RELEASE, ESTABLISHMENT AND SPREAD OF ASIAN NATURAL ENEMIES OF EUONYMUS SCALE (HOMOPTERA: DIASPIDIDAE) IN NEW ENGLAND

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ABSTRACT

Between 1990 and 1995, the USDA/APHIS National Biological Control Laboratory in Niles, MI, Texas A&M University, and the University of Massachusetts conducted a biological control introduction program against the Asian diaspidid scale insect *Unaspis euonymi* (Comstock), a pest of woody landscape plants. Two species of predators (*Chilocorus kuwanae* Silvestri, Coleop.: Coccinellidae and *Cybocephalus* sp. nr. *nipponicus* Enrody-Younga, Coleop.: Cybocephalidae) and three aphelinid parasitoids (*Encarsia* sp. nr. *diaspidicola* [Silvestri], *Coccobius* sp. nr. *fulvus* [Compere et Annecke], and *Aphytis* sp.) were collected near Beijing, China and released in southern New England. We report establishment of *C. kuwanae*, *C.* sp. nr. *nipponicus* and *Coccobius* sp. nr. *fulvus* in Massachusetts. *Chilocorus kuwanae* has spread throughout southern New England and the proportion of euonymus shrubs in landscape-level surveys bearing *C. kuwanae* stages was positively related to scale density, with the coccinellid present on 1.1%, 6.3%, 12.5%, and 26.3% of shrubs whose scale populations were classified as none, light, medium, and heavy, among 4843 plants examined from 1992-1994 in Massachusetts, Connecticut, and Rhode Island. *Cybocephalus* sp. nr. *nipponicus* and *C.* sp. nr. *fulvus*, while established at some release sites, have been observed to spread to new locations in only one and two instances, respectively. *Encarsia* sp. nr. *diaspidicola* was recovered at some release locations, but establishment is uncertain. No recoveries were made of the *Aphytis* sp. parasitoid, but this species was released later than the other species and further recovery efforts are needed.

Key Words: *Chilocorus kuwanae*, *Cybocephalus* sp. nr. *nipponicus*, *Coccobius* sp. nr. *fulvus*, biological control, establishment

RESUMEN

Entre 1990 y 1995 el Laboratorio Nacional de Control Biológico del USDA/APHIS en Niles, Michigan, la Universidad de Texas A&M, y la Universidad de Massachusetts dirigieron un programa de control biológico de introducción en contra de la escama asiática *Unaspis euonymi* (Comstock) (Diaspididae), una plaga que ataca arbustos leñosos utilizados en arreglos de jardinería. Dos especies de depredadores (*Chilocorus kuwanae* Silvestri, Coleop.: Coccinellidae y *Cybocephalus* sp. nr. *nipponicus* Endrody-Younga, Coleop.: Cybocephalidae) y tres parasitoides de Hymenoptera: Aphelinidae (*Encarsia* sp. nr. *diaspidicola* (Silvestri), *Coccobius* sp. nr. *fulvus* (Compere et Annecke), y *Aphytis* sp.), fueron colectados cerca de Beijing, China, y liberados en el sur de New England. Reportamos el establecimiento de *C. kuwanae*, *C.* sp. nr. *nipponicus* y *Coccobius* sp. nr. *fulvus* en Massachusetts. *Chilocorus kuwanae* se ha extendido por todo el sur de New England; las proporciones de arbustos de euonymus muestreados

en jardines con estadíos de *C. kuwanae* resultaron estar relacionados estadísticamente en forma positiva con la densidad de la escama, con la presencia de la coccinela en 1.1%, 6.3%, 12.5%, y 26.3% de los arbustos con poblaciones de escamas clasificadas como nula, ligera, mediana, y fuerte en 4,843 plantas examinadas en 1992-1994 en Massachusetts, Connecticut, y Rhode Island. *Cybocephalus* sp. nr. *nipponicus* y *C.* sp. nr. *fulvus*, aunque se establecieron en algunos sitios donde se realizaron liberaciones, han sido observados en otros sitios en sólo una y dos ocasiones respectivamente. *Encarsia* sp. nr. *diaspidicola* fué recolectada en varias localidades donde liberaciones fueron realizadas, pero su establecimiento no está confirmado. Recolectas del parasitoide *Aphytis* sp. no se han logrado, pero como esta especie fué liberada más tarde que las otras especies, es necesario que se realicen más esfuerzos de recolección en el futuro.

Euonymus scale, *Unaspis euonymi* (Comstock), is an exotic diaspidid scale of Asian origin that feeds on foliage and stems of woody landscape plants in the United States. In New England, the species overwinters as mated adult females, and eggs are produced in the spring. Three generations occur yearly. Major host plants are species of *Euonymus*, many of which were imported from Asia (Flint 1983) and are widely planted in urban areas (Gill et al. 1982). Effective natural enemies of euonymus scale were not present in North America before 1980, when USDA and state cooperating entomologists began the importation of predators and parasitoids from Korea (Drea & Hendrickson 1988, Hendrickson et al. 1991). From 1991-1994, collections of euonymus scale were made in the vicinity of Beijing, China and sent to M. Rose at Texas A&M University for quarantine and initiation of natural enemy cultures. Five species of scale natural enemies were recovered and released in New England: *Chilocorus kuwanae* Silvestri (Coleop.: Coccinellidae), *Cybocephalus* sp. nr. *nipponicus* Endrody-Younga (Coleop.: Cybocephalidae), two internal parasitoids, *Coccobius* sp. nr. *fulvus* (Compere et Annecke), *Encarsia* sp. nr. *diaspidicola* (Silvestri), and an external parasitoid, *Aphytis* sp. (all, Hymenoptera: Aphelinidae).

Chilocorus kuwanae is a multivoltine coccinellid that feeds on high density populations of various species of diaspidid scales (Nohara & Iwata 1989, Bull et al. 1993). *Cybocephalus* sp. nr. *nipponicus* is a much smaller predator that oviposits under individual scales, with the larva feeding sequentially on a small number of scales over the course of its development (Alvarez et al. in press). Of the three aphelinid parasitoids, only one, *Coccobius* sp. nr. *fulvus*, has received previous study. An internal parasitoid, this species parasitizes adult female scales, both before and after development of scale eggs (Takagi 1991).

Chilocorus kuwanae and *C.* sp. nr. *nipponicus* from Korea were established in the northeastern United States earlier (Drea & Carlson 1987, 1988). We report further releases of these predators in New England, releases of three species of parasitoids, establishment of *C. kuwanae*, *C.* sp. nr. *nipponicus*, and *C.* sp. nr. *fulvus* in Massachusetts, and estimates of rates of occurrence of *Chilocorus kuwanae* in southern New England on landscape euonymus plants in relation to scale density.

MATERIALS AND METHODS

Collection and Laboratory Rearing

In 1990, adult *Chilocorus kuwanae* feeding on euonymus scale on *Euonymus* spp. near Beijing, China were collected and shipped to the USDA/ARS quarantine facility

in Newark, DE, where the coccinellid was identified and bred for one generation prior to release from quarantine. This colony was then used to make releases in southern New England starting in 1991.

Each year from 1991-1994, 3-5 shipments of *Euonymus* sp. branches infested with euonymus scale were sent by collectors in China to a quarantine laboratory at Texas A&M University. Collections were made in various locations within 200 km of Beijing, by Mr. Shen Zhicheng (1991), Mr. Du Yongjun (1992, 1993) and Mr. Zhao Youfou (1994). In each year, collections were made in late April-early May of overwintered adult female scales and then again in summer and early fall. In this manner, different life stages of scale predominated in different collections, allowing opportunity to encounter parasitoids associated with various life stages.

Four of the five species of natural enemies obtained could be reared under laboratory conditions on San José scale (*Quadraspidiotus perniciosus* [Comstock]) and field collected stock was used to initiate laboratory cultures on this alternate host. Both parasitoids emerging from field-collected scales and from laboratory rearing were used for field releases, except for *C.* sp. nr. *fulvus* which could only be reared on euonymus scale. All *C.* sp. nr. *fulvus* released were adults that emerged from immatures collected in China.

In addition to the euonymus scale natural enemies obtained from China, two species of natural enemies (*C. kuwanae* and *C.* sp. nr. *nipponicus*) originally collected in Korea were also obtained from USDA entomologists from earlier sites of establishment in the Washington, D.C. area. Releases of these predators were made in Massachusetts in 1988 and 1989. Subsequent collection of these same natural enemies in China was intended to find populations of these agents from areas more climatically similar to southern New England, as well as to locate new agents.

Field Releases

Releases were made in Massachusetts, Connecticut, and Rhode Island on *Euonymus fortunei* (Turz.) Hand.-Mazz. and *Euonymus europaeus* L. plants infested with medium to heavy populations of euonymus scale in urban or suburban locations from 1991 to 1995. Releases of *C. kuwanae* included adults, older larvae, and pupae. Mobile stages were allowed to crawl from opened 0.5 liter cardboard containers placed in euonymus plants. All other agents were released as adults by fixing open vials or cups containing parasitoids or *C.* sp. nr. *nipponicus* onto infested shrubs and allowing adults to walk or fly out.

Assessment of Establishment

For *C. kuwanae*, establishment was confirmed by visual inspection of shrubs at release sites to detect larvae, pupae, or adults, which were readily observed. *Chilocorus* adults were identified to species by examination of the pronotal punctation pattern to separate the released species from the native species *Chilocorus stigma* Say, which was occasionally encountered feeding on euonymus scale (Drea & Carlson 1987). Fifteen release sites (three each in western, central and eastern Massachusetts, Connecticut and Rhode Island) were visited every three weeks for 1-3 years, depending on survival of the shrub at each site, and the number of *C. kuwanae* life stages (larvae, pupae, adults) seen in three 5-min counts was recorded as an index of coccinellid population increase.

For *C.* sp. nr. *nipponicus*, establishment was confirmed by recovery of adult specimens, relying primarily on detection of males, which have a beige head and pronotum

and black body, and their comparison to voucher specimens. At some locations, establishment of *C.* sp. nr. *nipponicus* was detected by holding scale-infested twigs in cardboard cartons for three weeks and later noting the presence of adults or pupae in the rearing container.

Establishment of released parasitoid species was assessed by either rearing or dissection. Rearing of scales was done by holding cut twigs in 0.5 liter cardboard containers with ventilated tops at 21-27°C for three weeks. Material in the bottom of the rearing containers was then examined for dead adult parasitoids. Parasitoids were identified by comparison with voucher specimens.

In 1994, dissection of fully developed third stage female scale insects from 18 locations in Massachusetts was used to detect immature parasitoids. Samples were collected every three weeks from April through October. Larval and egg stages of *Encarsia* sp. nr. *diaspidicola* could not be separated from those of *Aspidiotiphagus* sp., a preexisting euonymus scale parasitoid in the United States that was common in southern New England, and therefore dissection was not useful in detecting this species. However, larvae of *Coccobius* sp. nr. *fulvus* were distinctively longer and more thread-like than larvae of either *Aspidiotiphagus* sp. or *Encarsia* sp. nr. *diapsidicola*, and could be reliably recognized. Pupae of *Coccobius* sp. nr. *fulvus* were black in contrast to the yellow-brown or striped pupae of *Encarsia* sp. nr. *diaspidicola* and *Aspidiotiphagus* sp. Pupal exuviae of *Coccobius* sp. nr. *fulvus* were completely black and easily distinguished from those of the other parasitoids. Therefore, *C.* sp. nr. *fulvus* could be reliably detected in samples from release sites by finding their larvae, pupae, or pupal exuviae. Rates of parasitism of the preexisting parasitoid *Aspidiotiphagus* sp. in these samples were also recorded and are reported to provide comparisons to future samples after introduced parasitoids have had sufficient time to reach their maximal levels of impact. Voucher specimens of all five natural enemies have been deposited in the insect collection of the U.S. Natural History Museum.

Rates of *C. kuwanae* Presence on Landscape Plants

To determine how widespread *C. kuwanae* had become following its establishment, a total of 4843 euonymus plants were examined in surveys conducted in Massachusetts, Connecticut, and Rhode Island from 1992 to 1994. Landscape euonymus plants (*E. fortunei* and *E. europaeus*) were located throughout each state. In Massachusetts, where three quarters of all surveyed plants were located, surveys were conducted yearly in an average of 54 towns in eleven counties. Each shrub was classified by scale infestation level category and the presence or absence of *C. kuwanae* life stages in 2 minute inspection periods was noted. Scale infestation categories were as follows: *none*—close inspection fails to reveal any scales; *light*—the shrub from one meter away appears uninfested but close inspection of the undersides of leaves reveals the presence of scattered second stage male scales (the most abundant, easily visible stage); *medium*—the shrub is visibly infested in casual inspection, but scales do not encrust stems, nor is die back of limbs present; and heavy-scales encrust stems and foliage and are immediately visible from a distance, die back of limbs is common.

Statistical Analysis

Chi square tests were performed on data to determine relationships between presence of *C. kuwanae* beetles and scale infestation levels on shrubs; and, for other data, to determine the relationship between the presence of the parasitoid *Aspidiotiphagus* sp. and scale infestation levels on shrubs (Daniel 1987). Simple linear regression was

used to assess the relationship between the post-release counts of the number of *C. kuwanae* and numbers released at sites to determine if numbers of beetles released or differences in site features were more important to *C. kuwanae* population growth.

RESULTS

Releases

In 1988 and 1989, 400 adults of a Korean population of *C. kuwanae* were released in Massachusetts at 15 sites. In 1991 and 1992, 2535 adults, larvae, or pupae of Chinese *C. kuwanae* were released at 25 sites in southern New England (Massachusetts, Connecticut, or Rhode Island). From 1991 to 1993, 675 adults of *C.* sp. nr. *nipponicus* from Korea and 945 from China were released at 17 sites in southern New England (Fig. 1b). From 1991 to 1994, 3862 adults of *C.* sp. nr. *fulvus* were released at 11 sites in Hampshire and Franklin Counties in Massachusetts (Fig. 1c, map shows towns used for releases; some towns had several release sites). From 1993 to 1995, a total of 12,966 adults of *E.* sp. nr. *diaspidicola* were released at 27 sites in eight counties in Massachusetts and one county in Connecticut (Fig. 1d). In 1994 and 1995, 801 adults of Aphytis sp. were released at five sites in two Massachusetts counties (Fig. 1e).

Establishments

Chilocorus kuwanae (Chinese strain) established at most release sites, but population increase at sites varied greatly. Of 15 sites followed in detail, the beetle established at all 15, following release of various numbers. Peak numbers of *C. kuwanae* life stages counted per five minute observation varied from 5 or fewer at three sites where the beetle scarcely persisted, to over 50 at five sites where beetle increased substantially. No relationship was observed in simple linear regression between numbers released per site and subsequent peak counts of beetles ($R^2 = 0.002$, $Y = 30.37 + 0.007X$, with X and Y as in Fig. 2), suggesting that site factors other than initial release rate were primarily responsible for beetle success at individual sites (Fig. 2).

Cybocephalus sp. nr. *nipponicus* was encountered at five of the seventeen release sites in the year following release, indicating successful establishment. Same-season reproduction of the beetle was observed at ten other sites (Fig. 1b).

Coccobius sp. nr. *fulvus* was recovered in 1994 from six of eleven release sites (from releases in either 1992 or 1993), indicating establishment (six sites, but only four towns, Fig. 1c). Same-season reproduction was observed at one additional location. Of 155 wasps obtained in rearing samples, 109 (70%) were female.

Encarsia sp. nr. *diaspidicola* was recovered in 1994 from two of ten 1993 release sites. Same-season reproduction was observed in 1993 or 1994 at twelve other sites (Fig. 1d—note, some locations marked on the distribution map include several sites). Establishment of this species remains uncertain.

Aphytis sp. releases were made later than those of other species. Recovery efforts in 1995, the last year of the study were unsuccessful and the status of this species is unknown.

Spread of Released Species

Of the five species released, *C. kuwanae* has achieved the most extensive range in southern New England (Fig. 1a). In 1992-1994 surveys of randomly selected land-

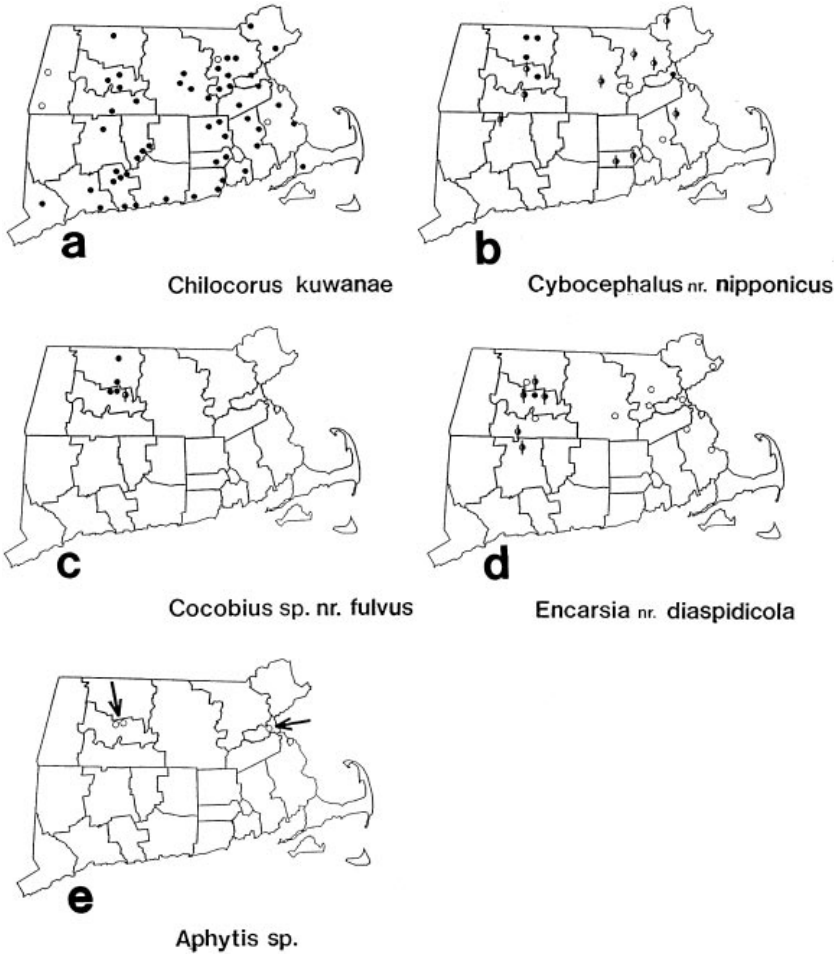

Fig. 1. Distribution in southern New England of *Chilocorus kuwanae* Silvestri (a); *Cybocephalus* sp. nr. *nipponicus* Enrody-Younga (b); *Coccobius* sp. nr. *fulvus* (Compere et Annecke) (c); *Encarsia* sp. nr. *diaspidicola* (Silvestri) (d); *Aphytis* sp. (e); open circles (releases with no recoveries), half filled circles with strike through lines (releases with recoveries, in the same year only) and filled circles (releases with recoveries one or more years after release).

scape euonymus plants in southern New England, the beetle was found on 14.1% of 3141 shrubs that were infested with euonymus scale, out of a total of 4843 plants examined. The proportion of shrubs with *C. kuwanae* present increased significantly with increasing scale density (Fig. 3) (df = 3, (χ^2 = 516.6).

Spread of other released species of natural enemies was rarely observed. One case of recovery at a nonrelease site was noted for *C.* sp. nr. *nipponicus* and two for *C.* sp. nr. *fulvus*.

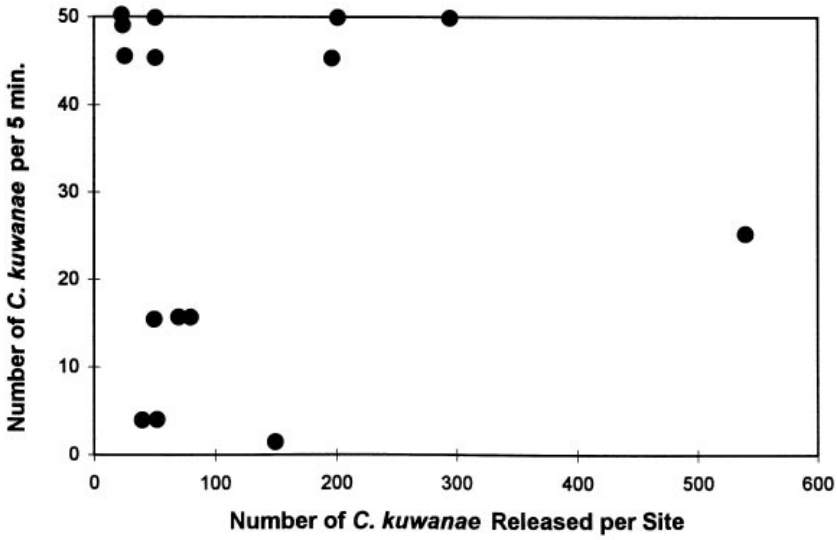

Fig. 2. Relationship between number of *Chilocorus kuwanae* Silvestri released at a site and the peak number of the coccinellid counted per five minutes subsequently at the same location, over the course of 1-3 years of observations every three weeks (April through October) ($R^2 = 0.002$, where $Y = 30.37 + 0.007X$).

Occurrence of Nonreleased Parasitoid Species

Aspidiotiphagus sp. was a preexisting aphelinid found parasitizing euonymus scale at 44% of 79 sites in southern New England from which euonymus scales were collected and reared in 1991 (Table 1). The presence of the parasitoid was not significantly related to the level of the scale infestation at a site (df = 2, $\chi^2 = 5.991$). Parasitism of third stage female euonymus scales, pooled by generation, for scales dissected in 1994 from 18 locations in Massachusetts was 13.4% (n = 2174 scales) for the overwintered spring adults, 33.6% (n = 1271 scales) for the summer generation, and 31.2% (n = 933 scales) in the fall generation.

DISCUSSION

The coccinellid *C. kuwanae* is now widespread throughout southern New England on euonymus scale plants infested with euonymus scale. Establishment of *C. kuwanae* at release sites was not related to numbers released. Likely influences were host density, bush size and degree of sunniness at sites. The later two factors were not, however, quantified. Coccinellids were recovered at sites ranging from shrubs at cool, moist sites surrounded by lawn, to hot, dry sites such as shrubs at shopping malls, where plants were often surrounded by concrete and bark mulch. Most sites examined were urban or suburban in nature and no attempt was made to assess rates of discovery by coccinellids of shrubs at isolated properties in non-urban areas. Rates of recovery of *C. kuwanae* on shrubs examined in the statewide surveys of euonymus scale were unlikely to have been influenced by proximity to release sites because release sites were few (45 over a five year period) and the number of shrubs in surveys was

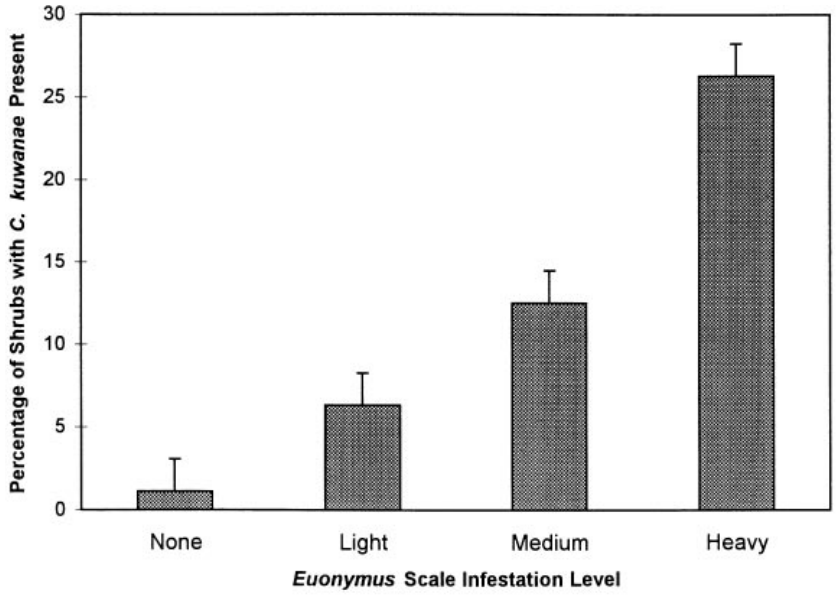

Fig. 3. Percentages (and SE values) of euonymus plants in various categories of scale density on which *Chilocorus kuwanae* Silvestri was present in surveys in southern New England in 1992-1994, where sample sizes by category were 1702 (N), 1401 (L), 745 (M), and 995 (H), and divided by state were 3220 (Massachusetts), 1076 (Connecticut), and 547 (Rhode Island).

large (4843) and shrubs were distributed widely over the three state area. Thus, while a few sites may by chance have been close (under 5 km) to release sites, most were not.

Chilocorus kuwanae life stages were most commonly encountered on plants with medium or heavy scale infestations, suggesting that the principal effect of this coccinellid will be to suppress scales at sites where scale densities are at or approaching damaging levels, rather than acting when scale densities are light. Because *Cybocephalus* sp. nr. *nipponicus* and *Coccobius*. sp. nr. *fulvus* require fewer hosts for repro-

TABLE 1. PRESENCE OF *ASPIDIOTIPHAGUS* SP. ON EUONYMUS PLANTS WITH DIFFERENT EUONYMUS SCALE DENSITIES IN SOUTHERN NEW ENGLAND IN 1991.

Scale Inf. Level	Samples with *Aspidiotiphagus*	Samples without *Aspidiotiphagus*
light	7 (64%)[a]	4
medium	7 (35%)	13
heavy	21 (44%)	27
total	35 (44%)	44

[a]Percentage samples with parasitoids was not significantly associated with scale density in a χ^2 test (df = 2, χ^2 = 5.991).

duction, these species may be more effective in causing mortality to low density scale populations. Establishment of these agents in Massachusetts makes possible a future evaluation of their effects on survivorship of scales in low density populations. The remaining parasitoids (*E.* sp. nr. *diapidicola* and *Aphytis* sp.) have not yet been shown to have established; future assessment of their status will be needed.

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