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SYMPATRY OF *ORIUS INSIDIOSUS* AND *O. PUMILIO* (HEMIPTERA: ANTHOCORIDAE) IN NORTH CENTRAL FLORIDA

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ABSTRACT

Two closely related species of Anthocoridae, the minute pirate bugs *Orius insidiosus* (Say) and *O. pumilio* (Champion), were collected together from false Queen Anne's lace/ large bullwort (*Ammi majus*) planted on an organic farm in Gainesville, Alachua Co., FL., over a period of 5 successive weeks. The presumptive prey on the false Queen Anne's lace was a single species of thrips, *Frankliniella bispinosa* (Morgan). In the first 4 weekly collections from the flower heads, the densities of *O. insidiosus* adults were 2.7-fold higher than those of *O. pumilio*. No eggs or nymphs of either species were observed on the plants. Sex ratios (males:females) of 2.7 and 1.0 were observed for *O. insidiosus* and *O. pumilio*, respectively. A colony of *O. insidiosus* was established from field-collected specimens. The sex ratio of the F_1 generation from this colony was 1.0, suggesting that the skewed field sex ratio was not a genetic phenomenon. These data demonstrate that these sympatric predators coexist at least temporarily, feeding on pests of the false Queen Anne's lace.

Key Words: minute pirate bug, Queen Anne's lace, *Ammi majus*, *Frankliniella bispinosa*, thrips, sex ratio

RESUMEN

Dos especies de Anthocoridae cercanamente relacionados, the minute pirate bugs *Orius insidiosus* (Say) y el *O. pumilio* (Champion), fueron colectados en la false Queen Anne's lace/ large bullwort (*Ammi majus*) plantadas en una granja orgánica en Gainesville, Alachua Co., FL., en un periodo de cinco semanas consecutivas. La presunta presa de la false Queen Anne's lace fue una especie de trips, *Frankliniella bispinosa* (Morgan). En las primeras cuatro semanas de colecta en las flores, las densidades de los adultos de *O. insidiosus* fueron tres veces mas altas que las de *O. pumilio*. No se observaron en las plantas huevos ni ninfas de estas especies. Se observaron proporciones sexuales macho:hembra de 2.7 y 1.0 para *O. insidiosus* and *O. pumilio* respectivamente. Se estableció una colonia de *O. insidiosus* a partir de especimenes colectados en el campo y se encontró que la proporción sexual era de 1.0, sugiriendo que el sesgo en la proporción sexual encontrada en el campo no era un fenómeno genético. Estos datos demuestran que estos depredadores coexisten por lo menos temporalmente en simpatria.

Translation provided by the authors.

The control of arthropod pests in organic farming is predicated on ecologically sound management without the use of synthetic chemical insecticides (Zehnder et al. 2007). In addition to modifying cultural practices and vegetation management, inundative or inoculative release of biological control agents can be used to reduce the populations of pests. The release of generalist feeders such as anthocorid predators has been used with some efficacy in the control of aphids, leafhoppers, psyllids, thrips, whiteflies, mealybugs, and sciarid flies in both field and greenhouse crops (Stiling & Cornelissen 2005). Prior to implementing a release of a biological control agent, the onus on the supplier and grower is to identify the interactions of the agent within the environment in order to provide targeted pest management with minimal collateral ecological disruption. Thus, it is critical to understand the natural history and niche of the intended biological control agent.

Although the genus *Orius* is cosmopolitan, interactions of the many species within the genus have not been explored extensively within their

natural ranges. Two closely related species of predacious minute pirate bugs, *Orius insidiosus* (Say) and *O. pumilio* (Champion) (Hemiptera: Anthocoridae), have been reported in Florida. Needham (1948) observed that *O. pumilio* and *O. insidiosus* were common in south Florida on *Bidens pilosa* L. (Spanish needles, beggar's ticks) flower heads. Funderburk et al. (2004) observed that *O. pumilio* co-occurs with *O. insidiosus* in southern Florida.

In geographical regions where 2 closely related species co-occur, it is critical to have good taxonomic references to provide clear identification of the 2 species. *Orius insidiosus* and *O. pumilio* are readily distinguished by coloration (Herring 1966; Salas 1995) notably by the lack of pigmentation in all femora and basal antennal segments and lack of pigmentation in the cuneus section of the wings in *O. pumilio*. In addition, Herring (1966) and Salas (1995) described male genitalia as differentiating the species.

Here we report co-occurrence of the 2 species, *O. insidiosus* and *O. pumilio*, in weekly collections from flower heads of false Queen Anne's lace/large bullwort (*Ammi majus* L.) planted on an organic farm for harvest as an ornamental crop. Our observations raise questions concerning relationships between the 2 species, especially regarding resource partitioning, sympatry, and range of overlap in North Central Florida.

Materials and Methods

Insect Collection

Insects were collected from *A. majus* flower heads growing in a row (43 m x 5 m) on a certified organic farm of 4 ha located near Gainesville, FL <http://www.plowsharescsa.org>. The *A. majus* grew in 2008 by natural reseeding from plants grown from seeds planted in 2007 (seeds from Johnny's Selected Seeds, Winslow, ME). Collections of the insects on the flowers were made between 10:00 AM and 12:00 PM EDT at 4 sites about 12 m apart along the row. For collections made during the first 3 weeks of the study, insects were sampled by gathering 3-5 flower heads and quickly shaking them approximately 10 times into a muslin insect net. *Orius* spp. were aspirated as quickly as possible from the net into plastic vials (8.5 × 4.8 cm diam.). Collection from the flower heads was repeated until approximately 100 *Orius* were collected at each site. On the fourth week of collection, 3 flower heads were shaken 10 times into the net, which was repeated 8 times per site. The aspirator vial was removed and placed on ice following collections at each site.

On the fifth week only, a 3.8-L plastic bag was placed around a group of 3-5 flower heads at each of the 4 sample sites and the flower stems were cut 13-25 cm below the heads. The bag was promptly sealed and refrigerated within an hour of collection. Carbon dioxide ($CO_{2)}$ was applied to each bag to anesthetize the insects, and flower heads were shaken sharply to remove all insects, which were placed into 70% ethanol for preservation and counting. Flower heads of 1 bag from each of the 4 sites were examined for *Orius* eggs.

Taxonomic Identification

All taxonomic identifications were based on the morphological characters described in Herring (1966) and Salas (1995). For examination in the laboratory, live insects in each collection vial were warmed to room temperature and anaesthetized with CO_2, subsequently placed on a CO_2 table (Genesee Scientific, San Diego, CA), and segregated by species and sex. *Orius insidiosus* was identified by dark (melanized) femora, basal antennal segment and wing cuneus. *Orius pumilio* was identified by straw-colored, translucent (non-melanized) femurs and basal antennal segments, and a lightly melanized wing cuneus. In addition, dissected male genitalia of ethanol-preserved specimens of each species were differentiated by (1) the relatively longer flagellum of *O. pumilio* than *O. insidiosus*, (2) a spatulate appearance of the cone (lame) of *O. pumilio* versus a more pointed tip on the cone of *O. insidiosus*, and (3) an increased twist of the cone of *O. pumilio* that is curled under and lateral to the flagellum as opposed to a more aligned cone and flagellum in *O. insidiosus*. For verification of our identifications, representative specimens of males and females of each species were sent to the USDA, ARS Systematic Entomology Laboratory, Beltsville, MD, for positive identification and voucher archiving. From the field collected adults, a permanent colony of *O. insidiosus* was established.

Because *Orius* spp. are important predators of thrips throughout Florida (Funderburk et al. 2000; Hansen et al. 2003), thrips collected in representative samples, but not every sample, were identified to species to determine potential prey available for *Orius* spp. at the study site. Adult thrips were identified under a stereomicroscope based on morphological characters. Representative specimens were slide mounted and identifications were confirmed based on morphological keys (Moritz et al. 2001; Reed et al. 2006) and comparison with reference specimens under a compound microscope.

Insect Rearing

To establish a continuous colony of *O. insidiosus*, all field-collected adults from each week were

placed in culture. The cultures were maintained in Rubbermaid® (Fairlawn, OH) boxes (16-cm × 16-cm × 5-cm) that had been modified by inserting a mesh nylon screen (12 cm × 12 cm × 50 cm) in the lid. The box contained a 2-cm layer of buckwheat hulls with the addition of a few grains of bee pollen (Sunflower Health Foods, Gainesville, FL), 1 mL Hydrocapsules® (ARS Inc., Micanopy, FL), 1 mL *Ephestia kuehniella* Zeller eggs (Beneficial Insectary, Redding, CA), and 1 fresh green bean every other day. The cultures were kept in incubators at 25°C, 75-80% RH, and photoperiod of 14:10 LD. The eggs deposited on each bean were counted, and the beans were maintained in culture boxes with food and Hydrocapsules for the F_1 generation.

Statistics

Data were analyzed by ANOVA and *t*-test in Statistica v. 7.1 (StatSoft Inc., Tulsa, OK).

Results

The collection of the *Orius* spp. was initiated subsequent to the initial flowering of the false Queen Ann's lace, so there are no data concerning the colonization of the plants either by the thrips, *Frankliniella bispinosa* (Morgan), or the *Orius*. Although there were numerous other arthropod and arachnid species present on the flower heads, only the thrips and anthocorid species were actively collected. During the first 3 weeks of collection, the flowers were in full bloom and large numbers of *F. bispinosa* were present and the only thrips species present in the flower samples. During the 4 weeks of collection, 3.6 times as many adult *O. insidiosus* as *O. pumilio* were collected, a highly significant difference (Table 1; $P = 4 \times 10^{-9}$, $df = 1, F = 68.5$). Due to the method of collection, no nymphs of either species were recovered, and none were observed. Numbers of males, females, all adults, and the sex ratios (males:females) were compared between the 2 species. Over the entire study, all measures showed significant differences between the 2 species (Table 2). On a week-by-week basis the numbers of males significantly differed by species on each date assessed by paired two-tailed *t*-tests, while total numbers differed for weeks 2, 3, and 4, and females and sex ratios differed on weeks 3 and 4 (Table 1). By the fifth week, most of the flower heads had begun seed production and very few *F. bispinosa* were present. The numbers of *O. insidiosus*, *O. pumilio*, and other predators were correspondingly small (results not presented), with highly variable populations. Examination of peduncles, petioles, and stems of these plants did not show the presence of *Orius* eggs.

The sex ratio markedly differed between the 2 species over the entire 4-week period. The overall mean sex ratios were 2.7 for *O. insidiosus* and 1.0 for *O. pumilio* (Table 1). To test the possibility that the skewed sex ratios were the result of a genetic phenomenon, field collected adults of *O. insidiosus* were placed in laboratory culture and the sex ratio of the F_1 generation was established. The cumulative mean sex ratio for the F_1 generation from weeks 1-3 was 1.0 (Table 3).

Table 1. Population size and sex ratios of adult *O. insidiosus* and *O. pumilio* collected weekly from *Ammi majus*.

	Date Collected	Adults Collected**	Males	Females	Sex Ratio (♂:♀)
O. insidiosus					
Week 1	4/25/2008	307	226*	81	2.8
Week 2	5/02/2008	296*	246*	50	4.9
Week 3	5/09/2008	346*	229*	117*	2.0*
Week 4	5/16/2008	312*	213*	99*	2.2*
	Mean ± SD	315 ± 22	229 ± 14	87 ± 29	2.7 ± 1.6
	Total	1261*	914*	347*	
O. pumilio					
Week 1	4/25/2008	71	33*	38	0.87
Week 2	5/02/2008	94*	50*	44	1.1
Week 3	5/09/2008	91*	45*	46*	0.98*
Week 4	5/16/2008	92*	47*	45*	1.0*
	Mean ± SD	87 ± 11	44 ± 7	43 ± 4	0.99 ± 0.09
	Total	348*	175*	173*	

*Differences between species, within a week or totaled for all four weeks, are significant ($P < 0.05$) by *t*-test.

**On Week 4, flower-heads were counted (24 flower-heads/site, 4 sites), and collections yielded 4.2 ± 0.9 (mean ± SD, $n = 4$ sites) *Orius* per flower-head of both species.

TABLE 2. STATISTICS FROM T-TESTS COMPARING SPECIES FOR DEPENDENT VARIABLES OVER ALL 4 WEEKS.

	t-value	*n*	*df*	*P*	*F*-ratio Variances
Males	9.39	16	30	<0.001	26.27
Females	3.74	16	30	<0.001	10.60
Total	8.38	16	30	<0.001	16.40
Ratio (F:M)	5.66	16	30	<0.001	7.69

DISCUSSION

The limited sampling of *Orius* spp. conducted from the flowers of false Queen Anne's lace growing within an organic farm has raised several important questions about the population dynamics and natural history of these hemipteran predators. The flower heads contained large numbers of the thrips *F. bispinosa*, which served as presumptive prey for both species (Reitz et al. 2006). Both species were present during the first 4 weeks of collecting. The numbers of *O. insidiosus* adults frequenting the flowers were considerably larger than those of *O. pumilio* and the interspecies ratio remained constant over this time period. Even though these 2 species of anthocorids might be considered competitors for the same food resource, the relative abundance of the thrips apparently eliminated food as limiting factor, thus sufficiently removing competitive pressures and permitting both species to coexist within the same microenvironment. In spite of the abundance of food, no eggs or nymphs of either species were observed in the collections. Finally, by the fifth week the flowers had mostly progressed to seed production, and few thrips were observed. This corresponded with collection of only a few *Orius* from the sites, suggesting that they had moved elsewhere, possibly following their prey or fresh pollen source to another host plant. These observations suggest that the flower heads were aggregation sites for adult feeding and mating, and that other behaviors (especially oviposition) and life stages occurred elsewhere. Because sampling was restricted to these flowers, no conclusions can be drawn about the absolute size or geographic distribution of either population on the farm.

Importantly, the co-existence of adults of 2 closely related species in the same habitat, feeding on the same prey, establishes these anthocorids as sympatric species. That these are bona fide separate species is supported by clear morphological differences between the male genitalia of the 2 species in scanning electron microscope studies, and attempts to interbreed them have been unsuccessful (Shapiro & Shirk, unpublished data). Thus, *O. insidiosus* and *O. pumilio* are almost certainly distinct species. As noted above, the nymphal habitats and ovipositional habits of the 2 species may distinguish their ecological niches, and North Central Florida may represent a climatic limit for *O. pumilio*. The sex ratios differed significantly between the 2 species in each sample. Two thirds of the *O. insidiosus* population was male, with 5 times more males than the number of *O. pumilio* males. Only twice as many *O. insidiosus* females were collected compared to *O. pumilio*. The skewed sex ratio of *O. insidiosus* adults was most likely due to a behavioral pattern, or to differential mortality rather than genetically biased birth rates. When *O. insidiosus* were collected in the field and placed into laboratory culture in the same sex ratio, they produced an F_1 generation with a sex ratio of 1.0. Thus, the observed sex ratio for field collected *O. insidiosus* was not a genetic phenomenon but most likely the result of sampling, differential hatch rates or survival of 1 sex. Production of the more dispersing sex may be favored in low-quality habitats, while the philopatric sex would be favored in high-quality habitats (Julliard 2000; Leturque & Rousset 2003). More specifically with regard to parasitic insects, Dick & Patterson (2008) noted some factors that might affect sex ratios: sampling bias, differential longevity of the sexes, mobility, and local mate competition. They noted that sex ratios of bat flies (Diptera: Streblidae) are commonly skewed toward males. As with bat flies, *O. insidiosus* populations in flowers may be skewed simply because females must leave to oviposit while males remain at the feeding/mating sites. In addition, sampling was limited to the late morning hours, and behaviors such as oviposition may vary according to a circadian rhythm. In our laboratory colony, male *O. insidiosus* are more short-lived than females and more mobile (Shapiro, per-

TABLE 3. F_1 *O. INSIDIOSUS* LABORATORY COLONY PROGENY, SURVIVAL, AND SEX RATIOS.

		Adults Observed			
F_1 Progeny	Eggs Added	Males	Females	% Survival	Sex Ratio
Week 1	775	316	294	78.7%	1.1
Week 2	1120	196	213	36.5%	0.9
Week 3	1425	295	282	40.5%	1.0

sonal observations), contrary to the observed bias in *O. insidiosus* sex ratio.

What limits the range of these 2 species is unclear. *Orius insidiosus* clearly demonstrates reproductive diapause (Ruberson et al. 1991, 1998, 2000), a strategy for survival in temperate climates. Historical observations imply that *O. pumilio* is a tropical species (Henry 1988; Carpintero 2002), but it has not yet been studied regarding diapause status. Variability in climate may result in a dynamic latitudinal change in the range and in the competitive advantage of each species, and therefore in locations where interspecies interactions occur. The organic farm in this study hosts a particularly suitable habitat in which to study interspecies interactions. Fortuitously, false Queen Anne's lace also proved to be a suitable host plant for aggregation of these anthocorid predators and might serve as a good reservoir for insect predators and their prey when incorporated into the foliage of the farm, e.g., in flower strips (Zehnder et al. 2007; Jonsson et al. 2008).

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