Digestive Enzymes and Stylet Morphology of *Deraeocoris nigritulus* (Uhler) (Hemiptera: Miridae) Reflect Adaptations for Predatory Habits

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ABSTRACT *Deraeocoris nigritulus* (Uhler) is often collected from the cones of Virginia pine, *Pinus virginiana*. Most species of *Deraeocoris* are predacious, but whether this plant bug is phytophagous or predacious is not known. To better understand the feeding adaptations of this mirid, the digestive enzymes from the salivary glands and anterior midgut were analyzed, and the mouthpart stylets were investigated with scanning electron microscopy. Evidence of a trypsin-like enzyme, a chymotrypsin-like enzyme, and pectinase were found in the salivary glands. Low levels of trypsin-like, chymotrypsin-like, elastase-like, pectinase, and α-amylase activities, as well as high levels of α-glucosidase activity, were found in the anterior midgut. The right maxillary stylet has two rows of at least seven strongly recurved teeth in front of at least three weakly recurved teeth on the inner surface, all pointing away from the head. Therefore, this insect is equipped with digestive enzymes and mouthparts mainly adapted for zoophagy.

KEY WORDS *Deraeocoris nigritulus*, digestive enzymes, Miridae, predator, stylet morphology

A CONSUMER'S ABILITY TO use plant or animal materials for food is indicated by the presence of specific digestive enzymes and by mouthpart morphology (Baptist 1941, Miles 1972, Cohen 1990, 1995, 1996, 1998a, b, 2000, Hori 2000, Zeng and Cohen 2000a, b). Digestive enzymes advantageous for zoophagy include proteases (e.g., trypsin, chymotrypsin, cathepsin), hyaluronidase, and phospholipase (Cohen 1998b, 2000). Specific digestive enzymes useful for phytophagy include amylase and pectinase (Cohen 1996).

Among heteropterans, morphological comparisons of both mandibular and maxillary stylets reveal differences between phytophages and zoophages. Cobben (1978) showed that the right maxillary stylets of predacious heteropterans (e.g., Nabidae, Anthocoridae) are more deeply serrated than those of phytophagous heteropterans (e.g., Tingidae). Cohen (1996) showed that the mandibular stylets of phytophagous and predacious pentatomids varied in relation to the direction of the barbs; those of phytophagous species point away from the head, whereas the barbs of predacious species point toward the head. The barbs on the mandibular stylets of reduviids and other predacious heteropterans are more numerous than the barbs on the mandibular stylets of phytophagous Heteroptera (e.g., Lygaeidae, *sensu lato*) (Cohen 1990).

Mirids, as well as all other heteropterans, use their digestive enzymes and stylets simultaneously to feed. They feed by piercing and cutting tissues with their stylets while injecting digestive enzymes through the salivary canal to liquefy food into a nutrient-rich slurry (Miles 1972, Hori 2000, Wheeler 2001). The food slurry is ingested through the food canal and passed into the alimentary canal where it is further digested and absorbed (Cohen 2000).

Deraeocoris nigritulus is a little known plant bug that has been collected on *Pinus virginiana* and *P. sylvestris* (Wheeler et al. 1983) and is often found by beating the cones over a beat net (A. G. Wheeler, Jr., personal communication). Members of the genus *Deraeocoris* are typically strict zoophages or facultative phytophages (Knight 1921, Razafimahatratra 1981), but both Knight (1921) and Razafimahatratra (1981) assume that strict phytophages exist within the genus. Because this mirid has been found only on two closely related hosts [unlike the predacious *D. nebulosus* (Uhler), which is found on over 80 plant species (Boyd 2001)] and is found in association with pine cones (potentially feeding on the seeds), it might be one of the strict phytophages that Knight and Razafimahatratra assume to exist.

The objective of this study was to infer the feeding habits of a cryptic species of an ecologically important genus of insects by analyzing the digestive enzymes in the salivary gland complex and anterior midgut and by observing its stylet morphology.

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Materials and Methods

Insects. *Deraeocoris nigritulus* was collected from Virginia pine, *P. virginiana*, in Anderson Co. and Pickens Co., SC, during the spring and summer of 2001. Voucher specimens were placed in the Clemson University Arthropod Collection.

Sample Preparation. Only adult females were used in these tests, and enzyme samples were prepared following the method of Cohen (1993) with modification. The mirids were starved for 24 h before dissections to standardize the insects and to allow an accumulation of digestive enzymes in the salivary glands. The insects were placed at −20°C for 4 min and dissected in ice-cold phosphate-buffered saline (pH 7.4) under a dissecting microscope. The salivary gland complex, including all lobes, accessory glands, and tubules, was exposed by holding the abdomen with fine forceps and gently pulling the head and prothorax away from the abdomen with another pair of fine forceps. The anterior midgut was exposed by holding the body with forceps, and with another pair of forceps, pulling the ovipositor and last three or four segments of the abdomen away from the rest of the abdomen.

The salivary glands of 20 insects were removed, placed in 1 ml phosphate buffer, homogenized, and centrifuged at 15,294 × *g* for 10 min at 4°C. The supernatant was placed in a 1.5-ml centrifuge tube and kept at 4°C until use (within 48 h). The anterior midguts of the same 20 insects were treated as the salivary glands. Protein concentrations of all enzyme samples were determined by bicinchoninic acid protein assay (Pierce, Rockford, IL) using bovine serum albumin as the standard. Three samples of 20 insects were used for each tissue, and each assay was repeated three times for each sample. Because of the relatively high protein concentration, each sample was diluted 1:1 in phosphate buffer.

***α*-Amylase Assay.** Amylase activity in the salivary glands and anterior midguts was determined with a diagnostic kit (No. 577–3; Sigma, St. Louis, MO) following modifications by Zeng and Cohen (2000a-c). The substrate was 4,6 ethylidene (G_7)-*p*-nitrophenyl (G_1)-α, D-maltoheptaside. Enzyme extracts (10 μl) were added to wells in a 96-well enzyme-linked immunosorbent assay (ELISA) plate. The substrate was equilibrated to 37°C for 10 min, and 200 μl was added to each well. The plate contents were mixed for 5 s and incubated at 37°C for 30 min. Absorbance was read at 405 nm in a plate reader (SPECTRA MAX Plus; Molecular Devices Corp., Sunnyvale, CA). Absorbance is directly related to amylase activity. Authentic α-amylase from barley malt (Sigma A-2771) was used as a positive control (1 mg/ml, 1 U/mg solid), and buffer and substrate only were used as a negative control. The relative amylase activity was calculated as absorbance units per milligram protein.

***α*-Glucosidase Assay.** The α-glucosidase activity of both salivary glands and anterior midguts was tested following Agustí and Cohen (2000), by adding 100 μl of extract to 100 μl of 10-mM solution of *p*-nitrophenyl α-D-glucopyranoside (Sigma, N-1377) in a 96-well ELISA plate and incubating at 37°C for 1 h. The reaction was stopped by adding 100 μl of 15% Na_2CO_3. Invertase from bakers yeast (Sigma I-4504) was used as a positive control (1 mg/ml, 400 U/mg solid), and buffer and substrate only were used as a negative control. Absorbance was read at 405 nm in a plate reader, and the relative α-glucosidase activity was calculated as absorbance units per milligram protein.

Protease Assays. Salivary glands and anterior midguts were analyzed for trypsin-like, elastase-like, and chymotrypsin-like enzymes, following methods of Agustí and Cohen (2000). The substrates for trypsin and elastase, *N*α-benzoyl-DL-arginine-*p*-nitroanilide (Sigma B-4875) and succinyl-alanyl-alanyl-alanyl-*p*-nitroanilide (Sigma S-4760), respectively, were dissolved in dimethylsulfoxide (DMSO) and added to phosphate buffer to make a 10 μM solution. The substrate for chymotrypsin, benzyol-L-tyrosine-*p*-nitroanilide (Sigma B-6760), was dissolved in dimethylformamide (DMF) and added to phosphate buffer to make a 10 μM solution. In wells of a 96-well ELISA plate, 50 μl of the salivary and gut extracts were added to 200 μl of the various specific substrates. Trypsin from bovine pancreas (Sigma T-8003), elastase from porcine pancreas (Sigma E-0258), and α-chymotrypsin from bovine pancreas (Sigma C-7762) were used as the positive controls (1 mg/ml; 10,000 BAEE units, 3–6 U, and 40–60 U/mg protein, respectively) and buffer and substrate only were used as a negative control. After a 20-h incubation at 37°C, absorbance was read at 405 nm in a plate reader, and the relative protease activity for each substrate was calculated as absorbance units per milligram protein.

Pectinase Assay. The pectinase activity of both salivary gland and anterior midgut extracts were tested following Agustí and Cohen (2000), with slight modification. Extract consisting of 100 μl was added to 4 ml of 0.5% pectin from citrus fruits (Sigma P-9135) in phosphate-buffered saline (pH 7.4) and incubated at 37°C for 20 h. The pectin-extract solution was passed through a no. 150 Cannon-Fenske routine type viscometer (Cannon Instrument Co., State College, PA) at 25°C. Heat-denatured extract was used as a negative control. Pectinase activity was measured as the reciprocal of viscosity (1/centistokes) per milligram protein because pectinase activity is indicated by viscosity reduction rather than a direct reading of viscosity.

Scanning Electron Microscopy Preparation. Six heads, including mouthparts, of *D. nigritulus* were placed in 95% ethanol for at least 24 h and then air dried. Dry heads were placed, labium up, on aluminum stubs with double-sided tape. Stylets were removed from the labium with an insect pin. Stylet bundles were kept together or were separated into their mandibular and maxillary stylets by placing a minuten pin at the base of the stylets and moving it toward the apex or by sliding forceps gently over the stylets from the base to the apex. Specimens were gold coated with a sputtering device and viewed in secondary emission mode in a Hitachi 3500 (London, England) scanning electron microscope at 10 kV.

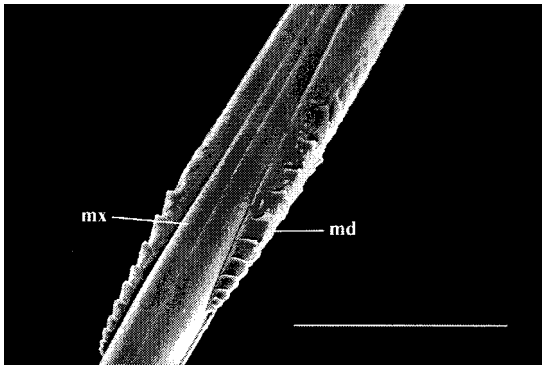

Fig. 1. Anterior view of the stylet bundle of *D. nigritulus*. The two mandibular (md) stylets are outside the two maxillary (mx) stylets. Scale bar = 50 μm.

Results

Digestive Enzymes. Amylase activity (0.16 ± 0.03 U/mg, $n = 3$) was detected in the anterior midgut but not in the salivary gland complex, and glucosidase activity was detected in the anterior midgut (4.62 ± 1.28 U/mg, $n = 3$) but not in the salivary gland complex. Trypsin-like activity was detected in the salivary gland complex (2.50 ± 0.08 U/mg, $n = 3$) and anterior midgut (0.30 ± 0.05 U/mg, $n = 3$). Chymotrypsin-like activity was detected in the salivary gland complex (1.22 ± 0.19 U/mg, $n = 3$) and anterior midgut (0.16 ± 0.06 U/mg, $n = 3$). Elastase-like activity was detected in the anterior midgut (0.16 ± 0.07 U/mg, $n = 3$) but not in the salivary gland complex. Pectinase activity was found in the salivary gland complex (0.20 ± 0.02/cSt/mg, $n = 3$) and in the anterior midgut (0.03 ± 0.00/cSt/mg, $n = 3$).

Mouthpart Morphology. As in all other heteropterans, the stylet bundle of *D. nigritulus* has two maxillary stylets inside two mandibular stylets (Fig. 1). The mirid's right maxillary stylet has two rows of at least seven strongly recurved teeth in front of at least three weakly recurved teeth on the inner surface pointing away from the head (Fig. 2).

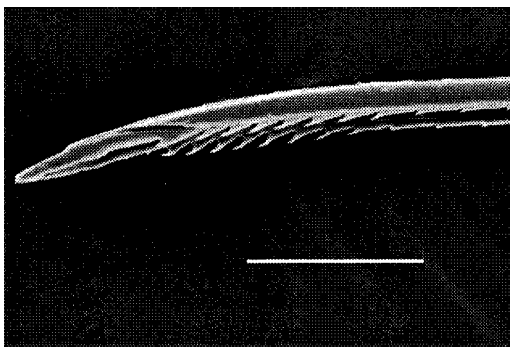

Fig. 2. Lateral view of the right maxillary stylet of *D. nigritulus*. Scale bar = 50 μm.

Discussion

Digestive Enzymes. No amylase or glucosidase activity was found in the salivary gland complex of this insect. Because these two enzymes are indicative of phytophagy (Cohen 1996) and plant-feeding mirids usually have high levels of amylase in the salivary gland complex (Agustí and Cohen 2000), this mirid is not a strict phytophage. Low levels of amylase and high levels of glucosidase were found in the anterior midgut, indicating that *D. nigritulus* might ingest starch granules or disaccharides from plants and digest them in the gut. The ability to use plants for food might enable *D. nigritulus* to survive in the absence of prey for short periods of time (Naranjo and Gibson 1996). The digestion of glycogen, obtained from arthropod prey, might also explain the presence of these enzymes in the gut. In comparison, *D. nebulosus*, a predator of many arthropods, has no amylase but low glucosidase activity in its salivary gland complex and equal levels of both in its midgut, indicating that it, too, might use plants for food (Boyd et al. 2002).

The presence of pectinase in the salivary glands and midguts also indicates an ability to feed on plants by attacking cells held together with pectin complexes. Hori (2000) suggested that, among the Heteroptera, pectinase is a fundamental enzyme only in the Miridae. However, Campbell and Shea (1990) demonstrated that *Leptoglossus occidentalis* Heidemann (Coreidae) possess pectinase using an electrophoretic pectinase assay. At least one species each of two other heteropteran families, Lygaeidae and Pyrrhocoridae, have been reported to possess pectinase (Hori 2000).

Pectinase might play a significant role in egg-laying behavior. Nearly all known mirids insert their eggs in plant material; a few deposit eggs on the plant surface (Wheeler 2001). Preoviposition behavior includes probing the plant with the rostrum—sometimes for >10 min (Knight 1941)—before inserting the ovipositor into the plant material (Ferran et al. 1996, Wheeler 2001). Hori and Miles (1993) found "strong" pectinase in saliva discharged without stimulation from the tip of the stylets of *Creontiades dilutus* (Stål). Pectinase found in mirid zoophages, such as *D. nigritulus* and *D. nebulosus* (Boyd et al. 2002), might be used to soften the plant tissue before oviposition.

The presence of protease activity in the salivary gland complex of *D. nigritulus* indicates that this mirid is primarily predacious. Trypsin-like and chymotrypsin-like proteases were detected in the salivary gland complex, but the anterior midgut had low levels of trypsin-like, elastase-like, and chymotrypsin-like proteases. The presence of trypsin-like and chymotrypsin-like enzymes demonstrates an insect's ability to access structural or other insoluble proteins (Cohen 1993, 1998a, 2000). Possession of trypsin-like enzymes and the lack of chymotrypsin-like and elastase-like enzymes in the salivary gland complex is common for heteropteran predators (Cohen 1993, 1998a, 2000, Agustí and Cohen 2000). The green mirid, *Creontiades dilutus*, was the first mirid shown to have chymotrypsin-like activity in the salivary glands (Colebatch et al.

2001, 2002). Trypsin-like enzymes are endoproteases that attack proteins at residues of arginine and lysine, whereas chymotrypsin-like enzymes, also endoproteases, attack proteins at aromatic residues (e.g., tryptophan). Elastase-like protease has been identified only in the salivary glands of one mirid, *Lygus hesperus* Knight (Zeng and Cohen 2001), and in the salivary glands of the reduviid *Zelus renardii* Kolenati (A. C. Cohen, personal communication). Zeng and Cohen (2001) found more elastase activity in field-caught insects than those reared in the laboratory. Individuals of *D. nigritulus* in this study were field collected, but another species, *D. nebulosus,* in a similar study, also had no detectable elastase activity (Boyd et al. 2002).

Low levels of activity of all three specific proteases—trypsin-like, elastase-like, and chymotrypsin-like—in the anterior midgut indicate the ability of *D. nigritulus* to synthesize proteases in the gut, the presence of symbiotic microorganisms in the gut, carryover from ingested salivary gland secretions, or starvation effects. These alkaline endoproteases help break down proteins once they are inside the gut, making the proteins absorbable after further hydrolysis by exopeptidases such as aminopeptidase and carboxypeptidase.

Mouthpart Morphology. The mandibular stylets of *D. nigritulus* are typical of the Miridae, whether phytophagous or zoophagous (Cobben 1978, Cohen 1996, Boyd 2001, Wheeler 2001). Many other heteropterans produce a salivary flange that is used as a fulcrum, among other functions, for stylet movement (Cohen 1998b, 2000). Because mirids do not produce a salivary flange, the apical serrations on the mandibular stylets are considered adaptive in holding onto tissues below the outer layer of the prey or host plant and in producing a fulcrum for movement of the maxillary stylets (Cobben 1978, Cohen 2000, Wheeler 2001).

Cobben (1978) suggested that the inner surface of the right maxillary stylets of heteropterans ranged from moderately serrated in predatory families (e.g., Anthocoridae, Nabidae) to smooth in strictly phytophagous families (e.g., Tingidae), with the Miridae representing an intermediate condition (Wheeler 2001). Boyd (2001) observed maxillary stylets from 20 mirids, ranging from strictly phytophagous to strictly zoophagous, and suggested that the serrations of the right maxillary stylets are deeper for predators than for herbivores. The deep serrations in the right maxillary stylet of *D. nigritulus,* which are similar to those of *D. nebulosus* (Boyd et al. 2002) and *D. olivaceous* (F.) (Cobben 1978), probably are used to disrupt prey by ripping and tearing tissues (Cohen 2000). The barbs point away from the head, indicating that the cutting action occurs when the stylet is thrust forward, unlike predacious pentatomids, which have barbs on the mandibular stylets pointing toward the head (Cohen 1996).

Conclusions. *Deraeocoris nigritulus* is suited primarily for zoophagy. The presence of tryspin-like and chymotrypsin-like enzymes in the salivary gland complex are essential in breaking down otherwise insoluble proteins in the prey. These enzymes and the deep serrations of the right maxillary stylet enable this plant bug to break down tissues of arthropods chemically and mechanically (Cohen 2000). The presence of pectinase in the salivary glands allows this mirid to break down plant cells, and glucosidase in the midgut enables it to break down sugars from plants or glycogen from prey into monomeric units for absorption. Low levels of amylase might enable this plant bug to ingest intact starch granules and break them down in the gut.

Deraeocoris nigritulus is a predator, even though it resides mainly in pine cones during the day and has a limited host range. This mirid probably is nocturnal and feeds on co-occurring soft-bodied arthropods on host pines. It has the digestive enzymes needed to use plants for food, but the multiple proteases and the deeply serrated right maxillary stylet indicates that it is only a facultative phytophage. Knight (1921) and Razafimahatratra (1981) postulated the existence of a species of *Deraeocoris* that is a strict phytophage. However, such a species has yet to be found. Further study into this mainly predacious genus might yet reveal that one exists.

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References Cited

Agustí, N., and A. C. Cohen. 2000. *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. J. Entomol. Sci. 35: 176–186.

Baptist, B. A. 1941. The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. Q. J. Microscop. Sci. 83: 91–139.

Boyd, D. W., Jr. 2001. *Deraeocoris nebulosus* (Uhler) (Hemiptera: Miridae): a potential biological control agent. Ph.D. dissertation. Clemson University, Clemson, SC.

Boyd, D. W., Jr., A. C. Cohen, and D. R. Alverson. 2002. Digestive enzyme and stylet morphology of *Deraeocoris nebulosus* (Uhler) (Hemiptera: Miridae), a predacious plant bug. Ann. Entomol. Soc. Am. 95: 395–401.

Campbell, B. C., and P. J. Shea. 1990. A simple staining technique for assessing feeding damage by *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) on cones. Can. Entomol 122: 963–968.

Cobben, R. H. 1978. Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. Meded. Landbouwhogesch. Wageningen. 78: 1–407.

Cohen, A. C. 1990. Feeding adaptations of some predaceous heteropterans. Ann. Entomol. Soc. Am. 83: 1215–1223.

Cohen, A. C. 1993. Organization of digestion and preliminary characterization of salivary trypsin-like enzymes in a predaceous heteropteran, *Zelus renardii.* J. Insect Physiol. 39: 823–829.

Cohen, A. C. 1995. Extra-oral digestion in predaceous terrestrial Arthropoda. Annu. Rev. Entomol. 40: 85–103.

Cohen, A. C. 1996. Plant feeding by predatory Heteroptera: evolutionary and adaptational aspects of trophic switching, pp. 1–17. *In* O. Alomar and R. N. Wiedenmann (eds.), Zoophytophagous Heteroptera: implications for life history and integrated pest management. Entomological Society of America, Lanham, MD.

Cohen, A. C. 1998a. Solid-to-liquid feeding: the inside(s) story of extra-oral digestion in predaceous Arthropoda. Am. Entomol. 44: 103–117.

Cohen, A. C. 1998b. Biochemical and morphological dynamics and predatory feeding habits in terrestrial Heteroptera, pp. 1–8. *In* J. R. Ruberson and M. Coll (eds.), Predaceous Heteroptera: implications for biological control. Entomological Society of America, Lanham, MD.

Cohen, A. C. 2000. How carnivorous bugs feed, pp. 563–570. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of economic importance. CRC, Boca Raton, FL.

Colebatch, G., P. East, and P. Cooper. 2001. Preliminary characterisation of digestive proteases of the green mirid, *Creontiades dilutus* (Hemiptera: Miridae). Insect Biochem. Mol. Biol. 31: 415–423.

Colebatch, G., P. Cooper, and P. East. 2002. cDNA cloning of a salivary chymotrypsin-like protease and the identification of six additional cDNAs encoding putative digestive proteases from the green mirid, *Creontiades dilutus* (Hemiptera: Miridae). Insect Biochem. Mol. Biol. 32: 1065–1075.

Ferran, A., A. Rortais, J. C. Malausa, J. Gambier, and M. Lambin. 1996. Ovipositional behaviour of *Macrolophus caliginosus* (Heteroptera: Miridae) on tobacco leaves. Bull. Entomol. Res. 86: 123–128.

Hori, K. 2000. Possible causes of disease symptoms resulting from the feeding of phytophagous Heteroptera, pp. 11–35. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of economic importance. CRC, Boca Raton, FL.

Hori, K., and P. W. Miles. 1993. The etiology of damage to lucerne by the green mirid, *Creontiades dilutus* (Stål). Aust. J. Exp. Agric. 33: 327–331.

Knight, H. H. 1921. Monograph of the North American species of *Deraeocoris* (Heteroptera: Miridae). Minn. State Entomol. 18: 76–210.

Knight, H. H. 1941. The plant bugs, or Miridae, of Illinois. Bull. Ill. Nat. Hist. Surv. 22: 1–234.

Miles, P. W. 1972. The saliva of Hemiptera. Adv. Insect Physiol. 9: 183–255.

Naranjo, S. E. and R. L. Gibson. 1996. Phytophagy in predaceous Heteroptera: effects on life history and population dynamics. *In* O. Alomar and R. N. Wiedenmann (eds.), Zoophytophagous Heteroptera: implications for life history and integrated pest management. Entomological Society of America, Lanham, MD.

Razafimahatratra, V. D. 1981. A revision of the genus *Deraeocoris* Kirschbaum (Heteroptera: Miridae) from western America north of Mexico. Ph.D. dissertation, Oregon State University, Corvallis. OR.

Wheeler, A. G., Jr. 2001. Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists. Cornell University Press, Ithaca, NY.

Wheeler, A. G., Jr., T. J. Henry, and T. L. Mason, Jr. 1983. An annotated list of the Miridae of West Virginia (Hemiptera-Heteroptera). Trans. Am. Entomol. Soc. 109: 127–159.

Zeng, F. and A. C. Cohen. 2000a. Comparison of α-amylase and protease activities of a zoophytophagous and two phytozoophagous Heteroptera. Comp. Biochem. Physiol. 126A: 101–106.

Zeng, F., and A. C. Cohen. 2000b. Demonstration of amylase from the zoophytophagous anthocorid *Orius insidiosus.* Arch. Insect Biochem. Physiol. 44: 136–139.

Zeng, F. and A. C. Cohen. 2000c. Partial characterization of α-amylase in the salivary glands of *Lygus hesperus* and *L. lineolaris.* Comp. Biochem. Physiol. 126B: 9–16.

Zeng, F., and A. C. Cohen. 2001. Induction of elastase in a zoophytophagous heteropteran, *Lygus hesperus* (Hemiptera: Miridae). Ann. Entomol. Soc. Am. 94: 146–151.

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