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論　文（Original article）

Experimental release of adult *Dastarcus helophoroides* (Coleoptera: Bothrideridae) in a pine stand damaged by pine wilt disease: Effects on *Monochamus alternatus* (Coleoptera: Cerambycidae)

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Abstract

I examined the effect of the parasitoid *Dastarcus helophoroides* (Fairmaire) on *Monochamus alternatus* Hope in *Pinus densiflora* Sieb. et Zucc. trees that had been killed naturally by pine wilt disease. Selected trees received either released *D. helophoroides* adults (treated trees) or no treatment (control trees). Vertical (within-tree) and horizontal (among-tree) movement of the released *D. helophoroides* adults was examined to elucidate their dispersal ability in a damaged pine stand. The release of *D. helophoroides* clearly had a detrimental effect on *M. alternatus* survival; *M. alternatus* mortality was 76.3% in 2002, 84.6% in 2003, and 57.2% in 2004 in treated trees, which was significantly higher than that in control trees (18.8%, 5.3%, and 26.7%, respectively). No parasitism of *M. alternatus* by dispersing *D. helophoroides* adults was observed on control trees in 2002 and 2003, but it was 16.7% in one of six control trees located 265 m from the nearest treated tree in 2004. No *M. alternatus* were parasitized in the other control trees, even in the vicinity of treated trees. Two adults that were released in May 2003 remained at the study site were collected in November 2003. Although parasitism by *D. helophoroides* released onto stems 1.2 m above the ground was observed up to 11 m in treated trees, both the percentage of parasitism and the mortality of *M. alternatus* decreased with increasing height in the tree.

Key words : biological control, *Dastarcus helophoroides*, dispersal ability, *Monochamus alternatus*, release

Introduction

Dastarcus helophoroides (Fairmaire) is a native parasitoid of cerambycid beetles. Adult females deposit egg clusters on the gallery walls bored into the stems of trees by host larvae (Gao & Qin, 1992). The hatchlings seek out and paralyze their hosts. Larvae that successfully parasitize hosts feed externally on the host and grow rapidly. Mature larvae spin cocoons and pupate, emerge in August and September (Urano et al., 2004), and overwinter as adults. *D. helophoroides* parasitizes the larvae, pupae, and adults of *Monochamus alternatus* Hope (Okamoto, 1999), the primary vector of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle in Japan. Parasitism of *M. alternatus* by *D. helophoroides* has been observed only in Okayama, Hiroshima, and Tottori prefectures under natural conditions (Taketsune, 1982; Inoue, 1993; Urano et al., 2004).

M. alternatus adults oviposit on dying pine trees in the summer. The larvae feed on the host's phloem and cambium, form oval-shaped entrance holes, bore into the xylem, and construct vertical galleries plugged with fibrous frass before winter. Pupal chambers, in which the larvae overwinter and pupate, are constructed at the ends of galleries. Adults emerge in June and July in central Japan (Kishi, 1995).

Dispersal ability is critical when considering a parasitoid as a potential biological control agent. In previous experiments, *D. helophoroides* was released on pine logs naturally infested by *M. alternatus* (Miura et al., 2000; Miura et al., 2003; Urano, 2003; Ishii, 2004) or on stems onto which released *M. alternatus* adults had been allowed to oviposit (Urano, 2004). Although Urano (2004) released *D. helophoroides* on stems that were 4.7 – 6.6 m in height and parasitism was observed up to 4.5 m in height, it is unknown whether *D. helophoroides* can move to and parasitize *M. alternatus* in pine trees over 4.5 m in height. In addition, previous studies showed that either no adults (Urano, 2004) or only a few adults (Miura et al., 2003) moved from the tree on which they were released to other trees. Both studies, however, were conducted in relatively small plots (196 – 229 m^2); thus, it is possible that some adults could have dispersed beyond the plots. A survey of adult dispersal ability over large areas is necessary.

We released *D. helophoroides* on trees of *Pinus densiflora*

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Sieb. et Zucc. that had been killed by pine wilt disease and infested by *M. alternatus* naturally, and the percentage of parasitism by *D. helophoroides* was determined. The dispersal of released *D. helophoroides* adults was examined by following the vertical (within-tree) and horizontal (among-tree) distribution of *D. helophoroides* parasitism on *M. alternatus*. Furthermore, methods for using *D. helophoroides* as a practical biological control agent against *M. alternatus* are discussed.

Materials and methods

Study site

A *D. helophoroides* release experiment was conducted in a *P. densiflora* stand in the Ohmi-Fuji Karyoku Park (Yasu City, Shiga Prefecture, Japan; 35°03′ N, 136°02′ E; 110–150 m a.s.l.) from 2002 to 2004. The park has a total area of approximately 59 ha; *Quercus serrata* Thunb. and *Chamaecyparis obtusa* Endl. are the other dominant canopy species at the study site. Mass mortality of *P. densiflora* caused by pine wilt disease has occurred in the park since the 1980s.

In total, 446 *P. densiflora* trees were numbered in the park in November 1999, and dead trees were counted in October and November 2000–2004. The number of dead trees was 82, 62, 71, 29, and 12, respectively, for each year from 2000 to 2004. At the end of the 2004 season, the cumulative mortality of these trees since 1999 reached 57.4%. Dissection of more than 30 dead *P. densiflora* trees in 2000 and 2001 showed no parasitism by *D. helophoroides*, suggesting that the parasitoids were not indigenous to the site (T. Urano, unpublished data).

Rearing of *D. helophoroides* in the laboratory

The released *D. helophoroides* adults 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 parasitoids were reared at the Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto. Collected adults were reared in plastic Petri dishes (9.5 cm in diameter, 2.3 cm in height) lined with filter paper and containing 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. A piece of artificial diet (1–2 mg/adult) was provided once a week. The main ingredient of the artificial diet was silkworm pupa powder (Marukyu Corp., Japan; Ogura et al., 1999). An absorbent cotton ball moistened with tap water was placed in a small plastic container (2.0 cm in diameter, 1.2 cm in height). The adults were maintained at 28°C under a 16-h light (L):8-h dark (D) photoperiod.

Wood blocks containing the eggs were transferred to new Petri dishes, and eggs were provided with live *M. alternatus* larvae (collected from dead *P. densiflora* at the Kansai Research Center) as hosts. When the parasitoid larvae reached the second instar, they were transferred to 2.5 g of artificial diet paste made of chicken liver and other ingredients (Ogura, 2001). Fully grown larvae were placed into new Petri dishes, allowed to form cocoons, and maintained at 28°C under a 16 L: 8 D photoperiod until emergence.

Adult *D. helophoroides* reared in Petri dishes emerged between March and September each year. They were maintained at 7–34°C under a natural photoperiod in the laboratory for release in April and May of the following year. They began to oviposit in March or April of the following year.

Release of *D. helophoroides* and tree dissection

Fifteen trees that had been killed by pine wilt disease were arbitrarily selected in autumn 2001 for the experiment in 2002. In total, 373 adult *D. helophoroides* were released on nine dead trees on the stem bark surface (treated trees) on 1 May 2002. The remaining six trees did not receive *D. helophoroides* (control trees). Distances from control trees to the nearest treated trees ranged from 1.4 to 650 m. On 2 May 2003, 1050 adult *D. helophoroides* were released on 9 of 18 dead trees that were selected in autumn 2002. Distances from control trees to the nearest treated trees ranged from 360 to 660 m. On 30 April 2004, 480 adult *D. helophoroides* were released on 10 of 16 dead trees that were selected in autumn 2003. Distances from control trees to the nearest treated trees ranged from 2.9 to 298 m.

The selected trees were 4.2–12.5 m in height and 6.6–16.2 cm in diameter at breast height (DBH). The release was performed by taping and stapling the wood blocks containing adults in artificial holes to the stems of treated trees at breast height (about 1.2 m) without felling the trees.

Between early June and mid-July, all trees were felled, cut into 1-m-long logs (each log was numbered from the bottom), hauled to the Kansai Research Center, and placed in outdoor cages (75 × 75 cm, 180 cm in height; 1-mm mesh). Each log was placed in a separate cage to avoid the transfer of insects among logs. Logs were debarked and dissected within 20 days of their removal from the study site, i.e., between early June and late July.

The numbers of live and dead *M. alternatus* in galleries within the xylem of the logs and their developmental stage were recorded. Most *M. alternatus* were in pupal chambers or had emerged when the logs were dissected. The numbers of gallery entrances and emergence holes were also recorded. Emergence holes were counted as live individuals. Mortality factors were classified into two categories: parasitism by *D. helophoroides* and unknown. Parasitism by *D. helophoroides* was determined by the presence of parasitoid larvae on the host body or

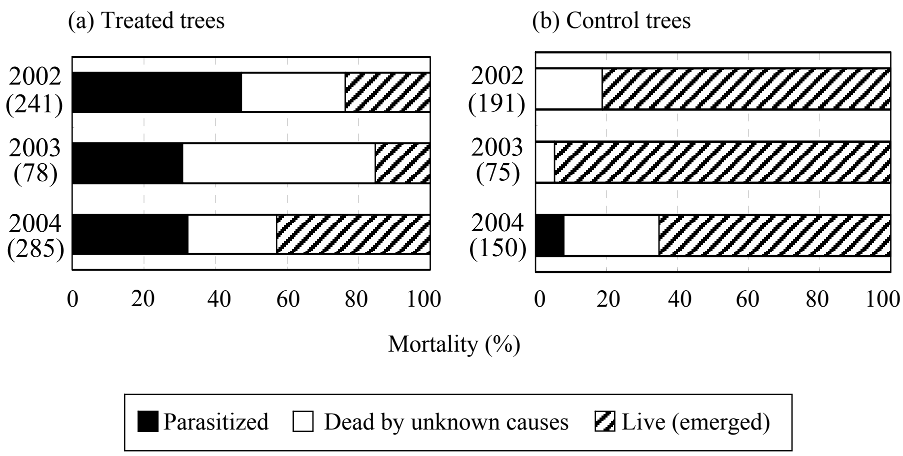

Fig. 1. Percentages of live and dead *Monochamus alternatus* in trees onto which *Dastarcus helophoroides* adults were released ((a) treated trees) or not released ((b) control trees) from 2002 to 2004. The number of live *M. alternatus* includes emerged adults, counted as the number of emergence holes observed on tree stems. Numbers in parentheses indicate the total number of live and dead *M. alternatus*.

parasitoid cocoons. The percentage of parasitism was calculated by dividing the number of parasitized *M. alternatus* by the sum of all *M. alternatus*. *M. alternatus* mortality was calculated by dividing the sum of parasitized and dead individuals from unknown causes by the sum of all *M. alternatus* individuals.

Traces of predation by an insect predator, *Trogossita japonica* Reitter (Coleoptera: Trogossitidae), and woodpeckers (*Picus awokera* Temminck and *Dendrocopos kizuki nippon* [Kuroda]) were frequently found in the pupal chambers of *M. alternatus*. They were easily discriminated from mortality caused by *D. helophoroides* parasitism and were excluded from further analyses.

To determine the movement and retention of the released *D. helophoroides* adults, 20 stems and 50 stumps of *P. densiflora* that had been dead for >2 years were debarked at the study site in October and November 2003.

Differences in *M. alternatus* mortality between treated and control trees were compared using the chi-square test.

Results

Detection of *D. helophoroides* adults and egg clusters from treated trees and their vicinities

The numbers of released *D. helophoroides* adults that were collected from treated trees during the process of dissection were 6 (1.6% of the total number of released adults) in 2002, 16 (1.5%) in 2003, and 10 (2.1%) in 2004. Eight adults were found on the bark surface, three in crevices on the outer bark, eighteen beneath the bark, and three in *M. alternatus* galleries in the xylem. In addition, on 12 November 2003, one *D. helophoroides* adult was collected from beneath the bark of an old *P. densiflora* tree that had died 2 – 3 years before. Another adult was collected under the bark of a *P. densiflora* stump on 21 November. Both were found within 10 m of the stump of the nearest treated tree.

Three clusters of 30–50 parasitoid eggs were found under the bark of a treated tree in 2003 at a height of 2–3 m above the ground. The clusters had already hatched, and were found at the surface of the xylem, within 2 cm of the entrance holes of short, empty galleries of *M. alternatus* larvae.

Proportion of parasitism by *D. helophoroides*

I found 268 – 610 gallery entrances on the xylem surface, and examined 75–285 pupal chambers containing live and dead *M. alternatus* in each treatment in each year (Fig. 1). Mean *M. alternatus* mortality in the treated trees (Fig. 1a) was 76.3% in 2002, 84.6% in 2003, and 57.2% in 2004; mortality varied greatly among trees (range: 48.5 – 94.3% in 2002, 25.0 – 100.0% in 2003, and 22.2–83.3% in 2004). *M. alternatus* mortality in the treated trees was significantly higher than that in control trees in 2002 (χ^2cal = 140.967, $P < 0.0001$), 2003 (χ^2cal = 96.832, $P < 0.0001$), and 2004 (χ^2cal = 19.950, $P < 0.0001$).

The percentage of parasitism of *M. alternatus* by *D. helophoroides* on treated trees was 47.3% in 2002, 30.8% in 2003, and 32.3% in 2004 (Fig. 1a), with considerable variation among trees (range: 16.1 – 68.3% in 2002, 0 – 80.0% in 2003, and 11.1 – 75.0% in 2004).

M. alternatus mortality from unknown causes in the treated trees accounted for 29.0% in 2002, 53.8% in 2003, and 24.9% in 2004. The corpses were decayed or dried and showed no signs of predation or disease. The percentages of larval, pupal, and adult corpses were 9.0%, 34.5%, and 56.5%, respectively.

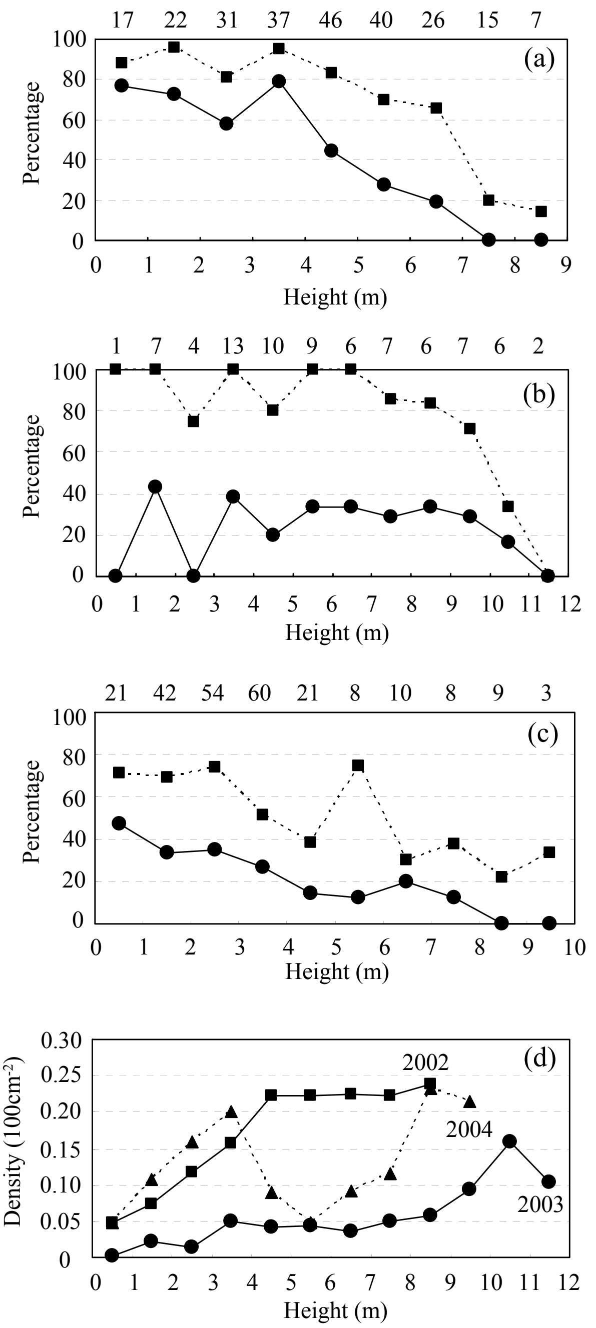

Fig. 2. Percentage of parasitism by *Dastarcus helophoroides* (circles) and overall percentage of *Monochamus alternatus* mortality (squares; sum of those parasitized plus dead from unknown causes) in 1-m sections of logs of the same height cut from treated trees in (a) 2002, (b) 2003, and (c) 2004. Numbers above the graph indicate total numbers of live and dead *M. alternatus* in the group of logs at each height. (d) Total density of live and dead *M. alternatus* in 1-m section logs per 100 cm^2 of log surface from 2002 to 2004. The uppermost logs were taken from 8 – 9 m in 2002, 11 – 12 m in 2003, and 9 – 10 m in 2004.

No *M. alternatus* in control trees were parasitized by *D. helophoroides* in 2002 and 2003. Mortality from unknown causes was as low as 18.8% in 2002 and 5.3% in 2003. In 2004, 16.7% of *M. alternatus* were parasitized in one of the six control trees, which was located 265 m from the nearest treated tree (Fig. 1b). In this tree, many *M. alternatus* died due to unknown causes, which accounted for 57.5% of the dead individuals from unknown causes found in the six control trees, and raised the overall percent mortality in the control trees up to 34.7%.

Vertical changes in percent parasitism and host density in trees

The heights of the 28 treated trees ranged from 4.5 to 12.5 m; thus, the number of 1-m-long logs per tree varied between 4 and 12 among trees. Parasitism by *D. helophoroides* was found in treated trees up to the seventh log (6 – 7 m above the ground; Fig. 2a) in 2002, the eleventh log (10 – 11 m above the ground; Fig. 2b) in 2003, and the eighth log (7 – 8 m above the ground; Fig. 2c) in 2004. In 2002, the percentage of parasitism by *D. helophoroides* was >50% up to 4 m above the ground and <50% above 4 m (Fig. 2a). The overall mortality showed a similar pattern to the percentage of parasitism, but was >80% up to 5 m above the ground. In 2003, the percentage of parasitism was <50% in total, and was low in the bottom and uppermost logs (Fig. 2b). The overall mortality was >70% up to 10 m above the ground and declined dramatically in the upper part of the trees. In 2004, the percentage of parasitism and overall mortality were both large in the bottom part and gradually decreased with height (Fig. 2c). The percentage of parasitism was <50% in total, but the overall mortality reached 70% in the lower part of the trees.

The total density of live and dead *M. alternatus* in each 1-m section (Fig. 2d), which was calculated by dividing the total number of *M. alternatus* by the total surface area of the group of logs at each height, showed different tendencies among the three years. In 2003, the density was continuously low, except in the upper part, because the number of *M. alternatus* individuals was generally small. In the other two years, the density was low in the bottom and increased up to 4 or 5 m in height, and then remained almost constant above this height in 2002, although the density decreased in the middle sections in 2004.

Discussion

The release of *D. helophoroides* on standing trees reduced the survival of *M. alternatus*, although *M. alternatus* mortality from parasitism by *D. helophoroides* was <50% (Fig. 1a). The mortality of *M. alternatus* was as high as 76.3% in 2002 and 84.6% in 2003, when mortality from unknown causes was included. As the occurrence of individuals that died of unknown causes was higher in treated trees than in control trees (Fig. 1b), mortality was probably related to the release of the parasitoids. I found several corpses of *D. helophoroides* hatchlings by dissecting dead *M. alternatus* originally classified as having been killed by unknown causes (Urano, unpublished data). Thus, at least some of the dead *M. alternatus* were parasitized by *D. helophoroides* hatchlings that died during early development for reasons such as shortage of available nutrients or decay of the host.

Several previous studies have examined the vertical movement of *D. helophoroides* on logs or tree stems. Urano (2004) released adult *D. helophoroides* on trees at 1.2 and 2.5 m above the ground and found parasitism up to 4.5 m. Ishii (2004) released adults on 4.0-m-long logs at 1.2 m above the ground and found parasitism up to 4.0 m. Ogura (2002) released hatchlings on logs and observed their movement up and down the surface for at least 1.5 m. In this study, I found parasitizing *D. helophoroides* that had originated from adults released at 1.2 m above the ground from the bottoms to the tops of trees, up to 11 m in height (Fig. 2b). The percentage of parasitism decreased with increasing height (Figs. 2a – c), although the density of *M. alternatus* showed different trends with increasing height among the three years (Fig. 2d). To examine the effect of host density on the performance of the parasitoids, the relationships between the density of *M. alternatus* and both percent parasitism by *D. helophoroides* and *M. alternatus* mortality in each 1-m section of log were analyzed (Fig. 3). Although the host mortality and percent parasitism tended to be smaller with increasing host density, the relationships were not necessarily significant. Thus I consider that the high host mortality and parasitism by *D. helophoroides* in the lower part of the trees (Fig. 2) were not attributed to the low host density but to the proximity of the release point of the parasitoids. It is likely that adults released at 1.2 m oviposited near the bottom part of a stem, and seldom reached the upper parts. Releasing adults on the middle or upper parts of a stem in closer proximity to the host is expected to increase the percentage of parasitism of *M. alternatus* by *D. helophoroides*.

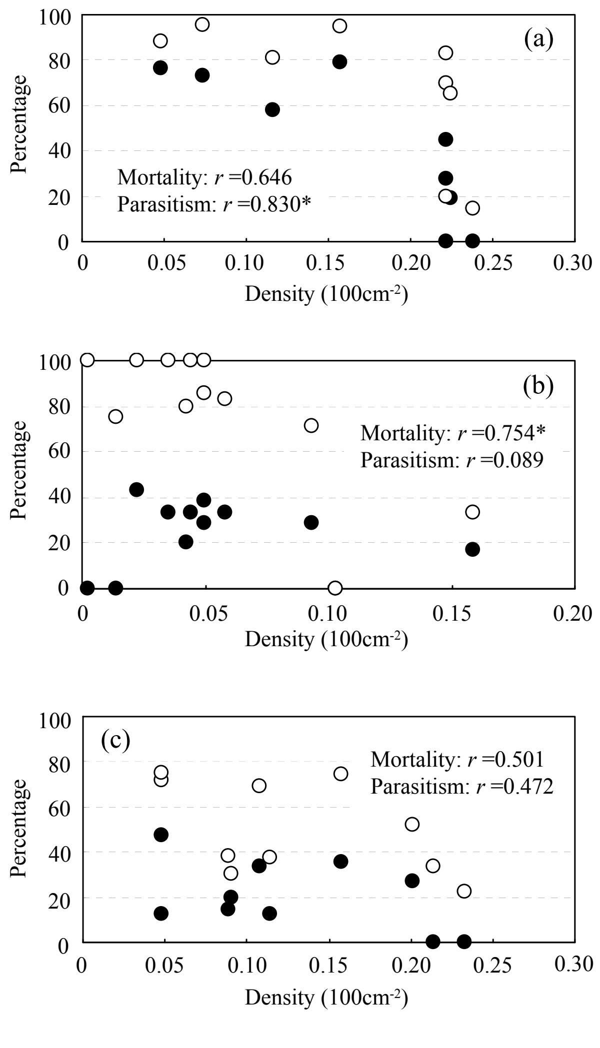

Fig. 3. Percentage of parasitism by *Dastarcus helophoroides* (solid circles) and overall percentage of *Monochamus alternatus* mortality (blank circles) in relation to the total density of live and dead *M. alternatus* per 100 cm^2 of log surface in treated trees ((a) 2002, (b) 2003, and (c) 2004). * $P < 0.05$ for the correlation coefficient (r).

Miura et al. (2003) and Urano (2004) examined the dispersal ability of adult *D. helophoroides* released on *P. densiflora* logs or on stems infested with *M. alternatus* in the field and found that few to no adults moved among trees. The relatively low dispersal ability of *D. helophoroides* was also demonstrated here. Because the number of adults re-collected upon dissecting the treated trees accounted for only 1.5 – 2.1% of the total number released, most of the released adults probably left the trees or died at the site. I found two adults near the stump of a treated tree in November 2003, indicating that at least a small number of the released adults had dispersed and moved to the vicinity of the treated trees some months after release. The longevity of *D. helophoroides* adults in the laboratory is 2–3 years (Inoue, 1993). If these adults survived to the next spring to find hosts, there must have been some *D. helophoroides* that successfully parasitized *M. alternatus* in dead trees away from the release point. In this study, however, no clear evidence of parasitism was found in control trees in 2002 and 2003 (Fig. 1b), indicating that the released *D. helophoroides* adults seldom moved to or colonized the control trees. In 2004, parasitism on *M. alternatus* was observed in one control tree located 265 m from the nearest treated tree. The overall mortality of *M. alternatus*, including that from unknown causes, was 34.7%. Adult *D. helophoroides* colonizing this tree may have been individuals released in 2004 or individuals released in 2002 or 2003, suggesting that establishment of released parasitoids in the field requires a multiple-year repetition of mass release. Continuous release and surveys could be used to examine the establishment of a *D. helophoroides* population at the study site.

To improve the effectiveness of the application, it is desirable that the released *D. helophoroides* adults not only reduce the survival of *M. alternatus* within the treated trees but also successfully disperse and colonize to the surrounding dead pine trees. However, dispersal of released *D. helophoroides* was reported to be rare in previous studies (Miura et al., 2003; Urano, 2004), as in this study. In all of these studies, the parasitoids were released once between April and May, corresponding to their oviposition period. The dispersal of parasitoids may be more successful if they are released at different times of the year, e.g., during their emergence period (August and September) or early spring just after they have undergone hibernation, because the adults are long-lived and their patterns of activity may change depending on age or seasonality. Some environmental factors may be favorable for the dispersal or colonization of *D. helophoroides*. Further studies are needed to examine the effects of time or environmental factors on the dispersal ability of adult *D. helophoroides* for the development of an effective method of application.

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マツ材線虫病被害林分におけるマツノマダラカミキリ穿入アカマツへのサビマダラオオホソカタムシの放飼試験

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要 旨

マツ材線虫病の被害を受けたアカマツ林分において、サビマダラオオホソカタムシ成虫のマツノマダラカミキリ穿入枯死木への放飼試験を行った。試験は 2002 年から 2004 年にかけて行い、各年の前年に枯死してマツノマダラカミキリの穿入を受けたアカマツを供試木とし、放飼を行った木（放飼木）と行わない無放飼木とを設定した。放飼虫の樹幹内垂直方向および供試木間の水平方向の移動を調査し、本種の林内における分散能力を検討した。放飼木におけるマツノマダラカミキリの総死亡率は 2002 年 76.3％、2003 年 84.6％、2004 年 57.2％と、無放飼木における死亡率（2002 年：18.8％、2003 年：5.3％、2004 年：26.7％）に比べ明らかに高かった。2002 年および 2003 年の無放飼木においては移動分散した成虫による産卵寄生は認められなかったが、2004 年は無放飼木 6 本中 1 本で 16.7％の寄生が認められた。この無放飼木は最も近い放飼木から 265 m離れていたが、放飼木の近くに位置する他の無放飼木では寄生は認められなかった。2003 年 5 月に放飼した成虫で林内に残留したと思われる個体が、2003 年 11 月に 2 個体採集された。放飼木の胸高部に放飼した成虫の次世代による寄生は、樹幹の高さ 11 mの部位まで認められたが、マツノマダラカミキリ死亡率およびサビマダラオオホソカタムシ寄生率は樹幹の高い部位では低下する傾向があった。

キーワード：生物的防除、サビマダラオオホソカタムシ、分散能力、マツノマダラカミキリ、放飼

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