EFFECT OF PREY SEX, DENSITY, AND AGE ON OVIPOSITION OF *CYBOCEPHALUS* SP. NR. *NIPPONICUS* (COLEOPTERA: CYBOCEPHALIDAE), A NATURAL ENEMY OF EUONYMUS SCALE (HOMOPTERA: DIASPIDIDAE)

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ABSTRACT

Cybocephalus sp. nr. *nipponicus* Endrody-Younga (Coleoptera: Cybocephalidae) females lay their eggs individually under the cover of scale insects, similar to some hymenopteran parasitoids. Because this beetle's oviposition occurs in close association with individual scale insects, qualities of individual scale insects or patches of scale insects are factors that can potentially be used by beetles to select oviposition sites. The effect of two such factors (scale sex and density) on the oviposition of *C.* sp. nr. *nipponicus* were evaluated in the laboratory using the euonymus scale *Unaspis euonymi* (Comstock) as the ovipositional prey. For comparison, the effect of scale sex, density, and age on beetle oviposition also was investigated for a second oviposition prey, San José scale (*Quadraspidiotus perniciosus* (Comstock)).

Oviposition rates for *C*. sp. nr. *nipponicus* were strongly affected by prey sex when ovipositing on euonymus scale, with 97% of all eggs being placed under covers of male scales. In contrast, beetles placed eggs in equal proportions under covers of mature (>29 days) female and the empty scales of emerged male San José scales. Oviposition rates were affected by San José scale density. There were significantly more beetle eggs laid in patches with more than 70 scales than in those with fewer scales. Increasing scale age positively affected beetle oviposition on San José scale. Fifty-five percent of all eggs were laid in the oldest group of scales (age 53-58 days), whereas no eggs were laid in the youngest group of scales (age 9-14 days).

Key Words: *Unaspis euonymi*, biological control, *Quadraspidiotus perniciosus*

RESUMEN

Las hembras de *Cybocephalus* sp. nr. *nipponicus* Endrody-Younga (Coleoptera: Cybocephalidae) ponen sus huevecillos enforma individual debajo de las cubiertas de insectos escama de escamas, en una forma similar a algunos parasitoides himenópteros. Como existe una fuerte asociación entre este coleóptero y las escamas, las características de dichas escamas, ya sea en forma individual o en grupo, son factores que potencialmente pueden ser utilizados por los coleópteros para seleccionar sus sitios de oviposición. El efecto de dos de estos factores (sexo y densidad de las escamas) sobre la oviposición de *C.* sp. nr. *nipponicus* fueron evaluados en el laboratorio usando la escama de “euonymus,” *Unaspis euonymi* (Comstock), como el huésped de oviposición. Para hacer comparaciones, los efectos del sexo, densidad, y edad de las escamas también fueron investigados sobre un segundo huésped de oviposición, la escama de San José (*Quadraspidiotus perniciosus* (Comstock)).

Cuando los coleópteros *C.* sp. nr. *nipponicus* ovipositaron en la escama de euonymus, la tasa de oviposición fué fuertemente afectada por el sexo de su huésped, con el 97% de todos los huevos siendo depositados debajo de escamas macho. En contraste, los huevos fueron puestos en igual proporción debajo de escamas hembra (>29 días) y bajo las cubiertas vacías de escamas macho cuando su huésped fue la escama de San José. Hubo un número significantemente mayor de huevos en grupos con más de 70 escamas, que en grupos con menos escamas. El aumento en la edad de las escamas de San José afectó positivamente la oviposición de los coleópteros. El 55% de todos los huevos fue ovipositado en el grupo más viejo de escamas (entre 53 y 58 días de edad), mientras que ningún huevo fue ovipositado en el grupo más joven de escamas (entre 9 y 14 días de edad).

Cybocephalus sp. nr. *nipponicus* Endrody-Younga was introduced from Korea and China into the United States as part of a USDA biological control project against euonymus scale, *Unaspis euonymi* [Comstock] (Homoptera: Diaspididae) (Drea & Hendrickson 1988). Drea & Carlson (1988) reported establishment of *C.* sp. nr. *nipponicus* at three release sites in the United States and the subsequent reduction of euonymus scale populations at these sites.

Cybocephalid beetles are predators that lay their eggs individually under the cover of diaspidid scales (Blumberg 1973, Blumberg & Swirski 1982, Nohara & Iwata 1988), similar to some hymenopteran parasitoids. Alvarez & Van Driesche (1998) studied aspects of the biology of *C*. sp. nr. *nipponicus*, which was in broad terms similar to biologies of *Cybocephalus nigriceps nigriceps* (Sahlberg), *Cybocephalus micans* Reitter, and *Cybocephalus gibbulus* (Erichson) (Blumberg & Swirski 1982; Nohara & Iwata 1988). *Cybocephalus* sp. nr. *nipponicus* females mate within two days after adult emergence and begin laying eggs beneath prey scales about four days later (Alvarez & Van Driesche 1998). Beetles lack ovipositors; however, *C.* sp. nr. *nipponicus* females possess a telescopic abdominal segment which they use to insert eggs under a scale insect's cover. After contacting prey scales, female beetles drum scale covers and then chew a hole through the cover of the scale. Beetles then turn around and insert one or more eggs under the cover of the scale insect through the holes that the beetles cut in the cover. Sometimes eggs are inserted instead under the edge of the cover without cutting a hole. Rarely females may lay eggs under the cover of scale insects that they have previously eaten. The total number of eggs laid per female varies substantially and lifetime fertility is largely a matter of how long a female survives (Alvarez & Van Driesche 1998). Daily fertility (calculated as the mean fertility, divided by the mean longevity) (Bellows *et al*. 1992) for *C.* sp. nr. *nipponicus* is 3.11 (Alvarez & Van Driesche 1998).

Because this beetle's oviposition occurs in close association with individual prey, qualities of individual scale insects or patches of scale insects could be factors used by beetles to choose oviposition sites. The objective of this study was to clarify how *C.* sp. nr. *nipponicus* oviposition varied in response to changes in scale sex and density, using *Unaspis euonymi* as the ovipositional prey. A second ovipositional prey, San José scale, was also examined for the effect of prey sex, density, and age.

MATERIALS AND METHODS

Source of Beetles and Colony Maintenance

A *C.* sp. nr. *nipponicus* colony was started at the University of Massachusetts with specimens imported from Beijing, China. Voucher specimens have been placed in the collection of the University of Massachusetts. San José scale, a natural prey species of *C.* sp. nr. *nipponicus*, reared on butternut squash (*Cucurbita* sp.), was used as prey for the beetles in the laboratory. Rearing procedures for San José scales and *C.* sp. nr *nipponicus* beetles are given in Alvarez & Van Driesche (1998).

Description of Experimental Arena and Environmental Conditions

All studies were conducted in temperature-controlled cabinets (22 ± 1°C) with a 14:10 L:D photoperiod. Because euonymus scale, the field prey of greatest interest, could not be reared apart from euonymus shrubs, naturally infested euonymus plants, *Euonymus fortunei* (L.) were collected in Amherst, Massachusetts and taken to the laboratory. Male euonymus scales were distinguished from female scales by their smaller size and distinct white color. Male scales develop a white, waxy protective covering over their bodies, while female scales develop a brown covering similar to an oyster's shell. Twig sections (10 cm long) bearing female and male euonymus scales were cut as needed for particular experiments and exposed to beetles in the laboratory. Each twig was placed in a 4-cm water vial and sealed with Parafilm®, leaving 6 cm of twig exposed. Vials with twigs were placed individually within ventilated plastic Petri dishes to confine test beetles.

A standard experimental arena was used for the experiments using San José scale. The arena consisted of 4-cm diameter circles delimited with modeling clay on test squash. Arenas were inoculated with first instar San José scale nymphs ("crawlers") as needed for particular experiments. Male San José scales were distinguished from female scales by their smaller size and distinct oblong shape of the scale covers. Adult male scales emerged approximately 20 days after crawler inoculation at 22°C. Scale covers remained intact, allowing exact counts of original numbers of scales in particular arenas, as needed for some experiments. Therefore, in these experiments, "male scales" consisted of scale covers from which an adult male had emerged. Arenas were numbered and covered with 4 cm diameter plastic Petri dishes ventilated with organdy to confine test beetles. Petri dishes were attached to the squash by pressing them into the rings of modeling clay.

Effect of Scale Sex

To assess the effect of euonymus scale sex on beetle oviposition, fifteen *C.* nr. *nipponicus* couples (one female and one male) were confined individually in ventilated plastic Petri dishes for two days. Each Petri dish contained a twig section as described previously, bearing euonymus scale females and males. After beetles were removed from the Petri dishes, euonymus twigs were examined by turning over covers of all scales to detect beetle eggs. The number of beetle eggs under covers of female and male scale insects was counted on each twig. A Chi-square test was performed to determine if beetle oviposition choice was affected by the sex of the euonymus scale preys.

To provide a comparison of *C.* nr. *nipponicus* oviposition on San José scale, mating pairs of beetles were chosen from laboratory colonies and confined in experimental arenas containing mixtures of adult male and female San José scales of standard age (25 ± 5 days). Beetles were removed from experimental arenas after two days and scale patches were examined by turning over covers of all scales to locate newly laid eggs. For every scale in the patch, we recorded the scale's sex and whether or not beetle eggs had been deposited under the scale cover. For scales with beetle eggs, the number of eggs per scale was also recorded. Sixty-nine scale patches were tested, each with a single pair of beetles. A Chi-square test was performed to examine if sex of adult San José scales affected beetle oviposition choice.

Effect of Scale Density

The effect of euonymus scale density on beetle oviposition rates (per 48 h) was examined using the same *E. fortunei* twig sections described earlier. Ten *C.* nr. *nipponicus* couples (one female, one male) were confined individually in ventilated Petri dishes on each twig. After two days, beetles were removed and the number of scale insects and the number bearing beetle eggs were counted on each twig. In addition, five pairs of beetles were placed individually as pairs, in twigs with no scales. To examine the effect of scale density on beetle oviposition a linear regression was performed.

The effect of San José scale density on beetle oviposition rates (per 48 h) was examined using the same experimental arenas described earlier and San José scale insects of a standard age (25 ± 5 days). The number of scale insects in each patch varied randomly, and was determined by the number of crawlers that successfully settled in each patch. Scales in patches consisted of mixtures of covers of emerged male scales and live adult female scales. One mating pair of beetles was chosen from laboratory colonies and confined over a patch of scale insects in an experimental arena for two days. Beetles were then removed and all scales examined. This process was replicated 70 times. In addition, ten pairs of beetles were placed individually as pairs, in arenas with no scales. Arenas with no scales included honey as food for the beetles. In arenas with scale patches, the number of scales in each patch and the number bearing beetle eggs were recorded. For scales with beetle eggs, the number of eggs per scale was recorded. Patches with scales were divided into five categories of scale density (11-40, 41-70, 71-100, 101-130, and >131) and the number of beetle eggs laid per patch was compared between groups in a one-way ANOVA. Significant differences were identified using a least significant difference (LSD) comparison test.

Effect of Scale Age

To test the effect of San José scale age on beetle oviposition, patches of scales of five different ages were exposed together in the same experimental arena. To construct a scale population with members of five different ages, each arena was divided into 5 equal wedges (72° each), and inoculated in five steps, one section every 11 days. Two days after a section was inoculated (at which time crawlers had settled), it was covered with organdy to prevent crawlers from later inoculations from entering previously inoculated sections. In this way, forty-five days after the first inoculation there were five scale patches in each arena with, respectively, scales that were 1, 12, 23, 34 or 45 days old. Following the last inoculation, scales were allowed to develop for eight more days. The experiment was replicated twenty-four times, with four replicates initiated daily over a period of six days. Each arena contained one mating pair of beetles. Ages of scales in the experiment fell into five ranges: 9 to 14 days, 20 to 25 days, 31 to 36 days, 42 to 47 days, and 53 to 58 days. Beetles were removed after two days and scale patches were examined and the number of scales per wedge and the number of beetle eggs were recorded within each age group and compared with one-way ANOVA and a least significant difference (LSD) comparison.

Results

Effect of Scale Sex

When presented with euonymus scale as prey, there were significantly more eggs laid under male scales (97.3%) than under females (2.7%) ($\chi^2 = 238.41$; $P < 0.01$). The percentages of male and female scale receiving *C.* sp. nr. *nipponicus* eggs were, respectively, 4.89 and 0.038%. The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on euonymus scales in a two day period was 4.9 ± 0.76 ($\bar{x}$ ± SE; n = 15). The total numbers of female and male euonymus scales across all replications in the test were, respectively, 5283 and 1544. All the 74 eggs laid by the 15 females occurred singly.

The total numbers of female and male San José scales across all replications in the test were, respectively, 2505 and 2930. There was no difference in the total number of eggs laid in a two-day period under female and male scales (48.5% and 51.5%, respectively). Of 715 eggs laid by 69 females, 662 (92.6%) occurred singly, and 46 (6.4%) were in pairs. One group of three eggs (0.4%) and one of four eggs (0.6%) were also found. Male San José scales never received more than one egg. The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid under San José scales in a two day period was 10.4 ± 0.8 ($\bar{x}$ ± SE; n = 69).

The percentage of female and male San José scales receiving *C.* sp. nr. *nipponicus* eggs were, respectively, 13.9 and 12.6%. Looking only at the scales that received *C.* sp. nr. *nipponicus* eggs, the average numbers of eggs per female and per male scale were, respectively, 1.09 and 1.00. Chi-square analysis suggested that there was no relationship between beetle oviposition and the sex of the San José scale prey ($\chi^2 = 0.12$; $P = 0.7258$).

Effect of Scale Density

The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on euonymus scales in a two day period was 5.8 ± 1.0 (n = 10). All *C.* sp. nr. *nipponicus* eggs occurred singly and no significant relationship was observed between scale density

TABLE 1. EFFECT OF SAN JOSE SCALE DENSITY ON *CYBOCEPHALUS* SP. NR. *NIPPONICUS* OVIPOSITION IN A TWO DAY PERIOD AT 22 ± 1°C.

Scale density groups	n	Scale density Mean number (S.E.)	Oviposition Mean number (S.E.)
0	10	0.0f	0.0c
11-40	11	29.9e (2.4)	5.5b (1.9)
41-70	24	55.8d (1.9)	8.3b (1.0)
71-100	17	85.4c (2.1)	12.7a (1.9)
101-130	12	115.9b (2.5)	15.3a (2.2)
>131	6	157.2a (9.0)	15.5a (1.8)

Column values with different letters are significantly different according to one-way ANOVA and LSD criterion at the 0.05 level.

per twig and beetle oviposition. The average number of euonymus scales per twig was 493.10 ± 55.38. No eggs were laid on twigs with no scales.

The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on San José scales in a two day period was 10.7 ± 0.9 (n = 70). The number of eggs laid increased with scale density, although there were only two groups which differed significantly, densities over 70 scales per patch and densities below 70 ($F = 4.49$ df = 6,64; $P < 0.05$). The highest oviposition rate (per 48 h) (15.5 ± 1.8 eggs) was recorded for the highest scale density (Table 1). No eggs were laid in arenas with no scales.

Effect of Scale Age

The age of San José scales affected oviposition rates by *C.* sp. nr. *nipponicus* beetles. Both the number and percentage of beetle eggs laid increased with increasing scale age (Table 2). Although scale density varied slightly between treatments (because scale numbers were not standardized after inoculations), there were no statistically significant differences among numbers of scales of the different ages across the experimental arenas ($F = 0.64$; df = 4, 115; $P = 0.637$) (Table 2). Therefore, effects of scale age on beetle oviposition rates could be analyzed without adjustments for potential effects of scale density. A statistically significant difference was observed in the preference of *C.* sp. nr. *nipponicus* to oviposit in scales of different ages. Fifty-five percent of the total eggs were laid in the oldest group of scales (age 53-58 days), whereas no eggs were laid in the youngest group of scales (age 9-14 days) ($F = 30.21$; df = 4,115 $P < 0.05$) (Table 2).

DISCUSSION

Species of Cybocephalidae have been successfully used for the biological control of diaspididae scales (Blumberg & Swirski 1982). One of the reasons for this success could be the placement of the eggs under scale covers and the subsequent feeding by larvae under scale covers, both of which are likely to protect these stages from attack by other predators and exposure to pesticides (Alvarez & Van Driesche, 1998).

Models of reproductive behavior of predators generally assume that egg production is directly regulated by the rate of food consumption (Beddington *et al*. 1976; Gutierrez & Baumgaertner 1984), which is unlikely to be the most important factor

TABLE 2. EFFECT OF AGE OF SAN JOSÉ SCALE INSECTS ON OVIPOSITION OF *CYBOCEPHALUS* SP. NR. *NIPPONICUS* BEETLES IN A TWO DAY PERIOD AT 22 ± 1°C.

	Mean number (S.E.)		
Scale age (Days)	Oviposition (No. eggs laid/wedge)	Total scales per wedge	% of all eggs found
9-14	0.0c (0.0)	43.5a (4.4)	0.0c (0.0)
20-25	1.1bc (2.2)	45.0a (0.1)	8.8bc (3.0)
31-36	1.9b (1.8)	41.0a (4.1)	18.1b (3.9)
42-47	2.5b (3.5)	40.3a (4.6)	18.3b (4.2)
53-58	5.3a (3.4)	50.5a (5.8)	54.8a (5.5)

Column values with separate letters were significantly different in a one-way ANOVA with an LSD criterion at the 0.05 level.

governing the oviposition strategy of *Cybocephalus* nr. *nipponicus* beetles. Availability of food is not the only factor involved in egg production and oviposition of *C.* nr *nipponicus*. Female beetles were able to respond to the absence of prey, withholding eggs for two days, even though they were supplied with honey and water as food sources. Therefore, oviposition is likely in part to be triggered by certain qualitative features of scale populations. Ladybird beetles have been shown to respond to cues indicating the presence of their prey (i.e., aphids). Some female coccinelids can withhold eggs for several hours in the absence of such cues (Evans & Dixon 1986). It is possible that the survival of *C.* nr. *nipponicus* in the field depends on the presence of scale prey at the time of oviposition.

Also, as demonstrated by Mills (1982) with ladybird beetles, *C.* nr. *nipponicus* females responded to increases in scale density abundance by increasing oviposition up to a maximum (71 scales per experimental arena). At still higher scale densities, the rate of egg production is constant and independent of prey abundance. Unlike coccinellids, cybocephalids are able to maintain their populations at low scale densities and thus help keep scale populations below damaging levels (Alvarez & Van Driesche 1998).

In some cases, *C.* nr. *nipponicus* eggs were found in groups under scale covers. The number of eggs laid by females under a given scale may depend on several factors, including the number of available scales in a patch. It seems that if females do not find new scales to oviposit under, they lay their eggs under scales on which oviposition has already occurred.

Oviposition rates for *C.* sp. nr. *nipponicus* beetles were strongly affected by the sex of the prey when oviposition occurred on euonymus scale, with nearly all eggs being laid under male scales. In contrast, beetles placed eggs in equal proportions under female and male San José scales. While cover thickness was not measured in these experiments, difference in cover thickness between these two scale species and their sexes could explain this oviposition preference. Female and male of San José scale are much more similar in size and thickness of covers than are the different sexes of euonymus scale (Alvarez, unpublished data). Honda and Luck (1995) noted that the physical characteristics of the scale cover affects the successful suppression of diaspidid scales by coccinellid predators.

When presented with scales of different ages in one patch, *C.* nr. *nipponicus* preferred to oviposit under the oldest scales. Alvarez & Van Driesche (1998) showed previously that beetle larvae need to consume more prey if feeding on younger scales than if feeding on older scales. Furthermore, the highest larval survival (to the adult) occurred when larvae fed on scales older than 30 days (Alvarez & Van Driesche 1998). Oviposition under covers of older scales may therefore suggest that the survival of beetle offspring depends on the placement of the eggs under scales best able to provide a suitable food resource to beetle larvae, resulting in enhanced larval survival. As noted by Blumberg & Swirski (1982) when working with *Cybocephalus micans* Reitter and *C. nigriceps nigriceps* (Sahlberg), *C.* nr. *nipponicus* eggs were found to be laid under bodies of dead female scales only rarely. The causal mechanism, which affects beetle selection of live, older scales, remains unknown.

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