Blattella asahinai (Dictyoptera: Blattellidae): A New Predator of Lepidopteran Eggs in South Texas Soybean

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ABSTRACT Large numbers of a cockroach that resembled the German cockroach, *Blattella germanica* (L.), were observed during winter 2005–2006 in home turf settings in Weslaco, TX, 11 km from the U.S. border with northeastern Mexico. In June and July 2006, this cockroach was observed at night on the foliage of soybean, *Glycine max* (L.) Merr., in numbers often exceeding 50 per m row. Because of its similarity to *B. germanica*, but with the characteristic of flying frequently, we suspected that our observations might be of the Asian cockroach, *Blattella asahinai* Mizukubo. Using a combination of morphological characters, cuticular hydrocarbons, and sequences of the mitochondrial cytochrome oxidase II gene, we definitively identified this cockroach as *B. asahinai*. *B. asahinai* was frequently observed feeding on sentinel eggs of *Helicoverpa zea* (Boddie) and *Spodoptera exigua* (Hübner). This cockroach was the dominant egg predator in soybean during 2006, making up 36.4% of all predators observed feeding on eggs. *B. asahinai* was only observed occupying the soybean canopy nocturnally, and it made up 53.7% of predators observed feeding at night. We speculate that *B. asahinai* may serve as an important beneficial insect in soybean and other crops.

KEY WORDS range extension, cuticular hydrocarbons, COII gene

Large numbers of a cockroach that resembled the German cockroach, *Blattella germanica* (L.), were observed during winter 2005–2006 in home turf settings in Weslaco, TX, 11 km from the U.S. border with northeastern Mexico. During nocturnal observations of arthropods in soybean, *Glycine max* (L.) Merr., 'Vernal' in June and July 2006, this cockroach was observed on foliage in numbers often exceeding 50 per m row (Fig. 1). Small numbers of cockroaches also were observed in a nearby cotton, *Gossypium hirsutum* L., field that was undergoing heavy pesticide inputs for eradication of the boll weevil. This cockroach was common at night in the foliage of the soybean canopy and during 5 yr of research on nocturnal activity of insects in soybean and cotton, had not been previously observed. Nocturnal observations in 2006 revealed that this cockroach frequently fed on sentinel lepidopteran eggs. Because this cockroach seems to be an important beneficial insect in soybean, individuals were collected and processed for definitive identification.

Observations of behavior of this cockroach, in particular a propensity to fly and a similarity in appearance to *B. germanica*, suggested that the cockroach we observed might be the Asian cockroach, *Blattella asahinai* Mizukubo (Dictyoptera: Blattellidae) (Brenner et al. 1988). *B. asahinai* was first described from Okinawa Island, Japan (Mizukubo 1981). Roth (1985) described the species as *Blattella beybienkoi*, but later the names were synonymized (Roth 1986). *B. asahinai* is most closely related to *B. germanica.* Observations of behavior and male morphology can definitively separate *B. asahinai* from *B. germanica.* However, to separate *B. asahinai* from other closely related *Blattella* species that may have entered the United States, nonmorphological evaluations of cuticular hydrocarbons and mitochondrial DNA had to be applied.

In this article, we present data supporting the identification of this invasive cockroach as *B. asahinai*, applying a combination of morphological characters, cuticular hydrocarbon profiles, and mitochondrial cytochrome oxidase subunit two (COII) sequence homologies. We also present data on predation of lepidopteran eggs by *B. asahinai* in soybean.

Materials and Methods

Field Observations. Observations of predators feeding on sentinel lepidopteran eggs were conducted in soybean plots of various sizes (0.025–0.50 ha.) from 2001 to 2006 as part of several different research projects. Because these studies all used the same technique for observing predation, the data on cockroach predation is pooled. In all years, Vernal soybean was planted using standard production practices during March on a 1-m row spacing with drip irrigation. There were 3.1-m bare ground buffers surrounding all plots or field edges. No pesticides were applied during the course of these studies.

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Fig. 1. Adults and nymphs of *Blattella asahinai* observed nocturnally near sentinel eggs in Vernal soybean, July 2006.

Predators feeding on lepidopteran eggs in soybean were observed using the methods of Pfannenstiel and Yeargan (2002), and they are summarized here with appropriate changes noted. During each year, sample stations in soybean were flagged at 3- or 5-m intervals as sites for sentinel placement. Sentinel eggs used in these studies were either *Helicoverpa zea* (Boddie) or *Spodoptera exigua* (Hübner) (both Lepidoptera: Noctuidae), although *S. exigua* was not used in all studies in all years. When both lepidopteran species were used, the sites within a row were alternately assigned to either *H. zea* or *S. exigua* by using different colored flags.

H. zea and *S. exigua* colonies were maintained in the laboratory by using modified methods of Ignoffo (1965). Adults were placed in ice cream cartons (3.8 liters) lined with green florist paper for oviposition. A 10% sucrose solution was provided as a food source. Florist paper on which eggs had been attached were collected daily; the paper on which eggs had been laid was then cut into small (3–20-cm^2) sections containing either 10 *H. zea* eggs or one *S. exigua* egg mass (range 20–160 eggs) each. Eggs were then placed in a refrigerator at 4°C immediately after oviposition until used or discarded after 4 d. Groups of 10 *H. zea* eggs were used to extend the amount of time that a predator feeds, thus increasing the probability of observing predation events.

Eggs were transported to the field in an ice chest with a cold pack, and then they were attached to plants at 3 p.m. by stapling the eggs in the desired location. Afternoon was used for deployment of eggs for purely logistical purposes. Paper sections containing eggs were not used if any of the eggs were dislodged during transportation. Sentinel eggs were attached to the top of a soybean leaf ≈55–70% of plant height. Lepidopteran pests of field crops such as soybean often deposit eggs on the foliage of the middle to upper parts of the plant (Terry et al. 1987; Sappington et al. 2001; R.S.P. unpublished data), although often on the undersides of leaves. Placing the eggs on the top of leaves was done to facilitate observation. Nuessly and Sterling (1994) found no differences in predation on *H. zea* eggs between the upper and lower leaf surfaces in cotton in central Texas.

Sentinel eggs were observed at 1800 hours, 2100 hours, 2400 hours, 0300 hours, 0600 hours, 0900 hours, 1200 hours, and 1500 hours (CDT). Sunrise occurred as the 0600 hours observation was being finished and sunset occurred just before the 2100 hours observation was initiated, allowing for four day and four night samples. *H. zea* and *S. exigua* eggs take ≈3 d to develop. Due to the prompt refrigeration after oviposition, eggs used during this study did not approach hatching at any time, and they were available to predators during the entire observation period. At each observation period, predators observed feeding on the eggs were visually identified or collected for identification. Sampling was initiated in mid-April and continued at 2- to 4-wk intervals into early August. One additional evaluation of nocturnal predation only was conducted. The observations of feeding were used to identify the

predators most frequently feeding on lepidopteran eggs and to determine diel periodicity.

Species Identification. Cockroaches were collected from a Vernal soybean field at the USDA–ARS research farm 3.2 km southeast of Weslaco, Hidalgo Co., TX (26° 08.12 N, 97° 57.26 W) in June and July 2007. Collections were made by sweep netting at night or by hand-collecting individuals hiding in leaf litter at the base of the soybean plants during the day.

Initial identifications of adult males were made based on external and internal morphological traits (Mizukubo 1981, Roth 1985), including interocular distance, tergal glands on the seventh and eighth tergites (T7 and T8), male paraprocts and supra-anal plate (T10), thorns on the left stylus, and morphology of the left phallomere.

To confirm morphological identifications, we also conducted tests using both hydrocarbon profiles and mitochondrial DNA. For hydrocarbon analysis, individual adult males were extracted in 1 ml of *n*-hexane for 5 min with occasional gentle mixing. The extract was fractionated on 500 mg of silica gel, the hexane fraction blown to dryness under a gentle stream of N_2 and then resuspended in 100 μl of hexane. Two microliters of extract was injected into a 300°C splitless inlet in a HP5890 GC, with a HP7673 autoinjector. Hydrocarbon separation was done on a DB-5 capillary column (30 m by 0.25 mm by 0.25 μm; He flow of 30 cm/s) (Agilent Technologies, Palo Alto, CA) coupled to a flame ionization detector at 320°C. The oven was programmed to start at 100°C for 2 min, and then increase at 15°C/min to 320°C and remain there for 20 min. ChemStation software, version A.09.09 (Agilent Technologies) was used for data acquisition and analysis.

Genomic DNA was isolated from 10 individuals from each of three sampling locations by using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). These samples were made up of the following: *B. germanica* collected in an apartment in Raleigh, Wake County, NC; *B. asahinai* from Gainesville, Alachua County, FL; and unknown *Blattella* collected from Weslaco, Hidalgo County, TX. The universal primers A-t Leu and B-t Lys (Liu and Beckenbach 1992) were used to amplify a segment of the COII region of the mitochondrial DNA from each individual following the protocol of Maekawa and Matsumoto (2000). Amplified polymerase chain reaction (PCR) products were purified. Both forward- and reverse-sequencing reactions were performed following the methodology outlined in Copren et al. (2005).

Sequence alignments were performed using the Vector NTI Advance 9.1.0 program (Invitrogen, Carlsbad, CA). Additional COII region sequences were obtained from GenBank for both *B. germanica* (accession no. AB011235) and *Blattella lituricollis* (Walker) (accession no. AB005906) (Maekawa et al. 1999, Maekawa and Matsumoto 2000). Phylogenetic reconstruction was conducted using MEGA, version 3.1 (Kumar et al. 2004). Robustness of the consensus tree was examined after 1,000 bootstrap replicates (Felsenstein 1985).

Results

Field Observations. Cockroaches, later identified as *B. asahinai*, were frequently observed feeding on *H. zea* and *S. exigua* eggs during 2006. Because research on predation of lepidopteran eggs in soybean had been conducted from 2001 to 2005, we were able to compare observations made in 2006 with previous years. No observations of any *Blattella* sp. feeding or otherwise present were made in soybean between 2001 and 2004 ($n = 195$ observations of predation). In 2005, a single individual was observed feeding on *H. zea* eggs (1.9% of all observations; $n = 52$), but this observation was not considered notable at the time. However, in 2006, this cockroach was the arthropod most frequently observed preying on lepidopteran eggs in soybean, representing 36.4% of all observations (16/44). As a proportion of nocturnal predators, *B. asahinai* made up 53.7% of all nocturnal predators observed feeding on eggs (22/41). On 11–12 July and 1–2 August, 24-h mortality of sentinel *H. zea* eggs was 68.2 and 84.9%, respectively (out of $n = 60$ sites, with a total of 600 eggs).

During late spring 2006, nymphs and adults of *B. asahinai* were observed foraging and undergoing courtship and mating activities at night throughout the field. Although daytime sampling using vertical beat sheets did not adequately reflect the densities of cockroaches present, visual samples of 1-m sections of row taken on 25 July showed highly conservative estimates of 32.5 per row m (range 18–55; $n = 6$). This estimate was conservative because the plants were not disturbed. On disturbance of the plants, it was clear that many cockroaches were present under the leaves and deep within the canopy. Additional *B. asahinai* were likely to be present in the leaf litter at the base of the soybean plants.

Identification. External and internal morphological traits of field-collected cockroaches from Texas soybean fields were consistent with those described by Mizukubo (1981) for *B. asahinai.* In adult males, the interocular distance was 0.55× the distance between the antennal sockets, T8 was deeply notched in the middle, only three thorns were visible on the left stylus, and the left phallomere had a shorter "hook" than in *B. germanica.* The Texas specimens were smaller and lighter in color than laboratory-reared *B. germanica;* wings were longer, and unlike *B. germanica*, the supra-anal plate in these specimens had brown markings. Adults also were observed to fly in the soybean field, a behavior that is consistent with *B. asahinai.*

The cuticular hydrocarbon profile of the Texas specimens was consistent with the profile reported for *B. asahinai* (Carlson and Brenner 1988). *B. asahinai* and *B. germanica* share a series of large alkanes and mono-, di-, and trimethylalkanes that elute after nonacosane (C_{29}). However, *B. asahinai* does not possess the earlier eluting homologous cluster of medium-sized hydrocarbons eluting after heptacosane (C_{27}), which are present in *B. germanica.* Only minor peaks occur before nonacosane, and the peaks eluting after

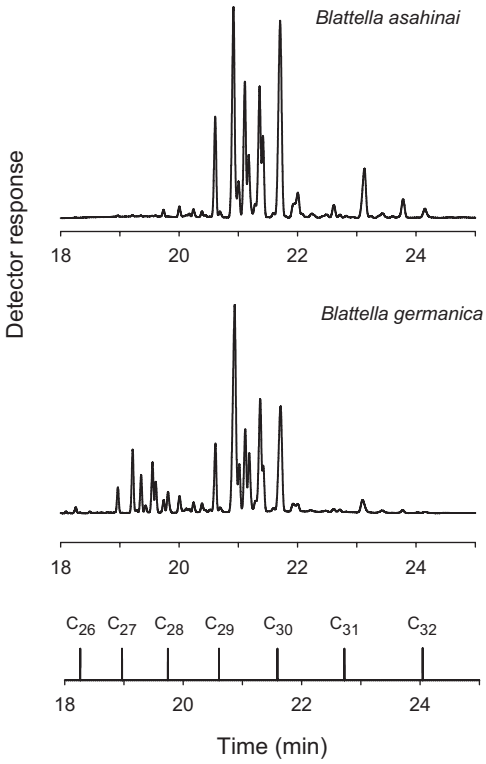

Fig. 2. Gas chromatograms of cuticular hydrocarbons of the Asian cockroach and the German cockroach. Retention times of standard *n*-alkanes are indicated (bottom).

hentriacontane (C_{31}) are larger than those of *B. germanica* (Fig. 2).

Sequence alignment of the mitochondrial COII gene resulted in 647 nucleotide sites. Of these sites, 49 (7.57%) characters were variable but uninformative. Five sites (0.77%) were informative. Three haplotypes were identified. *B. germanica* sequences from North Carolina were identical to each other and to the sequences found in GenBank (accession no. AB011235). Two haplotypes, occurring at a ratio of 8:2, were identified in *B. asahinai* collected in Florida (GenBank accession nos. EF492884 and EF492883, respectively). The *B. asahinai* haplotype differed at two nucleotide sites, representing 0.31% sequence variation. In comparison, the *B. asahinai* haplotypes differed from *B. germanica* at 11 nucleotide sites, representing 1.7% sequence variation. All sequence variation between *B. germanica* and *B. asahinai* occurred at third codon positions, and they did not alter the amino acid sequence. In contrast, *B. asahinai* samples from Florida diverged from *B. lituricollis* (accession no. AB005906) at 47 nucleotide sites, representing a 7.25% sequence divergence. Samples collected in Texas made up a single haplotype, which was identical to the most common *B. asahinai* haplotypic variant found in Florida (accession no. EF492884). Neighbor joining analysis resulted in a tree topology identifying three terminal clades, corresponding to the three *Blattella* species examined (Fig. 3).

Based on behavior, distribution, morphology, cuticular hydrocarbon profile, and DNA sequence comparisons, we conclude that the cockroaches collected in soybean fields near Weslaco, TX, were *B. asahinai.*

Discussion

At the time of our observations in 2006, *B. asahinai* was identified from Houston, TX, ≈580 km northeast of Weslaco (Schofield 2006), presumably based on gross morphology and behavior. Because other members of the diverse 45-species *Blattella* genus were not considered, and identification of *B. asahinai* from Texas was from specimens collected in residential settings, it was possible that our specimens may have represented a newly introduced *asahinai*-like species from Asia or Africa, where this genus is particularly diverse. No *B. asahinai* specimens have ever been definitively identified from agricultural fields, and there are no reports of this insect as a major predator on the eggs of lepidopteran pests. Three main reasons persuaded us not to dismiss the possibility of an *asahinai*-like introduction in Weslaco, TX. First, all *Blattella* species except *germanica* live outdoors and most species fly, like *asahinai.* Second, *B. asahinai* has been reported from South Carolina, Georgia, Florida, and Alabama (Richman 2006, Hu et al. 2005) but not in Mississippi or Louisiana. It was therefore possible that our observations represented a new introduction. Third, the difficulties of *Blattella* species identification based on morphological characters alone are high-

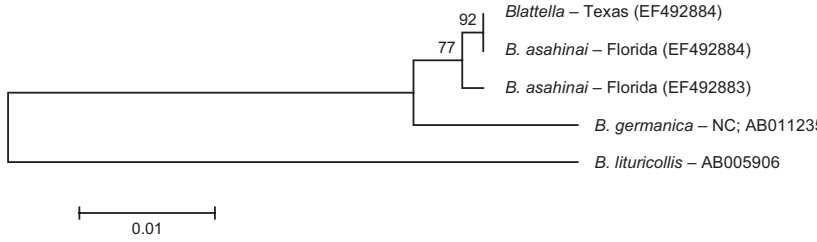

Fig. 3. Phylogenetic tree of *Blattella* spp. obtained from 647 bp of the COII gene of the mitochondrial DNA based on the neighbor-joining method (1,000 replications of bootstrapping). Numbers above branches correspond to the percentage of 1,000 bootstrap replicates.

lighted in Louis M. Roth's authoritative revision of the genus (Roth 1985).

Our results definitively identify the cockroaches we observed as *B. asahinai;* the cuticular hydrocarbon profile of our specimens matched the *B. asahinai* profile (Carlson and Brenner 1988, Brenner et al. 1993) and excluded the possibility that the Texas specimens were *Blattella vaga* Hebard, *Blattella bisignata* (Brunner), *B. lituricollis, Blattella sauteri* (Karny), or *Blattella karnyi* (Princis), whose cuticular hydrocarbon profiles have been documented (Brenner et al. 1993). DNA sequences of the Texas specimens also matched the sequences of authentic *B. asahinai* collected in central Florida. Unfortunately, specimens were not available from Okinawa, Japan, the type locality of *B. asahinai.* We used *B. germanica* and *B. lituricollis* sequences to examine the degree of divergence of sequences within the closely related *Germanica*-Group. Maekawa and Matsumoto (2000) demonstrated the utility of the COII gene sequence for species identification between *B. germanica* and *B. lituricollis.* Our results support these findings and report, for the first time, sequence divergence between *B. asahinai* and both *B. germanica* and *B. lituricollis.* Furthermore, DNA sequencing of the mitochondrial COII gene confirms the species identity of the Texas *Blattella* species as *B. asahinai* given the exact haplotypic match with that of the most common variant found in *B. asahinai* from Florida. *B. asahinai* and *B. germanica* are most closely related (Roth 1985), and they even can hybridize in the laboratory; yet, they show sequence divergence at the COII gene. Therefore, it is unlikely that a more distantly related *Blattella* would share sequence identity with our specimens.

While the present article was in review, *Blattella* specimens from residential sites in Harris County, TX, were identified as *B. asahinai*, based on sequence similarity of the ITS1 region of nuclear rRNA to GenBank sequences (Mukha et al. 2002, Austin et al. 2007). We suggest that the geographic range of *B. asahinai* has expanded dramatically not only to southern Texas but also from residential to agricultural environments.

B. asahinai has been observed near the border with Mexico in large numbers for at least 2 yr, and it is likely that this cockroach is well established in northeastern Mexico. It is probable that these populations represent the leading front of a range extension of the original introduction in Lakeland, FL, because populations have been found in Georgia, Alabama (Hu et al. 2005), and in Harris County in eastern Texas (Austin et al. 2007).

Exclusively nocturnal activity in the soybean plant canopy most likely explains why *B. asahinai* had not been observed feeding on pests in annual crops in the United States, despite *B. asahinai* being present since 1986. Research on nocturnal predation is rarely conducted. Predation by starved *B. asahinai* on parasitized citrus aphids (*Toxoptera citricida* Kirkaldy) in the laboratory was reported by Persad and Hoy (2004), although aphid mortality in the presence of *B. asahinai* was not significantly different from controls when other food was present. Our observations of predation on lepidopteran eggs in soybean represent the first known observation of *B. asahinai* behaving as a beneficial insect. It is unclear what role *B. asahinai* plays within a crop (e.g., soybean), but predation on lepidopteran eggs in 2006 was significant. We do not know what range of insect pests this species consumes or whether predation is a consistent life history trait of this cockroach. However, the high frequency of predation by *B. asahinai* in our soybean fields suggests that the impact of *B. asahinai* on insect pests in crops should be investigated further.

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