ARTICLE IN PRESS

Rangeland Ecology & Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Rangeland Ecology & Management

journal homepage: http://www.elsevier.com/locate/rama

Original Research

Evaluation of Plasma Cholinesterase Activity in Native Birds from Pesticide-Exposed Agricultural Lands☆

Irene Ruvalcaba-Ortega [a], Mario Bermúdez de León [b], Sandra Mendiola-Castillo [a], Laura González-Escalante [a,b], Ricardo Canales-del-Castillo [a], Roberto Mercado-Hernández [a], Antonio Guzmán-Velasco [a], José Ignacio González-Rojas [a,*]

[a] Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, 66455 San Nicolás de los Garza, Nuevo León, Mexico
[b] Departmento de Biología Molecular, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, 64720 Monterrey, Nuevo León, Mexico

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 10 March 2017
Accepted 13 March 2017
Available online xxxx

Key Words:
agricultural lands
Grassland Priority Conservation Area
native birds
pesticides
plasma cholinesterase activity

ABSTRACT

Wildlife near agricultural lands is exposed to pesticides, particularly organophosphorus and carbamates, where birds appear to be more sensitive to their toxic effects than other vertebrates. One of the main effects of pesticides is the disruption of the nervous system through the inhibition of cholinesterase enzymes. The aim of this study was to determine the plasma cholinesterase activity in native birds of pesticide-exposed agricultural lands within the Grassland Priority Conservation Area El Tokio, located in northeastern Mexico. The study was conducted during three summer seasons (2008 − 2010), when the reproduction of birds and pesticide spraying occurred. Forty-four birds of 13 different species were captured, sampled, and released. High variability values among individuals and species were found, ranging from 0.200 ± 0.055 to 4.960 ± 0.150 μmol/min/L. White-winged doves' values were significantly smaller than basal reference, showing 29 − 49% of plasma cholinesterase inhibition and possible pesticide exposure. Mean plasma cholinesterase activity values for 10 of the species had not been reported previously. These data can serve for future interpretations of plasma cholinesterase activity values in wild birds within agricultural lands and for decision making in priority conservation areas.

Introduction

Almost 5 billion ha of the world's land surface (≈38%) are used as agricultural lands, and since the 1950s, pesticides have been increasingly used to protect crops (Wood et al., 2000). However, the application of 3 million tons of pesticides has resulted in over 26 million nonfatal and 220 000 fatal human cases of pesticide poisoning and approximately 750 000 chronic illnesses per year (Hart and Pimentel, 2002; Ritchter, 2002). The disruption of the nervous system of invertebrates and vertebrates caused by the inhibition of cholinesterase enzymes is evidence of the toxicity of pesticides, mostly organophosphorus and carbamates (O'Brien, 1967). The main toxic effect of pesticides, as organophosphates and carbamates, is the phosphorylation of acetylcholinesterase enzyme and other esterases. The phosphorylation inactivates these enzymes, which provokes an excessive stimulation of target organs by acetylcholine (Tully et al., 2003). Both acetylcholinesterase and butyrylcholinesterase are the key enzymes involved in regulating the transmission at the nerve synapse, with acetylcholinesterase being the predominant enzyme performing this function. In animals, acetylcholinesterase activity in plasma has been demonstrated to be higher than butyrylcholinesterase activity (Askar et al., 2011), and it is therefore more widely used to monitor pesticide exposure.

Due to the fact that birds frequently forage in agricultural areas (Rodenhouse et al., 1993) and are highly sensitive to cholinesterase-inhibiting properties of pesticides (Hill, 1988; Grue et al., 1997), birds have been commonly used as indicators of the pesticide exposure (Pimentel, 2005). There is a mean of 1 237 documented cases of bird kills each year in the United States, which were mainly caused by fenthion, carbofuran, endosulfan, monocrototophos, diazinon, and parathion (Fleischli et al., 2004; American Bird Conservancy/US Environmental Protection Agency, 2005). However, it has been estimated that pesticide-induced mortality could reach 67 million per year only in the US breeding grounds (Pimentel, 2005). Other sublethal effects have been reported, such as reduced reproduction and survival through neurophysiological and behavioral alterations (Grue et al., 1997). Monitoring of pesticide impacts on birds or any other nontarget group, to the best of our knowledge, is lacking in Mexico. Throughout the past 30 yr, cultivated areas in Mexico have been oscillating between 18 and 25 million ha (Servicio de Informacion Agroalimetaria y Pesquera/Secretaria de Agricultura, 2013). Insecticide use in agriculture

☆ This study was supported by Pronatura Noreste, A. C. with Neotropical Migratory Bird Conservation Act funds through the project "Ecological Reserves at Llano de La Soledad Ejidos" (MX-N481). The authors declare no conflict of interests.

* Correspondence: Dr. José Ignacio González Rojas, Av. Universidad s/n, Ciudad Universitaria, 66455 San Nicolás de los Garza, Nuevo León, Mexico. Tel.: +52 81 8396 5812; fax: +52 81 8376 2813.
E-mail address: jose.gonzalezr@uanl.mx (J.I. González-Rojas).

http://dx.doi.org/10.1016/j.rama.2017.03.003
1550-7424/

Please cite this article as: Ruvalcaba-Ortega, I., et al., Evaluation of Plasma Cholinesterase Activity in Native Birds from Pesticide-Exposed Agricultural Lands, Rangeland Ecology & Management (2017), http://dx.doi.org/10.1016/j.rama.2017.03.003

has increased 150% in the past 20 yr, from 14.48 to 37.5 thousand tons (Secretaria de Medio Ambiente y Recursos Naturales, 2011; Instituto Nacional de de Estadistica y Geografia, 2013). In northeastern México, the municipality of Galeana, Nuevo León is the third most valuable potato producer in the country (Servicio de Informacion Agroalimetaria y Pesquera/Secretaria de Agricultura, 2013). Potatoes are one of the crops with the highest quantities of pesticide use (Instituto Nacional de Estadistica y Geografia/Secretaria de Medio Ambiente, 1997). Unfortunately, this region also harbors a Grassland Priority Conservation Area (GPCA) (Pool and Panjabi, 2011), El Tokio, with the most important grasslands in the region, and the remaining populations of endangered endemic fauna such as the Worthen's Sparrow *(Spizella wortheni)* (Canales-del-Castillo et al., 2010) and the Mexican prairie-dog *(Cynomys mexicanus)* (Treviño-Villarreal and Grant, 1998). These grasslands also support the highest densities of some resident and/or wintering grassland bird populations, the most endangered group of birds of North America (Herkert, 1995; Saue et al., 2012), such as the Mountain Plover *(Charadrius montanus)* (Western Hemisphere Shorebird Reserve Network, 2005), Sprague's Pipit *(Anthus spragueii)*, and Horned Lark *(Eremophila alpestris)* (Panjabi et al., 2013). The aim of this study was to determine plasma cholinesterase activity values in native birds from the pesticide-exposed agricultural lands within the GPCA El Tokio.

Materials and Methods

Birds were captured and sampled during three summer seasons (2008 − 2010) in nine localities of pesticide-exposed agricultural lands in Galeana, Nuevo León, within El Tokio GPCA (25°04′01.61″N, 100°43′10.61″W, elevation 1880 masl) (Figure 1) that are constantly sprayed with pesticides during this time of the year. Following the instructions of Rose et al. (2006), birds were captured in the morning using mist nets (6 × 3 m) and kept in a well-ventilated, cool, quiet environment to prevent overheating and to minimize stress. Individuals were weighted and measured (wing chord, tail, and tarsi length) and aged and sexed following Pyle (1997). Blood was collected by puncturing either the jugular or basilic (wing) vein with a 1-mL syringe and deposited in microtubes containing 3.8% sodium citrate. Blood sample volumes did not exceed 1% of the bird's body weight. Birds were released after sampling. Samples were centrifuged at 1 000 g for 10 min at 4°C. The plasma was recovered and stored at −20°C until analysis. Samples with a visible hemolysis were discarded. Total plasma cholinesterase was measured colorimetrically using the process as previously described (Gonzalez-Escalante et al., 2013) based on Ellman's method (Ellman et al., 1961). Briefly, 3 mL of DTNB/buffer solution (250 μM DTNB in phosphate buffer pH 7.9) were dispensed in a cuvette and preincubated in a water bath, previously maintained at 30°C by 1 h. When the DNTB/buffer temperature reached 27°C, 20 μL of plasma were added and gently mixed using a 25-μL Hamilton syringe. After the sample temperature reached 30°C, 100 μL of substrate (5 mM acetylthiocholine iodide) were added and scanned at 406 nm in a DU 800 UV/Vis spectrophotometer (Beckman Coulter, CA) for 30, 60, 90, and 120 s. The reagent acetylthiocholine iodide (A5751, Sigma-Aldrich) was used as the substrate for the cholinesterase enzymes, and enzyme activity was measured and expressed as the amount of acetylthiocholine iodide (μmol) hydrolyzed/min/L using the following equation: ΔA/min × 11 700 where ΔA/min is the change of absorbance per minute. Each

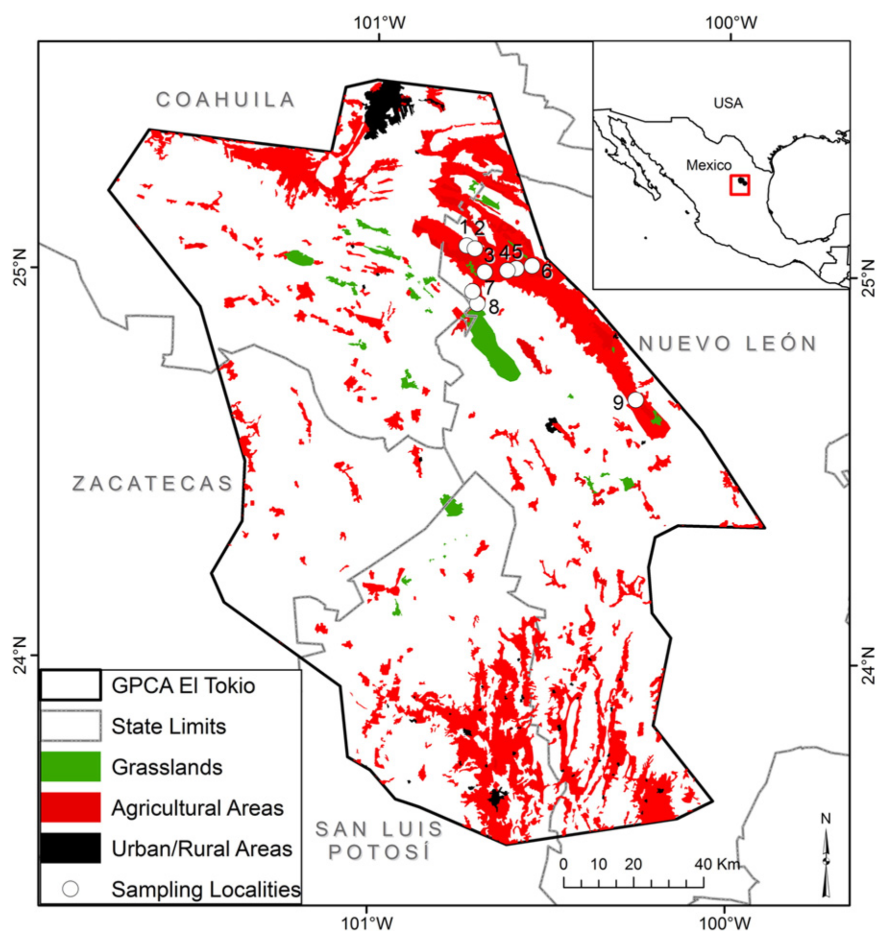

Figure 1. Map of the sampling localities within the Grassland Priority Conservation Area (GPCA) El Tokio. Localities where birds were captured have been numbered as follows: 1) La Casita, 2) San Juan del Prado, 3) El Erial, 4) El Uno, 5) La Concha, 6) San Rafael, 7) La Hediondilla, 8) La Soledad, and 9) El Tokio. Agricultural lands, grasslands, and urban and rural areas, as well as bordering states, are shown.