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Biology and oviposition behavior of Cybocephalus nr. nipponicus Endrody-Younga (Coleoptera: Cybocephalidae), a natural enemy of euonymus scale Unaspis euonymi (Comstock) (Homoptera: Diaspididae).

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BIOLOGY AND OVIPOSITION BEHAVIOR OF *CYBOCEPHALUS* NR. *NIPPONICUS* ENDRODY-YOUNGA (COLEOPTERA: CYBOCEPHALIDAE), A NATURAL ENEMY OF EUONYMUS SCALE *UNASPIS EUONYMI* (COMSTOCK) (HOMOPTERA: DIASPIDIDAE)

A Thesis Presented

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JUAN MANUEL ALVAREZ

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This thesis is dedicated to my wife: Pilar Andrea Gomez;
and to my parents: Aura Cecilia and Jorge Alberto.

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CHAPTER I

INTRODUCTION

The nursery industry is a major part of U.S. agriculture. In Massachusetts, the "green" industry (nursery and greenhouse) is one of the two most important sectors of agriculture in the state. Euonymus plants are one of the top ten ornamental plants in Massachusetts. Euonymus sales in the United States exceed $17,000,000 per year (APHIS 1994). The use of this group of plants in North America dates from the 19th century, following plant importations from Asia. However, euonymus plants are severely attacked by the Asian pest scale *Unaspis euonymi* [Comstock] (Homoptera: Diaspididae) (Drea 1987).

The Pest: *Unaspis euonymi* (Homoptera: Diaspididae), Euonymus Scale

Origin, host plants, distribution, and economic importance

The genus *Unaspis* appears to be of Korean origin (Drea & Carlson 1987). *Unaspis euonymi* is the primary pest of most species of *Euonymus* and some other members of the family Celastraceae (Gill *et al.* 1982). The scale infests species in 30 genera of plants, including *Pachysandra, Hibiscus, Celastrus* and *Prunus* (Drea 1987). The pest apparently occurs wherever euonymus is grown and is a major pest of the plant in all temperate regions of the world except Australia (Johnson & Lyon 1991). In 1936, the scale was reported killing *Euonymus* shrubs which were cultivated as a source of rubber in the old Russian union (Nikol'skii 1936).

In North America, the scale occurs throughout the eastern United States, California and the Pacific northwest, and parts of Canada (APHIS 1994). Lyne (1921) reported a *Chionaspis* sp. (euonymus scale was formerly known as *Chionaspis euonymi* [Comstock]) being found at various times infesting nursery stock imported from Japan to British Columbia. No definitive records exist of euonymus scale's invasion of the United States. It is believed to have been accidentally brought in with euonymus plants from Asia in the last century, before plant pest quarantine laws were established (APHIS 1993). In 1933, the scale was noted as having caused serious injury to euonymus in Virginia for the previous 50 years, and was considered "the only factor limiting the cultivation of this shrub" (Chapman *et al.* 1931). In New Jersey, euonymus has been eliminated as an ornamental plant in many nurseries because of damage from this scale (Drea 1987). In 1986, *U. euonymi* was the third most commonly reported pest arthropod in Pennsylvania nurseries and from 1975 to 1985, the scale was one of ten insects in Pennsylvania for which quarantines were issued (Drea 1987). Presently, interest in euonymus species by homeowners, nursery owners, and landscapers has decreased because of the damage caused by euonymus scale.

Effects of scale on plant

In general, armored scales feed by rupturing plant cells and ingesting their liquid contents (Beardsley & Gonzales 1975, Yasuda 1979). Cockfield *et al.* (1987) investigated the damage to leaves of *Euonymus fortunei* (L.) caused by *U. euonymi.* Leaf chlorosis resulted from chloroplast destruction, especially in the palisade parenchyma cells.

Cockfield *et al.* (1987) found that reduction in chlorophyll caused per scale was approximately 14 times greater for female scales than for males. Reduction in net CO_2 assimilation caused by *U. euonymi* is believed to be primarily due to chlorophyll loss (Cockfield *et al.* 1987). These authors also suggested that the degradation of leaf chloroplasts associated with *U. euonymi* feeding increases the suitability of plants for female scales by increasing the availability of soluble amino acids. The movement of nutrients such as nitrogen in plants is related to the performance of phloem feeders. In variegated plants, nutrients are more mobile than in green plants. Sadof & Raupp (1991) found that the fecundity of *U. euonymi* was greater on variegated plants than on green plants, which suggests that these insects are able to exploit increased nitrogen flux through the phloem.

Euonymus scales cause severe damage to leaves and stems and are able to kill whole plants, a serious problem in landscape plantings (Hendrickson & Drea 1988). Leaf abscission on plants infested by *U. euonymi* increases significantly during the second generation of the scale and infested plants suffer more winter injury than uninfested plants (Cockfield & Potter 1990). Economic losses due to euonymus scale were determined in separate research by Van Driesche and heavy scale infestations were found to increase the percentage of shrubs dying over a single year from 3% (for uninfested or lightly infested shrubs) to 12% (Van Driesche *et al.*#1 in press). Currently, the production and use of euonymus plants in the nursery industry are diminishing. If the pest were controlled, euonymus would be one of the preferred groups of plants for nursery production (Drea 1987).

Description and biology of euonymus scale

Euonymus scale belongs to the family Diaspididae, the largest within the homopteran superfamily Coccoidea, containing approximately 350 genera and 2000 species. Diaspidid species are present world-wide, causing serious pest problems (Kozár 1990). These insects have hard, armor-like body coverings throughout most of their development. These structures give rise to the common name of the group, the armored scales.

Adult female euonymus scales are brownish, about 1.5 mm long, and oystershell-like in shape. Eggs are retained under the armored covering, where they hatch in the spring to become mobile first instar nymphs, known as "crawlers". Crawlers disperse on and between plants, eventually settling to feed, inserting their mouthparts into leaves, stems or branches. Crawlers may be dispersed by wind to infest distant trees and shrubs. Once female crawlers settle, they lose their legs, produce an armored covering, and never move again (Hendrickson & Drea 1988). Immature female scales are brown, but less elongate than adult females. The stages of the female scale's life cycle (crawler, second stage female, and adult), can be determined by inspection of the scale's armor.

Male crawlers, after settling to feed on their host plants, form white, elongate scale coverings that are smaller than those of females. Eventually, winged males develop under these covers and emerge as free-living adults that mate with sedentary females. Because adult males lack mouthparts, they cannot feed and have very short lives. The male scale's life cycle can be divided into two feeding stages (crawler and second stage males), followed by the winged adult stage. Both sexes are found on the leaves and branches, but there are generally many more males than females on the leaves (Hendrickson & Drea

1988). Cockfield & Potter (1987) reported that more females than males initially settle on the stems, but about equal numbers settle on leaves. These authors found that the proportion of scales reaching maturity was higher for females on stems than on leaves, and higher for males on leaves than on stems. They concluded that there was either differential mortality or slower development between the sexes, depending on the settling site. Usually the white male scales are the most apparent stage, especially in the case of heavy infestations.

Euonymus scale has two generations a year in Massachusetts, with periods of crawler dispersal in June and August. In most southern locations, the pest has three generations per year on *Euonymus japonicus* (Thunb.), as for example in Louisiana (Brewer & Oliver 1984). *Unaspis euonymi* overwinteres as partly grown or full-grown females on euonymus and bittersweet shrubs (Felt & Bromley 1931, Pyenson 1941).

Host Plants (*Euonymus* spp.)

Euonymus plants belong to the family Celastraceae. There are more than 140 species in the genus *Euonymus* (APHIS 1993). The size, shape, foliage type, and fruit color of these different species are very diverse, including small or large shrubs, climbing vines, ground covers, and small trees (Bailey 1957), which enhances the usefulness of the genus as landscape plants. The attractiveness of *Euonymus* species is based not only on the colorful foliage that some species exhibit but also the bright color of their fruits, as in the American euonymus or strawberry-bush *Euonymus americanus* L. (Dirr 1983). The Easter wahoo, *Euonymus atropurpureus* Jacq., has bright crimson fruits (Flint 1983).

This species is a large shrub or small tree with wide, flat-topped, irregular crown (height of 3.6 to 7.2 m) which was introduced into the United States in 1756 (Dirr 1983). The European euonymus, *Euonymus europaeus* L., whose principal landscape value is its bright pink to red fruit capsules, is not recommended for landscape use because of its high susceptibility to euonymus scale (Dirr 1983).

Many species of euonymus are susceptible to euonymus scale. However, some species are resistant to the pest. The winged euonymus, *Euonymus alatus* Sieb., is native to northeastern Asia and central China and was introduced to the United States about 1860. This species of euonymus is one of the least susceptible members of the genus to this scale (Flint 1983). This plant is recognized by its green-to-brown, glabrous stems with two to four corky wings. Its most important landscape characteristic is its brilliant red foliage during the fall (Dirr 1983).

The two most common species of euonymus are the wintercreeper euonymus, *E. fortunei*, and the Japanese euonymus, *E. japonicus*. The wintercreeper euonymus presents a wide range of different characteristics according to the cultivar. Dirr (1983) lists 27 different cultivars among which are such popular forms as Winwed Gaiety. This species is native to China and was introduced into the United States in 1907 (Dirr 1983). Susceptibility to euonymus scale is the only important limitation of this species, a flaw which currently limits its use in North America (Flint 1983). The Japanese euonymus is a very dense oval shrub that may reach a height of 3 to 4.5 m and about twice that in width. This species has lustrous, waxy, dark green leaves. Its landscape usage has decreased because of its susceptibility to some significant diseases and to euonymus scale. Dirr

(1983) lists 8 different cultivars of *E. japonicus*. Native to southern Japan, this species was introduced to the United States in 1804. Brewer & Oliver (1987), investigating the occurrence and level of infestation by *U. euonymi* on different cultivars of *E. japonicus* plants, found that the variety *aureo-variegatus* Regel had higher levels of scale infestations than did the variety *aureo-marginatus* Rehd. These authors also found that the percentage of plants infested generally increased with age.

The spreading euonymus, *Euonymus kiautschovicus* Loes., is a semi-evergreen shrub and is less susceptible to euonymus scale than *E. fortunei* and *E. japonica* (Flint 1983). This species is native to eastern and central China and was introduced into the United States in 1860.

Chemical Control Methods

Many attempts to control the euonymus scale with chemicals have been reported. Sanders (1928) reported that sprays of Sunoco oil against euonymus scale showed promise as a summer control of this scale in Maryland. Osburn (1945) reported the use of DDT sprays against pest scales. However, the predaceous coccinellid, *Chilocorus stigma* Say, was also killed. A solution of a water soluble dinitrophenol (sodium dinitro-ortho-cresylate) mixed with sodium fluosilicate showed 85% control of euonymus scale, and when the same solution was used with a wetting agent such as sodium alkyl aryl sulphonate (0.25-0.5 %), the combination gave 100% control (Marcovitch 1951). Brewer & Oliver (1984) evaluated the effectiveness of nine insecticides for control of euonymus scale. They found that the most effective were 0.135% permethrin, 0.168% carbaryl, and

0.069% fenvalerate. These authors did not recommend the use of euonymus plants for landscape plantings in Louisiana due to the difficulty of control of euonymus scale. During studies in Georgia on the chemical control of euonymus scale on potted *E. japonicus* plants, the application of carbofuran did not cause any mortality of adult females. However, oviposition was reduced and the progeny from females kept on treated plants did not survive (Tippins 1981). Several pesticides such as malathion, diazinon, and acephate have been used to control euonymus scale, with applications directed against the crawlers. However, the effectiveness of those pesticides depends on the accuracy of timing in each scale generation (Drea 1991). Dormant oils combined with ethion have also been used to control euonymus scale (Drea 1991). An important feature of all chemical control of euonymus scale is that scale populations are suppressed but never eliminated because most life stages (other than crawlers) survive. Consequently annual treatments are required on an ongoing basis and scale infestations quickly rebound to damaging levels in two to three years if chemical suppression is discontinued. Therefore, the pursuit of more enduring suppression through biological control methods was desirable. Besides, euonymus plants are used as ornamental plants in urban areas (homes, schools, bussines and industrial places), and biological control of euonymus scale would be the most recommended method to control the pest in such areas.

Biological Control of Euonymus Scale

In the 1980s, personnel of the USDA/ARS Asian Parasitoid Laboratory in Seoul, Korea, were asked to collect natural enemies of euonymus scale for possible introduction into the United States. Several species of natural enemies were collected and released into North America (APHIS 1993). The coccinellid *Chilocorus kuwanae* (Silvestri) and the cybocephalid *Cybocephalus* nr. *nipponicus* Endrody-Younga were among the first species released (Drea & Hendrickson 1988). These two predators are widely distributed in China and have been recognized as effective natural enemies of diaspidid scales in China and Japan (Tachikawa 1974, Xia *et al.* 1986, Huang *et al.* 1988, Gao 1989). These species were first released by USDA scientists on euonymus scale-infested *E. europaeus* plants at the U.S. National Arboretum in Washington, DC in 1984. By the end of 1985, both predators were established in the small grove of euonymus which also included *E. europaeus*, *E. hamiltoniana nikoensis*, and *E. kiautschovicus*. By 1986, both beetle species were abundant and the site was used as an outdoor insectary from which beetles were collected and redistributed to other areas in the eastern United States. Two years after the initial release, the euonymus scale population at the National Arboretum was at a very low level. Both beetles have since been established on euonymus scale-infested plants in several parts of the country, including New England (Drea & Hendrickson 1988).

A proposal to implement a nationwide biological control project against euonymus scale was presented by John Drea to APHIS in 1991. Following the approval of the proposal, Roy Van Driesche at the University of Massachusetts, in cooperation with Mike Rose of Texas A&M University, and the APHIS National Biological Control Laboratory in Niles, Michigan, initiated the process of acquiring additional species of natural enemies through channels previously established with researchers in China (APHIS 1994). Three new species, all aphelinid parasitoids, were collected: *Coccobius* sp., *Encarsia* nr. *diaspidicola*, and *Aphytis* sp. The second and third of these species of natural enemies have been reared at the National Biological Control Laboratory in Niles, Michigan and at the University of Massachusetts in Amherst, Massachusetts, and have been released in several states (APHIS 1994). Evaluations of the effects of these natural enemies on euonymus scale populations were conducted in Massachusetts from 1991-94, and are being analyzed. Control by *C. kuwanae* has been observed (Van Driesche *et al.*#2 in press). For *C.* nr. *nipponicus* and the parasitoids, establishment has occurred at various sites and further study is needed.

Thesis Objectives

The objectives of this thesis have been to study one of these Asian predators, *Cybocephalus* nr. *nipponicus*, imported to control this scale. Studies were conducted in the laboratory on the biology of adult and larval *C.* nr. *nipponicus*, the oviposition strategy of adult beetles, and the interaction of adult beetles with the scale parasitoid *Encarsia* nr. *diaspidicola* (Hymenoptera: Aphelinidae), as follows:

CHAPTER 1: *Cybocephalus* nr. *nipponicus* biology. Most aspects of *C.* nr. *nipponicus* biology were unknown prior to this study; therefore the objective of this chapter was to measure basic aspects of the biology of both adults and larvae of *C.* nr. *nipponicus* under laboratory conditions, using an alternate host, *Quadraspidiotus perniciosus* [Comstock], (San José scale).

CHAPTER 2: Oviposition Behavior of *Cybocephalus* nr. *nipponicus*. The objective of this chapter was to clarify the oviposition behavior of *C.* nr. *nipponicus* in relation to scale sex, age, and density.

CHAPTER 3: Interaction of *Cybocephalus* nr. *nipponicus* with *Encarsia* nr. *diaspidicola*. The objective of this chapter was to determine if *C.* nr *nipponicus* adults laid eggs on scales previously parasitized by *Encarsia* nr. *diaspidicola,* to clarify if this predator would integrate well with parasitoids for control of the scale.

CHAPTER II

BIOLOGY OF *CYBOCEPHALUS* NR. *NIPPONICUS* (COLEOPTERA: CYBOCEPHALIDAE)

Introduction

Species of the family Cybocephalidae are considered important predators of diaspidid scales (Blumberg & Swirski 1982). Beetles in this family may rank second only to the coccinellids as predators of armored scales. However, their impact on scale populations has not been studied in depth (Drea & Carlson 1988). Diaspidid scale insects are the main food of the predatory beetle *Cybocephalus nipponicus* Endrody-Younga (Tanaka & Inoue 1983), and this beetle appears to be a promising natural enemy of the euonymus scale (APHIS 1994). However, most aspects of the biology of *C.* nr. *nipponicus* were unknown prior to this study. The biology, survival of adults, and diapause characteristics of several species of *Cybocephalus* such as *C. micans* Reitter, *C. nigriceps nigriceps* (Sahlberg), *C. aegyptiacus* Endrody-Younga, *C. binotatus* Grouvelle, and *C. gibbulus* (Erichson) have been the subject of several studies in Israel (Blumberg 1971; Blumberg 1973ab; Blumberg 1976; Blumberg & Swirski 1974a, 1974b, 1976, 1982) and Japan (Nohara & Iwata 1988). Drea & Carlson (1988) reported the establishment of *C.* nr. *nipponicus* at three release sites in the United States and the subsequent reduction of euonymus scale populations on the host plants at these sites. These researchers suggested that *C.* nr. *nipponicus* has a life cycle similar to those of *C. micans* and *C. nigreceps nigreceps*.

Work conducted here included studies on the biology of adults and larvae of *C.* nr. *nipponicus* (Chinese strain) in the laboratory on San José scale. Measurements were made of: (1) developmental times for stages from egg to adult (at one temperature only); (2) egg -through- adult survivorship rates by stage; (3) sex ratio of surviving progeny; (4) longevity of male and female beetles; (5) beetle fecundity and egg fertility; and (6) prey consumption by larvae and adults.

Materials and Methods

Source of beetles and San José scale colony maintenance methods

A colony of *C.* nr. *nipponicus* was started at the University of Massachusetts with specimens imported from Beijing, China into the United States with help from Larry Ertle of the USDA Beneficial Insects Research Laboratory in Newark, Delaware, Mike Rose of Texas A & M University, Mike Bryan of USDA-APHIS, and Thomas Dorsey of the N.J. Department of Agriculture. Voucher specimens have been placed in the University of Massachusetts collection of arthropods, Amherst, MA. Because euonymus scale could not be reared apart from euonymus shrubs, an alternate species of host scale, San José scale (*Quadraspidiotus perniciosus* [Comstock], Homoptera: Diaspididae) was used for these studies. This species could be reared conveniently on butternut squash, *Cucurbita* sp., in growth chambers.

Butternut squash were selected as rearing material for San José scale culture, because it stored well and infested squash supported growth of San José scales and *C.* nr *nipponicus* for about 3 months. Squash were washed and bleached, and both ends were waxed (with paraffin) before inoculation with scale crawlers. Waxing the ends of squash not only protects them from rotting near the stem, but also limits the surface area infested by scales, allowing easier handling. Waxing was accomplished by immersing the ends of the squash in melted paraffin.

Prepared squash were inoculated with scale crawlers collected from heavily infested squash (term "mother squash"). Collection was easily achieved because crawlers, the mobile first instars of *Q. perniciosus*, are highly phototropic (Rose 1990) and therefore could be attracted to the paraffined tops of mother squash by positioning these underneath lights. Crawlers were collected daily by brushing the tops of these squash onto a white paper sheet. Collected crawlers were examined under a stereomicroscope to eliminate all other organisms, especially mites. Scale cultures were maintained by transferring collected crawlers from mother squash onto new squash on a daily basis. Prior to infesting a squash, the date was recorded on the squash's surface with a wax marking pencil. On each succeeding day, the squash was rotated about one-seventh turn away from the already settled crawlers from the day before, and the newly exposed upper surface inoculated in turn. This process was continued for seven days. Approximately 35 days were required to complete a scale generation from crawler inoculation to mature reproducing females under the environmental conditions employed at 26° C. Scale-infested squash were used both to maintain and increase the pool of mother squash and to provide the beetle colony with food. As squash aged, they were eliminated from the pool of mother squash to reduce contamination by fungi and mites. The San José scale culture was maintained in rearing chambers (26° ±1° C , a 14:10 L:D photoperiod). Mother squash and newly inoculated squash were isolated from each other to minimize contamination of newly inoculated squash to be used in experiments. To avoid contamination with aphelinid wasps, newly-inoculated squash were further confined in ventilated plastic bags or ventilated plastic boxes sealed with masking tape.

Description of procedures to rear *C.* nr. *nipponicus* beetles

Beetles were maintained on San José scale on butternut squash. Infested squash were placed into Plexiglas cages (28 cm. on a side) with circular openings on both front and back. Cage openings were covered with organdy sleeves to provide access and ventilation. Four butternut squash infested with 30-40 day-old San José scale were placed into each cage. An initial population of 250 beetles was added to each cage. As squash deteriorated (in about 3 months), they were removed to different cages and replaced with new infested squash. The cages with old squash were observed daily, and adult beetles were collected as they emerged and were returned to the rearing colony. Adults continued to emerge for about four weeks following removal of squash from the colony. One ounce (29.6 ml.) plastic cups with cotton wicks were used to provide water for the beetles. Pieces of self-adhesive yellow paper (5x4 cm) were streaked with thin lines of honey and placed inside rearing cages to feed beetles.

Description of experimental arena and environmental conditions

Using this *C.* nr. *nipponicus* colony, laboratory studies were conducted in temperature-controlled cabinets (22±1°C) with a 14:10 (L:D) photoperiod. A standard experimental arena was used for all experiments (except where specified). Experimental arenas consisted of circles 4 cm in diameter which were delimited with modeling clay on the squash surface. The squash surface within circles was inoculated with crawlers as desired for particular experiments. Crawlers were obtained from mother squash and examined under the stereomicroscope to eliminate mites. San José adult male scales were distinguished from female scales by the smaller size and distinct oblong shape of their scale covers. San José scale males emerged approximately 20 days after crawler inoculation at 22°C. However, scale armor remained intact, allowing exact counts of original numbers of scales in particular arenas, as needed for some experiments. When female scales were 25±5 days old, clay circles were numbered and covered with plastic Petri dishes (4 cm. diameter), ventilated with organdy. Lids were attached to squash surfaces by using modeling clay.

Developmental life history studies

Studies on developmental rates and survivorship of specific *C.* nr. *nipponicus* life stages were conducted by isolating beetles from the rearing colony. *Cybocephalus* nr. *nipponicus* eggs were obtained from mating beetles chosen at random from the laboratory colony. These pairs of beetles were confined individually inside the circles (as described above) containing patches of adult San José scales (one month old) on butternut squash. Beetles were removed from circles after 24 hours and the scale patches were examined under a stereomicroscope, turning over scale covers to locate newly laid eggs (0-24 h old) which were collected for observation. Eggs were measured and placed in plastic Petri dishes (4 cm diameter) with a piece of moist sponge glued under the lids to ensure high relative humidity inside the closed cages. Petri dishes containing eggs were sealed with Parafilm®. Eggs were observed daily until hatch and the percentage of egg hatch was recorded.

Neonate beetle larvae, which had eclosed from eggs isolated as described above, were confined individually inside the same kind of experimental circles. A camel's hair brush was used to place the beetle larvae on the scale patches. Survivorship of larvae was determined by following the fate of individual larvae daily, noting the dates of completion of the larval stage. Circles in which larvae were observed to build pupal cells were marked and observed daily until adult emergence to determine the duration and survivorship of the pupal stage and the sex ratio of emerging adults.

Another experiment on pupation biology was conducted to determine whether *C.* nr *nipponicus* larvae were able to pupate in soil, apart from host scales. Thirty larvae, each one-day old, were reared individually on San José scale patches in the same experimental arena described above. One patch with a high scale density (approx. 100 adult scales/ patch) was supplied to each larva. After 10 days, the now nearly mature larvae were located by turning over scale armor (using a dissecting microscope and fine probes). Larvae were placed individually in a Petri dish (4 cm diameter) which contained a thin layer of moist vermiculite, which had been previously sterilized. Each dish was covered with a ventilated lid. All Petri dishes were placed in a growth chamber at 22° C and observed daily for emergence of adult beetles.

To determine lifetime fecundity of *C.* nr *nipponicus* adults, pairs of newly emerged adult beetles (0-24 h old) were isolated in the same kind of circles described above, in which there were low density patches of San José scale (approx. 50 scales, one month old). Each pair of beetles was transferred to a new patch every two days for as long as the female lived. Female survival and oviposition were recorded. Males were replaced as they died.

Larval consumption of San José scale

Scales which died from predation by *C.* nr. *nipponicus* larvae showed distinctive signs and could readily be distinguished from scales killed by other mortality factors. Although at high scale densities per patch the scale armors were not attacked, at low scale densities bitten armors by beetle larvae showed visible and distinctive broken areas and in most cases the scale body of those attacked armor were also seen with bounds of larval bites. This allowed the number of scales attacked by beetle larvae to be counted. Larval consumption of scales was recorded only for larvae that completed their entire developmental period up to initiation of the pupation cell. Neonate larvae were confined on patches containing scales of mixed age (range 17 to 44 d) and observed daily until pupation. The numbers of living and attacked scales were counted in each patch. Effects of both scale age and density on numbers of scales attacked during the larval stage were determined by linear regressions.

Adult consumption of San José scale

As with larvae, scales killed by adults of *Cybocephalus* nr. *nipponicus* bore distinctive signs and could be recognized and counted following exposure to beetles in test arenas. Consumption rates of adult *C.* nr. *nipponicus* beetles were measured using the same experimental arenas as described above. Separate tests were conducted on the effects of scale density and age on beetle consumption. For tests on scale density, patches were inoculated with San José 25±5 days before starting the experiment, so that all scales were the same age during the test. Five scale densities were considered in the experiment (15, 30, 65, 100 and 140 adult San José scales per patch) with 15 replicates per scale density. Seventy five pairs of beetles were chosen from laboratory colonies and placed individually on each replicate. Each pair was confined for two days on a patch. After beetles were removed from the experimental arenas, scale patches were examined and the number of scales attacked by beetles was recorded. To determine the effects of scale density on beetle consumption, a one-way ANOVA was used to compared numbers of scales attacked in each treatment, together with a least significant difference (LSD) comparison test.

For the test on the effect of San José scale age on scale consumption rates by adult beetles, five different ages of scale were exposed together in the same experimental arena as described previously. To construct a scale population with members of five different ages, each circular experimental 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 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 of ages of: 1, 12, 23, 34 and 45 days old. These scale patches were allowed to develop for eight more days. At this point, a mating pair of beetles was introduced into each of four arenas. This process was repeated daily for 6 days until beetles had been placed over all 24 replicates. Thus scale age treatments as used in the experiment were the following five ranges: 9 to 14 days, 20 to 25 days, 31 to 36 days, 42 to 47 days, and 53 to 58 days. (Staggered initiation of replicates was necessary because of the need to examine scale patches immediately after beetles were removed, and because of the length of time required for such examination). Beetles were removed after two days and scale patches were examined and the number of scales per wedge and the number of scales attacked were recorded within each age group and compared with one-way ANOVA and a least significant difference (LSD) comparison.

Adult consumption of euonymus scale

Fifteen twig sections, each bearing euonymus scale females and males, were collected from naturally infested euonymus plants (*E. fortunei)* in late November 1994. Each twig was placed in a 4 cm water vial and sealed with Parafilm®, leaving 6 cm of twig exposed. One female and one male beetle were confined in a ventilated plastic Petri dish together with each twig. Following a 2-day period, the number of live and dead scales were counted on each twig.

Results

Stage descriptions

Because descriptions of the life history stages of this species have not been published, the general appearances of these are given briefly here. *Cybocephalus* nr. *nipponicus* eggs are ovoid and measure 0.46±0.02 x 0.22±0.01 mm (n=35). Eggs are transparent when recently laid and greyish when mature. However, some eggs became purple when mature. No differences in size, hatching or sex were found between greyish and purple eggs. Recently emerged larvae are transparent with brownish mouth parts. There are three brown spots on each side of the head capsule. As larvae grow, they become yellow, due to the consumption of yellow San José scale bodies. Larvae bite scales, which results in visible and distinctive broken areas on the scale covers. Larvae hide under scale covers and consume scales, killing them in the process. The larvae are cannibalistic when food is scarce, consuming both *Cybocephalus* larvae and eggs. At 12 days of age (at 22°C), larvae become less active and darker, cease feeding and start the process of building a pupal cell. The pupal cell is dome-shaped and is constructed by joining together the armor coverings of three or more adjacent scales. Adults of *C. nipponicus* are hemispherical and measure 1.1± 0.07mm in length x 0.9 ± 0.04mm in width (n= 25) for females and 1.0 ± 0.05mm x 0.8 ± 0.04mm (n=30) for males. Females are completely black; males have a large beige-colored head and pronotum, with the rest of the body black.

Developmental and survivorship rates, and adult sex ratio

Developmental times for the life stages are given in Table 2.1. From 900 eggs that were collected and placed in the Petri dishes, 776 (86.2%) hatched (Table 2.2). From 776 larvae that were isolated and reared individually, 175 (22.6%) survived to pupal cell formation. Cannibalism and predation by populations of the mite *Tyrophagus putrescentiae* (Schrank) were the more evident factors affecting egg and larval survivorship. Cannibalism occurred in the Petri dishes where eggs were placed to hatch and no food was available for the hatched larvae. Predation by mites occured in the experimental circles where the high relative humidity and cadavers of partly consumed scales under armor were favorable for mite populations. Predation by mites occurred more frequently upon late larval instars and beetle pupae than on young larvae. Of 175 beetle pupae reared individually on patches of San José scale, adult beetles emerged from 161 (92 %). From 30 larvae reared individually on San José scale for 10 days and then removed to pupate in Petri dishes with vermiculite, 11 adult beetles were recovered (36.6%). The sex ratio of 161 individuals surviving to the adult stage was 5.3 : 4.7 (86 females: 75 males).

Adult longevity

Under the laboratory conditions used, the average longevity of females and males of *C.* nr. *nipponicus*, respectively, was: 99.14±79.14 d (n=14) and 77.8±47.29 (n=10) (Fig. 2.1).

Fertility

Mating was required before oviposition occurred. The preoviposition period of *C.* nr. *nipponicus* was four days. Beetle eggs were laid under scale covers. Puncture wounds in the armor of scales bearing beetle eggs were not usually detectable and therefore were not a reliable indicator of oviposition. However, a characteristic purple stain was sometimes left on or near scales which had beetle eggs and could be used as evidence of beetle oviposition. The total number of eggs laid per female varied substantially (288.57±236.45 eggs), with a range of 19-589 eggs per female. The mean life time egg production was 92.57±76.50 days (range: 9- 245). Egg production in 2-day periods ranged from 0 to 20 with a mean oviposition of 5.8±1.38 eggs per two day period (for 14 females for their entire lifetimes) (Fig. 2.2). Variation in total fecundity (per life time) was largely a matter of how long a female survived. Mean daily fertility (calculated as the total number of eggs laid per life time, divided by the mean life time egg production [days]) provides a measure of daily fertility (Bellows *et al.* 1992). The *C.* nr. *nipponicus* daily fertility value was 3.11 (n=14).

Larval consumption of San José scale

Young larvae of *C.* nr. *nipponicus* penetrate under the edge of the scale armor and feed on the liquid contents of scale bodies. The scale armor and the cuticle of the scale body were not eaten at high scale densities, but were sometimes consumed at low scale densities per patch. Consumption of scale bodies was often incomplete, but even partial consumption caused scale death.

The mean number of scales attacked per larva (over the whole larval life time) was 19.48±11.38. Larvae ate more scales if feeding on younger scale and fewer as scale age increased; this relationship was statistically significant (Fig. 2.3b). The relationship between scale age and the percentage of scales eaten (Fig. 2.3d) and relationships between scale density and both number eaten and percentage eaten, were not significant (Fig. 2.3ac).

Adult consumption of San José scale

Adult beetles of *C.* nr. *nipponicus*, after physically contacting a scale, drummed the scale cover with the antennae and then used their mandibules to chew a hole in the scale armor. Beetles then consumed the liquid contents of scale bodies under scale armor.

The number of San José scales attacked (wholly or partially eaten) by pairs of adult beetles (one female, one male) increased with scale density per patch. It was highest (19.92 scales) in arenas with 65 scales per patch (Table 2.3). There were significantly more scales attacked in arenas with 65,100 and 140 scales per patch than in arenas with 15 or 30 scales per patch ($F= 4.63$ df=[4,64] $p<0.05$). The highest percentage of scales attacked, however, occurred at the lower scale densities and decreased as scale density increased ($F= 17.07$ df=[4,64] $p<0.05$). Significant LSD comparisons for the five densities are presented in Table 2.3.

The age of San José scales affected attack rates by adult *C.* nr. *nipponicus* beetles. Both the number and percentage of scales attacked increased with increasing scale age (Table 2.4). 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] $p=0.6371$) (Table 2.4). Therefore, effects of scale age on beetle consumption rates could be analyzed without adjustments for potential effects of scale density. A statistically significant difference was observed in the preference of *C.* nr. *nipponicus* to attack different scale ages. Twenty-three percent of the oldest group of scales were attacked (53-58 days old), whereas only 0.5% were attacked in the youngest group of scales ($F=23.79$, df= [4, 115] $p<0.05$). The highest number of scales attacked occurred in the oldest group of scales (9.37 scales) (Table 2.4).

Adult consumption of euonymus scale

When presented with euonymus scales as prey, the average number of scales that a pair of adult *Cybocephalus* nr. *nipponicus* beetles (one female, one male) attacked was 91.8±33.68 per two day period (n=15), a 4.5 fold increase compared to San José consumption rates. The number of male scales attacked (56.33±30.25) was significantly higher than that of females (35.47±8.91). The relationship between scale density and number of euonymus scales attacked is presented in Fig. 2.4.

Discussion

Knowledge of the biology of cybocephalid species is very limited. Blumberg & Swirski (1982) and Nohara & Iwata (1988) studied aspects of the biologies of *C. nigriceps nigriceps*, *C. micans*, and *C. gibbulus*, which were in broad terms similar to the biology of

C. nr. *nipponicus*. At 22 °C, *Cybocephalus* nr. *nipponicus* beetles have a generation time of approximately seven weeks from egg to newly emerged adult, but adults live for many months. Scale populations persist all year long and, under these circumstances, *C.* nr. *nipponicus* could produce up to three generations per year in New England between May and October. The long lived adults of each generation could produce progeny over the entire season, leading to overlapping generations.

Placement of the eggs of cybocephalid species, such as *C.* nr. *nipponicus*, under scale armor and the subsequent feeding by larvae under scale armor is likely to protect these stages from adverse conditions such as exposure to pesticides. For example, *C. fodori* preying on *Q. perniciosus* was observed to survive in pesticide treated fruit orchards in northern Greece (Katsoyannos 1984). Kehat *et al.* (1974) also showed that *Cybocephalus* spp. were present in chemically treated date palm plantations in Israel, in contrast to coccinellid species (whose life stages are all exposed, rather than protected by scale armor) which were all killed in treated plantations.

Another advantage of cybocephalids as scale predators compared to coccinellids is the lower number of scales needed per larva for development, or per adult for reproduction. For example, when presented with *Ceroplastes japonicus* scale as prey, the average daily consumption rate of *Chilocorus kuwanae* was 199 scales per larva (Xia *et al.* 1986), ten times as large as the life time consumption of San José by *C.* nr *nipponicus* larvae. Therefore, while coccinellids are valuable at attacking high density scale populations, they are often unable to reproduce efficiently at low scale densities.

In contrast, at such low densities, cybocephalids are able to maintain their populations and thus help keep scale population below damaging levels.

Finally, the significance of beetle species as scale predators will vary with the defense mechanisms presented by different prey species. Honda & Luck (1995) noted that successful suppression of diaspidid scales by coccinellid predators depends on factors such as the physical characteristics of the scale cover. Diaspidid scales with thin, easily penetrated covers are more likely to be suppressed than those with thick covers. Scale armor thickness also strongly affects the success of cybocephalid species as scale predators. *Cybocephalus micans* and *C. n. nigreceps*, for example, are almost incapable of feeding on adult female diaspidid scales (Blumberg & Swirski 1974), in contrast to *C.* nr. *nipponicus*, which was able to feed on adult females of both San José and euonymus scales. For *C.* nr. *nipponicus*, consumption rates varied strongly between San José scale and euonymus scales, reflecting ready consumption of second stage male euonymus scale.

To fully exploit members of Cybocephalidae as predators of pest scales detailed and comparative studies are needed between cybocephalid species to identify suitability of potential prey scale species for particular predator species. Field measures of actual predation rates are needed to assess actual value of these beetles as biological control agents.

Table 2.1. Developmental time for life stages of *Cybocephalus* nr. *nipponicus* at 22±1°C., with San José scale as prey.

	# days / stage	(n)
Eggs	9.1±1.1	100
Larvae	14.5±1.6 [1]	100
Pupa	20.4±2.7 [2]	50
Oviposition to Adult Emergence		
Females	43.9±2.5 (range 40-50)	50
Males	45.7±2.8 (range 41-56)	46
Female Adult Longevity	99.14±79.14 (range 13-245)	14
Male Adult Longevity	77.8±47.29 (range 7-151)	10

[1] From egg hatch to formation by larva of pupation cell.
[2] From formation of pupation cell by larva to emergence of adult beetle.

Table 2.2. A laboratory life table prepared for *Cybocephalus* nr. *nipponicus* reared on San José Scale, at 22±1°C.

Stage	No. alive at start	No. dying in stage	Apparent mortality
Egg	900	124	0.14
Larva	776	601	0.77
Pupa	175	14	0.08
Adult	161		

17.9 % Survivorship from egg to adult.
Sex ratio= 5.34 : 4.66 (86 females: 75 males.)
Expected number of eggs per female: 287.5
Total expected eggs: 287.5 x 86 = 24,725.5
R_o (The net reproductive rate) = 27.47

Table 2.3. Numbers of San José scales attacked by pairs of adults of *Cybocephalus* nr. *nipponicus* (one female, one male) at five different prey densities in a 2 day-period at 22± 1°C.

Scale Density (n)	Scales Attacked Mean Number (S.E)	% of Scales Attacked Mean Number (S.E)
15 (12)	9.92c (1.12)	66.11a (7.5)
30 (15)	14.53bc (1.35)	48.44b (4.5)
65 (12)	19.92a (2.22)	30.64c (3.41)
100 (14)	18.31ab (1.85)	18.31cd (1.85)
140 (13)	19.42ab (2.88)	13.87d (2.06)
Average	16.42	35.48

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

Table 2.4. Numbers of San José scales attacked by pairs of adults of *Cybocephalus* nr. *nipponicus* (one female, one male) for scales of five different ages, presented in a choice-design, for a 2 day-period at 22±1°C.

	Mean Number (S.E.)		
Scale Age (Days)	Total Scales	No. of Scales Attacked	% of Scales Attacked
9-14	43.54a(4.43)	0.08d (0.06)	0.5c (0.3)
20-25	45.04a(0.06)	1.75cd (0.48)	3.5c (1.0)
31-36	41.04a(4.12)	3.25bc (0.50)	8.6b (1.3)
42-47	40.33a(4.57)	3.62b (0.74)	10.5b (2.4)
53-58	50.50a(5.81)	9.37a (1.06)	22.6a (2.6)
Average	44.09	3.62	9.1

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

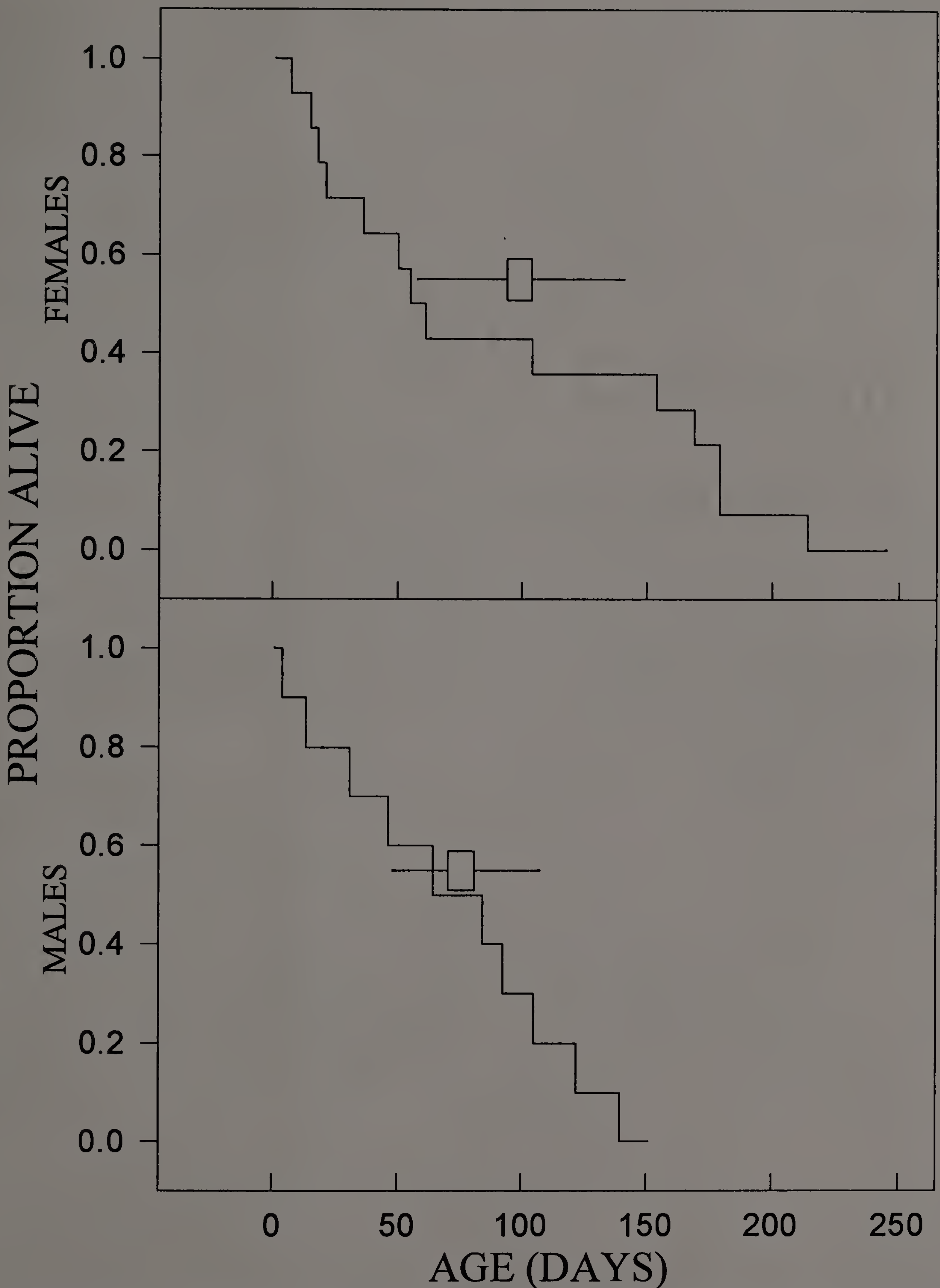

Figure 2.1. Longevity of *C.* nr. *nipponicus* adults reared on San José scale, at 22±1°C. Initial numbers for females and males were, respectively, 14 and 10. Only females that oviposited were included in the upper figure. Boxes (□) with horizontal bars give life span means ± 95% CIs.

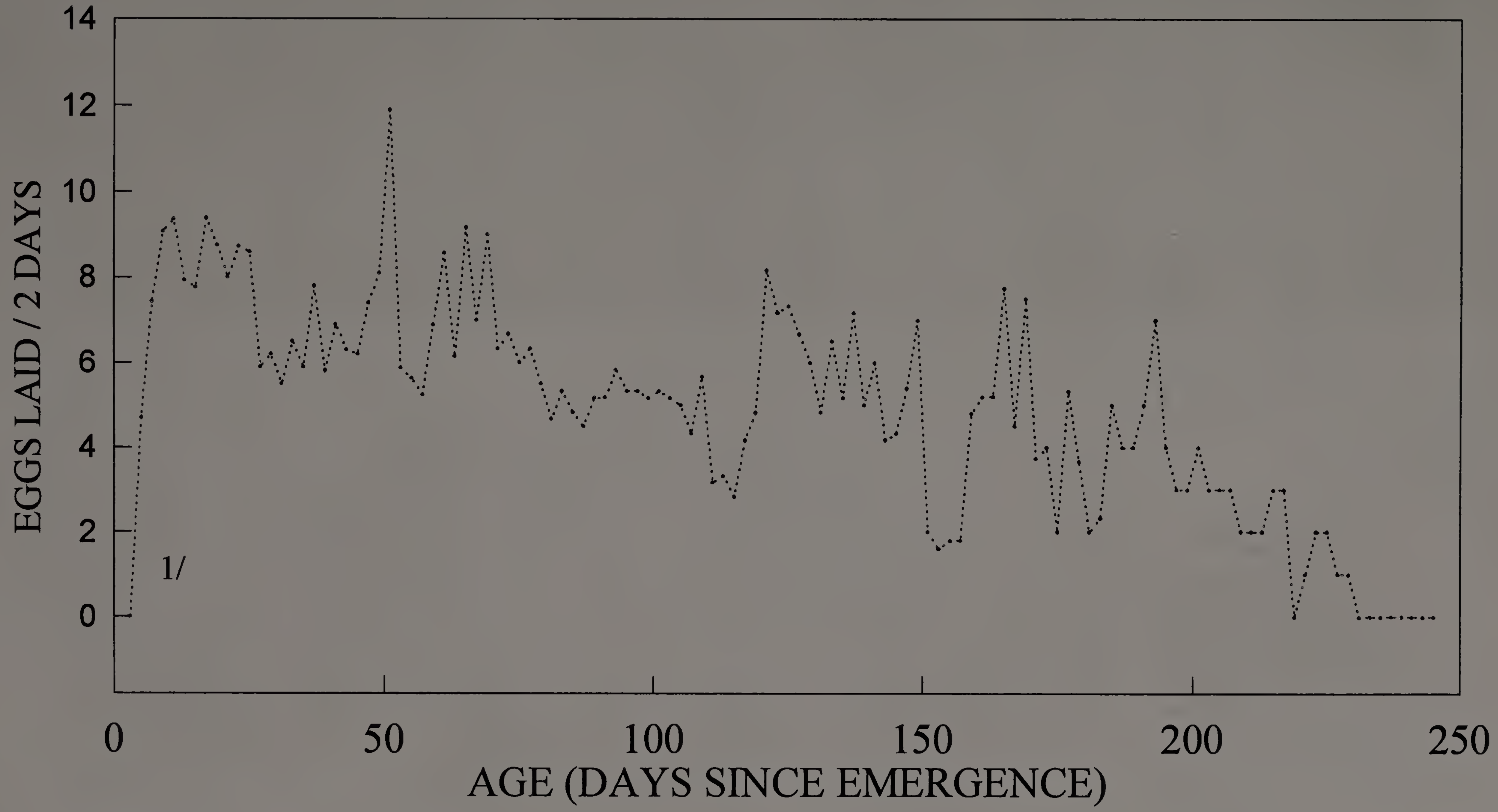

Figure 2.2. Mean number of eggs produced in a period of two days by *C*. nr. *nipponicus* during the whole life time. Female beetles were reared on San José scale at 22±1°C (n=14).

1/ Standard errors are not displayed for visual clarity.

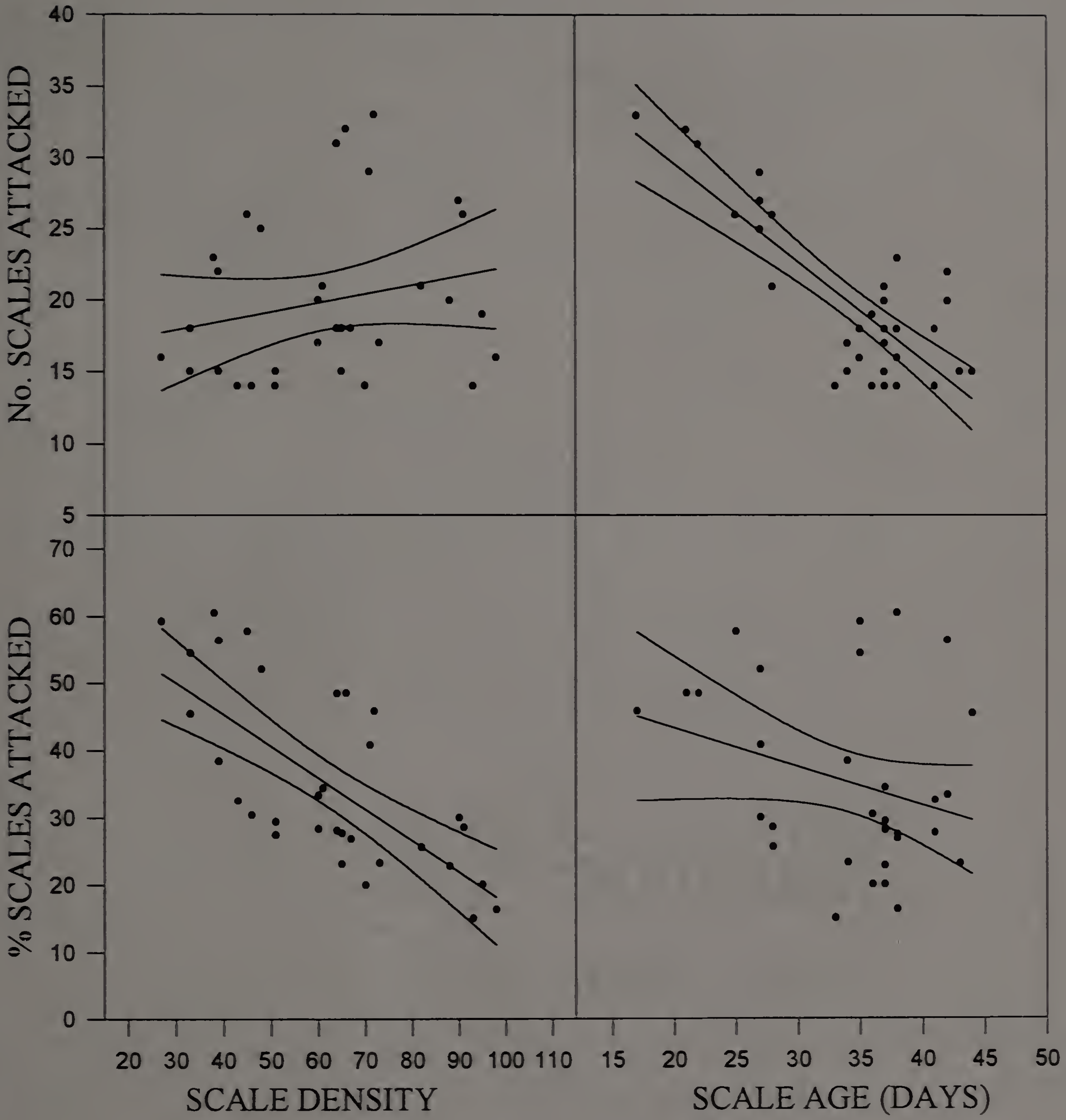

Figure 2.3. Effects of San José scale density and scale age on number and percentages of scales attacked by larvae of *C.* nr. *nipponicus* over the whole larval lifetime, at 22±1°C.

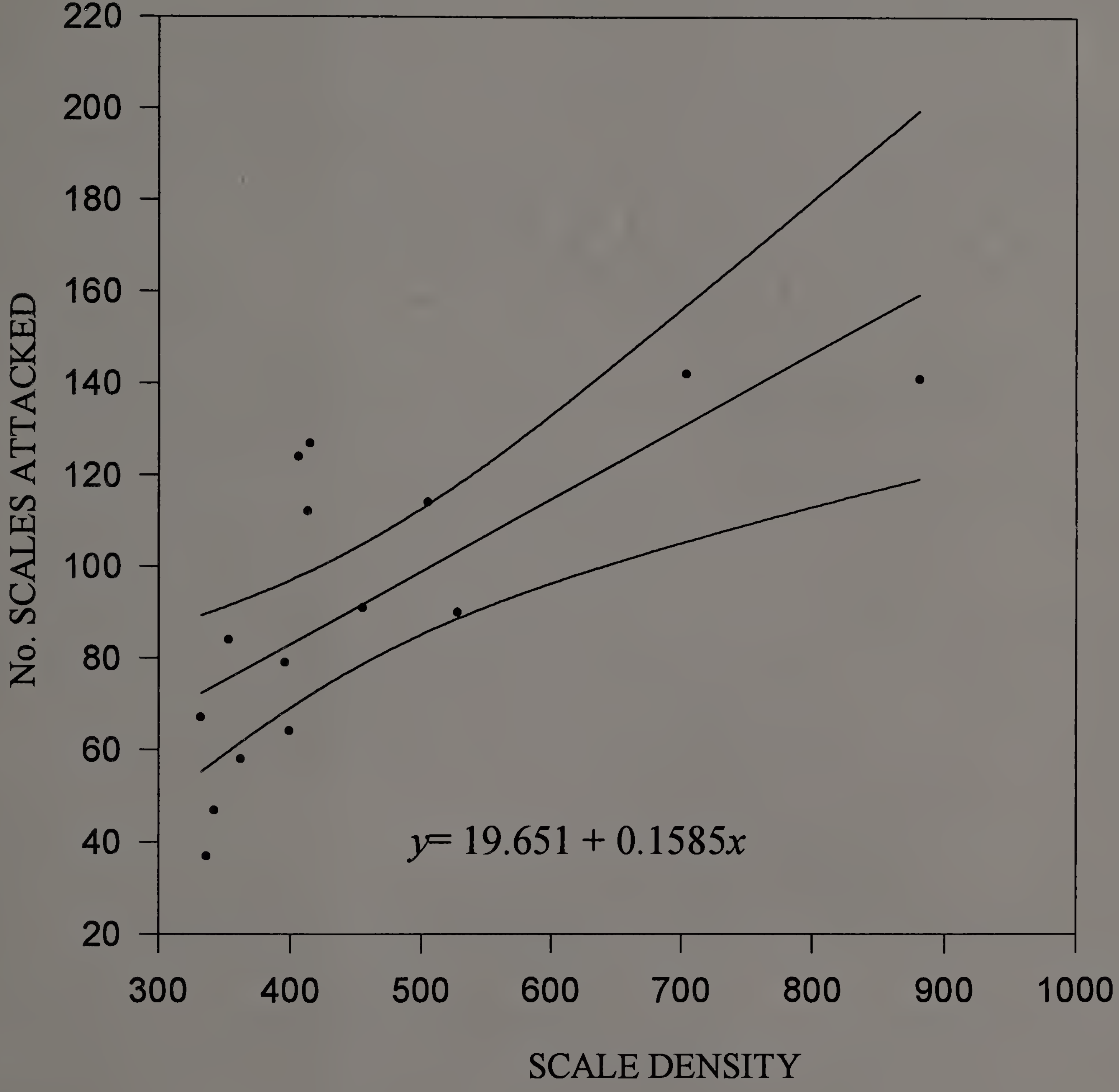

Figure 2.4. Effect of euonymus scale density on numbers of scales attacked by pairs of *C.* nr. *nipponicus* adults (one female, one male) in 2 day test period at 22±1°C.

CHAPTER III

OVIPOSITION BEHAVIOR OF *CYBOCEPHALUS* NR. *NIPPONICUS* (COLEOPTERA: CYBOCEPHALIDAE)

Introduction

Cybocephalid beetles are predators that lay their eggs one by one under the armor of diaspidid scales (Blumberg 1973, Blumberg & Swirski 1982, Nohara & Iwata 1988), in some sense like hymenopterous parasitoids. *Cybocephalus* nr. *nipponicus* females mate within two days after adult emergence and begin laying eggs beneath host scales about four days later (Alvarez & Van Driesche #1 in press). After contacting host scales, female beetles drum scale covers and then chew a hole through the armor of the scale. Beetles then turn around and insert one or more eggs under the armor of the scale. Sometimes females lay eggs under armor of scales that they have previously eaten. Beetles lack ovipositors; however, *C. nipponicus* females possess a telescopic abdominal segment which they can use to insert eggs under a scale's armor. Eggs are usually placed under armor through the holes the beetles cut in scales' armor, but sometimes eggs are inserted instead under the edge of the scale's armor without cutting a hole. Eggs are laid far from the site of insertion, presumably to protect them from both cannibalism and attack by other predators (Alvarez & Van Driesche #1 in press). Because this beetle's oviposition occurs in close association with individual scales, qualities of individual scales or patches of scales could be factors used by beetles to choose oviposition sites. The objective of this study was to clarify how *C.* nr. *nipponicus* oviposition behavior varied in response to changes in four such factors: scale sex, density, age, and species.

Materials and Methods

Source of beetles and San José scale colony maintenance methods

A colony of *C.* nr. *nipponicus* was started at the University of Massachusetts with specimens imported from Beijing, China into the United States with help from Larry Ertle of the USDA Beneficial Insects Research Laboratory in Newark, Delaware, Mike Rose of Texas A & M University, Mike Bryan of USDA-APHIS, and Thomas Dorsey of the N.J. Department of Agriculture. Voucher specimens have been placed in the University of Massachusetts collection of arthropods, Amherst, MA. Because euonymus scale could not be reared apart from euonymus shrubs, an alternate species of host scale, San José scale (*Quadraspidiotus perniciosus* [Comstock], Homoptera: Diaspididae), was used for these studies, which could be reared conveniently on butternut squash, *Cucurbita* sp., in growth chambers. Rearing procedures for San José scales and *C.* nr *nipponicus* beetles are given in Alvarez & Van Driesche (#1 in press).

Description of experimental arena and environmental conditions

Using this *C.* nr. *nipponicus* colony, laboratory studies were conducted in temperature-controlled cabinets (22±1°C) with a 14:10 (L:D) photoperiod. A standard experimental arena was used for all experiments (except in the experiment with euonymus scale). Experimental arenas consisted of circles 4 cm in diameter which were delimited with modeling clay on the squash surface. The squash surface within circles was inoculated with crawlers as desired for particular experiments. Crawlers were obtained from heavily infested "mother" squash and examined under the stereomicroscope to eliminate mites. San José adult male scales were distinguished from female scales by their smaller size and distinct oblong shape of their scale covers. San José males emerged approximately 20 days after crawler inoculation at 22°C. However, scale armor remained intact, allowing exact counts of original numbers of scales in particular arenas, as needed for some experiments. Clay circles were numbered and covered with plastic Petri dishes (4 cm. in diameter), which were ventilated with organdy. Lids were attached to squash surfaces by using modeling clay. Five experiments were conducted to clarify effects of scale sex, density, age, and species on oviposition behavior of *C.* nr. *nipponicus.*

Effect of scale sex

To assess the effect of host sex on beetle oviposition, mating pairs of beetles were chosen from laboratory colonies and confined for two days in experimental arenas containing mixtures of adult male and female scales (age 25±5 days). San José males emerged approximately 20 days after crawler inoculation at 22°C. However, scale armor remained intact, allowing exact counts of original numbers of scales in arenas. In this experiment scale armor from which an adult male had emerged was considered as a male scale. Beetles were removed from arenas after two days and scale patches were examined by turning over armor of all scales to locate newly laid eggs. For every scale in the patch its sex was recorded and whether or not beetle eggs had been deposited under the scale armor. For scales bearing 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: random scale densities

The effect of scale density on beetle oviposition rates (per 48 hr) was examined in this experiment using the same experimental arenas described earlier and San José scales of a standard age (25±5 days). The number of scales in each patch varied randomly, and was determined by the number of crawlers that successfully settled in each patch. Mating pairs of beetles were chosen from laboratory colonies and confined over scale arenas for two days. Beetles were then removed and all scales examined. 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. A total of 70 scale patches were exposed to one pair of beetles each. Patches 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 compared between groups in a one-way ANOVA. Significant differences were identified using a least significant difference (LSD) comparison test.

Effect of scale density: fixed scale densities

To further examine the relationship between scale density and beetle oviposition, and to overcome the problem of a differing number of replicates for each density category, a second experiment was performed in which the number of scales per patch was fixed by removing extra scales from patches four days after crawler inoculation. This procedure created fifteen scale patches for each of the six scale densities considered in this experiment (0, 15, 30, 65, 100 and 140 scales /patch). Scales in patches consisted of mixtures of armor of emerged male scales and live adult female scales. A few replicates were dropped because beetles died during the experiment. Patches with no scales (0 density treatment) included honey as food for the beetles. All the scales in this experiment were 25±5 days of age. Ninety female and 90 male beetles were chosen at random from laboratory colonies. Female beetles were assumed to have mated but were not actually mating when collected, in contrast to the case for the previous experiments. Each pair was confined for two days on a scale patch. After beetles were removed from the experimental arenas, scale patches were examined and the number of beetle eggs in each patch recorded. Differences in oviposition rates between scale densities were compared using a one-way ANOVA and a least significant difference (LSD) comparison test.

Effect of scale age

The effect of scale age on beetle oviposition was determined for San José scales of five different ages in the same experimental arena. To construct a scale population with members of five different ages, each circular experimental 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 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 of ages of: 1, 12, 23, 34 and 45 days old. These scale patches were allowed to develop for eight more days. At this point, a mating pair of beetles was introduced into each of four arenas. This process was repeated daily for 6 days until beetles had been placed over all 24 replicates. Thus scale age treatments as used in the experiment were the following five ranges: 9 to 14 days, 20 to 25 days, 31 to 36 days, 42 to 47 days, and 53 to 58 days. (Staggered initiation of replicates was necessary because of the need to examine scale patches immediately after beetles were removed, and because the length of time required for such examination). Beetles were removed after two days and scales were examined for beetle eggs. Number of scales per patch and numbers with beetle eggs were recorded within each age group and compared with one-way ANOVA and a least significant difference (LSD) comparison.

Effect of scale species: euonymus scales as hosts

To provide a comparison of oviposition behavior on the field host, euonymus scale, to the laboratory host (San José scale) used in this study, euonymus scale-infested plants were collected and exposed to beetle in the laboratory. Fifteen twig sections, each bearing euonymus scale females and males, were collected from naturally infested euonymus plants (*Euonymus fortunei* [L.]) in late November 1994. Each twig was placed in a 4 cm water vial and sealed with Parafilm®, leaving 6 cm of twig exposed. One female and one male beetle were confined in a ventilated plastic Petri dish together with each twig for two days. After beetles were removed from the Petri dishes, euonymus twigs were examined, turning over armor of scales to detect beetle eggs. The number of beetle eggs under female and male scales were 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 hosts.

Results

Effect of scale sex

The total numbers of female and male San José scales across all replications in the test were, respectively, 2477 and 2930; and the total number of eggs laid in a two-day period under female and male scales were 347 (48.5%) and 368 (51.5%). Of 715 eggs, 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 percentages of female and male scales receiving *C.* nr. *nipponicus* eggs were, respectively, 12.9 and 12.6. Looking only at the scales that received *C.* nr. *nipponicus* eggs, the average number of eggs per female and per male scale were, 1.08 and 1.00. Chi-square analysis suggested that there is no relationship between beetle oviposition and the sex of the San José scale host (Table 3.1a).

Effect of scale density: random scale densities

The highest oviposition rate (per 48 h) (15.50±1.84 eggs) was recorded for the highest scale density. However, the LSD analysis showed that there were only two groups which differed significantly, densities over 70 scales per patch and densities below 70 ($F=4.49$ $df=[6,64]$ $p<0.05$) (Table 3.2).

Effect of scale density: fixed scale densities

The highest oviposition rate (per 48 h) in this experiment (8.6 eggs) was recorded in arenas with 65 San José scales per patch. However, with exception of the treatment with zero scales, no statistically significant differences in oviposition existed among scale densities ($F= 6.76$ $df=[5,78]$ $p<0.05$) (Table 3.3).

Effect of scale age

Since there were no significant differences among the five scale densities in the previous experiment (Table 3.3), effects of scale age on oviposition rates could be analyzed without adjustments for scale density, which varied slightly between treatments because scale numbers were not standardized after inoculations to create standard age scale patches. A statistically significant difference was observed in the preference of *C.* 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 3.4).

Effect of scale species: euonymus scale as host

When presented with euonymus scales as hosts, the average number of eggs that a *Cybocephalus* nr. *nipponicus* female beetle laid in a two day period was 4.93 ± 2.93 ($n=15$). All *C.* nr. *nipponicus* eggs occurred singly and there were significantly more eggs laid under male scales (72 eggs [97.30%]) than under females (2 eggs [2.70%]) ($t=3.047$ $p<0.05$) (Table 3.1b). The percentages of female and male scales receiving *C.* nr.

nipponicus eggs were, respectively, 0.038 and 4.89%. No significant relationship was observed between scale density per twig and beetle oviposition.

Discussion

Oviposition rates for *Cybocephalus* nr. *nipponicus* beetles were strongly affected by the sex of the host euonymus scale. Beetles placed eggs randomly under female and male San José scales but laid nearly all eggs under male scales when attacking euonymus scale. While armor thickness was not measured in this experiment, difference in armor thickness between these two scale species and their sexes could explain this oviposition preference, female and male of San José scale being much more similar than the very different sexes of euonymus scale.

Experiments on the effect of scale density on *C*. nr. *nipponicus* oviposition rates produced conflicting results. When scale densities were not fixed and beetles were chosen while mating, scale density appeared to affect beetle oviposition. When scale densities were fixed and beetles were chosen at random, rather than in mating pairs, there was no evidence that oviposition rates responded to scale densities. This difference is unexplained. Therefore, it is concluded that oviposition is not affected by scale density.

Cybocephalus nr. *nipponicus* beetles preferred to lay eggs under older scales but the reason for this is unknown. Alvarez & Van Driesche (#1 in press) 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 #1 in press). Oviposition by beetles in older scales may be a mechanism to enhance larval survival.

Table 3.1. Effect of host sex on *Cybocephalus* nr. *nipponicus* oviposition in a two-day period at 22±1°C.

a. On San José Scale

	SCALES		
SCALE SEX	With beetle eggs	Without beetle eggs	Total
Females	319	2158	2477
Males	368	2562	2930
Total	687	4720	5407

Pearson's χ^2= 0.12 p=0.7258

b. On euonymus scale

	SCALES		
SCALE SEX	With beetle eggs	Without beetle eggs	Total
Females	2	5281	5283
Males	72	1472	1544
Total	74	6753	6827

Pearson's χ^2= 238.41 p<0.05

Table 3.2. Effect of scale density on *Cybocephalus* nr. *nipponicus* oviposition in a 2 day-period at 22±1°C, using an experimental design in which scale densities were not initially fixed.

Scale Density Groups	n	Scale Density Mean Number (S.E.)	Oviposition Mean Number (S.E.)
11-40	11	29.90e (2.35)	5.54b (1.86)
41-70	24	55.83d (1.88)	8.25b (1.04)
71-100	17	85.41c (2.13)	12.71a (1.88)
101-130	12	115.92b (2.53)	15.33a (2.23)
>131	6	157.17a (8.96)	15.50a (1.84)
Average		77.93	10.74

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

Table 3.3. Effect of scale density on *Cybocephalus* nr. *nipponicus* oviposition in a 2 day-period at 22±1°C, using an experimental design in which scale densities were initially modified to fixed levels.

Scale Density (n)	Oviposition Mean Number (S.E.)
0 (15)	0b (0)
15 (12)	6.80a (1.36)
30 (15)	6.64a (0.94)
65 (12)	8.63a (1.48)
100 (14)	7.58a (1.04)
140 (13)	7.08a (1.16)
Average	5.85

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

Table 3.4. Effect of age of San José Scale on oviposition of *Cybocephalus* nr. *nipponicus* beetles in a 2 day-period at 22±1°C.

	Mean Number (S.E.)		
Scale Age (Days)	Oviposition No. eggs laid / wedge	Total scales per wedge	% of all found eggs
9 - 14	0.00c (0.00)	43.54a (4.43)	0.0c (0.0)
20 - 25	1.08bc (2.18)	45.04a (0.06)	8.8bc (3.0)
31 - 36	1.87b (1.78)	41.04a (4.12)	18.1b (3.9)
42 - 47	2.46b (3.50)	40.33a (4.57)	18.3b (4.2)
53 - 58	5.29a (3.42)	50.50a (5.81)	54.8a (5.5)
Average	2.14	44.09	20.0

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

CHAPTER IV

EFFECT OF *ENCARSIA* NR. *DIASPIDICOLA* (HYMENOPTERA: APHELINIDAE) PARASITISM ON *CYBOCEPHALUS* NR. *NIPPONICUS* (COLEOPTERA: CYBOCEPHALIDAE) EGG LAYING CHOICES

Introduction

Encarsia diaspidicola (Silvestri) has proved to be an effective biological control agent of diaspidid scales. For example, in western Samoa this wasp was released against *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Homoptera: Diaspididae) and successfully controlled this pest of passion fruit vines (Sands *et al.* 1990). In many successful biological control projects, the effect of *Encarsia diaspidicola* has been combined with the action of insect predators. In one case, the wasp was released at two different places against *P. pentagona* and biological control was achieved at both sites. However, biological control of the scale was achieved faster at the site where an endemic coccinellid predator was present and kept scale populations at low levels until the parasitoid became established (Liebregts *et al.* 1989). In a different study, *Encarsia diaspidicola* was found in high numbers parasitising the scale *P. pentagona*, which itself was being attacked by five species of coccinellids and three species of cybocephalids in the same ecosystem (Guyot & Quilici 1987).

The predator *Cybocephalus* nr. *nipponicus* Endrody-Younga (Coleoptera: Cybocephalidae) and the wasp *Encarsia* nr. *diaspidicola* (Hymenoptera: Aphelinidae) were introduced into the United States as part of the biological control project of the euonymus scale *Unaspis euonymi* [Comstock] (Homoptera: Diaspididae) (Drea & Hendrickson 1988, APHIS 1994). In Massachusetts, both natural enemies have been recovered at some release sites. Alvarez & Van Driesche (#1 and 2, in press) have estimated the reproductive potential and oviposition behavior of *C.* nr. *nipponicus*. However, there are no studies on the possible interspecific competition between these two natural enemies. Of special interest is the potential ability of *C.* nr. *nipponicus* beetles to discriminate between parasitized and unparasitized scales in choosing scales on which to lay its eggs. Such discrimination would at least partially reduce harm to the parasitoid's population by reducing attacks of adult beetle on parasitized scale.

This study was designed in order to assess whether *Cybocephalus* nr. *nipponicus* female beetles can distinguish between parasitized scales and unparasitized scales when choosing scales as oviposition sites under laboratory conditions. In the same experiment, the effects of host age, density, and sex on parasitism levels by *E.* nr. *diaspidicola* were also determined.

Materials and Methods

Sources of wasps and beetles

Colonies of *Encarsia* nr. *diaspidicola* and *C.* nr. *nipponicus* were started at the University of Massachusetts with specimens imported from Beijing, China into the United States with help from the APHIS National Biological Control Laboratory in Niles, Michigan, Larry Ertle of the USDA Beneficial Insects Research Laboratory in Newark, Delaware, Mike Rose of Texas A & M University, Mike Bryan of USDA-APHIS, and

Thomas Dorsey of the N.J. Department of Agriculture. Voucher specimens have been placed in the University of Massachusetts collection of arthropods, Amherst, MA. Because euonymus scale could not be reared apart from euonymus shrubs, an alternate species of host scale, San José scale (*Quadraspidiotus perniciosus* [Comstock], Homoptera: Diaspididae) was used for these studies, which could be reared conveniently on butternut squash, *Cucurbita* sp., in growth chambers. San José scale is a pest scale of importance in the eastern United States and is attacked by both of these natural enemies in the field. Rearing procedures for San José scales and *C.* nr. *nipponicus* beetles are given in Alvarez & Van Driesche (#1 in press).

Description of procedures to rear *Encarsia* nr. *diaspidicola* wasps

Wasps were maintained on San José scale on butternut squash. Infested squash were placed into Plexiglas cages (28 cm. on a side) with circular openings on both front and back. Cage openings were covered with organdy sleeves to provide access and ventilation. Three butternut squash, moderately infested with 10-15 day-old San José scale, were placed into each cage. An initial population of 200 wasps was added to each cage. After two months, squash were removed to different cages and replaced with new infested squash. New colonies were started periodically by transferring old squash with parasitized scales into new cages. Wasps initially present usually died in three to six days and a new generation of adults emerged within 24-30 days. When emergence of new wasps began, two moderately infested squash were added to the cage. Emergence of adult wasps usually continued for one week. After wasp emergence was completed, old squash were removed and discarded. One ounce (29.6 ml.) plastic cups with cotton wicks were used to provide water for the wasps. Pieces of self-adhesive yellow paper (5x4 cm) were streaked with thin lines of honey and placed inside rearing cages to feed wasps.

Description of experimental arena and environmental conditions

Using these *C.* nr. *nipponicus* and *E.* nr. *diaspidicola* colonies, a laboratory study was conducted in temperature-controlled cabinets (22±1°C) with a 14:10 (L:D) photoperiod. Experimental arenas consisted of circles 2.4 cm in diameter, which were delimited with modeling clay on the squash surface. The squash surface within the circles was inoculated with San José crawlers. Crawlers were obtained from heavily infested squash and examined under a stereomicroscope to eliminate populations of the mite *Tyrophagus putrescentiae* (Schrank), a laboratory contaminant of the scale culture. San José adult male scales were distinguished from female scales by the smaller size and distinct oblong shape of their scale covers. Male scale emergence occurs between 16 and 20 days after crawler inoculation at 22°C. However, scale covers remained intact, allowing exact counts of initial numbers of male scales in particular arenas.

Clay circles were numbered and covered with plastic rings which were constructed from 2.4 cm-diameter plastic vials, 5.0 cm in height. Organdy was glued over the opening of one end of each ring. The other end was affixed to the squash surface by using modeling clay. A small hole (5 mm-dia.) was drilled on a side of each ring to provide access to introduce and remove wasps and beetles. This hole was closed with a small piece of foam (Fig. 4.1).

In order to create two test scale populations in each arena, one with and one without parasitism, a patch of scales was divided in two equal parts and one portion was covered with a small piece of organdy which was glued to the squash (Fig. 4.2). Over the other portion, there were introduced five *Encarsia* nr. *diaspidicola* wasps for 24 hours so that scales would be parasitized. Wasps were placed over three different ages of the host scale: 5 to 9, 10 to 19 and 20 to 29 days after crawler inoculation. Wasps were chosen by random from laboratory colonies by aspirating adults into screened Pasteur pipettes. After removing wasps from test arenas the organdy cover over the non-exposed group of scales was also removed. One pair of mating *Cybocephalus* nr. *nipponicus* beetles was then introduced to the arena and allowed to oviposit. The scale patch on which beetles foraged thus consisted of two halves, one with parasitism and one without. Beetles had access to all scales. Beetles were placed in arenas 2, 10 or 16 days after parasitoid oviposition. The experiment was replicated eleven times for each of the nine combinations (3 scale ages x 3 periods-post-parasitism) considered. After two days, beetles were removed and all scales examined. The number of scales in each patch, the number of scales parasitized (for the portion of the patch exposed to wasps) and the number of scales bearing *C.* nr *nipponicus* eggs in both portions of the patch (parasitized and unparasitized zones) were recorded and compared. San José scales parasitized by *E.* nr. *diaspidicola* showed distinctive signs and could be easily recognized eight days after parasitoid oviposition. Bodies of parasitized scales acquire an oily or brilliant appearance and their color darkens from yellow to light-brown or brown. Parasitized San José scales also swell, and the typical wrinkles on their bodies fade. The cuticle becomes thicker and harder. For scales with younger parasitoids (the two-day-post-parasitoid-oviposition group), it was necessary to dissect the scales and to find wasp larvae to verify parasitism. Because males of San José scales emerged 16-20 days after crawler inoculation, the effect of host sex on wasp oviposition was only evaluated in the youngest group of scales.

Analysis of data

To determine if sex of San José scales affected wasp oviposition choices, a Chi-square test was performed on the number of male and female scales in parasitized and unparasitized categories. To determine the effects of scale age on parasitism rates, a one-way ANOVA was used, together with a least significant difference (LSD) comparison test. To further examine the effects of both scale age and density on numbers of scales parasitized, linear regressions were performed.

To assess if sex of adult San José scales affected beetle oviposition, a Chi-square test was performed for each treatment using data only for unparasitized scales on the protected side of each test arena. The effect of San José scale age (in the various groups of scales of different ages) on beetle oviposition was determined using a one-way ANOVA and a least significant difference (LSD) comparison.

Once the age and sex groups of scales which were acceptable for oviposition by both parasitoids and beetles were determined by the above statistical tests, a Chi-square test was run for each group of scales that were acceptable to both organisms, to examine if beetles discriminate between parasitized and not parasitized scales.

Results

Parasitism and scale density

The number of scales in a patch varied and the number parasitized by *E.* nr. *diaspidicola* increased with increasing host density ($F= 160.61$ $df=[1,94]$ $p<0.05$) (Fig. 4.3). The average number of scales per patch, however, did not vary between experimental treatment groups for this study (Table 4.1) and thus scale density did not affect analysis of results.

Parasitism and scale age

Encarsia nr. *diaspidicola* reproduces parthenogenetically and no males were observed in the experiment. The parasitoid started to lay eggs immediately after being placed in the experimental arenas. Parasitism levels by *E.* nr. *diaspidicola* were fairly constant among experimental arenas within each scale age as shown by the standard errors for the number of parasitized hosts per arena (Table 4.1). The highest number of parasitized scales (per 24 h) in an individual patch in this experiment (69 parasitized scales) was recorded in the youngest group of scales. Statistically significant differences in parasitism existed among the 3 scale ages (Table 4.1). Wasps parasitized more scales of younger age and fewer as scale age increased ($F= 136.86$ $df= [1,94]$ $p<0.05$) (Fig. 4.4a). The relationship between scale age and the percentage of scales parasitized was also significant ($F=186.59$ $df=[1,94]$ $p<0.05$) (Fig. 4.4b). The percentage of scales parasitized in the youngest, middle, and oldest groups were, respectively, 29%, 19% and 1% (Table 4.1).

Parasitism and scale sex

There was no relationship between the level of parasitism and the sex of San José scale hosts, when scales were 5-9 days old (Pearson's Chi-square: 0.44, p= 0.5066). In older scale groups, male scales had emerged and were not subject to parasitism.

Beetle oviposition, arena portion, and scale sex and age

There was no significant difference in scale densities between the two halves of the experimental arenas (8399 scales in the protected sides and 8981 scales in those exposed to parasitism) (F=1.222, df=[1, 190], p=0.2703). The number of beetle eggs laid in the halves of the experimental arenas that were exposed to wasps (526 eggs) was lower than that in the halves that were protected (653 eggs). Chi-square analysis suggested that beetles preferred to lay eggs in the half of the experimental arenas that was not exposed to wasps (Table 4.2).

The total number of female and male San José scales across all replications in the experiment were, respectively, 10157 and 7223; and the total number of eggs laid in a two-day period under female and male scales were, respectively, 457 (38.8%) and 722 (61.2%). Chi-square analysis suggested that there is a relationship between beetle oviposition and the sex of the San José host (Table 4.3). The percentages of females and male scales receiving *C.* nr. *nipponicus* eggs were, respectively, 5% and 10%.

Data from the portions of experimental arenas not exposed to parasitism (protected halves) were used to determine the effects of scale age on beetle choice between the scale sexes. In all treatments where scales were younger than thirty days, the number of eggs laid under males was significantly higher than the number laid under female scales, with exception of the treatment 1:16 (scales of age 1 [5-9 days after crawler inoculation], and beetles placed 16 days after parasitism) (Table 4.4). The highest average of beetle oviposition on protected halves (8.91 eggs per 2 days) was found in the treatment of scales of age 2 (10-19 days after crawler inoculation) and beetles placed 10 days after parasitism (Table 4.3). The two highest percentages of scales receiving *C.* nr. *nipponicus* eggs were found in the oldest groups of scales, when beetles were placed 16 days after parasitism. However, no significant differences were observed in the percentages of San José scales receiving beetle eggs for groups of scales of different ages (Table 4.4).

Beetle oviposition, and the effect of previous paratization of scale by *Encarsia* nr. *diaspidicola*

A list of the groups of scales of ages and sexes that were suitable for both parasitoid oviposition and beetle oviposition is given in Table 4.5. Treatments (see Table 4.5) were grouped into blocks based on which scale sexes were selected by parasitoids and beetles for oviposition. These blocks were subjected, by block, to Chi-square tests. Results show that beetle oviposition is related to parasitism in the groups of scale age 1 and 2 (5-9 and 10-19 days after crawler inoculation) (Table 4.6a, b, and c). For these groups, beetles strongly avoided ovipositing in previously parasitized scales. Beetle oviposition was not related to parasitism in the oldest group of scales (Table 4.6d), a result that may be due to the small number of parasitized scales in this group in the test, due to the low preference of this group by parasitoids.

When beetles were placed 16 days and 10 days after parasitism, no eggs were placed under parasitized scales (Table 4.6c, d). When beetles were placed 2 days after parasitism, one beetle egg was found under a parasitized male scale which corresponded to a 0.5% of the parasitized male scales (Table 4.6a), compared to a beetle oviposition rate of 15% on the unparasitized male scales in this groups. Across all replicates for all nine original treatments, 1178 beetle eggs were found under non-parasitized scales and only one egg was found under a parasitized scale. Although beetle oviposition was significantly higher in the halves of the experimental arenas that were protected from wasps, no significant relationship was found between percentage of parasitism and beetle oviposition (Fig. 4.5).

Discussion

Gravid females of *C.* nr. *nipponicus* were able to assess whether or not a scale was parasitized and chose to lay eggs only under unparasitized scales, even as soon as two days after parasitoid oviposition. Visual signs of parasitism on the scales were observed under the stereomicroscope only about seven days after parasitoid oviposition. Although the mechanism of this host discrimination by *C.* nr. *nipponicus* was not investigated, it seems that beetles might be able to recognize a chemical cue on parasitized scales, as discrimination occurs before the scale body develops detectable features of color or texture that indicate parasitism.

In a previous study, it was noted that *Cybocephalus* nr. *nipponicus* prefers to lay its eggs under older scales (Alvarez & Van Driesche #2 in press), in contrast to *E.* nr. *diaspidicola*, which is shown here to prefer to oviposit in younger scales. This fact, plus the avoidance of adult beetle of oviposition in parasitized scales, suggests that field competition between this parasitoid and adults of this predator for hosts is unlikely. Whether larvae of this beetle consume or avoid parasitized scales was not investigated, and such an interaction might lead to some competition with *E.* nr. *diaspidicola*, but overall, the behavior of the ovipositing adults of these two species should minimize competition between the natural enemies.

Table 4.1. Effect of age and sex of San José scale on parasitism by five *Encarsia* nr. *diaspidicola* wasps in a 24 hour-period at 22±1°C.

Scale Age[1] (n)	Female Scales Parasitism Number[2]	Female Scales Parasitism Percentage	Male Scales Parasitism Number[2]	Male Scales Parasitism Percentage	Total (Both sexes combined) Scale Density	Total Parasitism Number[2]	Total Parasitism Percentage
1 (30)	16.5 (1.2)a	29 (1.7)a	10.7 (1.2)a[3]	32 (2.8)a	98.7 (9.0)a	27.2 (2.1)a	29 (1.6)a
2 (33)	18.1 (1.6)a	30 (1.8)a	0.6 (0.2)b[4]	1(0.6)b	98.0 (6.6)a	18.7 (1.7)b	19 (1.3)b
3 (33)	0.8 (0.2)b	2 (0.6) b	0 (0.0)b	0 (0.0)b	84.2 (5.0)a	0.76 (0.24)c	1 (0.3)c

[1] Scales age groups 1, 2 and 3 are respectively, 5-9, 10-19, and 20-29 days after crawlers inoculation. (n) is the number of experimental arenas evaluated for each scale age.

[2] Mean and, in parenthesis, standard error, for number of scales parasitized in test.

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

[4] Male scale emergence occurs between 16 and 20 days after crawler inoculation, making males of this age or older not available as potential hosts.

Table 4.2. *Cybocephalus* nr. *nipponicus* oviposition in San José scale in the two different halves (protected and exposed to wasps) of the experimental arena, in a two-day period at 22±1°C.

Portion of the Experimental Arena	San José Scales		
	With beetle eggs	Without beetle eggs	Total
Exposed to wasps[1]	526	8455	8981
Protected	653	7746	8399
Total	1179	16201	17380

Pearson's $\chi^2 = 25.25$ p (Pearson's)< 0.05

[1] These scales were in part of the arena exposed to parasitism, but were not necessarily parasitized.

Table 4.3. Effect of host sex on *Cybocephalus* nr. *nipponicus* oviposition in San José scale in a two-day period at 22±1°C.

	San José Scales		
Scale Sex	With beetle eggs	Without beetle eggs	Total
Females	457	9700	10157
Males	722	6501	7223
Total	1179	16201	17380

Pearson's $\chi^2 = 201.67$ $p<0.05$

Table 4.4. Effect of age and sex of unparasitized San José scale (protected sides only) on *Cybocephalus* nr. *nipponicus* oviposition in a 2 day-period at 22±1°C.

Treatment		Total Beetle Eggs in Protected Patch Under Scales of Given Sex		Pearson's Test[3]		Beetle Oviposition Mean (S.E.)	Percentage of Scales Bearing Beetle Eggs Mean (S.E.)
(n)[1]	Scale Age[2] Days (S.E.)	Female	Male	X^2	p		
1:2 (10)	10.8 (0.25)	0	69*	90.13	0.000	6.9 (1.42)ab[4]	8.16 (2.3)a
1:10 (10)	17.1 (0.23)	8	58*	83.22	0.000	6.6 (1.07)ab	7.35 (1.3)a
1:16 (10)	21.2 (0.13)	32	36	3.78	0.0518	6.8 (1.04) ab	9.15 (1.7)a
2:2 (11)	20.0 (0.36)	27	48*	8.66	0.0033	6.8 (0.72)ab	8.34 (1.15)a
2:10 (11)	23.45 (0.21)	40	58*	32.10	0.000	8.9 (1.42)a	9.33 (1.3) a
2:16 (11)	33.36 (0.39)	61	22	1.59	0.2079	7.55 (1.01)ab	11.22 (2.0)a
3:2 (11)	25.5 (0.78)	13	44*	23.19	0.000	5.18 (0.83)b	6.66 (2.1)a
3:10 (11)	33.91 (1.07)	36	31	0.60	0.4395	6.09 (0.79)ab	9.02 (1.8)a
3:16 (11)	42.0 (0.54)	37	33	1.06	0.3021	6.36 (1.21) ab	11.21 (2.8)a

[1] Treatments consist of scale patches of three ages (1, 2 and 3 are respectively, 5-9, 10-19, and 20-29 days after crawlers inoculation and three interval after parasitism (2, 10, 16); n is the number of experimental arenas evaluated for each treatment.

[2] Scale age when beetles were placed in the experimental arena.

[3] Pearson's Chi-square tests were run for each row to examine whether the number of scales bearing beetle eggs was related to the scale sex in each treatment. Numbers with an asterisk (*) indicate a significant relationship between the scale sex and beetle oviposition.

[4] Values within columns with different letters are significantly different according to one-way ANOVA and LSD criterion at the 0.05 level.

Table 4.5. Host acceptability for parasitoid and beetle oviposition for groups of scales used in experiment, given different ages and sexes.

Scale Age at Time of Parasitoid Oviposition		Number of Days After Parasitism		
		2	10	16
5-9		B[1].1	B.2	B.3
	S.A. (S.E.)[2]	10.8 (0.25)	17.1 (0.23)	21.2 (0.13)
	Wasp	females and males[3]	females and males	females and males
	Beetle	males only[4]	males only	females and males
10-19		B.4	B.5	B.6
	S.A. (S.E.)	20.0 (0.36)	23.4 (0.21)	33.4 (0.39)
	Wasp	females only	females only	females only
	Beetle	males only	males only	females and males
20-29		B.7	B.8	B.9
	S.A. (S.E.)	25.5 (0.78)	33.9 (1.07)	42.0 (0.54)
	Wasp	few females	few females	few females
	Beetle	males only	females and males	females and males

[1] Block number.

[2] Scale age when presented to beetles for oviposition, mean and standard error.

[3] Sexes acceptable for parasitism at different scale ages are based on data in Table 4.1.

[4] Sexes acceptable for beetle oviposition at different scale ages are based on results of Chi square tests given in Table 4.4; if beetles preferred a sex, only that sex was considered suitable for analysis in the experiment of beetle discrimination between healthy and parasitized scales.

Table 4.6. Effect of previous parasitism of San José scales by *Encarsia* nr. *diaspidicola* on *Cybocephalus* nr. *nipponicus* oviposition in scale groups of ages and sexes that were suitable for both parasitoid and beetle oviposition in a two day period at 22±1°C.

	SCALES					
	[1] a.Blocks 1 & 2			[2] b.Block 3		
SCALES	With Beetle Eggs	Without Beetle Eggs	Total	With Beetle Eggs	Without Beetle Eggs	Total
Parasitized	1	220	221	0	253	253
Not parasitized	199	1115	1314	130	1497	1627
Total	200	1335	1535	130	1750	1880

	[3] c.Blocks 4, 5 & 6			[4] d.Blocks 7, 8 & 9		
SCALES	With Beetle Eggs	Without Beetle Eggs	Total	With Beetle Eggs	Without Beetle Eggs	Total
Parasitized	0	599	599	0	25	25
Not parasitized	169	3155	3324	217	2476	2693
Total	169	3754	3923	217	2501	2718

[1] a.Blocks 1 & 2, based only on male scales.

[2] b.Block 3, based on both female and male scales.

[3] c.Block 4, 5 & 6, based only on female scales

[4] d.Blocks 7, 8 & 9, based only on female scales.

Figure 4.1. Experimental arena on butternut squash covered by ventilated plastic rings, where beetles and parasitoids were placed together.

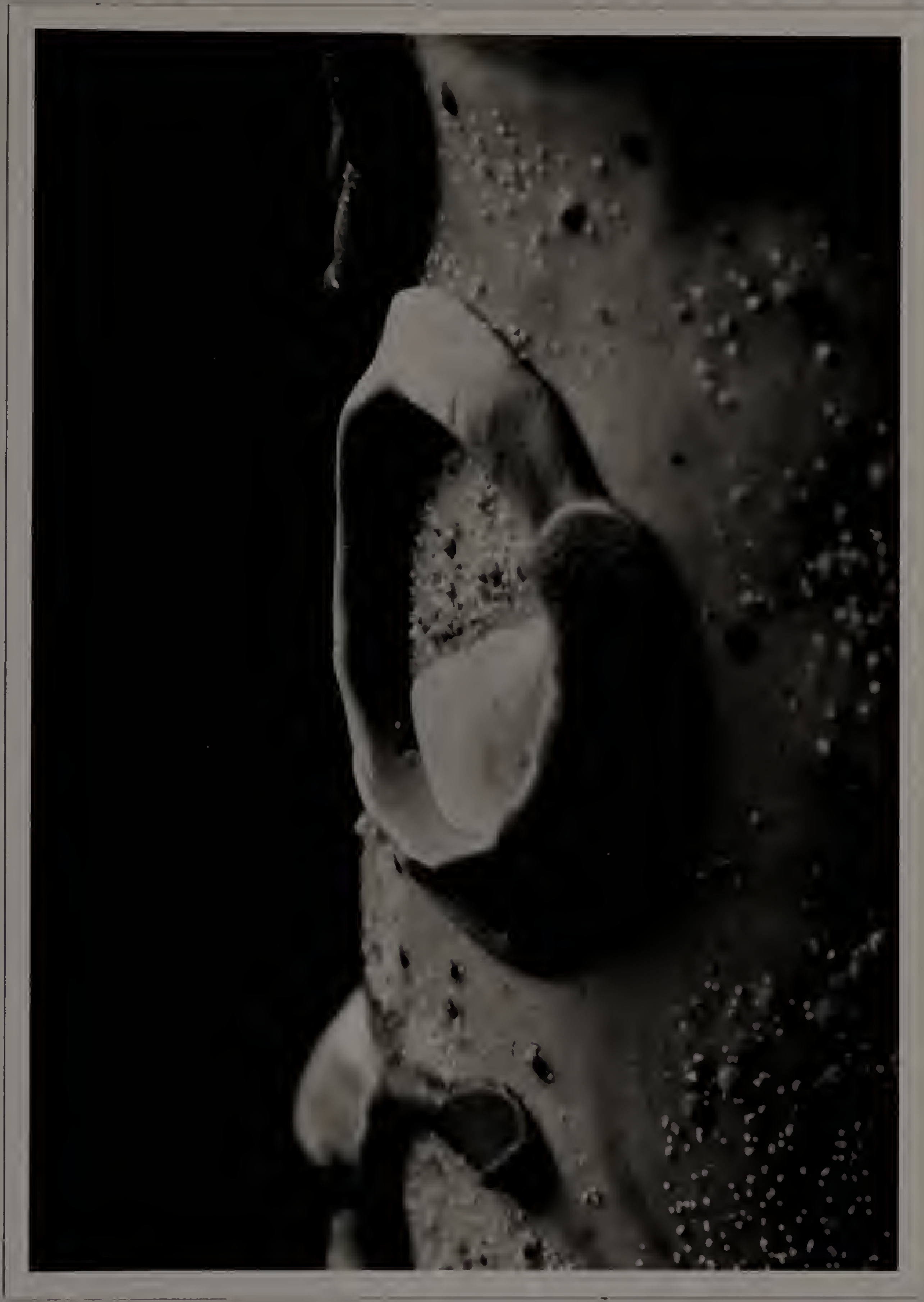

Figure 4.2. Experimental arena (without the plastic cover) was divided in two equal parts: one portion was covered with organdy which was glued to the squash, and the other portion was exposed to parasitoids.

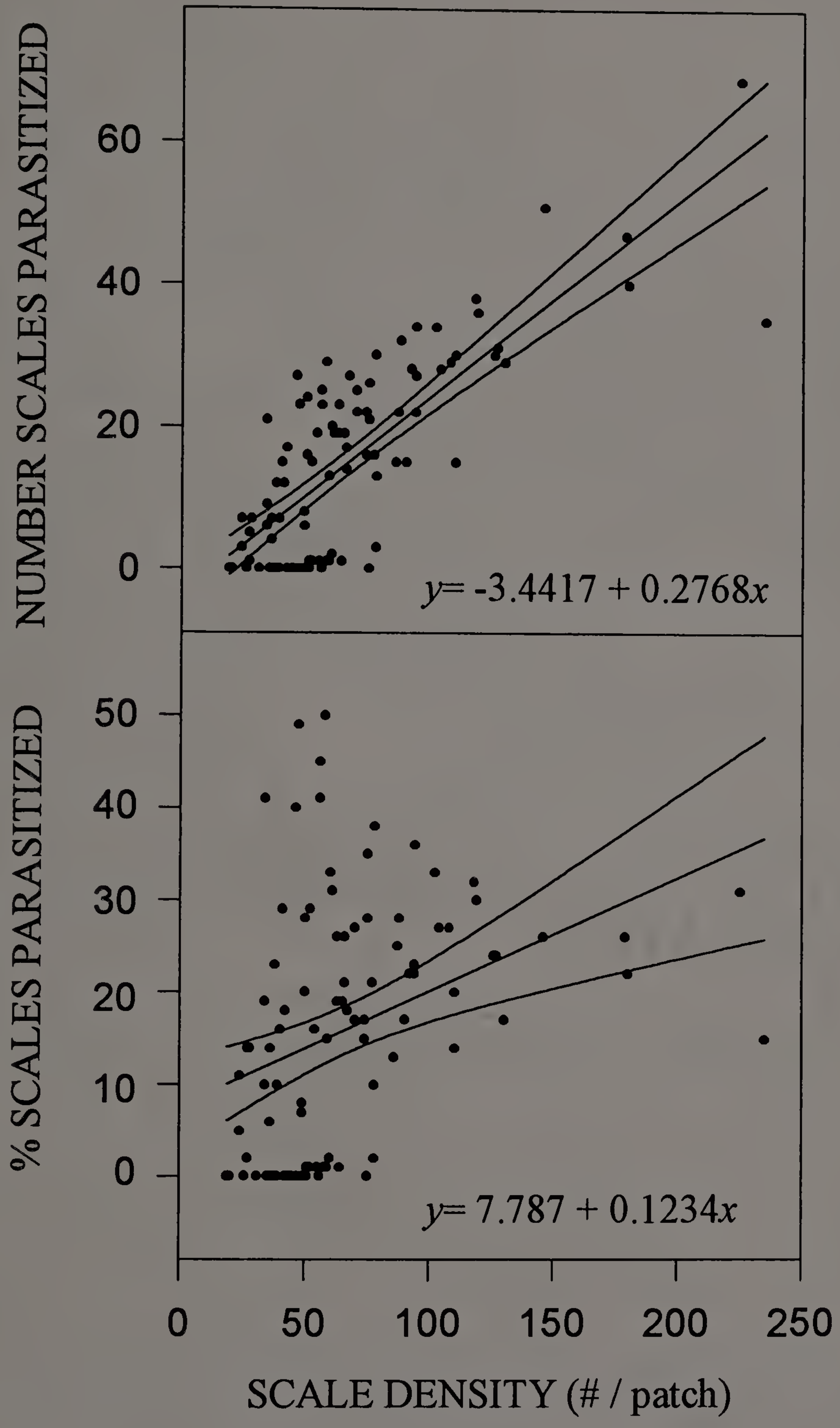

Figure 4.3. Effect of San José scale density on the number and percentage of scales parasitized by five *Encarsia* nr. *diaspidicola* wasps in 24 hours at 22±1°C.

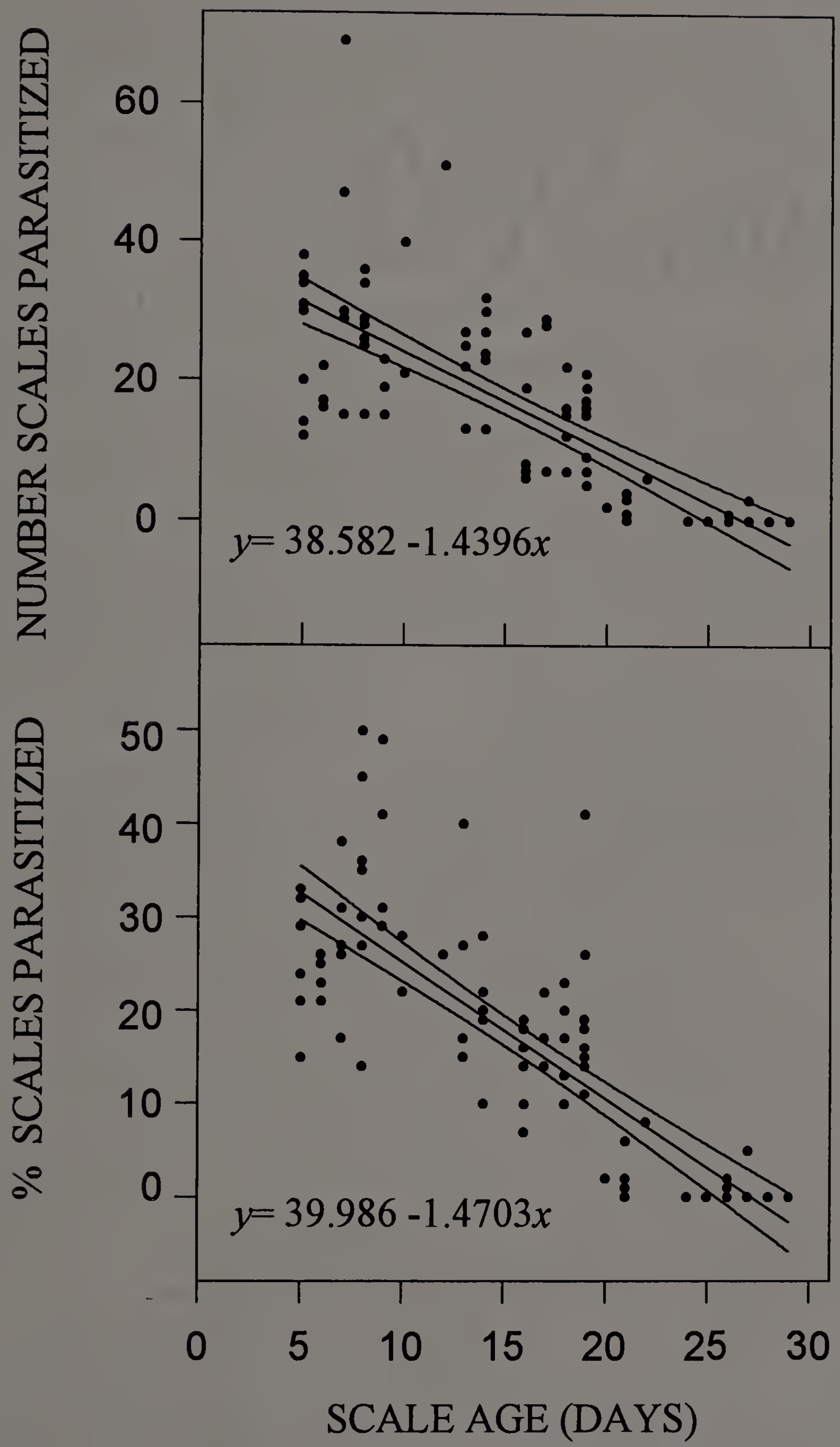

Figure 4.4. Effect of San José scale age on the number and percentage of scales parasitized by five *Encarsia* nr. *diaspidicola* wasps in 24 hours at 22±1°C.

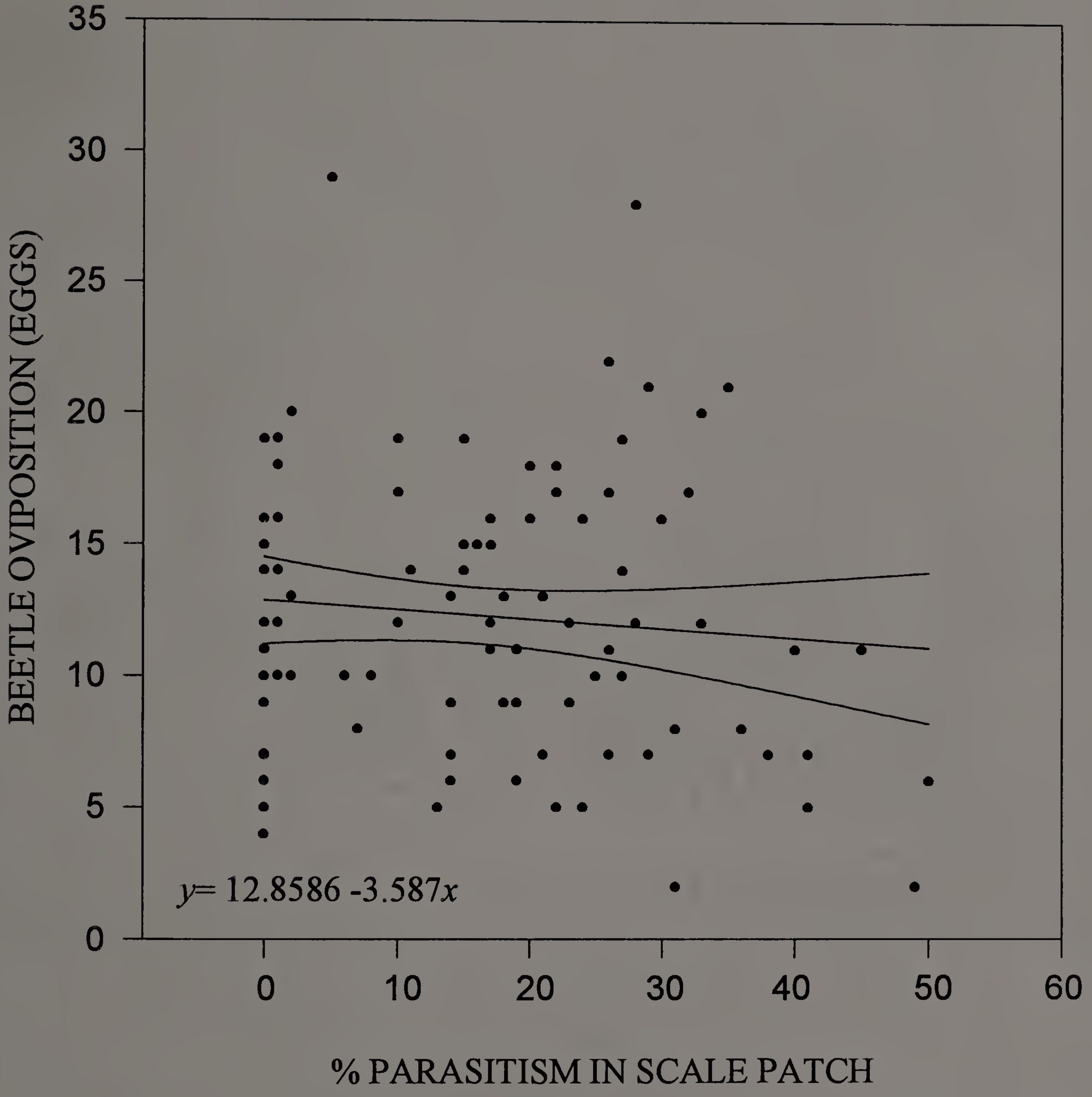

Figure 4.5. Effect of parasitism rate (%) in a scale patch by *Encarsia* nr. *diaspidicola* wasps on number of eggs subsequently laid in the patch by *Cybocephalus* nr. *nipponicus* at 22±1°C in 2 days.

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