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ORIGINAL CONTRIBUTION

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Improved monitoring of oriental fruit moth (Lepidoptera: Tortricidae) with terpinyl acetate plus acetic acid membrane lures

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Abstract

Male and female moth catches of *Grapholita molesta* (Busck) in traps were evaluated in stone and pome fruit orchards untreated or treated with sex pheromones for mating disruption in Uruguay, Argentina, Chile, USA, and Italy from 2015 to 2017. Trials evaluated various blends loaded into either membrane cup lures or septa. Membrane lures were loaded with terpinyl acetate (TA), acetic acid (AA) and (*Z*)-3-hexenyl acetate alone or in combinations. Two septa lures were loaded with either the three-component sex pheromone blend for *G. molesta* alone or in combination with codlemone (2-PH), the sex pheromone of *Cydia pomonella* (L). A third septum lure included the combination sex pheromone blend plus pear ester, (*E*,*Z*)-2,4-ethyl decadienoate (2-PH/PE), and a fourth septum was loaded with only β-ocimene. Results were consistent across geographical areas showing that the addition of β-ocimene or (*Z*)-3-hexenyl acetate did not increase moth catches. The addition of pear ester to the sex pheromone lure marginally increased moth catches. The use of TA and AA together significantly increased moth catches compared with the use of only one of the two components. Traps with the TA/AA lure outperformed the Ajar trap baited with a liquid TA plus sugar bait. The emission rate of AA was not a significant factor affecting the performance of the TA/AA lure. The addition of TA/AA significantly increased moth catches when combined with the 2-PH lure. The TA/AA lure also allowed traps to catch both sexes. Catch of *C. pomonella* with the 2-PH lure was comparable to the use of codlemone; however, moth catch was significantly reduced with the 2-PH/PE lure. Optimization of these complex lures can likely further improve managers' ability to monitor *G. molesta* and help to develop multispecies tortricid lures for use in individual traps.

KEYWORDS

apple, *Cydia pomonella*, *Grapholita molesta*, mating disruption, peach

1 | INTRODUCTION

The use of sex pheromones for the management of oriental fruit moth, *Grapholita molesta* (Busck), has been broadly adopted throughout its worldwide distribution in both stone (*Prunus* spp.) and pome (*Malus domestica* Borkhausen, and *Pyrus* spp.) fruits and now includes the use of hand-applied dispensers, aerosols, sprayables and attract and kill formulations (De Lame & Gut, 2006; Evenden & McLaughlin, 2004; Il'ichev, Stelinski, Williams, & Gut, 2006; Knight, Light, Pickel, Kovanci, & Molinari, 2008; Kovanci, Schal, Walgenbach, & Kennedy, 2005; Steliniski, Gut, Haas, McGhee, & Epstein, 2007; Steliniski et al., 2005; Trimble, Pree, Barszez, & Carter, 2004; Vickers, Rothschild, & Jones, 1985). A key component in the management of this important pest, as with other tortricid tree fruit pests, is effective monitoring to establish both action thresholds and to time supplementary insecticide treatments under either conventional or certified organic practices (Charmillot & Vickers, 1991; Knight & Light, 2005a,b). Unfortunately, the use of sex pheromone lures to monitor the seasonal population density of male *G. molesta* is problematic in sex pheromone-treated orchards, but the absence of moth catch can be a good indicator of effective disruption of sexual communication (Rice & Kirsch, 1990; Vickers, 1990). Many growers, instead, rely on bucket traps with liquid baits (terpinyl acetate plus brown sugar, TAS) to monitor populations (Il'ichev, Williams, & Gut, 2007; Kovanci & Walgenbach, 2005; Rice & Kirsch, 1990; Rothschild, Vickers, & Morton, 1984). TAS-baited traps catch both sexes and can reflect the proportion of mated females within a population (Vickers, 1990), but are cumbersome to use and maintain due to evaporation, spillage and required sorting of various nontarget species (Kovanci & Walgenbach, 2005). Development of dry terpinyl acetate lures that can be used in standard traps with sticky liners has been reported, but these traps were not as effective as bucket traps using the liquid TAS bait (Knight et al., 2011).

Development of the novel Ajar traps where the liquid bait was placed in a screen container inside the trap and moths were retained on standard sticky liners surrounding the screen offered an improved monitoring technique, and these traps achieved comparable performance to TAS-baited bucket traps (Cichon et al., 2013; Knight, Cichon, et al., 2014; Padilha, Arioli, Boff, Rosa, & Botton, 2017). However, the nonselectivity (high catches of dipterans, noctuids, and vespids) of Ajar traps was often found to require frequent liner changes. Unfortunately, the addition of screens to Ajar traps, while significantly reducing the nontarget catch, also reduced moth catches in some studies (Knight, Cichon, et al., 2014). While Ajar traps eliminated the sorting of nontargets in the liquid bait, the required periodic handling of the bait still made these traps relatively cumbersome to use, and they have not been commercialized.

Alternatively, various studies have focused on identifying plant volatiles as potential attractants for adult *G. molesta*. Individual and blends of various peach, pear and apple volatiles have been evaluated in laboratory assays, with some blends being identified as attractive (Molinari et al., 2010; Najar-Rodriguez, Orschel, & Dorn, 2013; Natale, Mattiacci, Hern, Pasqualini, & Dorn, 2003, 2004; Natale, Mattiacci, Pasqualini, & Dorn, 2004; Piñero & Dorn, 2007; Varela, Avilla, Anton, & Gemeno, 2011). Field studies have also reported that several volatile blends were attractive, including in Australia (Il'ichev, Kugimya, Williams, & Takabayashi, 2009) and in China (Lu, Huang, & Wang, 2012; Lu, Wang, Wang, Luo, & Qiao, 2015; Lu et al., 2014). However, these blends were not shown to be attractive in subsequent field trials conducted in Chile (Barros-Parada, Ammagarahalli, Basoalto, Fuentes-Contreras, & Gemeno, 2018). Studies in the United States found that the addition of either (*E*)-β-farnesene, (*E*)-β-ocimene or butyl hexanoate septa lures in the Ajar trap with the TAS bait increased total moth catches, and (*E*)-β-ocimene also increased female moth catches compared with the TAS bait alone (Knight et al., 2013). The addition of the green leaf volatile (*Z*)-3-hexen-1-yl acetate to (*E*)-β-ocimene in combination with either (*E*)-β-farnesene or butyl hexanoate did not increase moth catches. Previously, the addition of (*Z*)-3-hexen-1-yl acetate was shown to increase the number of *G. molesta* caught in a clear trap with a dry terpinyl acetate lure (Knight et al., 2011). Acetic acid is typically present in sugary baits (Utrio & Erikson, 1977) and has been directly used with ethanol in liquid bait traps in China (He, Qin, & Zhu, 2009). However, the use of acetic acid colures with plant volatiles has not previously been effective with *G. molesta* (Knight, Hilton, Basoalto, & Steliniski, 2014). Studies with traps baited with a combination of terpinyl acetate and acetic acid have not been previously reported.

Septa lures formulated with the three-component sex pheromone of *G. molesta* plus the major sex pheromone component of *Cydia pomonella* (L.), codlemone, (*E*,*E*)-8,10-dodecadien-1-ol, significantly increased moth catches in nondisrupted orchards (Allred, Croft, & Riedl, 1995). However, these lures were only intermediate when compared with a number of commercial sex pheromone lures in orchards treated with sex pheromone (Knight, Basoalto, & Stelinski, 2015). Also, the combination sex pheromone lure was significantly less attractive to *C. pomonella* than standard sex pheromone lures for this species, and this may limit its use in pome orchards managing both *C. pomonella* and *G. molesta* (Evenden & McLaughlin, 2005; Il'ichev et al., 2007; Steliniski, Il'ichev, & Gut, 2009; Steliniski et al., 2007). The addition of acetic acid, (*E*)-β-ocimene, or pear ester with this combination sex pheromone only marginally increased moth catches, including female moths (Knight, Cichon, et al., 2014).

Despite this large and recent body of research to develop new attractants and trap designs for monitoring *G. molesta*, further refinements are likely still needed. Furthermore, the potential effectiveness of various attractants should be tested over a wide geographical area to consider intraspecific variation in sensory communication channels, as was recently performed with male *G. molesta* to its sex pheromone blend (Knight, Barros-Parada, et al., 2015). Geographical differences in host range expansions could also provide selective pressure for moths' response to various chemical signals. For example, initial studies with pear ester in various stone fruits in California found that it was not attractive to *G. molesta* (Knight & Light, 2004). Yet, studies in Italy reported that antennae in both sexes are responsive to pear ester and, in an olfactometer assay, primarily males were more attracted to pear ester than to apple or pear shoot volatiles, but not in comparison with peach shoot volatiles (Molinari et al., 2010).

Herein, we report studies from Uruguay, Chile, Argentina, Italy and in the eastern and western United States evaluating several long-lasting membrane lures used alone and in combination with septa lures for *G. molesta* in apple and stone fruit orchards untreated or treated with sex pheromones for mating disruption. Various kairomonal combinations were tested and included terpinyl acetate, acetic acid, pear ester, β-ocimene and green leaf volatiles. Kairomones were tested alone and in combination with the sex pheromone of *G. molesta* and *C. pomonella*. Bisexual lures were developed which can significantly improve monitoring of *G. molesta* in sex pheromone-treated orchards. Further studies fine-tuning lure blends for bisexual catch of multiple tortricid species is warranted.

2 | MATERIALS AND METHODS

2.1 | Traps, lures and field study protocol

Studies were conducted with delta-shaped traps and several commercial and experimental lures during 2015–2017. Traps included the standard orange or white Pherocon VI trap (28 × 20 × 20 cm) used with paper liners coated with a polybutane adhesive (Trécé Inc., Adair, OK). The commercial sex pheromone lures Pherocon OFM-L2 (93:6:1 blend of (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenyl acetate and (*Z*)-8-dodecenol) and Pherocon CM L2 loaded with codlemone were provide by Trécé Inc. A vial lure loaded with 5.0 ml of glacial acetic acid (99.7%, Sigma-Aldrich, St Louis, MO) was made by drilling a 3.2-mm hole into the cap of 8-ml nalgene vials (Nalge Nunc International, Rochester, NY) and loading each vial with two small cotton balls.

Experimental lures including grey halobutyl septa, red rubber septa and white plastic membrane lures loaded with sex pheromones or host plant volatiles alone or combined were prepared by Trécé Inc. chemists and shipped to the various researchers. The three components of *G. molesta* pheromone (*E*)-8-dodecenyl acetate (98.6% purity), (*Z*)-8-dodecenyl acetate (98.1% purity) and (*Z*)-8-dodecenol (98.3% purity) and the sex pheromone of *C. pomonella*, codlemone, (*E,E*)-8,10-dodecadien-1-ol (97% purity), were purchased from Bedoukian Research, Danbury, CT. Grey halobutyl septa lures were prepared with a 93:6:1 ratio of this three-component pheromone blend alone (OFM-PH) or with codlemone (2-PH). A third grey septum lure was loaded with pear ester, (*E,Z*)-2,4-ethyl decadienoate (>92% purity); and a fourth septum lure combined the combination sex pheromone with pear ester (2-PH/PE). A red rubber septum (BO) was loaded with a racemic mixture of β-ocimene (>90% (*E*)-isomer, Sigma-Aldrich). Several proprietary plastic membrane cup lures (1.8 cm diameter) were also prepared by Trécé Inc. These included TRE1114 loaded with terpinyl acetate (TA); TRE1237 loaded with (*Z*)-3-hexenyl acetate (Z3); TRE3321 loaded with acetic acid (AA); TRE1370 loaded with a 1:1 ratio of terpinyl acetate and acetic acid (TA/AA); and TRE1367 loaded with a 1:1:1 ratio of terpinyl acetate, acetic acid and (*Z*)-3-hexenyl acetate (TA/AA/Z3). Two additional acetic acid lures with different membranes used to achieve higher emission rates than TRE3321 were also evaluated: TRE1468 (AA2) and the larger (36 cm^2) red TRE1531 (AA3) lure. The weight loss of all membrane lures loaded with terpinyl acetate or acetic acid ($N = 6$) was measured in the laboratory at 25°C. Lures previously kept at −15°C were removed from their packaging, aired for 24 h and weighed. Lures were then spaced 15 cm apart on paper-lined trays and reweighed weekly for 4 weeks. Data were expressed as mg loss per day.

A general protocol for all field studies was adopted across the various geographical regions and during different years. Treatments were randomized in each orchard with five replicates. Traps were placed in the mid-canopy, ≥30 m apart and >20 m from the borders of orchards. Moths were removed, sexed and counted weekly or biweekly. Count data were collected for both *G. molesta* and *C. pomonella*. Traps were rotated among positions on each date they were checked. Studies lasted from 1 week to 3 months. Liners were replaced as needed and at least every 4 weeks in the longer studies. Captures of nontargets on liners were recorded in a few studies. Lure replacement schedules varied: no replacement in shorter experiments, beta ocimene septa replaced every 2 weeks and other lures replaced at least every 4 weeks. Lures were placed at the centre of the liner. A few exceptions to this general protocol occurred in different trials and will be noted.

2.2 | Oregon 2015

This study was repeated over two periods in a mixed-cultivar peach orchard situated near Medford, OR (42°14′47.37″N, 119°52′21.88″W).

Trees were >30 years old and planted at a density of 600/ha. The orchard was not treated with sex pheromone during 2015. Moth catches in 10 lure treatments were compared in this study, including the combination sex pheromone lure (2-PH) alone and in binary and ternary lure combinations. The binary lure treatments included 2-PH plus one of the following lures: the PE or BO septa, or the Z3, AA or TA membrane lures. Ternary lure combinations included the two septa lures 2-PH and BO, and the addition of either the PE septum or one of the three membrane lures: Z3, AA or TA. The study was conducted twice from 25 July to 8 August and repeated from 20 August to 3 September.

2.3 | Uruguay 2015–2016

A study was conducted in peach (34°44′8″S, 56°17′11″W) situated near Moizo, Uruguay. The orchard was planted primarily with the cultivar Pavia Canario, trees <20 years old and planted at 1,000/ha. The orchard was treated with Isomate OFM dispensers (Pacific Biocontrol) at 250/ha and was adjacent to commercial apple orchards. This study compared five lures, including OFM-PH, 2-PH, 2-PH + BO, 2-PH + TA + AA and 2-PH + TA + AA + BO. The study was conducted from 15 December and terminated on 18 January.

2.4 | Oregon 2016

The same Medford orchard used in 2015 was treated with Cidetrak OFM-L dispensers (Trécé Inc.) applied at 250/ha in late May. Dispensers were loaded with 250 mg a.i. of the three-component blend. This study was repeated on three sets of dates, including 12–26 June, 14–29 July and 29 July to 8 August. Six lure treatments were compared in these studies, 2-PH alone and in binary and ternary lure combinations. The binary lure treatments included 2-PH plus the BO septum, 2-PH plus a membrane lure loaded with either AA or TA, or the two membrane lures TA and AA. Two ternary lure combinations included the septa with 2-PH and BO plus a membrane lure with either TA or TA/AA.

2.5 | South America 2015–2016

Studies were conducted in Argentina, Chile and Uruguay comparing moth catch in traps with eight different lure treatments. Treatments included the individual septa lures OFM-PH and 2-PH, and the membrane lures AA, and TA; binary lures including 2-PH plus either AA or TA and AA plus TA; and a ternary lure combining 2-PH, AA and TA. Two sets of studies were conducted in Argentina, including in a mixed-cultivar (Flamekist and Arctic Snow and O-Henry) nectarine/peach block (39°5′14.64″S, 67°38′27.31″W) and in a mixed-cultivar (Williams, Red Bartlett, and Abate Fetel) pear block (39°6′2.98″S; 67°35′32.80″W). Both orchards were treated with Isomate M100 (Pacific Biocontrol, Vancouver, WA) at 400 dispensers per ha applied in mid-October. These dispensers were loaded with 232 mg a.i. of a 93:6:1 blend of (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenyl acetate and (*Z*)-8-dodecenol. The pear block was also treated with Isomate C Plus (Pacific Biocontrol) at 1,000 dispensers per ha. These dispensers were loaded with 182 mg a.i. of a 60:34:6 blend of codlemone, 1-dodecanol and 1-tetradecanol. Both studies were initiated on 7 February and terminated on 23 March. Traps were checked and rotated every 3–7 days during the trial. In addition to the eight lure treatments established in the other two countries, traps baited with the Pherocon CM L2 grey septa (Trécé Inc.) to monitor *C. pomonella* were included in the pear study. Lures were pinned to the inside roof of the traps.

The same study conducted in Argentina was repeated in a peach orchard (cv. Carson) in Chile (35°33′29″S, 71°33′44″W). The peach orchard was treated with Cidetrak OFM-L (Trécé Inc.) at 450 dispensers per ha on 15 October 2015. These dispensers were loaded with 250 mg a.i. of a 93:6:1 blend of (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenyl acetate and (*Z*)-8-dodecenol. Mean tree height was 4.5 m, and trees were planted at a density of 1,111/ha. Traps were placed in the field on 20 February 2016 and checked weekly until 29 March 2016. The mating status of females was determined following dissection of the *bursa copulatrix*.

The study in Chile and Argentina was repeated in the same peach orchard treated with sex pheromone dispensers near Moizo previously described. Like the pear orchard in Argentina, an additional lure treatment was included, the Pherocon CM L2 lure (Trécé Inc.) for *C. pomonella*. This study was initiated on 1 February and terminated on 7 March 2016.

2.6 | Italy 2016

Three sets of trials were performed in peach orchards in Emilia-Romagna region of northern Italy (44°17′32.06″N–12°5′43.26″E to 44°28′45.56″N–12°5′5.40″E) with recent histories of fruit injury from *G. molesta* under sex pheromone mating disruption. The first trial included five treatments: the single lures OFM-PH, 2-PH, and 2-PH/PE; and the binary lures 2-PH + BO and 2-PH/PE + BO. The second trial included six treatments: the single lures OFM-PH, and 2-PH, the binary lures 2-PH + TA/AA and 2-PH/PE + TA/AA and the ternary lures 2-PH + TA/AA + BO and 2-PH/PE + TA/AA + BO. The third trial included six treatments: the single lures OFM-PH and 2-PH, the binary lures 2-PH + TA/AA/Z3 and 2-PH/PE + TA/AA/Z3 and the ternary lures 2-PH + TA/AA/Z3 + BO and 2-PH/PE + TA/AA/Z3 + BO. The three trials were conducted concurrently from 6 July to 1 August and then repeated in different orchards from 1 to 29 August.

Peach orchards in Italy were <10 years old and planted at 1,100–1,700 trees per ha. All orchards were treated with standard agronomic practices including the applications of insecticides for management of *G. molesta*. Sex pheromones were placed in each orchard (5–10 ha) for mating disruption of *G. molesta* beginning in late March. These included both the hand application of dispensers (Isomate-M Rosso, Pacific Biocontrol) loaded with 250.2 g a.i. per dispenser and applied at 600 dispensers per ha, the use of aerosol devices (CheckMate Puffer OFM-O, Suterra, Bend, OR) loaded with 48 g a.i. per unit with sprays applied at 4.8 mg a.i. every 15 min for 12 hours/day at 3 units/ha, and spray applications (CheckMate OFM F, Suterra) of 22.2 g a.i./ha every 3–4 weeks at 45–50 ml in 1,000 L water per ha.

2.7 | North Carolina 2016

The same three trials conducted in Italy were repeated in a mixed variety block of apple trees at the Mountain Horticultural Crops Research Station (Mills River, NC). Tree spacing varied from 2.4 to 4.2 m within rows, rows were planted 6 m apart, and tree height ranged from approximately 2.0 to 3.5 m. Each treatment was replicated six times. A replicate of lure treatments was placed in a row of trees at eye level (about 1.7 m). The orchard was treated uniformly with a standard fungicide and insecticide programme applied at approximately two-wk intervals during the season. The three experiments were run consecutively from 1 to 19 July, 29 July to 26 August and 26 August to 3 October, respectively. Traps were checked weekly during the experiments, and moths were not sexed.

2.8 | Uruguay 2016–2017

The same three trials conducted in Italy and North Carolina were also repeated in Uruguay. Trial 1 was conducted from 25 January to 10 February in a "Fuji" apple block (34°39′14.22″S, 56°39′14.22″W). The orchard was treated with OFM/CM puffers (Suterra) at 2.5 units/ha. These units released 6.6 mg a.i. of a 3:4 ratio of codlemone to the three-component *G. molesta* pheromone per puff every 15 min for 12 hours/day. The second trial was conducted from 26 January to 16 February in peaches (34°44′2.36″S, 56°17′5.80″W) treated with Checkmate OFM (Suterra) at 250 dispensers per ha. The third study was conducted from 26 January to 16 February in peaches (34°42′49.85″S, 56°05′19.85″W) treated with the paste formulation, SPLAT OFM (ISCA Technologies, Riverside, CA) at 3.7 kg/ha.

2.9 | Pennsylvania 2016

A longer-term study was conducted from 15 July to 11 October in apple blocks at the Penn State University Fruit Research Extension Center near Biglerville, PA (39°55′49″N–77°14′49″W), to evaluate lures that included the addition of pear ester and/or (*Z*)-3-hexenyl acetate. Studies were conducted in orchards both untreated (*N* = 5) and treated (*N* = 5) with sex pheromones. Six lure treatments were compared: the individual lures 2-PH and 2-PH/PE; and the binary lures 2-PH + TA/AA, 2-PH/PE + TA/AA, 2-PH + TA/AA/Z3 and 2-PH/PE + TA/AA/Z3. All apple blocks were planted as mixed cultivars (Delicious, Golden Delicious, Gala, Fuji and Yorking) and were unsprayed for *G. molesta* during the season. Four blocks were >15 years old and planted at 400–550 trees per ha. The other six blocks were <10 years old and planted at tree densities from 1,500 to 2,200/ha. One block was treated with Isomate CM/OFM TT dispensers (Pacific Biocontrol) at 500/ha. These dispensers were loaded with 268 mg of codlemone and 105 mg of the three-component sex pheromone of *G. molesta*. Four blocks were treated with CheckMate Puffer CM/OFM (Suterra) at 5 units/ha. Lures were placed on the liner, and liners were replaced when cumulative catches exceeded *ca.* 50 moths. All lures were replaced once after 4 weeks on 12 August. Moths were not sexed.

2.10 | California and Washington 2017

Moth catch was compared in Ajar traps (described in Cichon et al., 2013) and delta traps baited with the TA/AA membrane lure. A single peach orchard situated near Clarksburg, CA (38°25′14″N, 121°31′38″W), was treated with Isomate CM/OFM TT dispensers at 500/ha on 17 April. Five replicates of each lure/trap combination were randomized in the orchard with traps spaced 30 m apart. Traps were first checked on 24 April and weekly for three additional weeks (15 May). Moths were not sexed in this study.

Two studies were conducted in Washington to evaluate the membrane TA/AA lure. The first study was run from 18 May to 1 June and was similar to the previous study in California comparing moth catches in an Ajar trap baited with TAS and in delta traps baited with the TA/AA membrane lure (*N* = 10); but in a Moxee (46°30′5″N, 120°10′16″W) apple orchard not treated with mating disruption dispensers, and moths were sexed. This orchard is situated near a dairy and the number of muscid flies was recorded in both traps. The second study in this orchard compared moth catches in traps (*N* = 5) with the TA/AA lure against traps with the TA lure alone and in combination with three different acetic acid lures: AA, AA1 or AA2. These studies were established in two blocks ("Fuji" and "Delicious") on 30 June and 3 July and both ended on 10 July.

2.11 | Statistical analyses

Catch data for each of the 5–15 replicates of each treatment were summarized across each test's time period and analyzed with Statistix 9 (Analytical Software Inc., Tallahassee, FL). A square-root transformation was used to normalize count data prior to analysis of variance. The normality of data was inspected with a Shapiro–Wilks test. If the transformed data could not be normalized, they were analyzed with a nonparametric Kruskal–Wallis test (W) followed by an ANOVA of ranks if the test was significant, $p < 0.05$. Normalized data were analyzed with either a completely randomized or complete block ANOVA. A *p*-value of 0.05 was used to establish significance, Tukey's test.

3 | RESULTS

3.1 | Oregon 2015

Significant differences in total *G. molesta* moth catches occurred among the 10 lure treatments compared in this untreated peach orchard (Table 1). The two lure treatments with the highest mean catches were 2-PH + BO and 2-PH + BO + AA. However, traps with neither lure treatment caught significantly more total moths than traps with just the 2-PH lure (1.5-fold increase). The addition of a septum loaded with pear ester to traps baited with 2-PH or 2-PH + BO significantly reduced total catch. The addition of membrane lures

TABLE 1 Comparison of total and female *Grapholita molesta* adults caught in traps baited with the sex pheromone blend for *G. molesta* and *Cydia pomonella* (2-PH) either alone or in binary and ternary lure combinations with β-ocimene (BO) or pear ester (PE) loaded in septa or (*Z*)-3-hexenyl-acetate (Z3), acetic acid (AA) or terpinyl acetate (TA) loaded in membrane cup lures in two studies (*N* = 10) conducted in August and September in an untreated peach orchard, Oregon, USA, 2015

Lures						Mean (*SE*) moth catch[a]	
Septa	Membranes					Total	Females
2-PH	–	–	–	–	–	9.1 (1.5)ab	0.0 (0.0)
2-PH	BO	–	–	–	–	13.9 (3.5)a	0.0 (0.0)
2-PH	–	PE	–	–	–	4.4 (1.2)c	0.0 (0.0)
2-PH	–	–	Z3	–	–	6.4 (1.0)abc	0.0 (0.0)
2-PH	–	–	–	AA	–	9.2 (3.1)abc	0.5 (0.3)
2-PH	–	–	–	–	TA	9.0 (2.4)abc	1.2 (0.3)
2-PH	BO	PE	–	–	–	5.4 (1.8)c	0.0 (0.0)
2-PH	BO	–	Z3	–	–	5.5 (1.5)bc	0.0 (0.0)
2-PH	BO	–	–	AA	–	14.0 (4.0)a	0.6 (0.2)
2-PH	BO	–	–	–	TA	10.2 (4.5)abc	1.8 (0.8)
Stats						$F_{9,89} = 2.36$, $p < 0.05$	$W = 4.90$, $p = 0.18$

Note. Column means followed by a different letter were significantly different, $p < 0.05$; Tukey's test, $p < 0.05$. [a]Treatments with no female moth catch were excluded from the analysis.

with either Z3, TA or AA to traps baited with 2-PH did not increase moth catch. The addition of a membrane lure with Z3 to traps baited with 2-PH + BO reduced moth catch. There was no significant difference in moth catch in traps baited with either 2-PH or 2-PH + BO when either TA or AA was added. Low numbers of female *G. molesta* were caught in this study and only in the four lure treatments that included either TA or AA (Table 1). Female moth catch in traps was not significantly different among these four lure treatments.

3.2 | Uruguay 2015–2016

Significant differences in the total numbers of *G. molesta* were found among the five treatments, $F_{4,20} = 53.96$, $p < 0.0001$ (Figure 1). Adding the TA + AA lures to traps with 2-PH increased total catches 41-fold. Adding TA + AA lures to traps baited with 2-PH + BO increased moth catch nearly 20-fold. Traps with either OFM-PH or 2-PH did not catch any female *G. molesta*, and the two treatments including TA + AA lures caught significantly more female moths than traps with 2-PH + BO lures, $F_{2,12} = 33.16$, $p < 0.0001$. The percentage of female *G. molesta* ranged from 48% to 64% among these three lure treatments. No *C. pomonella* were caught in traps baited with the OFM-PH lure. Mean total moth catches of *C. pomonella* ranged from 3.6 (2-PH + TA + AA + BO) to 14.2 (2-PH) moths, and the proportion of female moth catch was >40% in traps with all four attractive lures. Cumulative catch of nontargets (primarily dipterans) was low in traps across all lure treatments, means = 11–17, including in blank traps, mean = 15.2, and nontarget catch was not significantly different, $F_{5,24} = 0.65$, $p = 0.66$.

3.3 | Oregon 2016

Mean total moth catches varied 10-fold across the six lure treatments compared in this study (Table 2). Moth catch was significantly greater in traps baited with 2-PH + TA/AA lures with or without the addition of the BO septum compared with 2-PH alone and 2-PH + BO. The two lure treatments combining 2-PH with the TA lure and with or without the BO septum were intermediate. No female *G. molesta* were caught in traps baited only with 2-PH, and catches were only incidental when the BO septum was added. The two lure treatments including 2-PH + TA/AA caught significantly more females than the two lure treatments with 2-PH + TA. The addition of BO did not affect female moth catch when used with either 2-PH + TA or 2-PH + TA/AA lures.

3.4 | South America 2015–2016

Significant results occurred in the total catch of *G. molesta* among eight lure treatments tested concurrently in Uruguay, Chile and Argentina (Table 3). The highest moth catch occurred in all three countries with traps baited with 2-PH + TA + AA lures. However, the significant mean separation of lure treatments varied among countries. For example, the AA + TA combination performed as well as 2-PH + TA + AA in Chile and Uruguay, but not in Argentina. Also, 2-PH + TA + AA outperformed 2-PH + TA only in Chile, although mean catch was *ca.* threefold higher in Uruguay. Adding the 2-PH lure with TA + AA to traps compared with TA + AA only significantly increased moth catch in Argentina. Total moth catch was low (defined here as <90% of catch in traps baited with 2-PH + TA + AA) in traps baited with the OFM-PH lure in Uruguay and Chile and low with 2-PH in Chile (Table 3). Across the four studies, total moth catch was 16-fold higher in traps baited with 2-PH + TA + AA than in traps with OFM-PH and nearly fivefold higher than in traps baited with only 2-PH. Incidental catches (≤0.6 moths per trap) occurred with several lures, including AA in all three countries, OFM-PH in Uruguay and Chile, TA in Uruguay and 2-PH and 2-PH + AA in Chile.

Differences in the catch of female *G. molesta* only occurred among lure treatments in Chile (Table 3). Here, traps baited with 2-PH + TA + AA caught significantly greater numbers than traps with TA or 2-PH + TA. Traps with TA + AA caught an intermediate number of females. In general, females were only caught at numbers >1 moth per trap in lure treatments including TA. The percentage of female moths caught with lure treatments including TA was high in both Uruguay (34%–55%) and Chile (64%–71%), and somewhat lower in Argentina (17%–26%). In Chile, the proportion of female moths (*N* = 332) that were mated in traps baited with terpinyl acetate was 37.7%; and in Argentina, mating was 17.4% in pears and 23.6% in peaches.

In Argentina, low numbers of *C. pomonella* were caught (≤1.2 moth per trap) across all treatments in both orchards, and no moths were caught in traps baited with OFM-PH, AA or in the blank. Mean (*SE*) catch of *C. pomonella* in traps baited with codlemone in the pear orchard was 1.2 (0.4). Mean (*SE*) catch of *C. pomonella* was higher in Uruguay, and no moths were caught in traps with OFM-PH, TA, AA or TA + AA. Mean moth counts were similar in traps baited with codlemone (10.0 [2.2]), or 2-PH alone (10.2 [2.7]) or 2-Ph in combination with either AA (13.2 [6.1]) or TA (9.2 [4.0]). However, total catch was significantly lower in traps baited with 2-PH + TA + AA, (7.0 [4.8]), $F_{4,20}$ = 3.54, $p < 0.05$.

The cumulative mean catches of nontargets in traps in Argentina over the 6-week study was 19–39 among lure treatments, and no significant difference was found, $F_{8,80}$ = 0.70, p = 0.69. Nontargets included low numbers of bees, lacewings, noctuids and coccinellids; and 96% of the total catch were dipterans, primarily muscids. Cumulative catch of nontarget dipterans in the peach orchard in Uruguay were low and consistent across all treatments with means ranging from 11 to 20 flies across lure treatments, $F_{9,40}$ = 0.92, p = 0.51. It is interesting that the most abundant nontarget caught in traps were thysanopterans, with cumulative mean catches ranging from 38 to 102 thrips across lure treatments; however, these means were not significantly different, $F_{9,40}$ = 1.06, p = 0.41.

3.5 | Uruguay 2016–2017

No significant differences were found for total moth catch in Trial 1 (Table 4). Female moths were either not caught or only incidentally in the OFM-PH lure treatment. Total moth counts were fairly low in Trial 2, especially in traps baited with OFM-PH and 2-PH. Mean total catch was >eightfold higher in traps with the TA/AA lure added

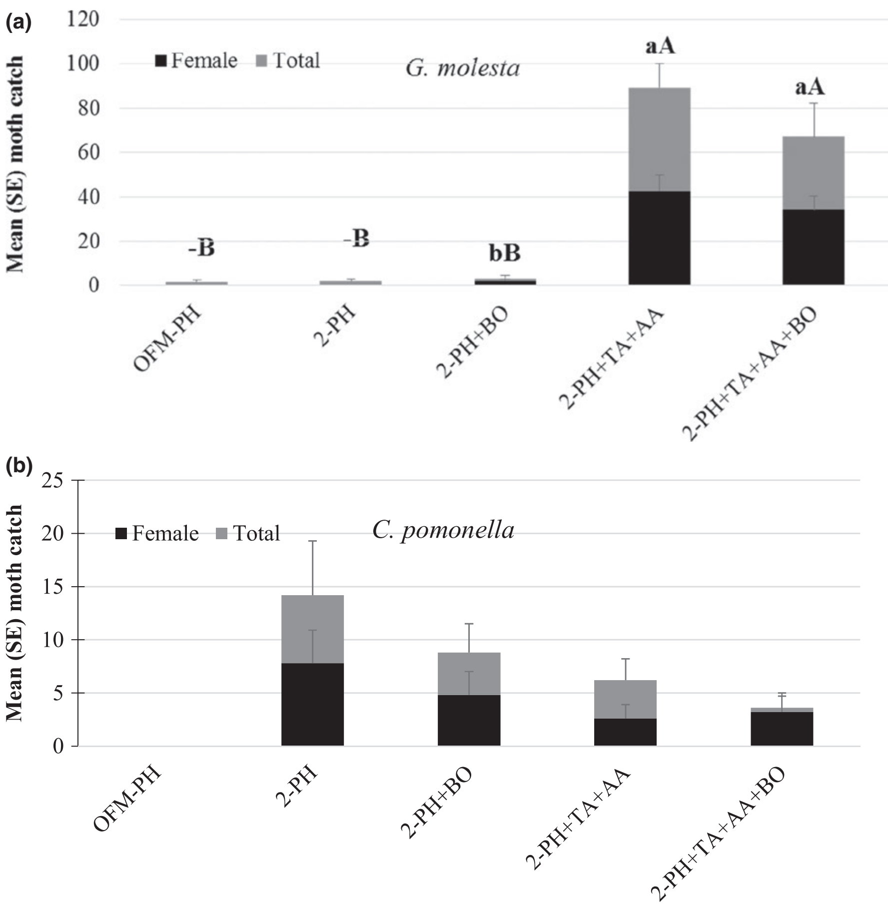

FIGURE 1 Comparison of female and total moth catches of *Grapholita molesta* (a) and *Cydia pomonella* (b) in traps baited with individual and combinations of the sex pheromone (OFM-PH), the combination sex pheromone (2-PH), β-ocimene (BO), terpinyl acetate (TA) and acetic acid (AA) lures, Uruguay 2015–2016. No female *G. molesta* were caught in traps baited with the two pheromone-only lures, and these data were not included in the analysis, denoted "–". Treatment histograms with different lower case and upper case letters were significantly different in female and total moth catches, $p < 0.05$. Catches of *C. pomonella* did not differ among lure treatments, $p > 0.05$

compared with the use of 2-PH only, and no significant difference was found for total or female catch among the four lure treatments including TA/AA. The proportion of females caught in these four lure treatments was high, 0.62–0.86. In comparison, moth catches were very high in all lure treatments in Trial 3 and few females were caught. These data were collected from a peach orchard several months after the SPLAT paste was applied and suggest that the formulation was providing scant disruption of these traps.

TABLE 2 Comparison of total and female *Grapholita molesta* adults caught in traps baited with a septum loaded with a combination sex pheromone blend for *G. molesta* and *Cydia pomonella* (2-PH) either alone or in combination with a β-ocimene (BO) septum or membrane cup lures loaded with terpinyl acetate (TA) or TA plus acetic acid (AA) in three serial studies (*N* = 15) conducted in a peach orchard treated with sex pheromone dispensers, Oregon, USA, 2016

Lures Septa		Membranes	Mean (SE) moth catch Total	Females
2-PH	–	–	0.9 (0.3)c	0.0 (0.0)
2-PH	BO	–	4.1 (1.1)bc	0.2 (0.1)
2-PH	–	TA	4.5 (1.4)abc	0.7 (0.3)b
2-PH	BO	TA	5.3 (1.3)ab	0.7 (0.3)b
2-PH	–	TA/AA	9.7 (1.5)a	3.3 (1.2)a
2-PH	BO	TA/AA	9.2 (1.7)a	3.3 (0.8)a
Stats			$F_{5,82}$ = 6.97, p < 0.0001	W = 15.91, p < 0.001 $F_{3,56}$ = 6.89, p < 0.001

Note. Column means followed by a different letter were significantly different, p < 0.05. Total moth catch was analysed with a randomized complete block ANOVA, and female catch was analysed with a Kruskal–Wallis test followed by an ANOVA of ranks, p < 0.05.

3.6 | Italy 2016

Catch of *G. molesta* in these commercial orchards was low with the five lure treatments tested in Trial 1, and means did not differ (Table 4). However, significant differences were found in total moth catch among lure treatments in Trial 2. The four treatments including 2-PH or 2-PH/PE plus TA/AA had the highest moth catches and did not differ. All of these treatments caught significantly more total moths than traps with either OFM-PH or 2-PH lures. The highest total catch was in traps with 2-PH/PE + TA/AA + BO, and this was 30-fold higher than traps with OFM-PH. Female moth catches occurred in all traps baited with TA/AA but did not differ among these four lure treatments. In Trial 3, significant differences in both total and female moths catches were found among lure treatments (Table 4). All of the lure treatments with TA/AA/Z3 except with 2-PH + TA/AA/Z3 + BO caught significantly more total moths than traps with 2-PH. Traps with 2-PH/PE + TA/AA/Z3 caught nearly fivefold more moths than traps with OFM-PH. No female moths were caught in traps with either OFM-PH or 2-PH. Female moth catch was significantly higher in traps with 2-PH/PE + TA/AA/Z3 than with 2-PH + TA/AA/Z3, and the other two lure treatments had intermediate female catches.

3.7 | North Carolina 2016

Significant differences in total moth catch occurred in Trial 1 with traps baited with 2-PH/PE + BO catching more than traps with OFM-PH. The other three lure treatments caught intermediate numbers of moths. Similarly, in Trial 2, significant differences in moth catch occurred among the five lure treatments. Moth catch in traps baited with either 2-PH/PE + TA/AA with or without the addition

TABLE 3 Comparison of total *Grapholita molesta* adults caught in unbaited traps (Blank) and traps baited with acetic acid (AA), terpinyl acetate (TA), sex pheromone (OFM-PH), a combination sex pheromone blend for *G. molesta* and *Cydia pomonella* (2-PH), and this combination blend plus either AA, TA or AA + TA in repeated studies in Uruguay, Chile and Argenina, 2015–2016

Lures			Mean (SE) moth catches[a]					
			Uruguay		Chile		Argentina	
Septa	Membranes		Total	Females	Total	Females	Total	Females[b]
OFM-PH	–	–	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.1 (0.8)c	0.1 (0.1)
2-PH	–	–	3.2 (2.0)b	0.0 (0.0)	0.2 (0.2)	0.0 (0.0)	6.2 (1.2)abc	0.7 (0.3)
–	AA	–	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.2 (0.2)
–	–	TA	0.4 (0.2)	0.0 (0.0)	10.4 (3.8)bc	6.6 (2.8)b	2.4 (0.8)bc	0.4 (0.2)
–	AA	TA	4.6 (1.9)ab	2.4 (1.2)	21.0 (5.5)ab	14.4 (3.8)ab	3.5 (1.2)bc	0.9 (0.5)
2-PH	AA	–	2.0 (0.7)b	0.0 (0.0)	0.6 (0.4)	0.4 (0.4)	5.2 (1.5)abc	0.3 (0.2)
2-PH	–	TA	4.4 (1.2)ab	2.4 (1.1)	7.6 (2.1)c	5.0 (1.1)b	10.5 (3.1)ab	2.0 (1.1)
2-PH	AA	TA	12.8 (3.3)a	4.4 (1.0)	27.2 (6.8)a	19.4 (4.6)a	15.2 (4.3)a	3.6 (1.8)
Stats			$F_{4,20}$ = 4.57 p < 0.01	$F_{2,12}$ = 1.28 p = 0.31	$F_{3,16}$ = 3.91 p < 0.05	$F_{3,16}$ = 4.15 p < 0.05	$F_{6,62}$ = 5.58 p < 0.0001	W_4 = 5.60 p = 0.23

Note. Column means followed by a different letter were significantly different, Tukey's test, p < 0.05. [a]Treatments with no moth catch or only an incidental catch (means ≤0.6 moth per trap) were excluded from the ANOVAs. Blank traps placed in the study did not catch any moths. [b]Following the significant Kruskal–Wallis nonparametric test, an ANOVA of the ranks was conducted, $F_{43,76}$ = 4.00, p < 0.05, and this was followed by a means separation test, p < 0.05.

TABLE 4 Comparison of lures with various blends in a series of three trials conducted in Uruguay, Italy and North Carolina testing septa lures loaded with the sex pheromone of *Grapholita molesta* (OFM-PH), the combination sex pheromone of *G. molesta* and *Cydia pomonella* (2-PH), the combination sex pheromone plus pear ester (2-PH/PE) and/or β-ocimene (BO), and a membrane cup lures loaded with terpinyl acetate (TA) and acetic acid (AA) together (TA/AA) and with (Z)-3-hexenyl acetate (TA/AA/Z3), 2016

	Lures			Mean (*SE*) moth catches[a]				
				Uruguay		Italy		NC
Trial No.	Septa	Membranes		Total	Females	Total	Females	Total
1	OFM-PH	–	–	6.6 (1.6)	0.2 (0.2)	1.4 (0.6)	0.0 (0.0)	1.7 (1.3)b
	2-PH	–	–	17.6 (4.7)	0.0 (0.0)	1.7 (0.9)	0.4 (0.3)	4.5 (0.9)ab
	2-PH/PE	–	–	18.0 (5.9)	0.0 (0.0)	4.6 (1.6)	0.2 (0.1)	3.3 (0.8)ab
	2-PH	BO	–	13.8 (3.3)	0.0 (0.0)	2.6 (0.9)	0.3 (0.2)	5.0 (1.6)ab
	2-PH/PE	BO	–	18.0 (5.7)	0.0 (0.0)	4.3 (1.2)	0.6 (0.3)	6.8 (0.9)a
Stats				$F_{4,20} = 1.37$ $p = 0.28$	–	$F_{4,44} = 2.53$ $p = 0.05$	–	$F_{4,20} = 4.21$ $p < 0.05$
2	OFM PH	–	–	0.2 (0.2)	0.0 (0.0)	0.9 (0.4)b	0.0 (0.0)	–
	2-PH	–	–	0.2 (0.2)	0.0 (0.0)	4.8 (2.0)b	0.0 (0.0)	7.7 (1.9)b
	2-PH	–	TA/AA	2.6 (0.7)ab	1.6 (0.9)	13.5 (4.7)a	3.7 (0.8)	12.2 (3.8)ab
	2-PH/PE	–	TA/AA	2.4 (0.9)ab	1.6 (0.6)	17.2 (2.3)a	4.8 (1.2)	14.2 (3.0)a
	2-PH	BO	TA/AA	2.8 (1.2)a	2.4 (1.3)	14.9 (4.0)a	6.0 (1.4)	11.3 (4.2)ab
	2-PH/PE	BO	TA/AA	1.6 (0.4)ab	1.2 (0.4)	27.4 (7.1)a	9.4 (2.1)	15.7 (4.6)a
Stats				$F_{3,16} = 0.31$ $p = 0.82$	$F_{3,16} = 0.05$ $p = 0.99$	$F_{5,53} = 14.37$ $p < 0.0001$	$F_{3,35} = 2.56$ $p = 0.07$	$F_{4,20} = 4.02$ $p < 0.05$
3	OFM-PH	–	–	20.0 (7.8)	0.0 (0.0)	4.0 (1.4)c	0.0 (0.0)	–
	2-PH	–	–	45.6 (7.5)	0.0 (0.0)	6.7 (2.5)c	0.0 (0.0)	9.7 (3.6)
	2-PH	–	TA/AA/Z3	33.6 (5.1)	1.0 (0.4)	13.5 (5.1)ab	1.3 (0.3)b	9.3 (3.5)
	2-PH/PE	–	TA/AA/Z3	31.6 (5.4)	0.4 (0.2)	19.5 (3.9)a	3.7 (0.9)a	7.8 (4.4)
	2-PH	BO	TA/AA/Z3	35.0 (4.4)	0.2 (0.2)	6.3 (1.4)bc	1.9 (0.5)ab	8.8 (5.2)
	2-PH/PE	BO	TA/AA/Z3	33.6 (4.6)	0.6 (0.4)	15.5 (3.7)a	3.1 (0.5)ab	9.7 (4.4)
Stats				$F_{5,24} = 2.21$ $p = 0.09$	–	$F_{5,53} = 14.07$ $p < 0.0001$	$F_{3,35} = 3.85$ $p < 0.05$	$F_{4,20} = 0.88$ $p = 0.49$

Note. Column means followed by a different letter were significantly different, $p < 0.05$. [a]Data were excluded from the analyses if the mean was ≤0.6 moths per trap.

of BO caught significantly more moths than traps baited with 2-PH. Moth catch in traps with the 2-PH + TA/AA with or without BO caught intermediate number of moths. Moth counts were fairly consistent across the five lure treatments tested in Trial 3. Low numbers of *C. pomonella* were caught in these apple blocks during all three trials. The most *C. pomonella* were caught during Trial 1 and the five traps baited with 2-PH/PE caught four compared with 13 in traps baited with 2-PH.

3.8 | Pennsylvania 2016

A large number of *G. molesta* were caught in traps in the replicated apple orchards not treated with sex pheromones (Table 5). Significant differences occurred among lure treatments in these blocks and traps baited with 2-PH/PE + TA/AA caught significantly more moths than the other five lure treatments. Also, traps baited with 2-PH/PE + TA/AA/Z3 caught significantly more moths than traps with 2-PH. Moth catch in blocks under sex pheromones were 98% lower than in the untreated blocks, and no significant difference was found among lure treatments. *C. pomonella* was caught at fairly high levels in all lure treatments in the nondisrupted apple blocks. The highest catch was in traps baited with 2-PH. Catches were significantly lower when pear ester was added to the septum lure and when either TA/AA or TA/AA/Z3 was added to the trap. Traps including the TA/AA/Z3 lure had the lowest catches. The catch of *C. pomonella* in blocks treated with sex pheromone was much lower than in the untreated blocks. Only traps baited with 2-PH or 2-PH/PE caught >1.0 moth.

3.9 | California and Washington 2017

Significant differences were found in moth catch between the Ajar and delta trap baited with the TRE1370 lure in California and Washington. In California, the mean (*SE*) moth catch over the 4 week trial in the disrupted peach orchard averaged 7.0 (2.8) and 29.8 (10.9)

TABLE 5 Comparison of lures with various blends testing septa lures loaded with the combination sex pheromone of *Grapholita molesta* and *Cydia pomonella* (2-PH), the combination sex pheromone plus pear ester (2-PH/PE) and membrane cup lures loaded with terpinyl acetate and acetic acid together (TA/AA) or in combination with (*Z*)-3-hexenyl acetate (TA/AA/Z3), *N* = 5 apple orchards treated with (MD) and without sex pheromones (no MD) for both *G. molesta* and *C. pomonella*, Pennsylvania, USA, 2016

Lures		Mean (*SE*) moth catch[a]			
		G. molesta		*C. pomonella*	
Septa	Membranes	MD	No MD	MD	No MD
2-PH	–	1.0 (0.6)	169.8 (61.6)c	3.8 (2.2)	105.8 (25.6)a
2-PH/PE	–	0.8 (0.4)	246.4 (89.0)bc	2.0 (0.6)	43.6 (11.6)b
2-PH	TA/AA	4.8 (2.9)	267.2 (100.7)bc	0.4 (0.4)	25.6 (6.7)bc
2-PH/PE	TA/AA	4.6 (1.5)	448.4 (151.0)a	1.0 (0.3)	36.6 (11.3)bc
2-PH	TA/AA/Z3	1.6 (0.7)	231.0 (76.9)bc	0.4 (0.4)	12.4 (5.8)cd
2-PH/PE	TA/AA/Z3	1.8 (1.1)	282.8 (104.6)b	0.0 (0.0)	5.6 (3.2)d
Stats		$W_6 = 5.51$ $p = 0.35$	$F_{5,20} = 13.54$ $p < 0.0001$	$W_3 = 5.33$ $p = 0.15$	$F_{5,20} = 20.74$ $p < 0.0001$

Note. Column means followed by a different letter were significantly different, $p < 0.05$. [a]Data were excluded from the analyses if the mean was ≤0.6 moths per trap.

in the Ajar and delta trap baited with the TRE1370 lure, respectively; and this difference was significant. $F_{1,8} = 30.94$, $p < 0.001$. In comparison, two traps baited with sex pheromone lures and placed in the same orchard but adjacent (distances of 30–50 m) to our study both caught two moths during this time period.

In Washington, significant differences in moth catch occurred in the nondisrupted apple orchard between the Ajar and delta traps baited with the TRE1370 lure, $F_{1,18} = 21.73$, $p < 0.001$. Mean (*SE*) moth catch over a 2-week period averaged 1.2 (0.5) and 6.0 (1.1) in the two trap types, respectively. Both trap-lure types caught >40% female moths. In addition, the mean (*SE*) catch of muscid flies was significantly higher in the Ajar traps (28.0 [5.6]) than the delta traps (10.2 [2.0]), $F_{1,18} = 11.75$, $p < 0.01$.

Significant differences in female and total moth catches occurred among traps baited with TA, TA/AA and TA plus three different acetic acid lures (Table 6). While the three membrane lures varied widely in their release of acetic acid, mean moth catches did not differ among these three lures when used in combination with the TA lure. Also, moth catches were not different between the TA/AA lure and the binary use of TA plus any of the three acetic acid lures.

TABLE 6 Comparison of the weight loss of membrane lures over a 2-week period at 25°C and catches of *Grapholita molesta* in delta traps baited with terpinyl acetate (TA) and acetic acid (AA) lures, *N* = 10, Moxee, Washington, USA, 2017

Lures	Active: Mean (*SE*) daily weight loss (mg)	Mean (*SE*) moth catch	
		Total	Females
TA	TA: 12.6 (0.2)	1.1 (0.2)b	0.4 (0.3)b
TA/AA	TA + AA: 13.1 (0.3)	4.0 (1.4)ab	2.0 (0.9)ab
TA + AA	AA: 5.2 (0.05)	6.9 (2.8)ab	2.1 (1.1)ab
TA + AA2	AA: 27.8 (0.5)	8.6 (3.1)a	3.7 (1.1)a
TA + AA3	AA: 73.1 (1.0)	10.3 (3.3)a	4.2 (1.4)a
ANOVA		$F_{4,44} = 4.33$, $p < 0.01$	$F_{4,44} = 3.86$, $p < 0.01$

Note. Mean moth catch in each column followed by a different letter were significantly different in a randomized complete block. ANOVA, Tukey's HSD test, $p < 0.05$.

4 | DISCUSSION

Grapholita molesta is a key worldwide insect pest of stone fruits and of pome fruits in some geographical areas (Rothschild & Vickers, 1991). The ability to effectively monitor the adult stages of this pest allows farm managers to develop effective programmes that minimize the use of insecticides (Charmillot & Vickers, 1991). Work reported by our research group over the past 5 years has detailed new combination lures, including sex pheromones and host plant and microbial volatiles that can monitor both sexes in orchards treated with sex pheromones for mating disruption (Cichon et al., 2013; Knight et al., 2013; Knight, Cichon, et al., 2014; Knight, Barros-Parada, et al., 2015). One prerequisite included in our development of new, low-cost monitoring tools has been the manager's ability to use any new lures in standard traps with removable sticky liners (Cichon et al., 2013). Development of the Ajar trap was an intermediate step that allowed bisexual monitoring using sticky liners with catches comparable to the bucket traps but with fewer nontargets to sort, but was still a cumbersome design due to the replacement of the liquid baits (Knight et al., 2013; Knight, Cichon, et al., 2014; Padilha et al., 2017). Our new results demonstrate that *G. molesta* can now be monitored with a combination of volatiles released by septa and membrane lures within standard sticky traps. Also, the numbers of nontargets caught in traps baited with the TA/AA lure did not appear to be a problem across the various studies.

Earlier studies demonstrated that moth catches of *G. molesta* in traps baited with the combination sex pheromone lure (2-PH), which includes codlemone, were only intermediate in comparison with a number of commercial sex pheromone lure for *G. molesta* when used in disrupted orchards (Knight, Basoalto, et al., 2015). Here, among our various studies, the mean catch in traps was consistently higher (3–5×) in traps baited with the 2-PH lure vs.

a standard sex pheromone lure, but these means were never statistically significant. Previously, we found that a significant trade-off with using the combination sex pheromone lure was that male catch of *C. pomonella* was reduced in comparison with codlemone-baited traps (Knight, Cichon, et al., 2014; Knight, Basoalto, et al., 2015). Conversely, studies reported from Argentina and Uruguay found that *C. pomonella* catch was similar in traps baited with either codlemone or the 2-PH lure. However, significant negative effects on the catch of *C. pomonella* occurred when other lures were added to boost the catch of *G. molesta*, for example TA + AA lures in Uruguay and TA/AA, TA/AA/Z3 or pear ester in Pennsylvania. Similarly, previous work had reported that the addition of either acetic acid or the TAS liquid bait to traps baited with the 2-PH lure reduced catch of *C. pomonella* by 50% (Knight, Cichon, et al., 2014). However, no previous study has the use of pear ester been shown to reduce the catch of *C. pomonella*.

Initial field screening of target lepidopteran pest species in North America found that pear ester was not attractive for *G. molesta* (Knight & Light, 2004). Yet, later work in Italy suggested that both sexes of *G. molesta* can detect pear ester and that males in laboratory choice olfactometer bioassays were behaviourally attracted to pear ester (Molinari et al., 2010). Here, the importance of adding pear ester to the grey septa loaded with the combination sex pheromone (2-PH/PE) was somewhat inconclusive: reduced catch in Oregon in 2015, a marginal increase ($p = 0.05$) in Italy in 2016 and clearly no effect in the 2016 studies in Uruguay, North Carolina and Pennsylvania. However, combining the 2-PH/PE septum with the TA/AA membrane lure did significantly increase catch of *G. molesta* in Pennsylvania, but this did not occur in the other trials. Overall, including pear ester in the binary lure for *G. molesta* does not seem to be critical. However, our finding that inclusion of pear ester in the septa lure reduces catch of *C. pomonella* is problematic for any future commercialization of these lures, especially in orchards where both pests are present and managers want to monitor both in the same trap.

A number of laboratory and field studies have evaluated various host plant volatiles for *G. molesta* and achieved variable results. For example, either β-ocimene or (*Z*)-3-hexenyl acetate alone or in various HPV blends was found to increase moth catches (Il'ichev et al., 2009; Knight et al., 2011; Natale et al., 2003; Piñero & Dorn, 2007), or provided variable results, such as β-ocimene improved the performance of TAS-baited traps (Knight et al., 2013); but pear ester plus β-ocimene did not improve the 2-PH lure (Knight, Cichon, et al., 2014). Similar to this later study, we found that neither β-ocimene nor (*Z*)-3-hexenyl acetate affected moth catches when used with either 2-PH or TA/AA lures, and likely neither volatile will be developed further through our trials.

The addition of acetic acid lures to traps did not improve the performance of host plant volatile-based lures or 2-PH for *G. molesta* in previous studies (Knight, Cichon, et al., 2014; Knight, Hilton, et al., 2014). However, acetic acid had been used effectively in a liquid bait with sugar and ethanol in China (He et al., 2009). Here, we found in several studies that acetic acid did not improve the 2-PH lure. Similarly, adding a TA lure to traps with the 2-PH lure did not increase moth catches. In comparison, moth catches with the TAS liquid bait were significantly increased with the addition of the 2-PH lure (Knight, Cichon, et al., 2014). These disparate results could likely be due to the undefined and temporally sensitive volatile profile released by the TAS bait from open containers when compared with the closed TA membrane lure.

The most effective lure for *G. molesta* in our trials was clearly the 2-PH septum and TA/AA membrane used in traps together. Moth catches with this binary lure compared with the 2-PH lure alone increased from <twofold to >100-fold, and the proportion of females caught in traps baited with this binary lure ranged from 0.24 to 0.71 across the various studies. At present, we have no hypothesis to explain this high degree of variation in the relative performances of these lures, and further studies might address lure efficacy in different host crops (stone fruits, apple and pear) or possible geographical differences in these behavioural responses among *G. molesta* populations (Knight, Barros-Parada, et al., 2015). Clearly, the TA/AA membrane lure has not yet been optimized. For example, there was a clear positive trend in both total and female moth catch as a function of emission rate of acetic acid. Also, the weight loss data suggest that the loading of terpinyl acetate in the TA/AA lure may be too low and the binary emission profile of this lure should be characterized over time as well.

One focus of our recent work to develop bisexual lures for key tortricid pests of tree fruits has been to test whether multiple components that attract different species that co-occur in individual orchards can be formulated and used in individual traps (Knight, El-Sayed, Judd, & Basoalto, 2017; Knight, Hilton, et al., 2014). This work is motivated by the rapid advances in smart technologies, such as remote monitoring of traps through cameras (Kim, Jung, Kim, & Lee, 2011; Wen, Guyer, & Li, 2009). To date, our work has focused primarily on *C. pomonella* and several tortricid leafrollers present in the western United States (Basoalto et al., 2017; Knight, Hilton, et al., 2014; Knight et al., 2017). However, due to the frequent co-occurrence of *G. molesta* and *C. pomonella* plus a suite of leafroller species in orchards, it will likely be important to expand these studies to include 2-PH and TA/AA lures. Fortunately, acetic acid has been found to increase moth catches of all species when used in specific ways, such as in combination with pear ester for *C. pomonella* (Knight, Hilton, et al., 2014), or in combination with 2-phenylethanol for *Choristoneura rosaceana* Harris (Knight et al., 2017). Interestingly, it appears that the emission rate of acetic acid is an important factor affecting moth catches; *C. pomonella* was more attractive to low rates (AA lure) and *C. rosaceana* is more attracted to higher rates (AA2 and AA3 lures) in these studies. To date, we do not have enough information on the influence of acetic acid rate on catch of *G. molesta*, but our preliminary data suggest that this species may have a broader response than the other two species.

There may be serious drawbacks in developing a lure that can effectively catch several species in the same trap. For example, the inclusion of (*E*)-4,8-dimethyl-1,3,7-nonatriene to enhance the catch of *C. pomonella* was found to depress the catch of *C. rosaceana* when used with 2-phenylethanol and acetic acid (Knight et al., 2017). Our unexpected finding in the Pennsylvania trial where adding pear ester to a septum loaded with the sex pheromone of both species depressed the catch of *C. pomonella* suggests that a heuristic approach will not be effective to develop multispecies trapping systems. Unrealized factors such as moth behaviours near traps, including the role of vision or other sensory cues that may cause repellency and are used by related sympatric species to maintain mating isolation have not been addressed with kairomones. Many factors can impact the relative effectiveness of kairomone lures, including geography, season, crop and cultivar as shown for pear ester with *C. pomonella* (Knight & Light, 2005c). Likely, the complexity of combining sex pheromones with host plant, and microbial volatiles for a suite of species would be too great to create one optimized lure. Instead, perhaps developing suboptimal lures with some limited attractiveness across all species might be sufficient to establish action thresholds and to reduce monitoring costs.

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AUTHOR CONTRIBUTION

ALK conceived research, analysed the data, conducted statistical analyses and wrote the manuscript. All authors conducted research. All authors read and approved the manuscript.

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