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Daily Rhythm of Pheromone Production and Release by Females of the Black Pine Bast Scale, *Matsucoccus thunbergianae* (Homoptera: Coccoidea: Margarodidae)

일주기와 관련된 솔껍질깍지벌레 암컷성충의 성훼로몬 체내생산 및 발산

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ABSTRACT The daily rhythm of sex pheromone production and release by females of the black pine bast scale, *Matsucoccus thunbergianae* Miller and Park, was demonstrated by studying the amounts of pheromone possessed and released by females, periodically after emergence. Cycles of both pheromone production and release had daily peaks between 8 a.m. and 2 p.m., and had marked decreases after 4 p.m. It appeared that the amounts of pheromone gradually decreased three days after the emergence. Significance in synchronization of the daily rhythm of female pheromone release and activities of males and females with reference to reproductive success in this species is discussed.

KEY WORDS Black pine bast scale, *Matsucoccus thunbergianae*, Homoptera, Coccoidea, Margarodidae, pheromone, daily rhythm

초 록 솔껍질깍지벌레 암컷성충의 일주기와 관련된 성훼로몬의 체내생산과 발산 습성을 밝히기 위하여 우화후 시간별로 성훼로몬의 보유량 및 발산량을 조사하였다. 체내 생산량 및 발산량은 공히 매일 오전 8시부터 오후 2시사이에 가장 많았으며 오후 4시 이후는 현저히 줄어드는 양상을 나타내었고 또한 우화후 3일이 경과하면 점점 감소하였다. 본 곤충의 생식활동에 있어 성훼로몬의 발산과 암수성충활동의 일주기가 일치하는 것의 의미가 검토되었다.

검 색 어 솔껍질깍지벌레, 매미목, 깍지벌레상과, 짚신깍지벌레과, 성훼로몬, 일주기

The first report of sex pheromone in the superfamily Coccoidea was for the red pine scale (*Matsucoccus resinosae* Bean & Godwin) (Doane 1966). Since then, sex pheromone studies including female pheromone release were conducted. Female pheromone release in some species was found to be continuous while others exhibited periodic changes in amount of pheromone released. Citrus mealybug [*Planococcus citri*(Risso): Pseudococcidae] and *Pseudococcus calceolariae* females appeared to continuously release pheromone without daily rhythm (Rotundo & Tremblay 1980). Contrarily, Japanese pine bast scale [*M. matsumurae*(Kuwana)]females showed periodic changes in pheromone release which peaked at 5-9 a.m. (Qi et al. 1983). Tashiro and Chambers (1967) reported periodic release of pheromone in California red scale [*Aonidiella aurantii*(Maskell) : Diaspididae] females; they speculated that pheromone is continuously present in the sexually mature virgin females which have

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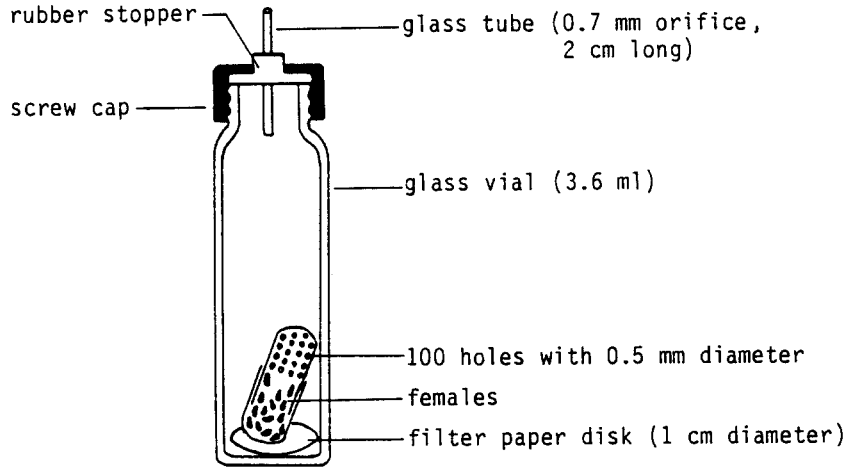

Fig. 1. Collection of pheromone volatilizing through the gelatin capsule holes in a filter paper disk.

the ability to release or withhold it. Moreno et al. (1972) also surmised that the sex pheromone is continuously present in yellow scale [*Aonidiella citrina* (Coquillett)] virgin females, and that only release is controlled.

Duration of pheromone release varies among species. *M. matsumurae* virgin females release a pheromone which elicits close range attraction of walking males for one week before the amount of pheromone release drastically decreases (Qi et al. 1983). *P. calceolariae* virgin females appeared to be attractive for approximately 3-4 weeks in the field (Rotundo & Tremblay 1977), and those without food kept releasing pheromone for 14-16 days (Rotundo et al. 1979). San Jose scale [*Quadraspidiotus perniciosus* (Comstock): Diaspididae] females attracted males for 30 days in field tests (Rice 1974), and sexually mature virgin females of the California red scale were attractive for as long as 84 days (Tashiro & Moffitt 1968).

The pheromone structure of the black pine bast scale(*M. thunbergianae* Miller & Park) was identified (Lanier et al. 1989). In collecting a large quantity of pheromone of this species for pheromone structure identification, some questions arose: Do females have the whole complement of pheromone when they emerge and only release is controlled, or do they release pheromone as they synthesize it? (If they have the whole pheromone at emergence, making crude solvent extracts by immersing the females in a solvent may be more effective than collecting volatiles from live females.) Do they release pheromone continuously, or periodically on a daily rhythm? And, for how many days do they release pheromone? In order to answer these questions, we studied the amounts of pheromone possessed and released by females with reference to elapsed time after emergence and daily rhythm.

MATERIALS AND METHODS

Preparation of pheromone samples

Infested Japanese black pine branches were collected in January 1987 and stored in the laboratory. At 5 p.m., February 14, all the females and male cocoons were discarded, and 40 newly emerged, virgin, average-sized(ca. 3 mm long) females were collected each morning from February 15 through February 18. Ninety females were collected each morning on February 19 and 20.

Surplus females and male cocoons were collected each day and discarded throughout the female sample collection period. Females were separated into daily emergence groups, which were stored in

gelatin capsules, each with 100 holes of 0.5 mm diameter. From February 15 through 18, 10 females were put in a capsule and the 30 remaining females were put in another capsule each morning. On February 19 and 20, 10 females were put in each of six separate capsules and the 30 remaining females were put in another capsule each morning.

Each 10 female sample was used to determine the amount of pheromone possessed by the female at each time interval. They were immersed in 1 ml hexane at 9 a.m., February 20 for the samples collected from February 15 to 18, and at six different times for those collected on February 19 and 20: 9 a.m., 1 p.m., 5 p.m. and 9 p.m. of February 20 and at 1 a.m. and 5 a.m. of February 21. The capsules holding 30 females were used for pheromone release rate studies (Fig. 1). Each capsule was placed in a vial with a filter paper disk of 1 cm diameter at the bottom and held for two hours from 8 to 10 a.m. February 20 for those that emerged from February 15 to 18. Pheromone collections were repeated at six different time intervals for those that emerged on February 19 and 20: at 8-10 a.m., noon-2 p.m., 4-6 p.m. and 8-10 p.m. of February 20, and at midnight -2 a.m. and 4-6 a.m. of February 21.

During each 2 hour interval of pheromone collection, the vial opening was plugged with a screw cap containing a hole in the center fitted with a glass tube(0.7 mm ID, 2 cm long). At the end of each time interval, the filter paper disk was extracted with 1 ml hexane. The number of hexane extract samples for each of the two sample preparation methods (dipping females into hexane or exposing a filter paper disk to volatilizing pheromone) was [2 days (newly emerged, one day old) × 6 times daily] + [4 days (2, 3, 4, 5 days old) × once daily] = 16.

Hexane extracts were stored for three days at room temperature. Then, 50 *ul* of each hexane extract was diluted in 950 *ul* hexane, and stored at -10 ± 5℃ until bioassayed.

Bioassay

General precedure of bioassay: The bioassay procedures were those of Park et al. (1986). Each test male was kept under a numbered, inverted, glass cup(ca. 5 ml) until tested. After each test, the male was covered with the same cup and kept for the next test. The diluted sample was delivered to walking males, on a sheet of white paper, in puffs from a medicine dropper that had been charged with 1 *ul* of the sample placed with a micropipet about 1 cm inside the tip. The dropper tip was positioned to one side of a walking male, about 8 mm from the antennae. Puffs of air were then forced from the medicine dropper by gently pressing the bulb at 1.5 sec intervals. The attraction was measured as degree of following towards the retreating dropper tip. Males that followed for one or more sides of a 4-cm-sided equilateral triangle were given scores of 1 to 3, according to the number of triangle sides completed; males responding to the attractant but following less than one side were given a score of 0.5, and those who did not follow were given a score of 0. The males used throughout the pheromone titer experiments were those which continuously walked approximately 0.5 cm/sec and showed a response score of 3 for 1 *ul* of the standard female hexane extract with 3.3×10^{-3} FE(female equivalent). Five males used for each diluted sample were allowed to rest approximately five minutes between tests. The males tested repeatedly until they became sluggish were replaced by fresh, actively walking males with a response score of 3 for 1 *ul* of the pheromone standard.

Bioassay I: Pheromone samples from storage were bioassayed without further dilution. The pheromone concentrations used in this bioassay were 5×10^{-4} FE and 3×10^{-3} FHE(female hour equivalent), respectively. The bioassay was done

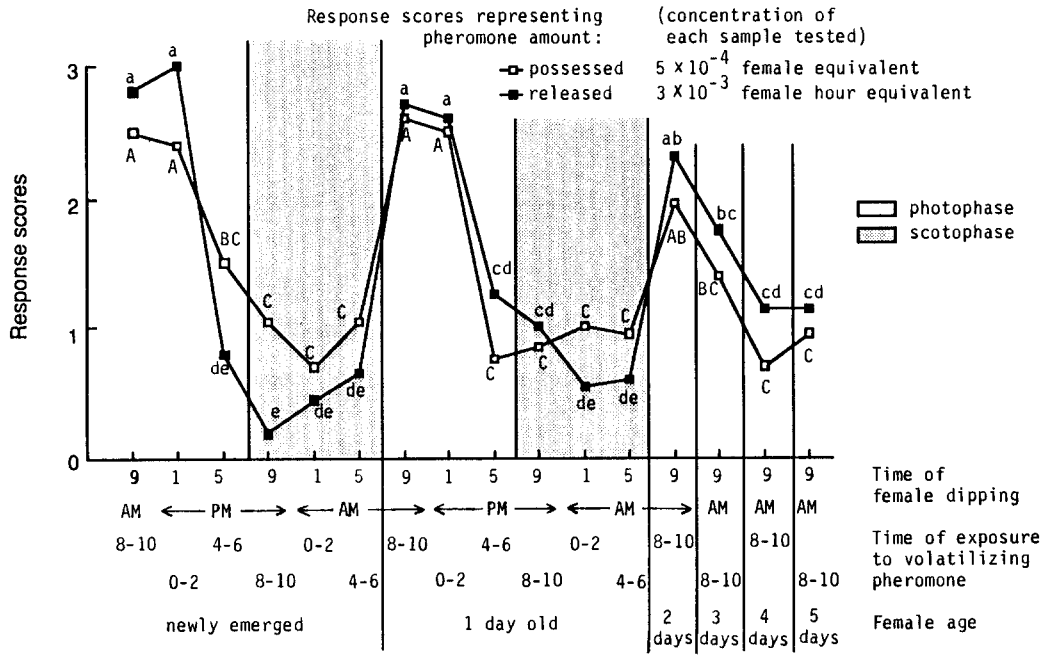

Fig. 2. Response scores of *M. thunbergianae* males representing temporal changes in the amounts of pheromone possessed and released by females. (Response scores with the same letter are not significantly different. $p<0.05$, Duncan's multiple range test. Capital letters are pheromone possessed; small letters are pheromone released.)

between 10 a.m. and 1 p.m. of February 27, 1987, and was replicated the next day.

Bioassay II: Eight pheromone dilutions were selected from the 16 samples for each of the two sample preparation methods that had been prepared with newly emerged females and one day old females at two different times each(9 a.m. and 1 p.m. for the study of pheromone possessed; 8-10 a.m. and noon-2 p.m. for pheromone released), and with 2, 3, 4 and 5day old females at one time each (9 a.m. for pheromone possessed; 8-10 a.m. for pheromone released). They were further diluted to 20% concentrations; the pheromone concentration in 1 *ul* of each diluted sample was 1×10^{-4} FE or 6×10^{-4} FHE. These samples were bioassayed in the same manner as in bioassay I between 10 a.m. and noon of March 1 and 2.

The whole procedure of sample preparation and bioassays was done at 21 ± 2℃, 65 ± 10% RH and LD 12 : 12 with photophase between 7 a.m. and 7 p.m. of 500-700 lux fluorescent light. The response were analyzed in a completely randomized design, and subjected to Duncan's multiple range test.

RESULTS

Temporal changes in the amount of pheromone possessed and released by the females are represented by scores of male response to crude female hexane extracts and to hexane extracts of filter paper substrate held with females, respectively. In both series of bioassays, score curves indicated approximately the same temporal changes in the amounts of pheromone(Fig. 2 and 3). Daily rhythm of pheromone production and release is suggested by the peak responses to samples made from newly emerged and one day old females between 8 a.m. and 2 p.m., and marked decreases to those made

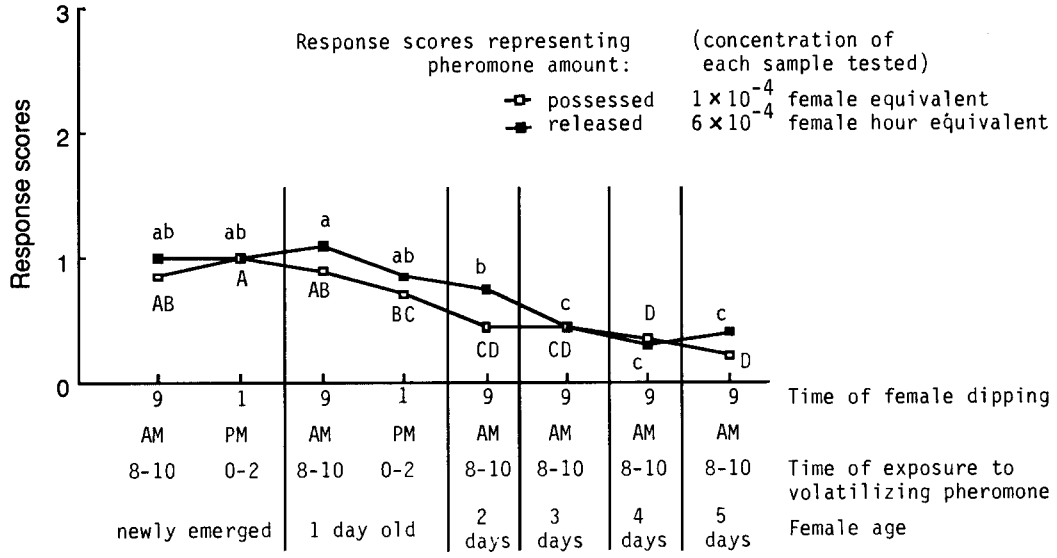

Fig. 3. Response scores of *M. thunbergianae* males representing changes in daily peaks of pheromone possessed and released by females. (Response scores with the same letter are not significantly different. $p<0.05$, Duncan's multiple range test. Capital letters are pheromone possessed; small letters are pheromone released.)

after 4 p.m. (Fig. 2). It appeared that the amount of pheromone gradually decreased three days after female emergence.

DISCUSSION

Pheromone production and release

Females release their pheromone as soon as they emerge in the morning. The amount of pheromone released was greatest between 8 a.m. and 2 p.m. This corresponds to peak pheromone release, between 5 and 9 a.m., by *M. matsumurae* females(Qi et al. 1983). It was surmised that females of the California red scale(Tashiro & Chambers 1967) and the yellow scale(Moreno et al. 1972) possess pheromone continuously, but they control its release. On the contrary, the temporal changes in pheromone concentration possessed by *M. thunbergianae* females paralleled those of pheromone release. It is obvious that, for *M. thunbergianae* females, the pheromone is released as it is synthesized on a daily rhythm. To our knowledge, demonstration of these comparative daily rhythms of female pheromone production and release is new to insect pheromones research.

Duration of female pheromone release in *M. thunbergianae* and in *M. matsumurae*(Qi et al. 1983) was much shorter than that in previously studied species of Diaspididae and Pseudococcidae. Females of diaspidids and pseudococcids are able to feed and live considerably longer than *Matsucoccus* females, which do not have functional mouthparts.

Daily rhythm of pheromone production and fertilization

Daily rhythm of pheromone release by females coincides with male activity. Both males and females emerge in the morning, after the onset of photophase, and males are able to fertilize females shortly after emergence(Park & Abrahamson, submitted). A male adult lives only about 12 hours, but mobility appears to greatly decrease after the first half of its life.

Females appear to utilize their energy very effectively. Shortly after copulation, most of them

crawl down the branches and settle in bark crevices and begin to lay eggs. The pheromone release drastically decreases after the copulation(Park 1988). If unmated, they continue releasing pheromone on a daily rhythm. Virgin females have the daily rhythm of mobility as well. They continuously walk about moving their antennae regularly during daylight hours, but become sluggish late in the afternoon. They stay in bark crevices or around branch nodes overnight, and resume mobility next morning; this cycle of activity is repeated for a few days (Park 1988). This cycle of pheromone production and mobility in females apparently enhances the reproductive ability of these short lived females.

Daily rhythm of female mobility also appears to protect them from adverse weather conditions and from natural enemies. Many females, emerged from sexually matured nymphs (neotenic), are fertilized by males on the first day of emergence. Unmated females repeat the above stated cycle of pheromone production and mobility. Females walking on the branches are frequently preyed upon by various natural enemies, whereas neither fully grown nymphs nor egg laying females were observed to be preyed upon. And it appears that females staying in their resting places overnight are better protected from adverse environmental factors than those continuously walking on the branches. Neoteny in females along with the synchronization of these daily rhythms of male activity, female pheromone release, and female mobility apparently maximizes reproductive success in this species.

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