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論　文（Original Article）

Experimental release of a parasitoid, *Dastarcus helophoroides* (Coleoptera: Bothrideridae), on *Monochamus alternatus* (Coleoptera: Cerambycidae) infesting *Pinus densiflora* in the field

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Abstract

Dastarcus helophoroides adults were released on pine stems infested by *Monochamus alternatus* in field plots. Twenty live pine trees were cut and the stems were set vertically in the plots without being cut into logs. *M. alternatus* was allowed to oviposit on the stems, and *D. helophoroides* adults were released on 10 of the 20 stems (released stems) in the following year. Mean percent parasitism on released stems was 34.8% but differed considerably among the stems (range 6–69%). Although released parasitoids had a negative effect on the host population, percent parasitism on adult *M. alternatus* was relatively low. No parasitism by dispersing *D. helophoroides* adults was observed on 8 non-released stems, which were 1.5–7.7 m away from the released stems. Generally, percent parasitism was maximized in the middle height range of stems and was low in the upper range.

Key words : biological control, *Dastarcus helophoroides*, field release, *Monochamus alternatus*, parasitoid

Introduction

Dastarcus helophoroides (Fairmaire) (Coleoptera: Bothrideridae) is a parasitoid of cerambycid beetles. Adult females deposit egg clusters on the walls of host galleries in tree stems (Gao and Qin, 1992), and hatchlings seek and paralyze hosts. Larvae that successfully parasitize a host lose their legs, begin to grow rapidly, and feed externally on the host. Mature larvae spin cocoons and pupate, then overwinter as adults.

D. helophoroides parasitizes the larvae, pupae, and adults of *Monochamus alternatus* Hope in dead pine trees, the primary vector of the pinewood nematode in Japan. Parasitism of *M. alternatus* has been observed only in Okayama and Hiroshima Prefectures in Honshu. Okamoto (1999) reported 58 % parasitism in pine stands in Okayama Prefecture. Release experiments of the parasitoid were conducted previously on pine logs infested by *M. alternatus* in the laboratory (Urano, 2003), in outdoor cages (Miura et al., 2000; Urano, 2003), and in the field (Miura et al., 2003). Results of these releases were effective: the parasitoid appears to be a good biological control agent for *M. alternatus*. In these experiments, however, the parasitoid was released on 1-m or 20-cm long logs. Release experiments should be done under conditions more similar to actual pine stands. In addition, disperse of the parasitoid larvae from a release point over the whole stem of a tree, which indicates host-searching ability of the parasitoid, cannot detected by using shortcut logs.

In advance of parasitoid release in damaged stands, I conducted a release experiment of *D. helophoroides* adults on standing pine stems inhabited by immature *M. alternatus* in the experimental forest at Kansai Research Center and recorded the percent *M. alternatus* parasitized.

Materials and methods

Field plots

The release experiment was conducted in the experimental forest at Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto, Japan. The total area of the forest is 2.56 ha. Three *Pinus densiflora* Sieb. et Zucc. stands were used for the experiment (Figure 1). Most of the forest is occupied by *Cryptomeria japonica* D. Don, *Chamaecyparis obtusa* Endl., foreign pine species, and bamboos. Except for the plots, there are only a few *P. densiflora* stands. Plots A and B were release plots and plot C, situated between A and B, was the control. The distance from A to C and B to C was 90 and 160 m, respectively. Characteristics of each plot are listed in Table 1.

Preparation of the stems for release

Twenty healthy *P. densiflora* trees were felled at the Kansai Research Center (not in the experimental forest)

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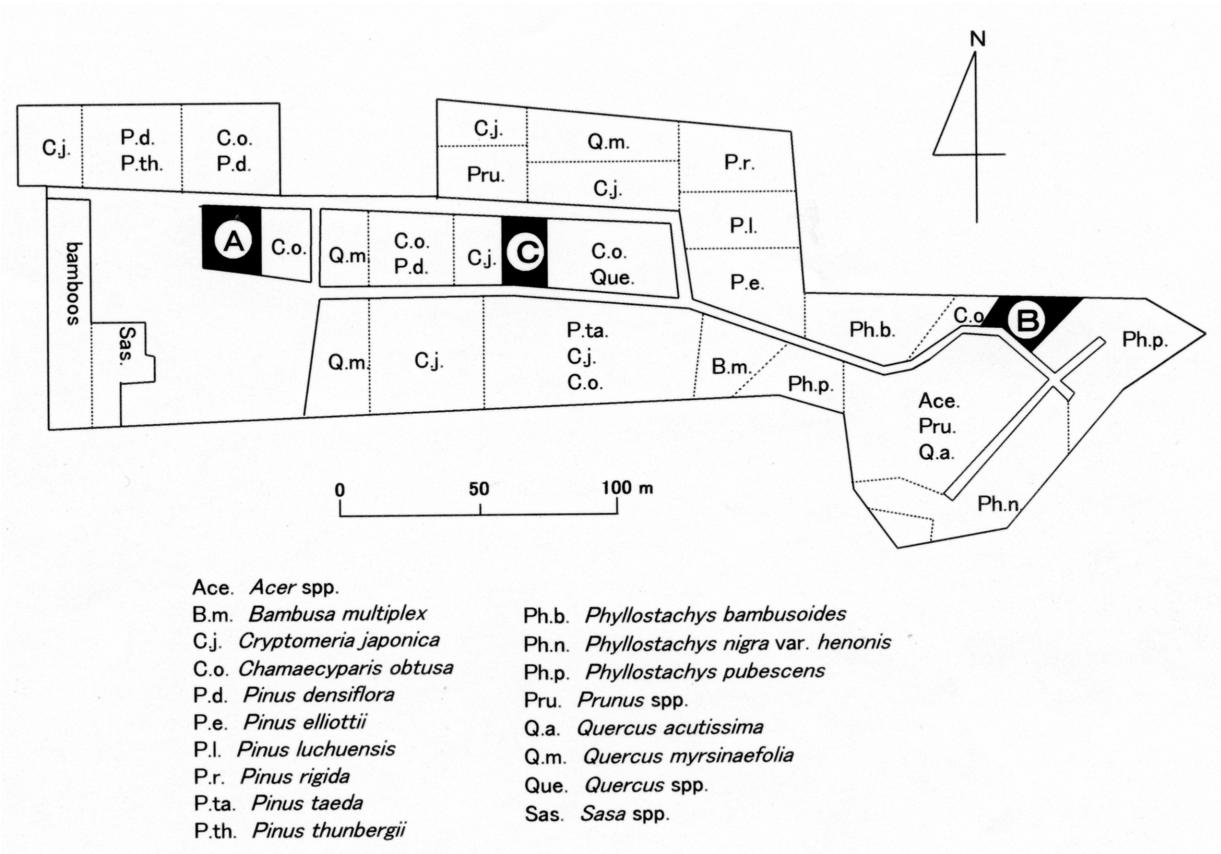

Fig. 1. Location of the three study plots (A, B, and C) and tree species in the experimental forest of Kansai Research Center.

Table 1. Characteristics of the plots

	Plot A	Plot B	Plot C (control)
Area (m^2)	208	229	124
No. of *Pinus densiflora*	120	108	31
Age (years)	10	18	20
Mean height (m)	5.6	8.3	12.0
Mean DBH (cm)	7.3	10.6	12.8

in July 2000. Mean height of the trees was 5.26 m (range 4.67–6.55 m) and mean DBH was 6.9 cm (range 6.3–10.2 cm). They were pruned and hauled into the plots without being cut into logs. Nine stems each were set in plots A and B, and two stems were set in plot C. Each stem was leaned against a live pine tree and the two fastened together with a rope. Stems were arranged randomly in each plot. Mean distance between two adjacent stems was 2.9 m (range 1.0–4.5 m).

Each stem was covered with white cotton screen at 0.5–1.5 and 2.0–3.0 m height in mid-July 2000. One mated female *M. alternatus* was released under each screen section and allowed to oviposit for two weeks. Two or three current shoots of *P. densiflora* were placed in the screen for adult's food. The screen cover was then removed, and the stems were kept in place until the following year.

Rearing and release of the parasitoid

D. helophoroides adults used for the experiment were laboratory-reared offspring of adults collected from dead *P. densiflora* trees at Wake-cho in Okayama Prefecture (34° 49'N, 134° 08'E) in May 1999. The method used for rearing the adults was as described by Ogura et al. (1999). Briefly, the adults were reared in a plastic petri dish (9.5 cm in diameter, 2.3 cm in height) with a dried *M. alternatus* larval corpse for food, a block of pine wood (1.5 × 1.5 × 5.0 cm) with a hole drilled in it (0.9 cm in diameter, 4.0 cm in depth) as an oviposition site, and absorbent cotton moistened with tap water which was provided in a plastic container (2.0 cm in diameter, 1.2 cm in height) on filter paper. The adults were maintained at 28 ℃ under 16L8D.

Dastarcus helophoroides eggs were moved to other petri dishes with the wood blocks. When the eggs eclosed, live *M. alternatus* larvae (collected from dead *P. densiflora* at Kansai Research Center) were put in the dishes. Hatchlings of *D. helophoroides* parasitized the hosts and were reared at 25 ℃ under 16L8D until the adults emerged.

A total of 300 *D. helophoroides* adults for the release experiment emerged from July 1999 to August 2000. In total, 215 adults were maintained at 28 ℃ and began to oviposit in the laboratory 8–10 months after adult eclosion. An additional 85 adults were maintained under laboratory conditions at 7–34 ℃ and began to oviposit in March 2001. I did not determine the sex ratio of all adults because the sex of *D. helophoroides* cannot be determined by observing the morphology of adults (Ogura et al., 1999). Nevertheless, the sex ratio of released adults was expected to be almost 1:1 because the proportion of males among 43 *D. helophoroides* determined was 0.56, which equates to a sex ratio of nearly 1:1 ($\chi^2_{cal} = 0.58$, $P > 0.05$) (Urano, 2003).

On April 20, 2001, I released 30 adults each on five stems (released stems) randomly chosen in plots A and B (i.e., 10 stems total). I cut the bark and sapwood with a hatchet and made crevices on each stem at 1.2 and 2.5 m high; 15 adults were then placed in each crevice. Plots A and B contained four non-released stems each but could have been parasitized by adults from the released stems nearby. The mean distance from each non-released stem to its nearest released stem was 4.8 m (range 1.5–7.7 m).

Because emergence of *M. alternatus* normally begins in late May, stems were taken in from the plots on May 18, 2001 to avoid release of *M. alternatus* adults in the field and occurrence of pine wilt disease in and around the experimental forest. All the stems, including controls, were cut into 1.5-m long logs, numbered on each log from the bottom, and then placed in outdoor cages (75 × 75 × 180 cm; 1-mm mesh). The cages were 100–300 m away from each plot. Stems were debarked and dissected from early June until early July. Diameter and bark thickness of each 1.5 m logs were measured. For *M. alternatus* in the dissected stems, I counted the following: live larvae, pupae, adults, emergence holes, parasitized individuals, and individuals that died due to unknown factors. Percent parasitism was calculated by dividing the number of parasitized *M. alternatus* by the sum of all categories.

Surface area of each log was calculated to determine the density of *M. alternatus* and vertical distribution of the parasitoids. Surface area and bark thickness in each released stem were shown in Figure 2. Most released stems were cut into three 1.5-m long logs while only 2 stems (A-1 and A-3) included 4 logs.

Dastarcus helophoroides is a facultative gregarious parasitoid, i.e. one or multiple larvae parasitize one host (Urano, 2003). I counted the number of the parasitoid's larvae and cocoons parasitizing one host individual for surveying the host utilization of the parasitoid.

Results

A total of 348 *M. alternatus* infested the 10 released stems (range 19–49 on each stem); among these, 121 individuals were parasitized by *D. helophoroides*. Mean percent parasitism was 34.8 % but varied considerably among the stems (range 6–69 %; Fig. 3a). The proportion of live *M. alternatus* was 55.7 %, and 9.5 % had died due to other factors. Fourteen of the 300 released *D. helophoroides* adults were collected; these were all found on the surface of or under bark.

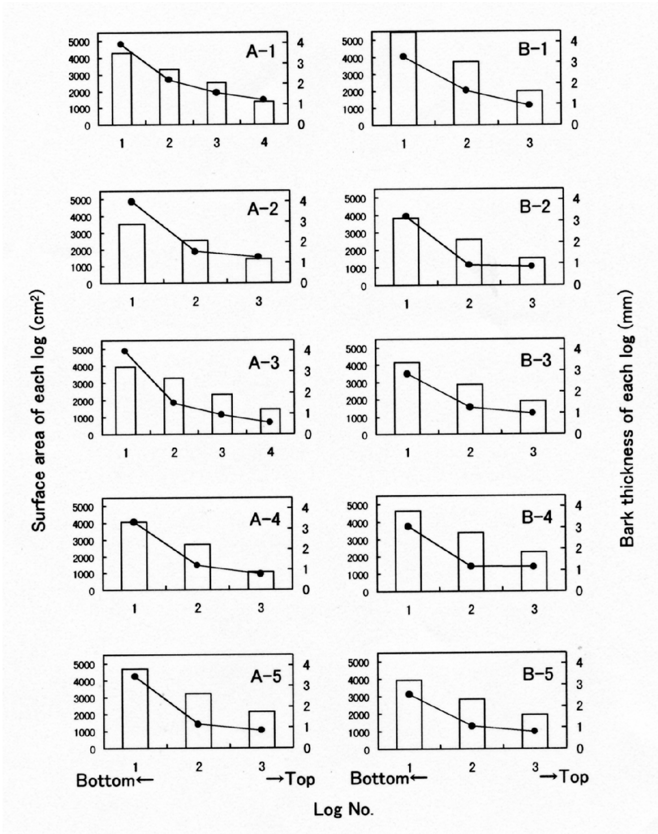

Fig. 2. Surface area (bars) and bark thickness (lines) of each 1.5-m long log cut from stems in plots A and B.

In total, 253 and 71 *M. alternatus* were observed in non-released and control stems, respectively (Fig. 3b). None of them were parasitized by *D. helophoroides*. The proportion of live *M. alternatus* was 92.9 % in non-released stems and 88.7 % in control stems. The proportion of live *M. alternatus* in released stems was significantly lower than that in the control stems ($\chi^2_{cal} = 27.055$, $P < 0.0001$).

Because *D. helophoroides* hatchlings paralyze their host and feed on it, developmental stage of the host remains collected from the stems indicates the stage when the host was paralyzed by the hatchlings. Most of the parasitized *M. alternatus* observed in the stems were larvae or pupae, and the number of adults was relatively small throughout the dissecting period (Fig. 4a). In addition, there was no progress of the host stage during

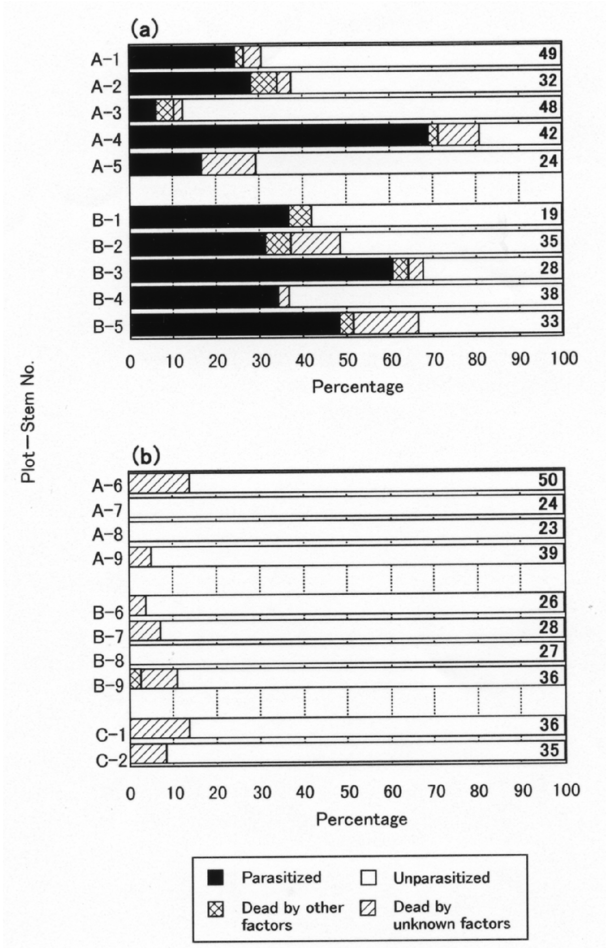

Fig. 3. Percentages of live (unparasitized) and dead *Monochamus alternatus* in stems onto which *Dastarcus helophoroides* adults were released (a) or were not released (b) in plots A and B. Plot C was the control. Live *M. alternatus* includes emerged adults, counted by the number of emergence holes on the stems. Numbers on each bar indicate the total number of *M. alternatus* larvae in each stem. The category dead by other factors includes *M. alternatus* killed by predators or microorganisms.

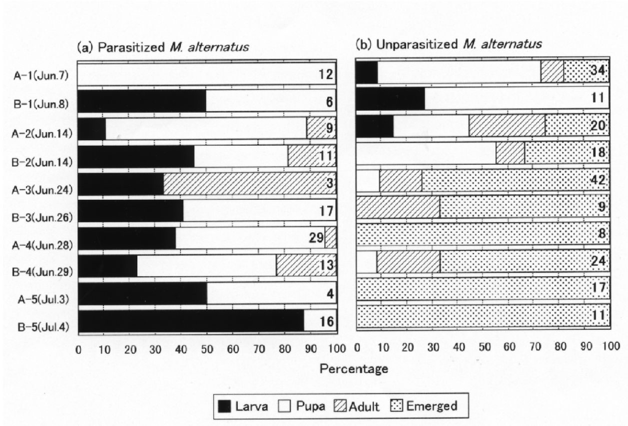

Fig. 4. Percentage of each developmental stage of *Monochamus alternatus* (a) parasitized by *Dastarcus helophoroides* and (b) unparasitized in each stem. Date of each stem dissection is shown in parentheses. Numbers on each bar indicate the total number of *M. alternatus* larvae in each stem. The category adults include adults in pupal chambers in the stems, and emerged includes adults emerged from the stems (as indicated by emergence holes).

about one month of the dissecting period, whereas the developmental stage of unparasitized *M. alternatus* clearly advanced during this period (Fig. 4b). Larvae and pupae accounted for a large number of unparasitized individuals in early June, and the percentage of adults became larger after mid-June. All *M. alternatus* had emerged from the stems by early July.

Total number and density of *M. alternatus* boring in each height of released stems was largest in No. 2 logs, which were 2nd logs from the bottom (Table 2). I observed 163 individuals in No. 2 logs that occupied 46.8 % of whole *M. alternatus*. Although number of surviving individuals was also largest in No. 2, the proportion of live *M. alternatus* in No. 2 (survival rate in Table 2) did not differ significantly from that in the other logs (χ^2_{cal} = 2.339, P = 0.3106). Number of parasitized *M. alternatus* and percent parasitism were also largest in No. 2 logs and no parasitism was observed in No. 4 logs. Proportion of parasitized *M. alternatus* differed significantly among Nos. 1- 3 logs (χ^2_{cal} = 10.583, P = 0.0050).

Although density of *M. alternatus* was greatest in the middle part of most stems, it was greatest in the upper part for others (A-1, B-1, and B-2) when the density in each stem was shown in Figure 5. Percent parasitism was also maximized in the middle part of stems but was low in the upper part. In fact, in six stems there was no parasitism in the upper part. In three cases, the percent parasitism was highest in the bottom part (B-2, B-4, and B-5 in Fig. 5).

Figure 6 shows the frequency distribution of the number of *D. helophoroides* larvae or cocoons found parasitizing a single host. Fifty-five of 117 hosts (47.0 %) supported a single parasitoid. Although I observed one host with 11 cocoons in maximum, each parasitoid was small. Average number of *D. helophoroides* per host was approximately two at each height of the stems (Table 2).

I collected 86 host-feeding larvae and 159 cocoons (full-grown larvae or pupae) of *D. helophoroides* from the stems. Average number of the parasitoid per host in each stage was 1.95 and 2.27, which did not differ significantly (Mann-Whitney U = 1516.5, P = 0.8912).

Total numbers of the parasitoid that parasitized each stage of the host were 109 on larva, 137 on pupa, and 9 on adult. As shown in Fig. 4, the small number of host adults affected the number of the parasitoids on them. Average number of the parasitoid per host was 2.32 on larva and 2.17 on pupa. There was no significant difference between them (Mann-Whitney U = 1340.5, P = 0.3976).

Table 2. Number, density, and percent parasitism of *M. alternatus* and number of *D. helophoroides* parasitizing a single host in the logs of each height.

Log No.[1]	Height (m)	Total No. of M.a.[2] (A)	Density of M.a. ($100cm^{-2}$)	No. of live M.a. (B)	Survival rate of M.a. (B/A)	No. of parasitized M.a. (C)	Percent Parasitism (C × 100/A)	No. of D.h.[3] per host
1	0-1.5	118	0.28	70	0.59	33	28.0	1.85
2	1.5-3.0	163	0.54	82	0.50	73	44.8	2.30
3	3.0-4.5	55	0.29	31	0.56	15	27.3	2.36
4	4.5-6.0	12	0.43	11	0.92	0	0	-

[1]Eight stems were cut into three logs (Nos. 1 – 3) while other two stems (A-1 and A-3) included four logs.
[2]*Monochamus alternatus*
[3]*Dastarcus helophoroides*

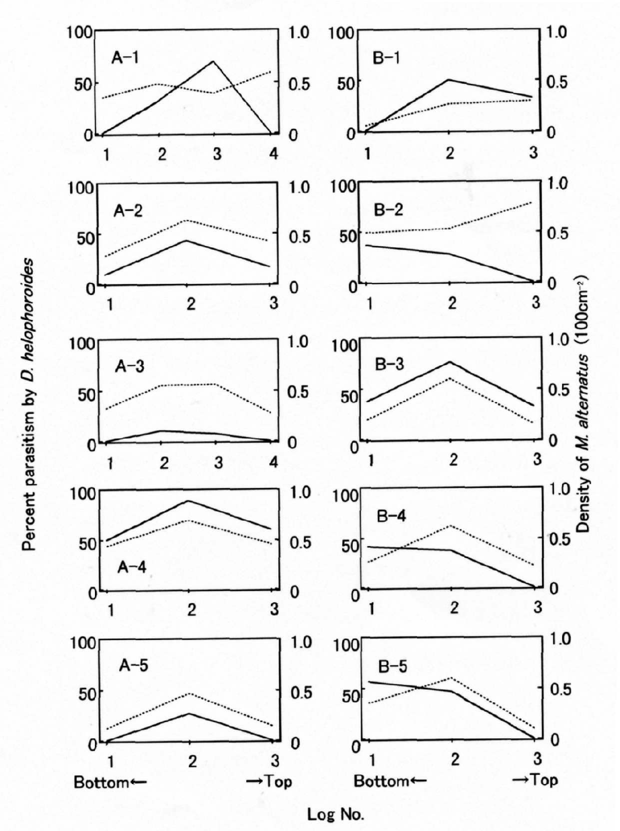

Fig. 5. Percent parasitism by *Dastarcus helophoroides* (solid lines) and density of *Monochamus alternatus* (dotted lines) in each 1 .5-m long log cut from stems in plots A and B, each of which were numbered from the bottom.

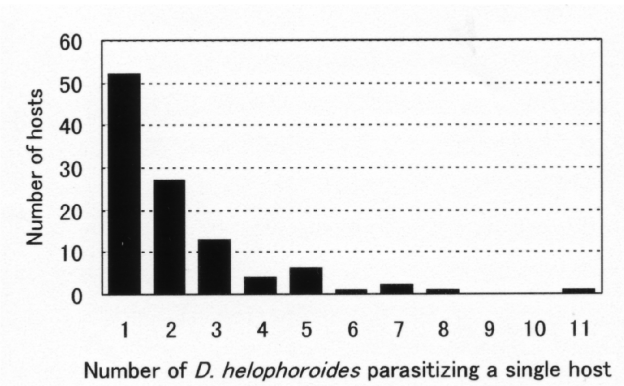

Fig. 6. Distribution of the number of *Dastarcus helophoroides* larvae or cocoons parasitizing a single host.

Discussion

Urano (2003) performed a release of *D. helophoroides* eggs in outdoor cages, with the result that the percent parasitism was 49.7 %. In that experiment, many *M. alternatus* had already emerged before hatching of the released eggs on several logs onto which the eggs were released in late May. Thus the parasitoids were released earlier on April 20 in the present study. Nevertheless, percent parasitism was 34.8 % and lower than the previous study. Miura et al. (2003) also released *D. helophoroides* in the field, although they released adults on 1-m logs set on living pine trees. In their study, mean percent parasitism of *M. alternatus* in 14 logs was 68.9 %.

I carried the stems from the plots on May 18 and put them into outdoor cages. Released adults should have been able to oviposit during the period the stems were kept in outdoor cages, from May 18 to July 4. Nevertheless, only 14 adults (5 % of all released adults) were collected from the stems in the cages. Relatively few *M. alternatus* were parasitized in the adult stage compared with other stages (Fig. 4a). In the previous study (Urano, 2003), when *D. helophoroides* eggs were released in outdoor cages at the end of May, most *M. alternatus* were parasitized in the adult stage. Because *M. alternatus* emerge from late May to July, the majority of live hosts in the stems must have been adults during the period the stems were kept in the cages. However, most adults were not parasitized and 106 adults emerged (Fig. 4b) in the present study. Thus, it appears that only a small number of eggs were laid by the parasitoid in outdoor cages.

Parasitism in non-released stems, which were 1.5–7.7 m away from released stems, was not observed (Fig. 3b). *D. helophoroides* adults rarely fly, and locomotor activity is low (Li and Wu, 1993). Miura et al. (2003) revealed that more than 20 % of the adults remained on the logs for 40 days after their release and a small portion of adults could disperse and oviposit only in a narrow range. Because only 5 % of released adults were collected in outdoor cages, most of adults had left their released stems during the release period but none of them did not oviposit on non-released stems in the present study.

Alternatively, most of the adults that remained on the stems may have fallen off the stems due to the impact of cutting and carrying when the stems were taken from the plots.

Percent parasitism of *D. helophoroides* was maximized in the middle of most stems and was not necessarily related to the density of *M. alternatus* (Fig. 5). Ogura (2002) reported that *D. helophoroides* hatchlings move up and down on the surface of pine logs for a minimum of 1.5 m. In the present study, adults were released at 1.2 and 2.5 m on each stem above ground level. Thus, the uppermost part of the stem was within the range of hatchlings' movement on most of the stems consisting of three 1.5-m logs (4.5 m at the top), even if released adults oviposited at the release point without any movement. Nevertheless, percent parasitism was always less in the uppermost part of stems (Fig. 5). There may have been physical factors, such as temperature or texture of the bark surface that prevented oviposition or host searching behavior on upper parts of stems. Percent parasitism in the bottom part of stems (No. 1 logs) was also lower than that in the middle part. Gao and Qin (1992) reported that adults oviposit on the walls of host galleries in stems although I have not observed any eggs of *D. helophoroides* in the field. If released adults had to enter under the bark for oviposition in the host galleries, the thick bark in the bottom part (Fig. 2) seemed to prevent their entry into the host galleries.

A total of 255 parasitoids were collected from the stems that were host-feeding larvae and cocoons (Fig. 6). Although I could not directly count the number of eggs laid in the stems, one female deposited about 20 eggs in average during one month from late April in the laboratory (Urano, 2003). I can estimate that released adults (150 females) laid about 3000 eggs during the release period if they oviposited at the same oviposition rate as the adults in the laboratory. Thus more than 90 % of parasitoids died before they succeeded in feeding host. Most of dead individuals are probably 1st-instar larvae, because they disperse from egg clusters and enter the pupal chamber of *M. alternatus* in xylem, and then paralyze their host. Mortality in the stages after host-feeding larva is relatively low because average numbers of the parasitoid per host did not differ significantly between the two developmental stages, host-feeding larva and cocoon.

Adult release of *D. helophoroides* on pine stems infested by *M. alternatus* was effective in the field because the total mortality of *M. alternatus* was reached to 44.3 % in released stems, whereas that in control stems was only 11.3 % (Fig. 4). However, carrying the stems from the plots into cages were so early that percent parasitism on adult *M. alternatus* was low in the present study. When I conduct a release experiment in damaged pine stands in the future, this point should be considered. Effects of both the height of release point and the number of *D. helophoroides* adults on vertical change of percent parasitism along the stem may be revealed more clearly by using longer stems in future experiments.

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マツノマダラカミキリ穿入アカマツ樹幹を用いたサビマダラオオホソカタムシの野外放飼試験

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要 旨

マツノマダラカミキリ穿入樹幹へのサビマダラオオホソカタムシ成虫の野外放飼試験を、森林総合研究所関西支所構内で行った。健全なアカマツ 20 本を伐倒し、樹幹を玉切らずに試験地内に立て掛け、そこにマツノマダラカミキリ成虫を放って産卵させた。翌年供試木の内 10 本（放飼木）にサビマダラオオホソカタムシ成虫を放飼した。放飼木を割材した結果、材内に穿入したマツノマダラカミキリに対する寄生率は平均 34.8 ％であった。寄生率は供試木ごとに 6 ～ 69 ％とばらつきが大きかった。放飼による寄主個体群抑制の効果は認められたものの、寄主成虫に対する寄生率は比較的低かった。放飼木から 1.5 ～ 7.7 m離れたところに無放飼木を設置したが、放飼木から無放飼木へ移動したサビマダラオオホソカタムシ成虫による寄生は認められなかった。樹幹の高さ別に寄生率を調べた結果、全体的に樹幹中央部において寄生率は最も高く、上部において低くなる傾向が認められた。

キーワード：生物的防除、サビマダラオオホソカタムシ、野外放飼、マツノマダラカミキリ、捕食寄生者

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