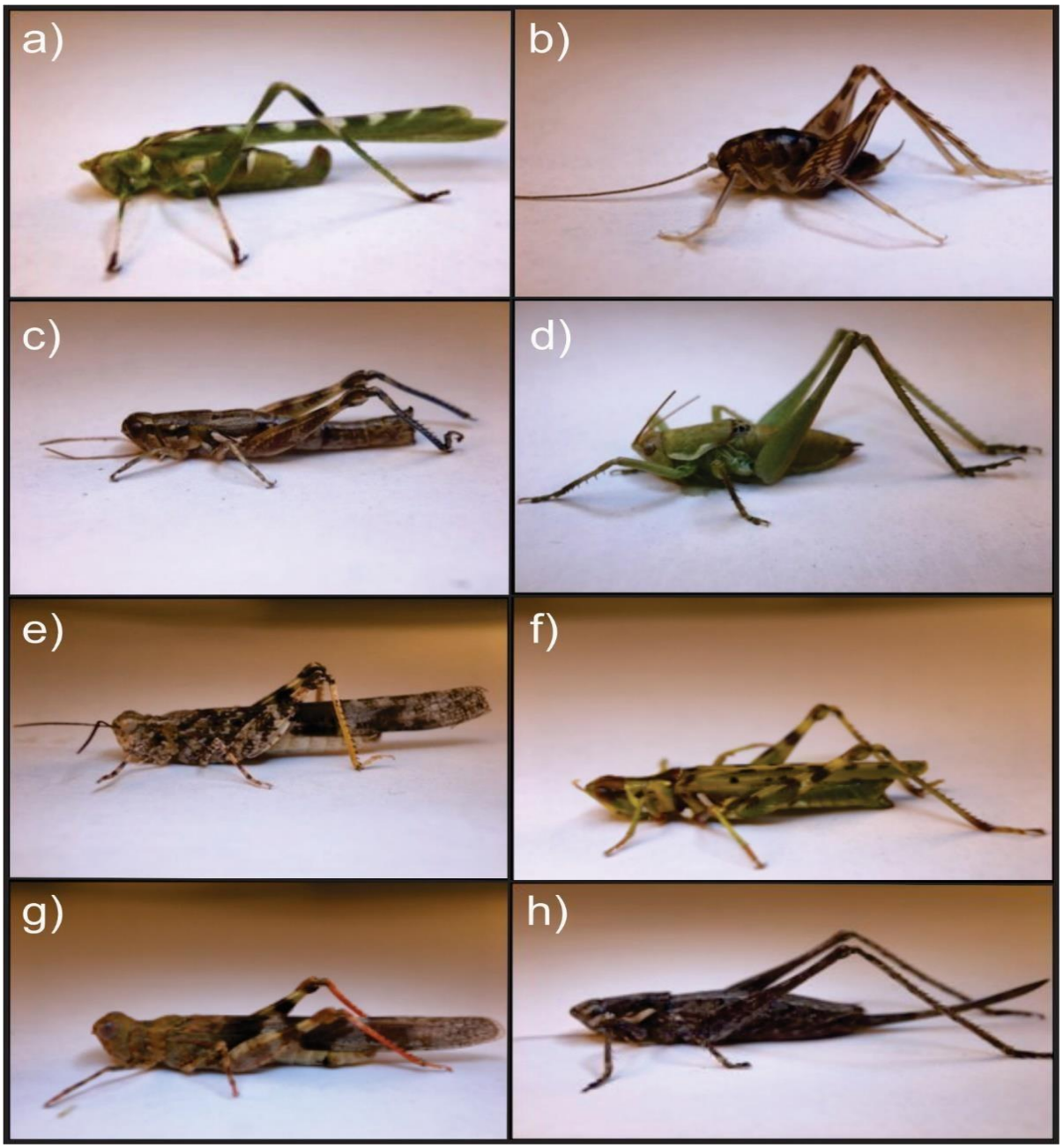

Figure 2. (a) *Insara covilleae*, (b) *Ceuthophilus maculatus*, (c) *Aulocara elliotti*, (d) *Hesperotettix speciosus*, (e) *Leprus wheeleri*, (f) *Schiatocerca alutacea albolineata*, (g) *Trimerotropis californica*, (h) *Ateloplus hesperus.* Photographs by Brianda Alirez.

DISCUSSION

The results of this study differ significantly from the 1993 survey by NMSU, and for that there are several potential reasons. They are as follows; (1) the differing scopes of the studies (2) climate change, and (3) natural adaptations and seasonal fluctuations in populations.

The areas of the studies are notably different. This report reflects the first part of a fiveyear study, and only covered five hectares of a drought ridden area at the base of the Florida Mountains. The study included the identification and documentation of Orthopteran species, but also approximated the density of the populations. The NMSU survey covered the each county in the state, but the study did not state where the

collection sites were, how many collections sweeps were made, how often the sweeps were made, or the number of specimens captured for each specie. For this study, collection sweeps were made daily for six months between 10:00 am and 2:00 pm. It is appropriate to compare the two studies because the goals of both studies were to document all or some Orthopterans. However, there is insufficient information to completely validate the differences.

Climate change is the most probable reason for the differences in the Orthopteran species captured in Luna County. In 1993, the National Oceanic and Atmospheric Administration (NOAA) only had one weather station in Luna County, which was near Columbus. Columbus is 49.89 km from our study site. The precipitation at that site in 1993 was 18.44 cm (NOAA, 2021). In 2020, NOAA had three weather stations, all in the same proximity, with an average precipitation of 6.97 cm (NOAA, 2021). That is a 62% decrease in precipitation, which would have a major impact on the florae and faunae in Luna County.

Populations of Orthopterans are known to fluctuate naturally regardless of seasonal changes and precipitation. However, a 62% decrease in precipitation will have a major impact on any population, including Orthoptera. Numerous species cannot adapt to such a drastic change. Many florae, especially the wild grasses, have died in the Chihuahua Desert due to the decrease in precipitation. A decrease in desert florae naturally leads to a decrease in Orthopterans, particularly grasshoppers. *Leprus wheeleri* is well adapted to blending in with the desertous soil and gravel in the 2020 study area. The grasshopper is perfectly camouflaged. *Leprus wheeleri* (Table 1) was the most abundant specie captured, comprising of 83% of the specimens. It was the only specie captured by both studies in Luna County. As mentioned earlier, *Leprus wheeleri* blends in so perfectly with the desert soil and gravel that it is almost impossible to see unless it moves. The visible parts of the grasshoppers when stationary are mottled tan and black. When flying, *Leprus wheeleris'* yellow wings are visible, but also blend in with the desert soils. All of the specimens captured in this study had yellow wings. Besides blending in with the habitat, the yellow wings absorb less energy than the blue wing dimorphism. The NMSU study did not mention if the specimens captured exhibited the blue or yellow dimorphism, however, the photograph in their report showed a yellow-winged *Leprus wheeleri.*

The majority of Orthopteran species captured were a multi-colored mixture of tan, brown and black to blend with the habitat (Figures 2b,2c,2e,2g,2h). All of those specimens were captured on the soil. They were not observed feeding. The two katydid species, *Insara covilleae* and *Schiatocerca alutacea albolineata* (Figures 2a, 2f), were captured while feeding on creosote bushes, which are mottled brownish green allowing the grasshopper protection from predators. *Hesperotettix specious* was captured while feeding on a honey mesquite tree, which has bright green foliage almost the same color as the grasshopper (Figure 2d). Though there is overlap between the two studies, they are different. The NMSU study intended to document Orthopterans only in the Acrididae and Romaleidae families in each New Mexican County. The 2020 study documented all Orthopterans found in the study site. Due to the sparsity of information on Orthopterans in this county, our study will continue for the next five years. The area of the study site will be increased 10-fold each year. Precipitation levels will be recorded along with the identification of the Orthopterans, plus their population densities.

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