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ORIGINAL CONTRIBUTION

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# Development of multi-component non-sex pheromone blends to monitor both sexes of *Cydia pomonella* (Lepidoptera: Tortricidae)

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## Abstract

Nineteen host plant volatiles (HPVs) were screened for attractivity to adult codling moth *Cydia pomonella* (L.) as a fourth component of core blends (3K) including (*E*,*Z*)-2,4-ethyl decadienoate, (*E*)-4,8-dimethyl-1,3,7-nonatriene and acetic acid. Each new quaternary combination was compared with a previously reported attractive bisexual lure (4K), consisting of the 3K blend plus 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, pyrLOX). All lure evaluations were conducted in apple, *Malus domestica* (Borkhausen). Several compounds were found to significantly lower total and/or female catches when added to the 3K blend, including (*Z*)-3-hexenol, (*E*)-2-hexanal and hexyl butanoate (female and total moths), and (*Z*)-3-hexenyl acetate and linalool (female moths). Other compounds when added to the 3K blend did not increase or decrease moth catches, including methyl salicylate, (*E*)-β-ocimene, limonene, β-caryophyllene, butyl hexanoate, farnesol, terpineol, terpinen-4-ol and α-pinene. A few added compounds significantly increased moth catches compared with the 3K blend, including β-pinene (male moths), (*Z*)-jasmone (male and total moths), (*E*)-β-farnesene and β-myrcene (female and total moths), and (*E*,*E*)-α-farnesene (male, female, and total moths). In addition, each of these five compounds when added to the 3K core blend performed similarly to the 4K lure (male, females, and total moths). Further studies should expand these results through tests of these and other new blends with a range of component ratios and total loading amounts. Field trials should also be replicated within all host crops of codling moth and across major geographical production regions.

KEYWORDS

acetic acid, codling moth, kairomones, linalool oxide, nonatriene, pear ester

# 1 | INTRODUCTION

Management of codling moth (*Cydia pomonella* L.), a key insect pest of apple, *Malus domestica* (Borkhausen), pear, *Pyrus* spp., and walnut, *Juglans regia* (L.), relies on the intelligent integration of several tactics whose deployment is typically cued from accurate monitoring (Knight et al., 2019a). The identification of pear ester, (*E*,*Z*)-2,4-ethyl decadienoate (PE) (Knight et al., 2018; Light et al., 2001), and subsequent improvements from adding either acetic acid (AA) (Landolt et al., 2007) or (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (Knight et al., 2011) alone and together (Knight & Light, 2012) created a series of new and increasingly more powerful lures to track both moth sexes. Most recently, a four-component kairomone blend (4K) comprised of PE, DMNT, AA and 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, pyrLOX) significantly increased the performance of previous lures 3- to 4-fold in orchards treated with or without sex pheromones for mating disruption (MD) (Knight et al., 2019b and 2019c). These results suggested that a mass trapping strategy exploiting 'female removal' could be improved (Jaffe et al., 2018; Jaffe & Landolt, 2018, 2019; Knight et al., 2019c). The serendipitous find of adding pyrLOX also suggested that a field review of other volatiles, such as key host plant volatiles (HPVs) in combination with the core three-component blend of PE, DMNT and AA (3K), could potentially identify additional attractive blends for codling moth. Nineteen HPVs reported from apple, pear and walnut were evaluated in combination with the 3K ternary blend and compared with the 4K blend in a series of studies conducted in apple during 2019.

# 2 | MATERIALS AND METHODS

## 2.1 | Chemicals and lures

Trécé Inc. (Adair, OK, USA) provided all lures used in these studies in proprietary formulations. Grey halobutyl elastomer septa were loaded with 4.0 mg of PE, (*E*,*Z*)-2,4-ethyl decadienoate (Pherocon® DA). Acetic acid (720 mg) was loaded in 3.2 $cm^2$ white plastic membrane cup lures (Pherocon® AA). Proprietary black PVC rectangles (2.7 cm × 2.4 cm) were loaded with 10 mg of DMNT, (*E*)-4,8-dimethyl-1,3,7-nonatriene, while proprietary black PVC discs (diameter 3.3 cm) were loaded with 10 mg of 6-ethenyl-2,2,6-trimethyloxan-3-ol (linalool oxide pyranoid, pyrLOX). Lure discs were also manufactured with 10 mg of one of 18 different volatile organic compounds (VOCs): (i) the green leaf volatiles (GLVs) (*E*)-2-hexenal, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate; (ii) the mono- and sesquiterpenes (*E*)-β-ocimene, β-myrcene, linalool, limonene, α-pinene, terpineol, and terpinen-4-ol, β-caryophyllene, (*E*,*E*)-α-farnesene, (*E*)-β-farnesene, and farnesol; and (iii) and other VOCs, including methyl salicylate, (*Z*)-jasmone, hexyl butanoate and butyl hexanoate. The monoterpene β-pinene was loaded in a 16.3 $cm^2$ white plastic membrane cup lure.

## 2.2 | Field trials

A series of 25 field trials evaluated the attractiveness of 19 new volatile blends for codling moth. In each trial, both a standard three-component lure (3K: PE +DMNT + AA) and four-component lure (4K: PE +DMNT + AA +pyrLOX) were included for comparison. One to six new lure blends were evaluated within each trial, and individual trials were initiated and conducted over 7- to 10-day periods from 12 May to 1 June and from 25 July to 4 August 2019. Some volatile trials were repeated 2 to 4 times on different dates. All trials were performed in a four-hectare unsprayed apple orchard (cultivar Red Delicious) situated near Wapato, WA, USA (46°24'48.99"N–120°28'30.12"W) with a uniform, high infestation level of codling moth overwintering from the previous year. Trials used a similar experimental design and included 8 to 12 replicates per lure treatment. Orange delta traps (Pherocon® VI, Trécé Inc.) were attached to poles and placed at 3.0 m of height or ca. 1.5 m below the top of the canopy. Lure treatments were randomized in each trial, and traps were placed down and across tree rows at ≥25 m spacing and always >20 m from the physical borders of the orchard. Lures were placed near the centre of the sticky liner, and liners were not replaced during trials. Liners were coated with a hot-melt pressure sensitive adhesive (Clean-Brake™, Trécé Inc.). Moths were counted and sexed in the laboratory.

## 2.3 | Statistical analyses

Count data were analysed with analysis of variance (ANOVA) (Statistix 9, Analytical Software). Data from experiments conducted only once were analysed as a completely randomized ANOVA. Trials repeated on different dates were analysed with a randomized block design with date as the blocking variable. A square-root transformation was used to normalize count data (Shapiro–Wilk test) prior to ANOVAs. Tukey's HSD test was used to detect significant pair-wise mean comparisons following significant ANOVAs. A *p*-value of .05 was used to establish a significance in all tests.

# 3 | RESULTS

Trials with the new four-component lure blends were organized into three categories based on their relative performance compared with the standard 3K blend: negative, neutral, and positive results (Tables 1-3). Five of the six compounds included in Trial 1 when added to traps with the 3K lure had a significant impact on reducing female moth catches: (*Z*)-3-hexenol, (*E*)-2-hexanal, (*Z*)-3-hexenyl acetate, hexyl butanoate and linalool (Table 1). No compounds significantly affected male catches. Three compounds significantly reduced the total catches: (*Z*)-3-hexenol, (*E*)-2-hexanal and hexyl butanoate. All six compounds had significantly lower female and total moth catches compared with the 4K lure. In addition, three

**TABLE 1** Summary of mean (+ *SEM*) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination (3K) of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene and acetic acid, a quaternary blend with pyranoid linalool oxide (4K) and quaternary blends with several different volatiles, $N = 8$ lure replicates, 1 trial

| Trial # | Volatile added | Mean ± *SEM* moth catch per trap: Females | Males | Total |
|---|---|---|---|---|
| 1 | 3K | 9.6 ± 2.5 ab | 2.0 ± 0.4 ab | 11.6 ± 2.7 ab |
| | 3K + (*Z*)–3-Hexen-ol | 1.8 ± 0.5 c | 1.0 ± 0.4 b | 2.8 ± 0.8 c |
| | 3K + (*E*)–2-Hexanal | 1.3 ± 0.6 c | 1.1 ± 0.5 b | 2.4 ± 0.8 c |
| | 3K + (*Z*)–3-Hexenyl acetate | 2.8 ± 0.8 c | 1.9 ± 0.4 ab | 4.6 ± 0.9 bc |
| | 3K + Methyl salicylate | 4.0 ± 0.9 bc | 2.3 ± 1.0 ab | 6.3 ± 1.7 bc |
| | 3K + Hexyl butanoate | 1.3 ± 0.5 c | 0.9 ± 0.4 b | 2.1 ± 0.8 c |
| | 3K + Linalool | 2.5 ± 1.1 c | 2.6 ± 1.0 ab | 5.1 ± 1.8 bc |
| | 4K | 14.1 ± 2.6 a | 5.0 ± 1.4 a | 19.1 ± 3.8 a |
| ANOVA, $df = 7, 56$ | | $F = 11.10$, $p < .0001$ | $F = 2.87$, $p = .012$ | $F = 8.21$, $p < .001$ |

*Note:* Column means within each trial followed by the same letter are not significantly different, Tukey's test, $p < 0.05$.

**TABLE 2** Summary of mean (+*SEM*) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination (3K) of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene and acetic acid, a quaternary blend with pyranoid linalool oxide (4K) and other quaternary blends with the addition of a fourth volatile, $N = 8–10$ lure replicates, trials conducted on 1–3 dates

| Trial # | Volatile added | Mean ± *SEM* moth catch per trap: Females | Males | Total |
|---|---|---|---|---|
| 2 | 3K | 4.6 ± 0.6 a | 2.0 ± 0.3 a | 6.6 ± 0.8 a |
| | 3K + (*E*)-β-Ocimene | 4.9 ± 0.8 a | 2.8 ± 0.4 a | 7.7 ± 1.1 a |
| | 4K | 5.9 ± 0.9 a | 3.2 ± 0.5 a | 9.1 ± 1.2 a |
| RCB ANOVA, $df = 2, 85$ | | $F = 0.92$, $p = .401$ | $F = 2.18$, $p = .120$ | $F = 2.03$, $p = .138$ |
| 3 | 3K | 3.7 ± 0.7 a | 1.8 ± 0.3 a | 5.5 ± 0.9 a |
| | 3K + Limonene | 5.3 ± 1.2 a | 2.7 ± 0.5 a | 8.0 ± 1.5 a |
| | 4K | 4.9 ± 0.9 a | 3.1 ± 0.6 a | 8.0 ± 1.4 a |
| RCB ANOVA, $df = 2, 57$ | | $F = 0.50$, $p = .601$ | $F = 1.45$, $p = .244$ | $F = 2.01$, $p = .143$ |
| 4 | 3K | 5.5 ± 0.8 b | 1.9 ± 0.4 a | 7.4 ± 1.0 b |
| | 3K + β-Caryophyllene | 5.2 ± 0.9 b | 3.6 ± 0.8 a | 8.8 ± 1.5 b |
| | 4K | 9.4 ± 1.1 a | 4.1 ± 0.8 a | 13.6 ± 1.5 a |
| RCB ANOVA, $df = 2, 50$ | | $F = 6.77$, $p = .003$ | $F = 2.04$, $p = .141$ | $F = 5.62$, $p = .006$ |
| 5 | 3K | 4.1 ± 1.2 b | 1.4 ± 0.3 a | 5.5 ± 1.2 b |
| | 3K + Butyl hexanoate | 6.3 ± 1.5 ab | 4.3 ± 1.1 a | 10.5 ± 2.0 ab |
| | 3K + Farnesol | 6.4 ± 1.6 ab | 3.8 ± 1.1 a | 10.1 ± 2.5 ab |
| | 4K | 10.9 ± 1.9 a | 3.5 ± 1.2 a | 14.4 ± 2.4 a |
| ANOVA, $df = 3, 28$ | | $F = 3.05$, $p = .045$ | $F = 1.53$, $p = .230$ | $F = 3.41$, $p = .031$ |
| 6 | 3K | 6.6 ± 0.9 a | 2.5 ± 0.8 ab | 9.1 ± 1.4 a |
| | 3K + Terpineol | 5.4 ± 1.2 a | 1.8 ± 0.5 ab | 7.1 ± 1.1 a |
| | 3K + Terpinen−4-ol | 5.9 ± 2.4 a | 1.1 ± 0.4 b | 7.0 ± 2.4 a |
| | 3K + α-Pinene | 8.4 ± 1.3 a | 2.6 ± 0.5 ab | 11.0 ± 1.5 a |
| | 4K | 8.9 ± 1.8 a | 3.5 ± 0.8 a | 12.4 ± 2.3 a |
| ANOVA, $df = 4, 35$ | | $F = 1.43$, $p = .245$ | $F = 2.71$, $p = .046$ | $F = 2.07$, $p = .106$ |

*Note:* Column means within each trial followed by the same letter are not significantly different, $p < .05$, Tukey's test, $p < .05$. Trials 2–6 were analysed with a randomized or a randomized complete block (RCB) ANOVA with date as the blocking variable. Trials 5 and 6 were conducted once, trials 3 and 4 were conducted twice, and trial 2 was repeated on three dates.

compounds had significantly lower male catches than the 4K lure: (*Z*)-3-hexenol, (*E*)-2-hexanal and hexyl butanoate.

A second group of HPVs had no effect on moth catches when added to traps baited with the 3K lure (Table 2). These included (*E*)-β-ocimene, limonene, β-caryophyllene, butyl hexanoate, farnesol, terpineol, terpinen-4-ol and α-pinene. Only two HPVs from this group had significantly lower moth counts than the 4K lure: β-caryophyllene (females and total) and terpinen-4-ol (males).

A third group of HPVs when added to traps with the 3K lure performed similarly to the 4K lure for male, female and total catch (Table 3). However, these four-component blends had variable significant positive effects compared with the 3K lure. When added to traps baited with the 3K lure, (*Z*)-jasmone, (*E*,*E*)-α-farnesene and β-pinene significantly increased male catches. The same three HPVs plus (*E*)-β-farnesene significantly increased total moth catches when added to traps baited with the 3K lure. Only two HPVs significantly increased female catches over the 3K blend: β-myrcene and (*E*,*E*)-α-farnesene. Two of the four-component blends (with β-myrcene or β-pinene) had ca. 20% and 10% higher numerical catches of females and total numbers of moths than the 4K lure, respectively, but these were not statistically significant.

**TABLE 3** Summary of mean (+ *SEM*) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination (3K) of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene and acetic acid, a quaternary blend with pyranoid linalool oxide (4K) and quaternary blends with a fourth volatile added, *N* = 8–10 lure replicates, trials conducted on 2–4 dates

| Trial # | Volatile added | Mean ± *SEM* moth catch per trap | | |
|---|---|---|---|---|
| | | Females | Males | Total |
| 7 | 3K | 4.8 ± 0.6 b | 1.8 ± 0.3 b | 6.6 ± 0.8 b |
| | 3K + (*Z*)-Jasmone | 7.0 ± 0.8 ab | 4.7 ± 0.9 a | 11.7 ± 1.6 a |
| | 4K | 7.8 ± 0.9 a | 3.5 ± 0.6 ab | 11.2 ± 1.3 a |
| RCB ANOVA, *df* = 2, 73 | | *F* = 4.42, *p* = .015 | *F* = 5.11, *p* = .008 | *F* = 6.58, *p* = .002 |
| 8 | 3K | 5.5 ± 0.6 b | 2.2 ± 0.4 a | 7.7 ± 0.8 b |
| | 3K + β-Myrcene | 8.9 ± 1.0 a | 3.7 ± 0.6 a | 12.6 ± 1.5 a |
| | 4K | 7.2 ± 0.8 ab | 3.5 ± 0.5 a | 10.6 ± 1.2 ab |
| RCB ANOVA, *df* = 2, 73 | | *F* = 4.83, *p* = .011 | *F* = 2.99, *p* = .057 | *F* = 4.83, *p* = .011 |
| 9 | 3K | 3.7 ± 0.5 b | 1.6 ± 0.2 b | 5.3 ± 0.6 b |
| | 3K + (*E*,*E*)-α-Farnesene | 5.5 ± 0.8 a | 3.4 ± 0.6 a | 8.9 ± 1.2 a |
| | 4K | 5.9 ± 0.8 a | 2.9 ± 0.5 ab | 8.9 ± 1.1 a |
| RCB ANOVA, *df* = 2, 111 | | *F* = 3.25, *p* = .042 | *F* = 3.25, *p* = .042 | *F* = 5.24, *p* = .007 |
| 10 | 3K | 3.8 ± 0.6 b | 1.7 ± 0.3 a | 5.5 ± 0.7 b |
| | 3K + (*E*)-β-Farnesene | 6.8 ± 1.1 a | 2.8 ± 0.5 a | 9.6 ± 1.4 a |
| | 4K | 6.5 ± 1.0 a | 3.2 ± 0.6 a | 9.7 ± 1.3 a |
| RCB ANOVA, *df* = 2, 83 | | *F* = 4.38, *p* = .016 | *F* = 2.23, *p* = .114 | *F* = 5.73, *p* = .005 |
| 11 | 3K | 6.6 ± 0.7 a | 2.4 ± 0.5 b | 9.1 ± 0.9 a |
| | 3K + β-Pinene | 8.8 ± 1.3 a | 5.1 ± 0.8 a | 13.9 ± 1.8 a |
| | 4K | 8.6 ± 1.0 a | 4.1 ± 0.7 ab | 12.7 ± 1.5 a |
| RCB ANOVA, *df* = 2, 50 | | *F* = 1.09, *p* = .343 | *F* = 4.34, *p* = .018 | *F* = 2.47, *p* = .095 |

*Note:* Column means within each trial followed by the same letter are not significantly different, $p < .05$, Tukey's test, $p < .05$. Tests 7–11 were analysed with a randomized or a randomized complete block (RCB) ANOVA with date as the blocking variable. Trial 11 was conducted twice, trials 7, 8 and 10 were repeated on three dates, and trial 9 was repeated on four dates.

## 4 | DISCUSSION

Previously, because PE is only found in pear and not apple and walnut (Casado et al., 2006, 2008; Giacomuzzi et al., 2016), and only late in the season from mature fruits (Light et al., 2001), it was hypothesized that other HPVs or blends, which are released across the three primary hosts of codling moth, should also be attractive to codling moth (Witzgall et al., 2008). Antennal electrophysiology has been used to identify potential HPVs attractive to both moth sexes, including several of the ones tested in this study (Bäckman et al., 2001; Bengtsson et al., 2001; Casado et al., 2006, 2008; Chen et al., 2018; Hern & Dorn, 2003; Najar-Rodriguez et al., 2013; Witzgall et al., 2005). Subsequently, some of these HPVs were shown to have attraction to codling moth adults in laboratory behavioural assays as single compounds or in blends, including (*E*,*E*)-α-farnesene and (*E*)-β-farnesene (Ansebo et al., 2004; Coracini et al., 2004), butyl hexanoate (Hern & Dorn, 2004), *β*-caryophyllene, limonene (Vallat & Dorn, 2005), farnesol and (*Z*)-jasmone (Coracini et al., 2004). However, perhaps due to the constraints imposed on characterizing moth behaviour using moths reared on artificial diet in olfactometers/flight tunnels devoid of background host volatiles, these studies failed to identify any HPV or blend that was more effective than PE for codling moth when tested in field trials with wild moths (Coracini et al., 2004; El-Sayed et al., 2013; Knight et al., 2011; Witzgall et al., 2005).

Significant progress in the development of effective non-pheromone lures for codling moth began when the microbial volatile AA was combined with PE (Landolt et al., 2007). Additional studies have suggested that AA is important in several HPV-based blends for other tortricids (Jósvai et al., 2016b; Knight et al., 2014, 2017; Larsson Herrera et al., 2020; Mujica et al., 2018; Tasin et al., 2018) and for species in other Lepidoptera families (Jósvai et al., 2016a; Landolt, 2005; Landolt et al., 2013). Further improvements in lure development for codling moth included the addition of DMNT (Knight & Light, 2012; Knight et al., 2019b), but DMNT is relatively short-lived compared with PE and is costly to synthesize (Knight et al., 2014).

Earlier studies included linalool as a putative attractant for codling moth because of its abundance in the headspace of host plants (Ansebo et al., 2004; Bäckman et al., 2001; Bengtsson et al., 2001; Schmera & Guerin, 2012; Yang et al., 2004). However, the inclusion of pyrLOX in the four-component (4K) blend was serendipitous as it had not been reported previously in analyses of codling moth host plants' volatile profiles and was only suggested from ongoing research on *Lobesia botrana* L., in grape, *Vitis vinifera* L. (Tasin et al., 2007). Recently, pyrLOX in combinations with AA has also been shown to be an effective lure for both males and females of the cherry bark tortrix, *Enarmonia formosana* (Scopoli), a worldwide pest of Rosaceous tree species (Jaastad et al., 2020).

Linalool oxides can be synthesized by plants as well as through microbial-mediated hydrolysis of linalool (Demyttenaere & Willemen, 1998; Pichersky et al., 1994; Raguso & Pichersky, 1999). While recent studies have thoroughly investigated yeast volatomes and behavioural response in *Spodoptera littoralis* (Boisduval) (Ljunggren et al., 2019), earlier work also hinted at the importance of yeast volatiles in combination with host volatiles for codling moth (Witzgall et al., 2012). We hypothesize that further concerted efforts to map host volatomes associated with a wide array of microorganisms in codling moths could unravel other novel compounds that can increase attraction of multi-component blends.

Over the past 20 years, a core goal of our research developing new, more attractive blends for codling moth has been to increase catches of females (Light et al., 2001). Female-based monitoring has been shown to provide a more direct prediction of key life-history events, *that is* egg hatch, and to establish action thresholds used to trigger supplemental insecticide sprays (Knight & Light, 2005a,b). The continuous improvement of lures for female codling moth such as the 4K lure, as well as several of the combinations presented in this study, provides an opportunity to develop more effective 'lure and kill' strategies to manage populations (Jaffe et al., 2018; Knight & Mujica, 2019) and/or improve monitoring of both sexes' population densities.

None of the 19 experimental four-component blends evaluated in our trials were significantly more attractive than the original 4K lure. However, several HPVs were found to significantly increase moth catches when combined with the 3K blend of PE, DMNT and AA. Among this group, the addition of only three HPVs enhanced female codling moth catches compared to the 3K blend, specifically (*E*,*E*)-α-farnesene, (*E*)-β-farnesene and β-myrcene. The sesquiterpene (*E*,*E*)-α-farnesene was the first HPV identified as an attractant for adult codling moth (Ansebo et al., 2004; Bengtsson et al., 2001; Hern & Dorn, 1999; Wearing & Hutchins, 1973; Witzgall et al., 2008; Yang et al., 2004). However, efforts to develop long-lasting lures using (*E*,*E*)-α-farnesene have not been successful, mainly due to its instability in the presence of $O_2$ and its short residual activity (Anet, 1969; Cavill & Coggiola, 1971). Although (*E*)-β-farnesene is known to elicit an antennal response in both codling moth sexes (Bengtsson et al., 2001), it was only attractive for males in previous field trials (Ansebo et al., 2004; Coracini et al., 2004), and both field and laboratory studies showed that a blend of (*E*,*E*)-α-farnesene and (*E*)-β-farnesene enhanced attractiveness to males (Ansebo et al., 2004; Coracini et al., 2004). In comparison, the inclusion of (*E*)-β-farnesene in the four-component blend was found to significantly increase female catches. Our use of the proprietary PVC matrix perhaps offers new opportunities to utilize these rather unstable volatiles.

The monoterpene β-myrcene is present in flowers and foliage of apple and pear as well as in walnut foliage (Baraldi et al., 1999; Casado et al., 2006, 2008), but had only previously been tested with codling moth as part of a six-component walnut-based monoterpene blend that captured few (0.1 male and female) moths per day in apple trials (Light & Knight, 2005). In our study, the addition of β-myrcene significantly improved the performance of the 3K lure and the blend caught 70.6% females and ca. 23.6% more female moths than the 4K lure. Further studies should prioritize further testing of β-myrcene in a variety of multi-component blends.

Development of new lures to enhance total moth catches, particularly in MD-treated orchards, using HPVs in combination with sex pheromone should also be considered. Several earlier studies have tested blends of sex pheromone ((*E*,*E*)-8,10-dodecadien-1-ol, codlemone) with some of the HPVs tested here (Knight et al., 2019c; Light et al., 1993). For example, in flight tunnel assays, linalool, (*E*)-β-farnesene and (*Z*)-3-hexen-1-ol significantly increased the proportion of males flying towards and reaching the odour source (Yang et al., 2004). Similarly, limonene, linalool and (*E*)-β-farnesene when combined with codlemone increased the number of males flying towards the odour source and shortened the response time of males (Schmera & Guerin, 2012). However, combinational effects of codlemone and HPVs can vary depending on whether they are loaded together or into separate lure matrices (Casado, 2007; Knight et al., 2019c; Preti et al., 2021). For instance, loading high amounts of PE with codlemone together in one septum lure reduced male codling moth's response to sex pheromone, but not when each compound was presented in a separate septum lure (Casado, 2007). Similarly, adding a separate septum with codlemone to traps baited with the 4K blend composed of four separate lures increased male and did not affect female moth catches (Knight et al., 2019b), while adding codlemone into the same PVC lure together with PE, DMNT and pyrLOX significantly decreased female catch (Preti et al., 2021).

Five of the HPVs tested significantly reduced catches of female codling moth when combined with the 3K blend (Table 1). The use of HPVs that repel the target insect pest has been considered as a component of a 'push and pull' management approach where pests' behaviours are manipulated to force their movement away from susceptible hosts to either non-crop hosts or to localized sprayed areas (Cook et al., 2007; Pickett & Khan, 2016). Perhaps contradictory, GLVs, such as (*Z*)-3-hexenol and (Z)-3-hexenyl acetate, have been added to the sex pheromone of codling moth and oriental fruit moth, *Grapholita molesta* (Busck) to enhance male response to lures (Light et al., 1993; Yu et al., 2015). However, the effect of GLVs on tortricid moth behaviour is dose-dependent (Varela et al., 2011; Von Arx et al., 2012; Wei & Kang, 2011). GLVs are usually present in apple orchards at high and variable ambient levels due to foliar micro-abrasions and microbial and herbivore damage (Giacomuzzi et al., 2016, 2017). Thus, it is unclear whether a consistent behavioural response in codling moth could be achieved under these orchard conditions with these types of compounds released from synthetic lures.

In contrast, the effect found with the ester hexyl butanoate, a minor component of apple aroma (Mehinagic Royer et al., 2006; Vrhovsek et al., 2014), may prove to be a more amenable choice to disrupt moth behaviours. Although a blend of butyl hexanoate and hexyl butanoate was the second most attractive lure tested for codling moth in apple only behind the use of $C_{10}$ decadienoate (Light & Knight, 2005), a six-component ester blend including hexyl butanoate was not attractive (Hern & Dorn, 2004). Moreover, codling moth larvae-infested apples are more attractive than uninfested apples for adults and released greater amounts of characteristic volatiles including esters, but hexyl butanoate was not reported in these studies (Hern & Dorn, 2001, 2002; Landolt & Guedot, 2008). Thus, the background level of hexyl butanoate may be quite low in apple orchards. In contrast, apple leaves infested by rosy apple aphid (RAA), *Dysaphis plantaginea* (Passerini), release 6- to 8-fold more butyrate esters and 3.6- and 1.6-fold more (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate, respectively, than uninfested leaves (Van Tol et al., 2009). Studies conducted in a heavily RAA-infested apple block found a much lower occurrence of mated female codling moths compared to an uninfested block (25% vs. 74%) and a lower fruit injury caused by codling moth (<0.1% vs. 3.2%), despite similar codling moth adult densities and management programs (A.L.K., unpublished data). This might indicate that one or more of these compounds in our codling moth study that lowered the attraction of the 3K lure might partly explain the reduced levels of mating and fruit injury that occurred in this study. Potential development of a 'push and pull' management strategy for codling moth using one or more of these volatiles should be explored.

One concern to address in future studies is that the emission rates of the HPVs from the PVC lures are unknown, and different results could be obtained with either repellant or attractive blends if the emission rates were modified in either direction (Cha et al., 2011). For instance, Hern and Dorn (1999) reported that female codling moth attraction towards α-farnesene was dose-dependent with high dosages being repellent for female codling moth. They also showed that dosage of butyl hexanoate was the determinant in the response of virgin and mated codling moth females in the olfactometer. Loading rates of PE in septa were clearly shown to be a key determinant of moth catches and sex ratio of codling moth (Knight & Light, 2005c). Also, the residual longevity of the 4K lure appears to be determined by the drop in the rate of DMNT released over time (Preti et al., 2021). Finally, it is clear from the literature published with PE that the effectiveness of any new blends will need to be tested in different geographical areas (Preti et al., 2021) and within each host crop (Light et al., 2001).

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## CONFLICT OF INTEREST

None of the authors have any conflict of interest.

## AUTHOR CONTRIBUTION

Author 2 conceived the research, and authors 1, 2, 3 and 4 conducted the field trials. Author 2 conducted the statistical analyses. Authors 1, 2, 5, 6 and 7 wrote the manuscript. Author 2 secured funding. All authors read and approved the manuscript.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are openly available in a public repository at: https://doi.org/10.5281/zenodo.4398939.

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