Sex Attractant Blend for *Dioryctria mongolicella* (Lepidoptera: Pyralidae) in the Northeast of China[1]

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Abstract The Mongolian pine borer, *Dioryctria mongolicella* Wang & Sung (Lepidoptera: Pyralidae), is a major pest in Mongolian pine (*Pinus sylvetris* L. var. *mongolica* Litvinov) plantations in the northeast of China. A blend of 11-hexadecenal and 11-hexadecenyl acetate in a ratio of 2:1 was detected from extracts of its female sex pheromone glands by gas chromatography–mass spectrometry. Our field trapping experiments confirmed that the *D. mongolicella* pheromone consists of a blend of (E)-11-hexadecenal (E11-16:Ald) and (Z)-11-hexadecenyl acetate (Z11-16:Ac). The addition of (Z,E)-9,11-tetradecadienyl acetate (Z9E11-14:Ac) and (Z,Z,Z)-3,6,9-tricosatriene (ZZZ3,6,9-23:H) increased catches of male *D. mongolicella* in sticky traps baited with E11-16:Ald and Z11-16:Ac. However, the addition of (3Z,6Z,9Z,12Z,15Z)-pentacosapentaene or (Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene to lures loaded with E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac did not significantly increase trap catches of *D. mongolicella* at two locations in Heilongjiang province, China. The synthetic blend of 400 μg E11-16:Ald, 200 μg Z11-16:Ac, 200 μg Z9E11-14:Ac, and 500 μg ZZZ3,6,9-23:H will help in developing efficient strategies for monitoring and control of *D. mongolicella* populations in Mongolian pine plantations.

Key Words *Dioryctria mongolicella*, pheromone gland analysis, field trapping

The Mongolian pine borer, *Dioryctria mongolicella* (Wang & Sung) (Lepidoptera: Pyralidae) was initially discovered and identified in the northeast of China (Qian 1981, Wang and Sung 1982). Larvae feed mainly under the bark of conifers, including Mongolian pine (*Pinus sylvestris* L. var. *mongolica* Litvinov) and Korean pine (*Pinus koraiensis* Siebold & Zuccarini) (Gao 1987). Irregular, creamy-white, granular pitch masses on stems or branches mixed with frass is the typical

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symptom of infestations. The larvae cause stem deformity and reduced upward growth (Xiao and Li 2020). The damage has been especially severe in Mongolian pine plantations in the northeast of China since 2012 (Yan et al. 2018). The trees that had been pruned in the summer were significantly more infested than unpruned trees.

Tactics to manage the larvae are generally not efficacious because they are not exposed to parasitism or predation by natural enemies and insecticidal sprays cannot penetrate into their concealed habitats. Injecting insecticides into the tunnel or coating insecticides on the trunk may prove effective (Cook et al. 2013) but are cumbersome to practice.

Mate-finding in *Dioryctria* is mediated by female-produced sex pheromones (Fatzinger and Asher 1971). Synthetic sex attractants have been used for monitoring the seasonal activity of male *Dioryctria* moths (Hanula et al. 1985, Miller et al. 2010, Pasek and Dix 1989, Strong et al. 2008). In China, more than 15 species of *Dioryctria* have been described (Ding and Zhang 2016; Kuang and Li 2009; Li 1999; Wang and Sung 1982, 1985). The combination of (Z)-11-hexadecenyl acetate (Z11-16:Ac), (Z)-11-hexadecenal (Z11-16:Ald), and (Z,E)-9,11-tetradecadienyl acetate (Z9E11-14:Ac) is the sex attractant for *Dioryctria rubella* Hampson (Wu et al. 1986). Catches of *Dioryctria mendacella* (Staudinger) were highest in traps baited with Z9E11-14:Ac and (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene (ZZZZZ3,6,9,12,15-25:H) (Hall et al. 2017). *Dioryctria mongolicella* is primarily a stem borer, which reduces structural integrity of trees. The primary pheromone component of *D. mongolicella*, however, has not been reported (Ando and Yamamoto 2022).

Moth sex pheromones usually consist of structurally similar chemical components but combined in a specific ratio (Lassance et al. 2013). Some *Dioryctria* species share a pheromone blend (Whitehouse et al. 2011). They may use different calling times or host plants, even a behavioral antagonist, to avoid cross-attraction (Leal et al. 2005). We expected that the sex attractant blend for *D. mongolicella* would be similar to that for *D. rubella* or *D. mendacella*. The development of an effective synthetic lure for *D. mongolicella* would be a useful tool for determining flight periods as part of a pest management program. Even pheromone-based mating disruption has the potential to reduce damage caused by *D. mongolicella* (Svensson et al. 2018).

The aims of the present study were threefold: (a) to analyze pheromone gland extracts of female *D. mongolicella* from the western population in Heilongjiang, China; (b) to evaluate the effects of ZZZZZ3,6,9,12,15-25:H and ZZZZZ3,6,9,12,15-23:H on attraction of males when added to the blend of (E)-11-hexadecenal (E11-16:Ald), (Z)-11-hexadecenyl acetate (Z11-16:Ac), and Z9E11-14:Ac; and (c) to evaluate the effect of (Z,Z,Z)-3,6,9-tricosatriene was used in some scientific papers: https://link.springer.com/article/10.1007/s10886-017-0871-7; https://doi.org/10.1016/j.jinsphys.2015.09.006; https://doi.org/10.1016/j.jinsphys.2010.08.024. (Z,Z,Z)-3,6,9-Tricosatriene and ZZZ3,6,9–23:H were used in another scientific paper: https://link.springer.com/article/10.1007/s10886-017-0846-8 on attraction of males when added to the blend of E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac.

Fig. 1. Pupa of *Dioryctria mongolicella* (A), granular pitch masses (B), cut granular pitch mass from pine stem (C), collected insect material (D).

Materials and Methods

Insects. *Dioryctria mongolicella* specimens were collected as late-instar larvae or pupa (Fig. 1A) from granular pitch masses (Fig. 1B) in the New River Experimental Forestry Station (NR; Heilongjiang province, China). Granular pitch masses were cut from the main stem and branch whorls of *P. sylvestris* var. *mongolica* using a sharp knife (Fig. 1C). They were maintained in egg trays (Fig. 1D) and transferred to a nylon cage (4 m $\times$ 4 m $\times$ 3.5 m). The late-instar larvae were allowed to pupate under natural ambient conditions. After eclosion, the virgin female moths were immediately separated from male moths and placed in an aluminum screen cage (32 cm $\times$ 32 cm $\times$ 46 cm). The sex of eclosed adults was confirmed by examining the abdominal tip for brushes in the male and a genital slot in the female (Hall et al. 2017).

Pheromone extracts. The terminal abdominal segments from virgin females 2–3 d old were excised 7–8 h into the scotophase. An extract was prepared by keeping 10 excised abdominal tips in 30 μl of redistilled hexane (Alfa Aesar, Shanghai, China) for 3 d at room temperature (Grant et al. 1987). The extract was then analyzed with a Thermo Trace ISQ Single Quadrupole MS coupled to a Thermo Trace 1300 gas chromatograph (GC; Thermo Fisher Scientific Inc., Shanghai, China) equipped with a DA-5MS column (30 m $\times$ 0.25 mm-internal diameter [i.d.], and 0.25-μm film thickness; Agela Technologies, Torrance, CA). The oven temperature was programmed as 80°C for 1 min, 8°C/min to 210°C, held for 10 min, 10°C/min to 250°C, then held for 4 min. Helium was used as the carrier gas. Potential pheromone components in the extracts were identified by comparing their mass spectra and retention times with those of synthetic authentic standards. Analyses were conducted on an Agilent 6890 GC (Agilent Technologies, Inc., Beijing, China) with a DB-1301 GC column (30 m $\times$ 0.32-mm i.d. $\times$ 0.25-μm film; J & W Scientific, Folsom, CA) and helium carrier gas, splitless injection, and flame ionization detection (FID). The oven temperature was programmed as 100°C for 2 min, 4°C/min to 180°C, 10°C/min to 250°C, then held for 10 min.

Chemicals. (Z)-9-tetradecenyl acetate (Z9-14:Ac), (Z9E11-14:Ac), and (Z)-11-hexadecen-1-ol (Z11-16:OH) were purchased from Biohome Tech (Kunming Biohome Technology Co. Ltd., Kunming, China). An aliquot of Z11-16:OH was converted to (E)-11-hexadecen-1-ol (E11-16:OH) (Liu et al. 1986). (Z11-16:Ald and E11-16:Ald were obtained by pyridinium chlorochromate oxidation of its corresponding 11-hexadecenol. Z11-16:Ac was obtained by acetylation. An aliquot of Z11-16:Ac was converted to (E)-11-hexadecenyl acetate (E11-16:Ac) in an analogous method to the above (Liu et al. 1986). (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene (C25 pentaene) was synthesized in a similar manner to previously described methods (Millar et al. 2005). According to the protocol (Fig. 2A), ethyl all *cis*-5,8,11,14,17-eicosapentaenoate (Fig. 2A, compound 1) (TCI Shanghai, China) was reduced with $LiAlH_4$ to afford the expected primary alcohol (Fig. 2A, compound 2), which was then converted to its alkyl halide (Fig. 2A, compound 3) by reaction with PBr_3. Coupling of this alkyl halide with the Grignard reagent of 1-bromopentane using a copper catalyst (Cahiez et al. 2000) yielded the desired pentaene (Fig. 2A, compound 4). (Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene (C23 pentaene) (Fig. 2A, compound 5) was synthesized from ethyl all *cis*-

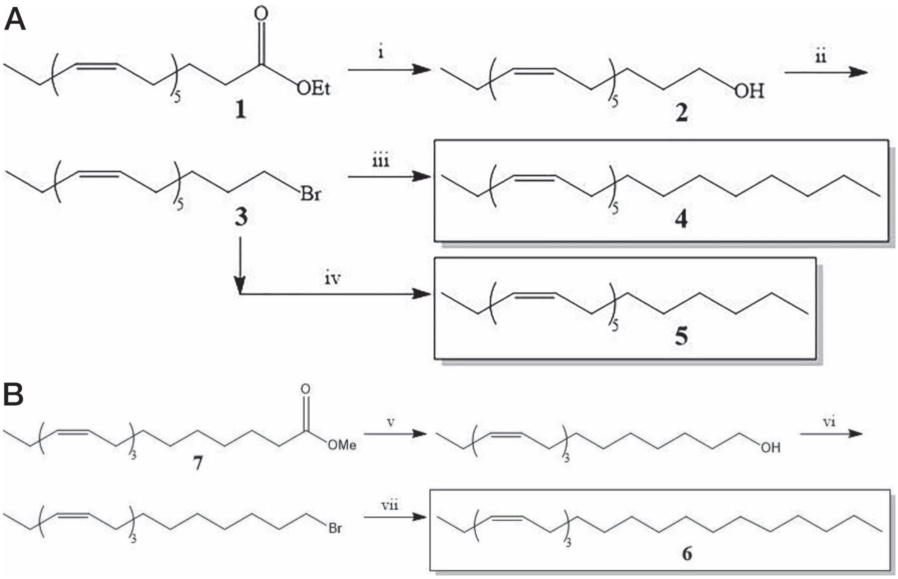

Fig. 2. Syntheses of (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene and (Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene (A): (1) $LiAlH_4$, ether, ice/sodium chloride; (2) PBr_3, petroleum ether, crushed ice; (3) $CH_3(CH_2)_4MgBr$, 2% Li_2CuCl_4, NMP, 2-MeTHF, ice/sodium chloride; (4) $CH_3(CH_2)_2MgBr$, 2% Li_2CuCl_4, NMP, 2-MeTHF, ice/sodium chloride. Syntheses of (Z,Z,Z)-3,6,9-tricosatriene (B): (5) $LiAlH_4$, ether, ice/sodium chloride; (6) PBr_3, petroleum ether, crushed ice; (7) $CH_3(CH_2)_4MgBr$, 2% Li_2CuCl_4, NMP, 2-MeTHF, ice/sodium chloride.

5,8,11,14,17-eicosapentaenoate by reduction, reaction with PBr_3, and reaction with propyl magnesium bromide in an analogous method to the above. (Z,Z,Z)-3,6,9-tricosatriene (ZZZ3,6,9-23:H) (Fig. 2B, compound 6) was synthesized from methyl linolenate (TCI) (Fig. 2B, compound 7) in an analogous method to the above. Mass spectra of C25 pentaene, C23 pentaene, and ZZZ3,6,9-23:H were tested by GC–mass spectrometry (GC-MS) (Thermo Fisher Scientific Inc.) and their characteristic ions (*m/z*) were compared with previous reports (Hall et al. 2017, Kuenen et al. 2010, Leal et al. 2005). The purity of all the chemicals was >95% as checked by GC-FID (Agilent).

Field trapping. Field experiments were conducted at two Mongolian pine plantations near Nenjiang River (Heilongjiang province, China), between N 48°1′, E 124°26′ and N 47°54′, E 124°23′, at 180 m elevation. The experimental stand consisted of young pines 10–15 yr old. Traps were sticky delta traps (25 cm × 20 cm × 15.6 cm; Pherobio Technology Co., Ltd., Beijing, China). Traps were suspended from tree branches at 1.8–2.0 m height and were at least 30 m apart, and redistributed after each check. Pheromone dispensers were white rubber septa (8 mm outer diameter; Geruibiyuan Technology Co., Ltd., Beijing, China). The dispenser was positioned at the center of sticky insert by pin. In addition to count trap catches, wing coloration of *Dioryctria* species on the sticky insert were checked at the same time.

We conducted three experiments over a 3-yr period (2019–2021). Experiment 1 was designed to test the effect of Type I pheromones on the attraction of male *D. mongolicella*. The experiment was conducted at NR, Heilongjiang province (13 July–28 July 2019). Five replicate blocks of six delta traps per block were set in the crowns (one trap per tree) of Mongolian pine. Traps were at least 30 m apart and nearest blocks were 200 m apart. One of the following six treatments was randomly assigned to each trap within a replicate: (a) Z9-14:Ac (100 μg), (b) Z9E11-14:Ac (100 μg), (c) E11-16:Ald (100 μg), (d) Z11-16:Ald + Z11-16:Ac (100 μg:100 μg), (e) E11-16:Ald + Z11-16:Ac (100 μg:100 μg), and (f) two virgin female moths. The two virgin females were not replaced. Trap catches were recorded twice a week. Lure replacement was unnecessary because the experiment ran for only 2 wk.

Experiment 2 was designed to verify the effects of C25 pentaene and C23 pentaene on the attraction of male *D. mongolicella* when added to the blend of E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac, respectively. The same experiment was conducted at NR and the Fu Yu Forestry Station (FY) in Heilongjiang province (31 July–30 September 2020). Five replicate blocks of seven delta traps per block were set in the crowns (one trap per tree) of Mongolian pine in the two forestry stations, respectively. One of the following seven treatments was randomly assigned to each trap within a replicate: (a) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C25 pentaene (500 μg:200 μg:200 μg:250 μg), (b) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C25 pentaene (500 μg:200 μg:200 μg:500 μg), (c) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C25 pentaene (500 μg:200 μg:200 μg:1000 μg), (d) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C23 pentaene (500 μg:200 μg:200 μg:250 μg), (e) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C23 pentaene (500 μg:200 μg:200 μg:500 μg), (f) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C23 pentaene (500 μg:200 μg:200 μg:1000 μg), and (g) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac (500 μg:200 μg:200 μg). Trap catches were recorded twice a week. Lures were replaced once after 4 wk. Sticky inserts in traps were replaced as needed.

Experiment 3 was designed to test the effect of ZZZ3,6,9-23:H on the attraction of male *D. mongolicella* when added to the blend of E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac. The experiment was conducted at NR, Heilongjiang province (29 July–29 August 2021). Six replicate blocks of four delta traps per block were set in the crowns (one trap per tree) of Mongolian pine. Traps were at least 30 m apart and nearest blocks were 200 m apart. One of the following four treatments was randomly assigned to each trap within a replicate: (a) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac (400 μg:200 μg:200 μg), (b) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C25 pentaene (400 μg:200 μg:200 μg:500 μg), (c) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C23 pentaene (400 μg:200 μg:200 μg:500 μg), and (d) E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZ3,6,9-23:H (400 μg:200 μg:200 μg:500 μg). Trap catches were recorded twice a week. Lure replacement was unnecessary because the experiment ran for only 4 wk.

Data analysis. Statistical tests were based on catches recorded per trap check. Total catches of moths of experiments in FY were transformed by $\ln(y + 1)$ to remove heteroscedasticity. For most experiments, differences in trap catches among treatments were compared using one-way analysis of variance (ANOVA),

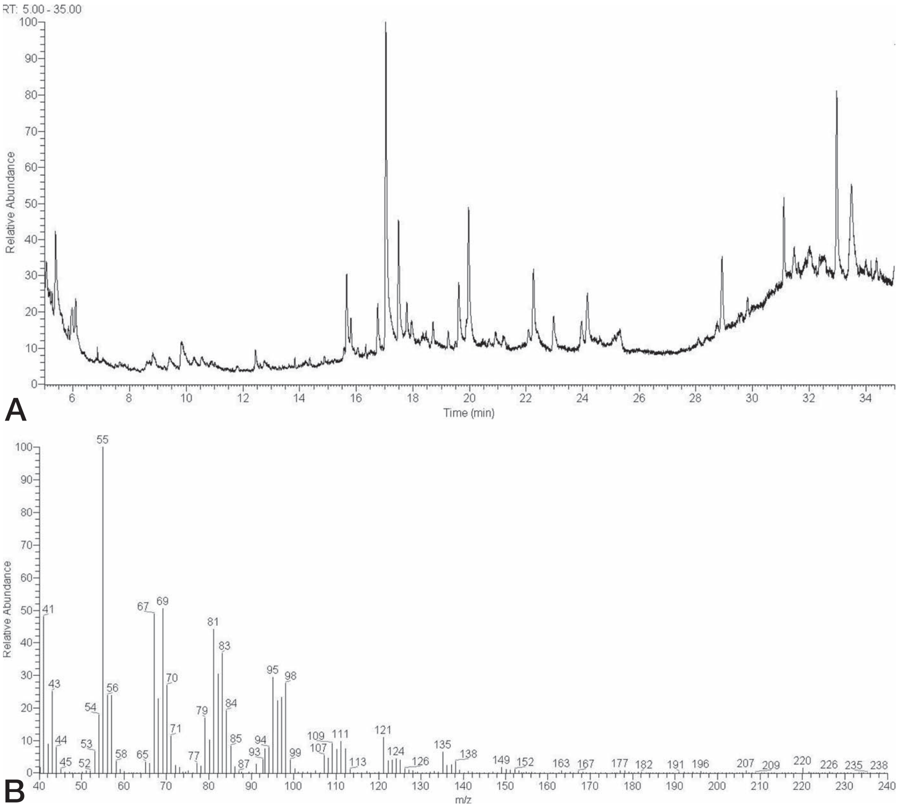

Fig. 3. Gas chromatography–mass spectrometry analysis of crude pheromone gland extracts of *Dioryctria mongolicella* on a DA-5MS column (A), mass spectrum of Peak 1 (B) and Peak 2 (C), and the retention times of synthetic E11-16:Ald and Z11-16:Ac coincided with that of crude pheromone gland extracts (D). E11-16:Ald = (E)-11-hexadecenal; Z11-16:Ac = (Z)-11-hexadecenyl acetate.

followed by multiple comparisons adjusted according to Fisher LSD method. Treatments with zero catches were omitted from the ANOVA. The numbers of male *D. mongolicella* captured are shown as the mean ± standard error (SE). All statistical analyses were conducted using SigmaPlot for Windows Version 14.0.

Results

Analyses of pheromone gland extracts. The GC-MS analysis of pheromone gland extracts from female *D. mongolicella* revealed the presence of 11-hexadecanal (Peak 1, 17.04 min) and 11-hexadecenyl acetate (Peak 2, 19.96 min) (Fig. 3A). The mass spectrum of Peak 1 showed a molecular ion m/z 238 and diagnostic fragment ions m/z 220 (M^+–H_2O), m/z 194 (M^+–McLafferty rearrange-

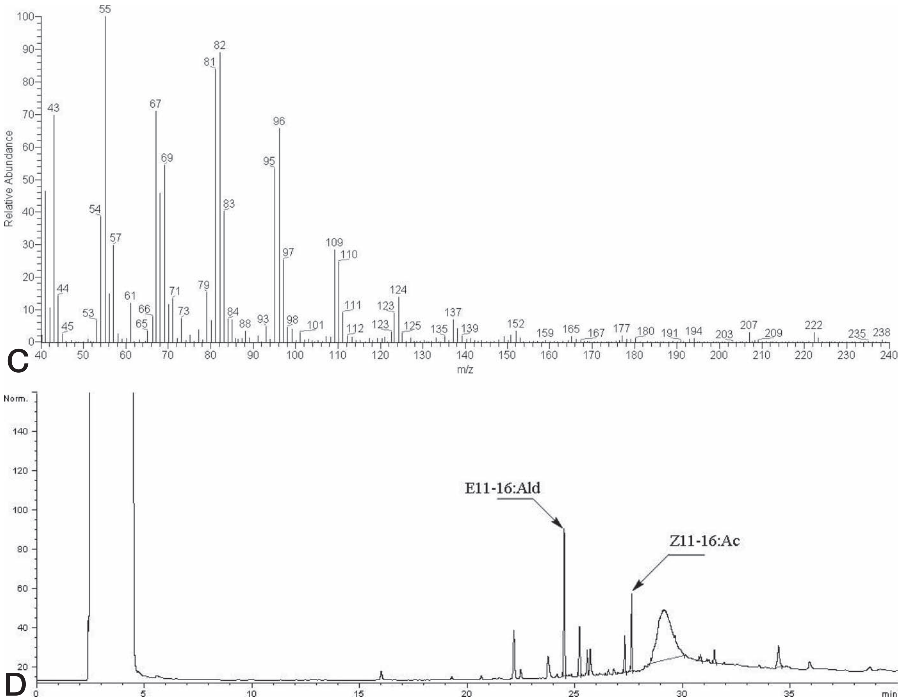

Fig. 3. Continued.

ment) and m/z 55 (base peak) (Fig. 3B). Peak 2 showed diagnostic ions m/z 222 (M^{+}–AcOH), m/z 61 ($[AcOH + 1]^{+}$) and m/z 55 (base peak) (Fig. 3C). These were tentatively identified as 11-hexadecenal and 11-hexadecenyl acetate by comparing the MS pattern with the MS library (National Institute of Standards and Technology 2017). To confirm the isomeric structures, pheromone gland extracts and authentic compounds were analyzed using GC-FID with a DB-1301 column. Among the four synthetic compounds (E11-16:Ald, Z11-16:Ald, E11-16:Ac, and Z11-16:Ac), the retention times of E11-16:Ald and Z11-16:Ac coincided with that of the crude pheromone extracts (Fig. 3D), and Peak 1 and Peak 2 increased in size. Therefore, Peak 1 and Peak 2 were identified as E11-16:Ald and Z11-16:Ac, respectively. The ratio of the compounds (E11-16:Ald:Z11-16:Ac) in the crude extract was 2:1 in 2020.

Field trapping. In 2009, we captured a total of 63 *D. mongolicella* in Experiment 1. There was a statistically significant treatment effect on catches of *D. mongolicella* ($F = 3.207$; df $= 4, 20$; $P < 0.05$; Table 1). Catches of moths in traps baited with two virgin female moths were higher than those in traps baited with Z9E11-14:Ac alone, E11-16:Ald alone, Z11-16:Ald + Z11-16:Ac, or E11-16:Ald + Z11-16:Ac. Traps baited with Z9-14:Ac alone were not attractive to *D. mongolicella*.

In 2020, we captured a total of 597 *D. mongolicella* in Experiment 2 (278 in NR and 319 in FY), but there were no statistically treatment effects on catches of *D. mongolicella* in either NR ($F = 0.956$; df $= 6, 28$; $P = 0.472$) or FY ($F = 0.318$; df $= 6,$

Table 1. Mean ± SE number of male *Dioryctria mongolicella* in traps baited with various blends of potential sex pheromone components in 2019.*

Treatment**	Ratio (μg)	Mean Trap Catch
Z9-14:Ac	100	0
Z9E11-14:Ac	100	1.0 ± 0.3b
E11-16:Ald	100	2.6 ± 1.2ab
Z11-16:Ald + Z11-16:Ac	100:100	1.2 ± 0.6b
E11-16:Ald + Z11-16:Ac	100:100	2.4 ± 0.9ab
Virgin females (two per trap)	—	5.4 ± 1.4a

* Field trapping conducted 13 July–28 July 2019, with five replicates counted twice a week. Trap catch data analyzed by ANOVA followed by Fisher's LSD Test. Means followed by different letters are statistically different at $P < 0.05$. Zero values were not included in the data analyses.

** Z9-14:Ac = (Z)-9-tetradecenyl acetate; Z9E11-14:Ac = (Z,E)-9,11-tetradecadienyl acetate; E11-16:Ald = (E)-11-hexadecenal; Z11-16:Ald = (Z)-11-hexadecenal; Z11-16:Ac = (Z)-11-hexadecenyl acetate.

28; $P = 0.922$) (Table 2). The addition of C25 pentaene or C23 pentaene to lures loaded with E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac did not significantly increase trap catches of *D. mongolicella* at either location.

In 2021, 394 *D. mongolicella* were captured in Experiment 3 in NR with catches significantly affected by treatments ($F = 3.331$; df = 3, 20; $P < 0.05$) (Table 3). Catches of *D. mongolicella* in traps baited with E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZ3,6,9-23:H were significantly higher than other traps baited with E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac or E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + C25 pentaene. The number (mean ± SE) of *D. mongolicella* in traps baited with E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZ3,6,9-23:H was 26.2 ± 5.2.

Discussion

Dioryctria mongolicella was found in the northeast of China and belongs to the genus *Dioryctria* Zeller including *Dioryctria abietella* Denis & Schiffermüller, *D. mendacella*, *Dioryctria sylvestrella* Ratzeburg and *D. rubella*, along with *D. mongolicella* (Wang and Sung 1982). Delineation of these species requires the identification of morphological traits and even molecular data. Despite their similarities, *D. mongolicella* can be separated from the others primarily by wing coloration, genital structures, and host association (Knölke 2008).

In GC-MS analyses of a single crude extract of approximately 10 pheromone glands of virgin female *D. mongolicella* obtained from China, 11-16:Ald and 11-16:Ac were detected in a ratio of 2:1. Our field trapping experiments confirm that the *D. mongolicella* pheromone consists of a blend of E11-16:Ald and Z11-16:Ac. However, traps baited with E11-16:Ald alone or binary blends of E11-16:Ald and Z11-16:Ac were less effective in attracting males than those baited with virgin

Table 2. Mean ± SE number of male *Dioryctria mongolicella* in traps baited with candidate sex attractants and pentaene at two sites in 2020.*

Treatment**	Ratio (μg)	Mean Trap Catch	
		NR	FY
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-25:H	500:200:200:250	8.8 ± 3.7	7.4 ± 3.0
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-25:H	500:200:200:500	6.2 ± 1.8	9.6 ± 4.1
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-25:H	500:200:200:1,000	8.0 ± 2.6	6.6 ± 1.4
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-23:H	500:200:200:250	6.2 ± 1.8	5.8 ± 2.0
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-23:H	500:200:200:500	6.6 ± 2.2	9.8 ± 4.6
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-23:H	500:200:200:1,000	12.0 ± 3.0	6.0 ± 2.9
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac	500:200:200	4.6 ± 1.2	8.0 ± 2.2

* Field trapping conducted 31 July–30 September 2020 in two sites, with five replicates counted weekly. Trap catch data analyzed by ANOVA followed by Fisher's LSD Test. Means without letters indicate no significant differences among treatments ($P = 0.05$).

** E11-16:Ald = (E)-11-hexadecenal; Z11-16:Ac = (Z)-11-hexadecenyl acetate; Z9E11-14:Ac = (Z,E)-9,11-tetradecadienyl acetate; ZZZZZ3,6,9,12,15-25:H = (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene; NR = New River Experimental Forestry Station; FY = Fu Yu Forestry Station.

Table 3. Mean ± SE number of male *Dioryctria mongolicella* in traps baited with candidate sex attractants and (Z,Z,Z)-3,6,9-tricosatriene in 2021.*

Treatment	Ratio (μg)	Mean Trap Catch
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac	400:200:200	12.3 ± 4.4ab
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-25:H	400:200:200:500	11.5 ± 2.3b
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZZZ3,6,9,12,15-23:H	400:200:200:500	15.7 ± 1.8ab
E11-16:Ald + Z11-16:Ac + Z9E11-14:Ac + ZZZ3,6,9-23:H	400:200:200:500	26.2 ± 5.2a

* Field trapping conducted 29 July–29 August 2021, with six replicates counted twice a week. Trap catch data analyzed by ANOVA followed by Fisher's LSD Test. Means followed by different letters are different at $P < 0.05$.

** E11-16:Ald = (E)-11-hexadecenal; Z11-16:Ac = (Z)-11-hexadecenyl acetate; Z9E11-14:Ac = (Z,E)-9,11-tetradecadienyl acetate; ZZZZZ3,6,9,12,15-25:H = (Z,Z,Z,Z,Z)-3,6,9,12,15-pentacosapentaene; ZZZ3,6,9-23:H = (Z,Z,Z)-3,6,9-tricosatriene.

females in Heilongjiang, China. This result suggests that the females produce an additional important pheromone component.

The sex attractant blend of *D. rubella* was characterized as the combination of Z11-16:Ac, Z11-16:Ald, and Z9E11-14:Ac. The addition of Z9E11-14:Ac increased catches of male *D. rubella* in sticky traps baited with Z11-16:Ald and Z11-16:Ac (Wu et al. 1986). Thus, the sex attractant blend of *D. rubella* seems to be similar to that of populations of *D. mongolicella* from the western population in Heilongjiang, China. C25 pentaene is a strong synergist to Z9E11-14:Ac, and both compounds are needed for significant attraction of some *Dioryctria* species (Hall et al. 2017, Löfstedt et al. 2012, Millar et al. 2005). Field trials that tested the attractiveness of C25 pentaene combined with Z11-16:Ac show an increase in male response in *Dioryctria amatella* (Hulst) (Miller et al. 2010). However, attraction of *D. mongolicella* male moths to E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac could not be greatly increased by addition of ZZZZZ3,6,9,12,15-25:H or ZZZZZ3,6,9,12,15-23:H. It is likely that pentaene is not a sex attractant for *D. mongolicella*.

There have been an increasing number of reports of lepidopteran sex pheromones containing both Type I components and Type II components (Ando et al. 2004). In addition to ZZZZZ3,6,9,12,15-25:H, mentioned above, ZZZ3,6,9-23:H was identified as a synergist in sex attractant pheromone of *Conogethes punctiferalis* (Guenée) (Lepidoptera: Crambidae) (Xiao et al. 2012). Silk et al. (2017) proposed that the sex pheromone blend of *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) includes primary components, with Z11-16:Ald, (Z)-5-tricosene (Z5-23:H), and ZZZ3,6,9-23:H fulfilling secondary roles in orientation and close-range courtship. ZZZ3,6,9-23:H and E11-16:Ac are pheromone components of *Leucinodes orbonalis* Guenée (Lepidoptera: Crambidae) (Vang et al. 2018). Hawaiin populations of *Omphisa anastomosalis* (Guenée) utilize

ZZZ3,6,9-23:H in mating communication (Lepidoptera: Crambidae) (McQuate et al. 2019), while ZZZ3,6,9-23:H was detected in the female body extract of pheromone components of *Hellula undalis* (F.) (Lepidoptera: Crambidae) (Vang et al. 2020).

Our field trapping experiment in 2021 confirms that attraction of *D. mongolicella* male moths to E11-16:Ald, Z11-16:Ac, and Z9E11-14:Ac could be greatly increased by addition of ZZZ3,6,9-23:H. It is likely that ZZZ3,6,9-23:H is a synergist in the sex attractant of *D. mongolicella*. Thus, lures containing E11-16:Ald (400 μg), Z11-16:Ac (200 μg), Z9E11-14:Ac (200 μg), and ZZZ3,6,9-23:H (500 μg) dispensed from rubber septa can be used to bait traps for *D. mongolicella*. Further work is in progress using the pheromone traps to monitor populations of *D. mongolicella* and gain a better understanding of its life cycle and population dynamics.

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