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- Note -

Field Bioassay for Longhorn Pine Sawyer Beetle *Monochamus alternatus* (Coleoptera: Cerambycidae) in Korea Based on Aggregation Pheromone 2-(Undecyloxy)ethanol

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The pinewood nematode *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae) poses a serious threat to pine forests in Europe and East Asia, leading to a debilitating pine wilt disease. Infected pine trees in Korea are generally fumigated or crushed to small wood chips after felling. Although pine wilt disease often recurs in pest management sites, there are no adequate means to monitor the effectiveness of pest control measures in those sites. Recently, a male-produced aggregation pheromone, 2-(undecyloxy)ethanol, was shown to be useful for attracting several *Monochamus* species, which are vectors for the pinewood nematodes. In this study, we investigated the abilities of 2-(undecyloxy)ethanol at three different doses (175, 350, and 700 mg), as well as host plant volatiles (α-pinene and ethanol), to attract *M. alternatus* (Coleoptera: Cerambycidae) at a pine forest in Pohang, Korea where infected pine trees had been cut down and fumigated. Twenty-seven *M. alternatus* were captured in cross-vane panel traps made of polyethylene terephthalate bottles and acrylic sheets. The results indicate that a high dose of 2-(undecyloxy)ethanol (700 mg per trap) is the most effective for attracting *M. alternatus*. The aggregation pheromone could be used to monitor the effectiveness of pest control measures as well as *M. alternatus* populations.

Key words : Aggregation pheromone, cross-vane panel trap, *Monochamus alternatus*, pine wilt disease, pinewood nematode

Introduction

Pine wilt disease, which blocks the circulation of water and nutrients in pine trees, is caused by the pinewood nematode *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae). Pinewood nematodes, native to North America, were accidentally introduced to Japan around 1905 [10], later spreading to China, Taiwan, and Korea. The nematodes were also detected in Europe (Portugal) in 1999 [16]. In Korea, where pine forest occupies approximately 23% of the land, pine wilt disease poses a serious threat to forest ecosystems ever since its first occurrence in 1988 [7, 14]. The Japanese red pine (*Pinus densiflora*), black pine (*P. thunbergii* Parl.), and Korean white pine (*P. koraiensis*) are all affected by pine wilt disease [14]. Nationwide efforts have been made to suppress the spread of pine wilt disease including fumigation or crushing of infected pine trees after cutting down, aerial spraying of insecticide, as well as injection of insecticide into pine tree trunks [7, 14]. However, no adequate means of monitoring pine wilt disease at pest management sites have been devised instead of detection of newly infected pine trees.

In East Asia, longhorn pine sawyer beetles, *M. alternatus* and *M. saltuarius* (Coleoptera: Cerambycidae), are primary vectors of pinewood nematodes [1, 11]. *Monochamus alternatus* is found mainly in the southern part of Korea with an annual mean temperature >13°C, whereas *M. saltuarius* is found in the mid-to-northern part of the country with an annual mean temperature <13°C [8]. Host plant volatiles such as α-pinene and ethanol are known to attract *Monochamus* species [4, 5]. Recently, 2-(undecyloxy)ethanol was identified as a male-produced aggregation pheromone of both *M. galloprovincialis* [13] and *M. alternatus* [15]. The

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hydroxyether structural motif found in 2-(undecyloxy)ethanol is considered to be conserved among many pheromones of Cerambycidae species [3] Attraction to aggregation pheromones is strongly synergized by host plant volatiles [2, 12, 13, 15].

In this study, we investigated the abilities of 2-(undecyloxy)ethanol along with the host plant volatiles α-pinene and ethanol to attract *M. alternatus* at a pine forest in Pohang, Korea. We used three different doses of 2-(undecyloxy)ethanol (175, 350, and 700 mg), whereas a single dose of 2-(undecyloxy)ethanol in the range of 25-95 mg per trap was used in other studies [9, 12, 13, 15]. To increase the surface area of the pheromone mixture, a superabsorbent polymer based on polyacrylic acids and water were added to the solution of 2-(undecyloxy)ethanol and host plant volatiles. As an alternative to commercial cross-vane panel traps or multi-funnel traps, we made cross-vane panel traps using polyethylene terephthalate (PET) bottles and acrylic sheets. Here, we report that a high dose of 2-(undecyloxy)ethanol (700 mg per trap) is the most effective for attracting *M. alternatus* at a pest management site in Korea.

Materials and Methods

Synthesis of 2-(undecyloxy)ethanol

The aggregation pheromone 2-(undecyloxy)ethanol was synthesized using the microwave-assisted organic synthesis technique.

((2-(Undecyloxy)ethoxy)methyl) benzene : 2-(benzyloxy) ethanol (0.50 g, 3.3 mmol) in 2 ml of N,N-dimethylformamide was added to sodium hydride (0.16 g, 6.6 mmol), and the reaction mixture was stirred for 5 min at 25°C. A microwave tube filled with the above mixture and 1-bromoundecane (0.85 g, 3.6 mmol) was heated in the microwave reactor at 100 W power and 80°C for 10 min. After removal of solvent *in vacuo*, the residue was treated with ethyl acetate and washed with water and saturated aqueous sodium chloride solution. The obtained organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel using hexane:ethyl acetate (20:1 and then 9:1) mixtures as eluents to obtain 0.72 g of product at 72% yield. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm): 7.35-7.23(m, 5H), 4.57(s, 2H), 3.60(s, 4H), 3.45(t, *J*=6.8 Hz, 2H), 1.59(quint, *J*=6.9 Hz, 2H), 1.30-1.26(m, 16H), 0.88(t, *J*=6.6 Hz, 3H).

2-(undecyloxy)ethanol: ((2-(undecyloxy)ethoxy)methyl) benzene (0.72 g, 2.3 mmol) in 10 ml of ethanol was added to a catalytic amount of palladium on activated carbon (10%), and the resulting mixture was stirred for 5 hr under a hydrogen gas atmosphere. After the reaction mixture was filtered with celite, the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel using hexane:ethyl acetate (5:1 and then 3:1) mixtures as eluents to obtain 0.37 g of product at 73% yield. ^{1}H NMR ($CDCl_3$, 400M Hz) δ (ppm): 3.73 (q, *J* = 5.1 Hz, 2H), 3.53 (t, *J* = 4.6 Hz, 2H), 3.47 (t, *J* = 6.6 Hz, 2H), 2.28 (t, *J* = 6.0 Hz, 1H), 1.59 (quint, *J* = 7.0 Hz, 2H), 1.30-1.26 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100M Hz) δ (ppm): 70.86, 70.46, 60.76, 30.92, 28.63, 28.51, 28.35, 25.10, 21.69, 13.10.

Construction of cross-vane panel traps

Cross-vane panel traps were constructed using 2-liter clear PET bottles and acrylic sheets as shown in Fig. 1. A PET bottle was cut into three pieces. The upper part, which serves as a funnel in an upside-down position, was assembled to the base part (capacity 1.2 liters). The cover and cross-vane panel were constructed using acrylic sheets.

Superabsorbent polymer-based pheromone lure

(±)-α-Pinene, ethanol, and isopropanol were purchased from Sigma (St. Louis, MO, USA). Superabsorbent polymer based on polyacrylic acid was purchased from a local per-

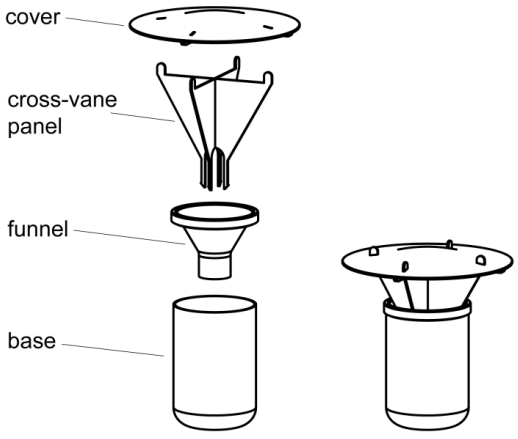


Fig. 1. Schematic diagram of cross-vane panel trap made of PET bottle and acrylic sheets. A 2-liter PET bottle was cut into three pieces. The upper part, which serves as a funnel in an upside-down position, was assembled to the base part. The capacity of the base part is 1.2 liters. The cross-vane panel and cover were made of acrylic sheets.

fume store. To increase the surface area of the pheromone mixture, α-pinene, ethanol, and 2-(undecyloxy)ethanol were mixed in a 50 ml conical tube, followed by addition of polyacrylic acid-based superabsorbent polymer (0.45 g) and water (approximately 27 ml). The volume of water was adjusted to maintain a ratio of pheromone mixture to water of 1:4. The lure was kept at room temperature overnight before use.

Field bioassay

A field experiment was carried out at a pine forest in Pohang, Korea for 2 weeks from June 24, 2014 to July 7, 2014 within 36°08′87″N, 129°33′03″E and 36°09′37″N, 129°31′88″E (Fig. 2). Black pine (*Pinus thunbergii* Parl.), native to Korea and Japan, was predominant at this site. The area was previously affected by pine wilt disease, wherein the infected pine trees had been cut down and fumigated using metham sodium [7, 14]. However, as control methods such as fumigation or crushing to small wood chips after felling of infected trees cannot remove all infected trees, including their branches, we speculate that there are still some living *M. alternatus* there. Daily monitoring of *M. alternatus* in an outdoor netted cage at Pohang since 2008 showed that *M. alternatus* emerges from the logs of infected pine trees on average on May 27, and its cumulative emergence ratio reaches 50% on June 21 (Gyeongsangbuk-do Forest Environment Research Institute, unpublished data). These data are consistent with the observations of Kim et al., who showed that the emergence of *M. alternatus* in Jinju, Korea occurs on average on May 15, and a 50% cumulative emergence ratio is reached at mid-June [6]. As Jinju is located 160 km south of Pohang, the emergence of *M. alternatus* in Jinju is 12 days earlier than Pohang.

Twenty traps were allocated in four blocks of five treatments (Fig. 2). Block 1 was located near the campus of Sunlin College while block 2 was close to a cemetery. Blocks 3 and 4 were close to a paddy field. As host plant volatiles α-pinene and ethanol (H) were reported to attract *Monochamus* species [4, 5], we used three different doses of 2-(undecyloxy)ethanol (P1, 175 mg; P2, 350 mg; and P3, 700 mg) along with α-pinene and ethanol for attracting *M. alternatus*. Treatments included (i) Ctrl, untreated control; (ii) H, α-pinene (3.78 g) and ethanol (1.26 g); (iii) H + P1; (iv) H + P2; and (v): H + P3. In each block, traps were randomly installed 50 m between traps, approximately 1.2 m high above the ground using poles. The pesticide Vaportape II (2,2-dichlorovinyl dimethyl phosphate) (Hercon Environmental) was placed in the trap to prevent escape of the captured beetles.

Statistical analysis

The linear model for the experiment is $x_{ij} = \mu + b_i + t_j + e_{ij}$, whereby μ is overall mean, b_i is the effect of block (i = 1, 2, 3, and 4), t_j is the effect of treatment (j = 1, 2, 3, 4, and 5), and e_{ij} is random error. The data collected were analyzed by two-way ANOVA. Data were transformed using the square root of (x + 0.5) before analysis for normality [17].

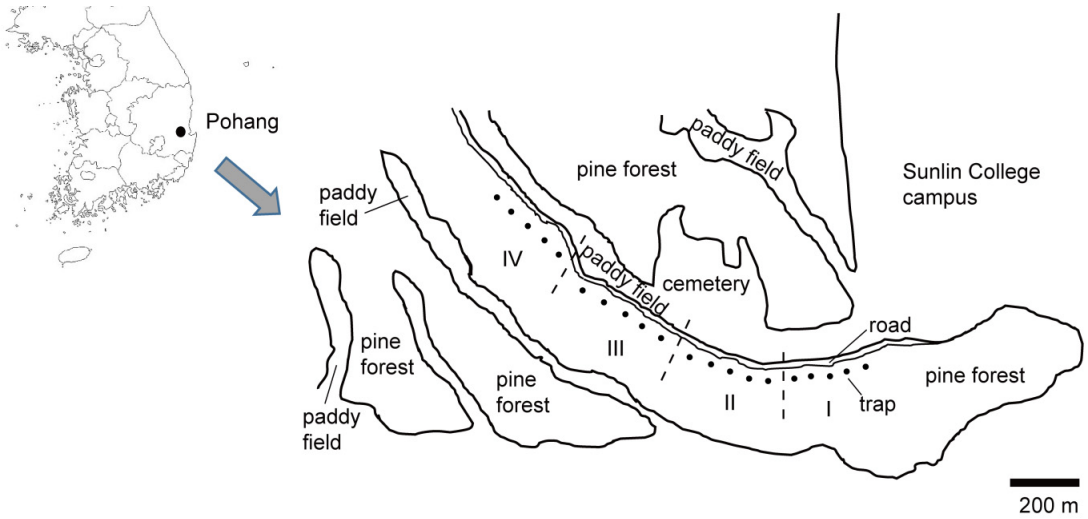
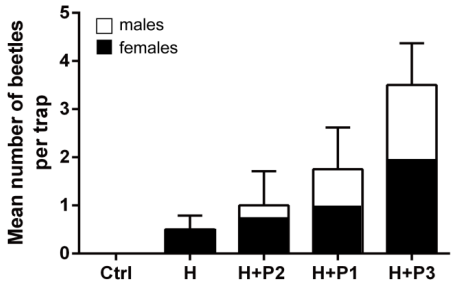


Fig. 2. Arrangement of traps at the experiment site at a pine forest in Pohang, Korea. Twenty traps were allocated into four blocks of five treatments. Traps were randomly installed in each block, 50 m between traps, and 1.2 m high above the ground. Blocks 1 to 4 are denoted as I~IV.

Results and Discussion

A total of 27 *M. alternatus* (10 males and 17 females) were captured from 20 traps which were allocated into four blocks of five treatments (Fig. 2). Blocks 1, 2, 3, and 4 had on average 0.75, 1.25, 3.0, and 1.75 beetles per trap, respectively. The block 1 located near the campus of a local college contained the least *M. alternatus* captured, whereas blocks 3 and 4 located close to a paddy field contained slightly more *M. alternatus*. There was no significant difference among the four blocks.

However, significant differences were detected among the five treatments (ANOVA: F=5.41; df=4, 12; $p<0.01$). On average, 0.5 beetles per trap were attracted to lure H containing α-pinene and ethanol, but the number was not significantly different from untreated control (Fig. 3). Lures H+P1 and H+P2 showed similar effectiveness with mean numbers of 1.75 and 1.0 beetles per trap, respectively, suggesting that the aggregation pheromone is more effective for attracting *M. alternatus* over α-pinene and ethanol (Fig. 3). Although lure P2 (350 mg) contained twice the amount of 2-(undecyloxy)ethanol per trap as compared with P1 (175 mg), less *M. alternatus* were captured in traps containing lure H+P2 than traps containing lure H+P1. These data suggest that a minimum 175 mg of 2-(undecyloxy)ethanol per trap could be used for recruitment of *M. alternatus* using cross-vane panel traps made of PET bottles. The highest number of *M. alternatus* (n=14, 52% of all beetles captured) was captured in traps with lure H+P3 containing 700 mg of 2-(undecyloxy)ethanol, 3.5 beetles per trap, versus any other treatments ($p<0.01$) (Fig. 3). In each treatment, both sexes of *M. alternatus* were attracted to lures containing α-pinene and ethanol (H) and/or 2-(undecyloxy)ethanol (P), but the numbers of males and females captured in each treatment were not significantly different. Our results are consistent with those of Pajares et al. (2010), who reported that a high release rate of 41 mg of 2-(undecylxoy)ethanol per trap is more effective than a low release rate for attracting *M. galloprovincialis*. As the infected pine trees in the experiment site had been cut down and fumigated or crushed to small wood chips every year, not many *M. alternatus* were living there.



Comparison of mean difference among treatments by Tukey's HSD

	Ctrl	H	H+P2	H+P1	H+P3
Mean	0	0.5	1.0	1.75	3.5

$D_{0.05} = 0.884$

Fig. 3. Two-week field bioassay results from June 24, 2014 to July 7, 2014. A total of 27 *M. alternatus* (10 males and 17 females) were captured. Ctrl, untreated control; H, α-pinene (3.78 g) and ethanol (1.26 g); P1, P2, and P3, 2-(undecyloxy)ethanol (175, 350, and 700 mg, respectively). Data represent mean ± S.E. of each treatment. White bars represent males and black bars represent females.

In conclusion, **w**e demonstrated that a high dose of male-produced aggregation pheromone 2-(undecyloxy)ethanol (700 mg per trap) is effective for attracting *M. alternatus* at a previously pine wilt disease-affected site in Korea using cross-vane panel traps made of PET bottles and acrylic sheets. Our results suggest that the aggregation pheromone could be used for monitoring of pest control measures for pine wilt disease as well as detection and population monitoring of pine sawyer beetles. Further study is needed to elucidate the effect of 2-(undecyloxy)ethanol along with host plant volatiles on recruitment of *M. saltuarius*.

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초록 : 집합페로몬 2-(Undecyloxy)ethanol을 이용한 솔수염하늘소 유인 실험

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소나무재선충(*Bursaphelenchus xylophilus*)은 소나무재선충병을 유발하여 한국의 소나무 숲에 심각한 영향을 미치고 있다. 소나무재선충에 감염된 소나무는 일반적으로 훈증 또는 파쇄처리되지만 소나무재선충병 피해지역의 방제효과를 검증할 수 있는 효과적인 방법은 아직 없다. 본 연구에서는 소나무재선충병 방제효과 검증에 적합한 솔수염하늘소 집합페로몬의 농도를 알아보기 위해 2-(Undecyloxy)ethanol, 알파-피넨, 에탄올을 이용하여 경상북도 포항시의 소나무재선충병 피해지역에서 필드테스트를 시행하였다. 총 27마리의 솔수염하늘소가 유인되었으며 고농도(700 mg)의 집합페로몬을 사용한 트랩이 가장 효과적이었다. 집합페로몬 2-(Undecyloxy)ethanol은 솔수염하늘소 개체수 조사와 소나무재선충병 방제효과 검증에 사용할 수 있을 것으로 사료된다.