Influence of Cations on the Intestinal Proteolytic Activity of *Anticarsia
gemmatalis* (Hübner) Larvae

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The digestive process of insects is key to developing mechanisms that regulate crop pests; various mechanisms have been sought to affect their activity, mainly, proteases and amylase inhibitors (Rodrigues and Machado 2011). Control strategies based on enzyme inhibition can be developed with knowledge of insect digestive physiology, particularly, enzyme diversity and digestive dynamics. Velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), is a voracious and abundant defoliator of soybean, *Glycine max* (L.) Merr., in tropical Mexico (Ávila and Rodríguez 2004). The most destructive larval instars are fourth through sixth. Mendonca et al. (2012) reported that larvae have trypsin-like serine proteinases and cysteine-proteinases in their digestive systems; these are possible control targets through their inhibition. However, reduction in the activity of digestive enzymes can be affected by other agents such as divalent cations including copper or zinc that can reduce hydrolytic capacity. Cations such as calcium, copper, manganese, and zinc have been used as foliar sprays to control insects (Fried et al. 2018). This indicates the potential of understanding the digestive process to develop control strategies against *A. gemmatalis*. The effect of cations on total intestinal proteolytic activity was evaluated from fourth-instar larvae of *A. gemmatalis* from soybean crops at Mante, Tamaulipas, Mexico, in September 2018. Enzymes were extracted according to Aguirrezabala-Campano et al. (2013).

Proteolytic activity was detected by using BApNA (Sigma-Aldrich, St. Louis, MO), a prototype substrate for trypsin-like enzymes and useful for detecting cysteine-proteinase (Mendonça et al. 2012, Torres-Castillo et al. 2016). Proteolytic activity was measured by 0.1 increases in absorbance at 410 nm after incubation for 20 minutes at 37°C. Serine proteinase activity was confirmed with 10 mM phenyl methanesulfonyl fluoride solution, and cysteine proteinase was confirmed with 10 mM trans-epoxysuccinyl-L-leucylamido(4-guanidino) butane (E-64) solution (Sigma-Aldrich, St. Louis, MO). Reactions were prepared with a 10 mM EDTA solution (Sigma-Aldrich) to determine their sensitivity to a chelating agent.

The effect of cations was determined by developing the proteolytic activity reaction using aliquots of 50, 100, and 200 μl of different cation solutions and adjusting final volumes as needed. Each cation was added using 100 mM solutions

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