**Bursaphelenchus xylophilus** is killed by homologues of 2-(1-undecyloxy)-1-ethanol

Junheon Kim1,*, Sang-Myeong Lee2,*, & Chung Gyoo Park1,3,4

2-(1-Undecyloxy)-1-ethanol, monochamol, is a male-produced aggregation pheromone of the *Monochamus* species, which are efficient vectors of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, which cause devastating damage to pines worldwide. The nematicidal activity of synthetic monochamol and its homologues (ROEtOH: R = C7-C13) were investigated to find potential alternatives to the currently used PWN control agents abamectin and emamectin. Compounds with C7-C13 chain length alkyl groups exhibited 100% nematicidal activity at a concentration of 1000 mg/L. At a concentration of 100 mg/L, 2-(1-nonyloxy)-1-ethanol (C9OEtOH), 2-(1-decyloxy)-1-ethanol (C10OEtOH), 2-(1-undecyloxy)-1-ethanol (C11OEtOH), and 2-(1-dodecyloxy)-1-ethanol (C12OEtOH) showed 100% nematicidal activity, but the others showed weaker activities. C11OEtOH showed similar nematicidal activity to abamectin in terms of LD90 values, which were 13.30 and 12.53 mg/L, respectively. However, C9OEtOH, C10OEtOH, and C12OEtOH (LC90 values: 53.63, 38.18, and 46.68 mg/L, respectively) were less effective than C11OEtOH and abamectin. These results indicate that monochamol could be an effective alternative agent against PWN. The relationship of insecticidal and nematicidal activity to different carbon chain lengths in compounds is discussed.

Pine wilt disease, caused by the pine wood nematode (PWN; *Bursaphelenchus xylophilus*), is a major threat to global pine forest ecosystems in locations, including Japan, Korea, China, Taiwan, Portugal, and Spain. The global impact of the presence of the PWN and its likelihood of invading different parts of the world are of increasing economic concern. Since its first report in Busan city in 1988, it has spread to several other parts of the Korean peninsula. Most damaged pine trees are red pine (*Pinus densiflora*) and black pine (*P. thunbergii*), and *P. koraiensis* has also recently been identified as a susceptible species in Korea. *P. nigra* and *P. radiata* have also been reported to be susceptible to PWN in Portugal and Spain, respectively. As *Pinus* species are also a predominant tree species in the Korean forest and are highly susceptible to PWN, the ecological and economic damage is substantial. PWN is predicted to have severe economic consequences for the conifer forestry industry in the EU.

Abamectin and emamectin benzoate, which both belong to the family of avermectins, are primarily used as trunk-injection agents for the control of PWN. These agents are known to be effective against PWN and safe for the environment. However, resistance of nematodes and insect pests to avermectins has been reported, although PWN has not yet been reported to show resistance. As in the case of other groups of insecticides, the continuous use of a single pesticide may induce resistance to those agents in PWN. To avoid the development of resistance and to achieve efficient control of PWN, the alternating use of diverse agents is recommended.

*Monochamus* species are known as efficient vectors of PWN in many countries: *M. alternatus* and *M. saltuarius* in Korea and Japan, *M. carolinensis* in the United States, and *M. galloprovincialis* in Europe. Male adults of *M. galloprovincialis*, *M. carolinensis*, and *M. alternatus* produce an aggregation pheromone, 2-(1-undecyloxy)-1-ethanol (monochamol), that attracts both conspecific females and males. *M. saltuarius* is also reported to be attracted to monochamol.

The nematicidal activity of monochamol was found by chance during our experiments on PWN. We were testing the attractiveness of synthetic monochamol to PWN and found the very interesting phenomenon that PWN was killed in monochamol solutions at very low concentration. We have investigated the nematicidal activity of monochamol.
Suggested that the steric hindrance of longer-chain analogs affects the toxicity of aliphatic isothiocyanates. The monoterpene's structural characteristics allow an easy transfer of the compounds through the insect cuticle. Li et al. examined the most toxic to two Drosophila species. The authors suggested that its higher toxicity might be linked to its structural characteristics, allowing an easy transfer of the compounds through the insect cuticle. Li et al. suggested that the steric hindrance of longer-chain analogs affects the toxicity of aliphatic isothiocyanates. The nematicidal activity of 2-(1-alkyloxy)-1-ethanol homologues, undecanol and abamectin against pine wood nematode, Bursaphelenchus xylophilus. The nematicidal activity of the test compounds varied with carbon chain length. Alkanols with C8-C11 carbon atom chains showed significantly higher nematicidal activity against PWN than compounds with shorter or longer carbon chain lengths. In this study, too, C11OEtOH showed higher nematicidal activity (13.30 and 15.78 mg/L, respectively) than the rest in terms of LC90 values, with no significant difference from abamectin (12.53 mg/L) (Table 2), followed by C10OEtOH, C12OEtOH and C9OEtOH. The undecanol has been reported to show nematicidal activity against PWN. Our synthetic monochamol also contained 1% undecanol. The mortality of PWN at 100 ppm of C11OEtOH which was containing 1 ppm undecanol was higher than that at 1 ppm of undecanol (40%) (Table 1). Therefore, the mortality of C11OEtOH was mainly due to C11OEtOH itself.

### Table 1. Nematicidal activity of 2-(1-alkyloxy)-1-ethanol homologues and undecanol against pine wood nematode, Bursaphelenchus xylophilus.

| Compound         | LC50 (mg/L) | 95% CI | LC90 (mg/L) | 95% CI | Slope ± SE | χ² (df) |
|------------------|-------------|--------|-------------|--------|------------|--------|
| C7OEtOH          | 133.28      | 121.92–145.96 | 774.75 | 672.17–905.78 | 0.73 ± 0.02 | 489.91(33) |
| C8OEtOH          | 101.39      | 89.31–115.33 | 543.02 | 448.68–676.27 | 0.76 ± 0.03 | 73.54(14) |
| C9OEtOH          | 19.35       | 18.07–20.75 | 53.65 | 48.79–59.47 | 1.26 ± 0.04 | 7514.83(45) |
| C10OEtOH         | 13.74       | 12.82–14.80 | 38.18 | 33.42–44.82 | 1.25 ± 0.07 | 292.75(26) |
| C11OEtOH         | 1.53        | 1.37–1.70 | 13.30 | 11.63–15.40 | 0.59 ± 0.02 | 648.93(42) |
| C12OEtOH         | 17.11       | 15.59–18.82 | 46.68 | 41.08–54.00 | 1.28 ± 0.06 | 1835.38(22) |
| C13OEtOH         | 231.2       | 205.20–270.07 | 414.61 | 343.20–534.25 | 2.19 ± 0.17 | 64.04(29) |
| Undecanol (C11OH)| 1.68        | 1.35–2.04a | 15.78 | 12.21–21.60a | 0.57 ± 0.04 | 7.09(10) |
| Abamectin        | 5.42        | 4.84–6.06b | 12.53 | 11.04–14.44a | 1.53 ± 0.08 | 7.72(9) |

### Table 2. LC50 and LC90 values of 2-(1-alkyloxy)-1-ethanol homologues, undecanol and abamectin against pine wood nematode, Bursaphelenchus xylophilus.

| Compound         | LC50 (mg/L) | LC90 (mg/L) | 95% CI | Slope ± SE | χ² (df) |
|------------------|-------------|-------------|--------|------------|--------|
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| C9OEtOH          | 19.35       | 53.65       | 18.07–20.75 | 1.26 ± 0.04 | 7514.83(45) |
| C10OEtOH         | 13.74       | 38.18       | 12.82–14.80 | 1.25 ± 0.07 | 292.75(26) |
| C11OEtOH         | 1.53        | 13.30       | 1.37–1.70 | 0.59 ± 0.02 | 648.93(42) |
| C12OEtOH         | 17.11       | 46.68       | 15.59–18.82 | 1.28 ± 0.06 | 1835.38(22) |
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| Undecanol (C11OH)| 1.68        | 15.78       | 1.35–2.04 | 0.57 ± 0.04 | 7.09(10) |
| Abamectin        | 5.42        | 12.53       | 4.84–6.06b | 1.53 ± 0.08 | 7.72(9) |

The results and discussion: The nematicidal activities of 2-(1-alkyloxy)-1-ethanol homologues, undecanol, and abamectin are shown in Table 1. There was a significant difference in nematicidal activities according to chain length. All 2-(1-alkyloxy)-1-ethanol homologues and undecanol showed 100% mortality at a concentration of 1000 mg/L. 2-(1-Alkyloxy)-1-ethanol homologues with C9-C12 chain length in the alkyl group and undecanol exhibited 100% mortality even at a concentration of 100 mg/L. However, other homologues with shorter or longer chain lengths, such as C6OEtOH, C8OEtOH, and C13OEtOH, showed less than 40% of mortality at the same concentration. At a concentration of 10 mg/L, only C11OEtOH and undecanol showed more than 80% nematicidal activity. The mortality of PWN at 100 ppm of C11OEtOH which was containing 1 ppm undecanol was higher than that at 1 ppm of undecanol (40%) (Table 1). Therefore, the mortality of C11OEtOH was mainly due to C11OEtOH itself.

The nematicidal activity of the test compounds varied with carbon chain length. Alkanols with C6-C11 carbon chain length and alkylamine with C8 showed significantly higher nematicidal activity against PWN than compounds with shorter or longer carbon chain lengths. In this study, too, C8OEtOH showed higher nematicidal activity than other 1-(2-alkyloxy)-2-ethanol homologues with shorter or longer carbon chains. The relationship between the structure of aliphatic carboxylic acids and their toxicity to nematodes has been sparsely reported. Among linear carboxylic acids with C6-C15 carbon atom chains, octanoic acid (C8 chain length) was the most toxic to two Drosophila species. The authors suggested that its higher toxicity might be linked to its structural characteristics, allowing an easy transfer of the compounds through the insect cuticle. Li et al. suggested that the steric hindrance of longer-chain analogs affects the toxicity of aliphatic isothiocyanates.
its homologues were synthesized following the method by Loffredo et al.33. A general procedure followed was:

\[
\text{R-Br} \quad \xrightarrow{\text{HO\text{\_}O\text{\_}OH}} \quad \text{R-O\text{\_}O\text{\_}OH} \quad \xrightarrow{\text{Na}} \quad \text{R-\text{\_}O\text{\_}OH}
\]

Figure 1. Synthetic scheme of 1-(2-alkyloxy)-1-ethanol. R is a straight-chain hydrocarbon with 7-13 carbon atoms.

toxicity of compounds increased when the electron population or electron accessibility (richness) increased27. In our study, C_{11}OEtOH exerted the most effective nematicidal activity, even though its total length is equivalent to tetradecanol (C_{14}OH) which showed weak nematicidal activity in a previous study23. The oxy group (-O-) in the structure of C_{11}OEtOH possibly contributed to the nematicidal activity. The addition of an oxy group to the structure would increase toxicity by increasing the compound’s transferability through insect cuticles. However, the mechanism of nematicidal activity of some homologues of 2-(1-alkyloxy)-1-ethanol remains unknown.

The different susceptibilities of invertebrates and vertebrates to these compounds provide an acceptable therapeutic ratio for pesticide use. In order to achieve resistance development of a pest against pesticides, the activities of the compounds must have different modes of action28. The mode of action of avermectin is related to the GABA-gated chloride channel29. Kang et al.30 estimated the inhibitory activity of C_{11}OH against acetylcholinesterase (AChE) and glutathione S-transferase (GST) of PWN to elucidate the mode of action30. Although C_{11}OH showed relatively high nematicidal activity, it showed no or little inhibition activity against AChE and GST of PWN30. This result suggests that AChE and GST may not be the targets of C_{11}OH. For the safe practical use of C_{11}OEtOH as a nematicide, the mode of action of C_{11}OEtOH should be investigated.

It is unclear why the pheromone of the Monochamus vector species has strong nematicidal activity against PWN, a parasite of *Pinus* spp. The reasons for PWN to spread through the Monochamus vector species that produce an aggregation pheromone that can kill them are worthy of future study.

For a trunk injection agent to be effective against PWN, it is necessary for the injected agent to be translocated throughout the tree at an effective concentration. For this purpose, the agent should have adequate water solubility and diffuse to every part of the tree. C_{11}OEtOH showed as effective a nematicidal activity as abamectin, which is in practical use as a trunk injection agent. Although C_{11}OEtOH has the advantage of being easily synthesized, it shows very low water solubility. Therefore, to develop C_{11}OEtOH as a commercial trunk injection against PWN, a water-soluble preparation of C_{11}OEtOH should be formulated.

In conclusion, 2-(1-undecyloxy)-1-ethanol (monochamol) can be used as an effective nematicide against pine wood nematode, and water-soluble monochamol formulations are required along with the identification of an effective concentration (dose) for the control of pine wilt disease. Studying the mechanisms of the nematicidal activity of monochamol is also valuable.

**Materials and Methods**

**Collection of pine wood nematodes.** PWN was extracted by Baermann funnel method41 from chips of PWN-infected pines collected from Jinju, Korea in 2014. The PWN were cultured as described by Park et al.32 until used for bioassay.

**Chemicals.** Authentic compounds used for bioassay were synthesized as shown in Fig. 1. Undecanol (99% pure) was obtained from Sigma-Aldrich (St. Louis, MO). Abamectin (1.8% of active ingredient) was purchased from Syngenta (Basel, Switzerland). BFC30, a surfactant, was generously provided by Dongbu Farm Hannon Ltd. (Daejeon, Korea).

**Instrumental analysis.** Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a GCMS-QP2010 coupled with a GC2010 (Shimadzu, Kyoto, Japan) equipped with a HP-Innowax (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA). The oven temperature was programmed as 40 °C for 1 min, then raised to 250 °C at 6 °C/min, and the temperature held for 4 min. Purities of synthesized compounds were checked by GC-FID (GC17A: Shimadzu; DB-5MS; J&W Scientific). \(^{13}H\) and \(^{13}C\) NMR (500 and 125 MHz, respectively) analysis was performed with a Bruker DRX-500 spectrometer using TMS in CDCl₃ as an internal standard at Center for Research Facilities of GNU.

**Synthesis of the monochamol and its homologues.** 2-(1-Undecyloxy)-1-ethanol (C_{11}OEtOH) and its homologues were synthesized following the method by Loffredo et al. (Fig. 1)33. A general procedure followed was:

2-(1-Hexyloxy)-1-ethanol (C_{6}OEtOH); Colorless liquid, yield: 72.2%, purity: 99.1%, MS m/z (% relative intensity, ion): 129 (0.9, M – CH₂OH), 115 (0.2), 97 (10.4), 83 (1.0), 70 (10.7), 63 (12.1), 57 (100.0), 45 (45.4), 43 (47.9).
2-(1-octyloxy)-1-ethanol (C₈OEtOH); Colorless liquid, yield: 69.0%, purity: 99.7%, MS m/z (% relative intensity, ion): 143 (6.6, M⁻²CH₂OH), 129 (0.8), 112 (18.6), 111 (45.9), 97 (2.5), 84 (38.5), 83 (22.4), 71 (99.3), 63 (48.6), 57 (100.0), 45 (75.0), 43 (88.5).

2-(1-nonyloxy)-1-ethanol (C₉OEtOH); Colorless liquid, yield: 59.8%, purity: 96.7%, MS m/z (% relative intensity, ion): 157 (3.5, M⁻²CH₂OH), 127 (1.8), 98 (12.5), 85 (37.5), 71 (58.5), 63 (21.2), 57 (60.9), 45 (59.9), 43 (100.0).

2-(1-decyloxy)-1-ethanol (C₁₀OEtOH); Colorless liquid, yield: 53.5%, purity: 99.4%, MS m/z (% relative intensity, ion): 171 (2.5, M⁻²CH₂OH), 157 (0.2), 140 (5.7), 112 (14.1), 97 (20.0), 85 (65.2), 71 (65.8), 63 (32.2), 57 (100.0), 45 (43.6), 43 (78.8).

2-(1-undecyloxy)-1-ethanol (C₁₁OEtOH); Colorless liquid, yield: 47.0%, purity: 99.0%, MS (%): 185 (4.3, M⁻²CH₂OH), 171 (0.3), 154 (6.7), 126 (12.6), 111 (16.0), 97 (39.9), 85 (50.6), 71 (66.6), 63 (28.0), 57 (100.0), 45 (37.5), 43 (59.2).

2-(1-dodecyloxy)-1-ethanol (C₁₂OEtOH); Colorless liquid, yield: 65.7%, purity: 98.0%, MS m/z (% relative intensity, ion): 199 (4.4, M⁻²CH₂OH), 185 (0.3), 168 (6.3), 140 (12.5), 125 (10.9), 111 (36.8), 97 (47.5), 85 (67.9), 71 (88.4), 63 (46.6), 57 (100.0), 45 (48.9), 43 (82.4).

2-(1-tridecyloxy)-1-ethanol (C₁₃OEtOH); white solid at 20°C; yield: 45.9%, purity: 98.2%, MS m/z (% relative intensity, ion): 213 (0.6, M⁻²CH₂OH), 182 (0.7), 154 (1.5), 125 (2.7), 111 (6.1), 97 (12.0), 85 (22.2), 71 (38.7), 63 (22.1), 57 (78.3), 45 (60.3), 43 (100.0).

**Nematicidal activity.** Solutions of 2-(1-alkyloxy)-2-ethanol homologues, undecanol, and abamectin (1.8%) were prepared by serial dilution with distilled water containing BFC30 (200 mg/L). The stock solutions were prepared in 1000 ppm, which were then diluted by 1/10 times in sequence in each step, if a concentration found somewhat effective to the PWN. Undecanol, which was reported to exhibit nematicidal activity was selected as one of the positive controls because of its 1.0% share in the synthetic monochol. The stock solution of abamectin (1.8%) was 100 ppm (active ingredient: 18 mg/L) which was also serially diluted. Test solutions (1 mL) were introduced into wells of 24-well plates (Falcon, USA). Each well was then inoculated with about 100 mixed stages (male: female: juvenile = 1:1:2:9.5) of PWN in 10 µL of distilled water. Controls were treated only with BFC30 solutions. The well plates were held under the same conditions as used for nematode colony maintenance. Mortality of nematodes was recorded after 24 h under a stereoscopic microscope. Nematodes were considered as dead if their bodies were straight and when they did not move, even after transferring to clean water. Two to four trials with three to four replicates were performed on different days. Data were pooled for analysis.

**Statistical analyses.** Nematode mortality was corrected using Abbott's formula and corrected mortality was transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Tukey-Kramer HSD test. The LC₅₀ value was estimated by probit analysis with dose-response data. Differences in LD₅₀ and LD₉₀ values between treatments were considered significant if the 95% confidence intervals did not overlap. Statistical analyses were performed using JMP ver. 9.0.2 (SAS Institute Inc., Cary, NC, USA). Mean (±SEM) values of untransformed data are reported.

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Author Contributions

J.K., S.-M.L. and C.G.P. designed the experiments. J.K. and S.-M.L. conducted the experiments. J.K. and C.G.P. wrote the main manuscripts. All authors reviewed the manuscript.

Additional Information

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