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Combining female removal with mating disruption for management of *Cydia pomonella* in apple

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With 2 figures and 4 tables

Abstract: Field studies tested the use of lure-baited traps for female removal (FR) of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) in apple, *Malus domestica* Borkhausen, treated with sex pheromone-based mating disruption (MD-FR). Four lures were evaluated, including the combination of (*E,Z*)-2,4-ethyl decadienoate (pear ester, PE), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX), these three components with (*E,E*)-8,10-dodecadien-1-ol (PH), and two lures loaded with PH/PE. All lures were used with a co-lure loaded with acetic acid (AA). Studies examined the importance of trap type and density on moth catches. Seventeen trials were conducted in 1-3 ha adjacent paired plots treated with or without 60 traps ha^{-1} in Washington State from 2018-2020. Paired plots were each treated with MD and a similar spray regime. The mating status of females in the MD-FR plots were compared with females collected from blocks not treated with MD. The PE/DMNT/LOX + AA lure caught significantly more females than any other lure. The most effective trap was a bucket trap with a green top and a clear bottom. Total or female moth catches per trap did not decline across trap densities from 37 to 99 ha^{-1}. MD-FR trials showed that levels of fruit injury could be significantly reduced 50–75% across each moth flight. The proportion of mated females was 8 to 19% lower in blocks treated with MD-FR than not treated with MD. Further studies are needed to refine key operational factors associated with MD-FR considering the economics of organic and conventional apple production across key varieties.

Keywords: Tortricidae; codling moth; *Malus domestica*; mass trapping; attract-and-kill

1 Introduction

Within a decade following the identification and commercial synthesis of (*E,E*)-8,10-dodecadien-1-ol (PH) as the major sex pheromone component of codling moth *Cydia pomonella* L. (Roelofs et al. 1971), applied studies conducted by key researchers working in apple, *Malus domestica* Borkhausen, explored whether mass trapping male moths could be used to suppress pest populations (Charmillot & Baggiolini 1975; Proverbs et al. 1975; Maitlen et al. 1976; Madsen et al. 1976; MacLellan 1976; Hagley 1978; Madsen & Carty 1979; Willson & Trammel 1980; Howell 1980). Both limited successes and gradual failures over several seasons occurred in these studies with respect to fruit protection by male removal. In summary, the key points from this body of research were that male removal could likely substitute for one insecticide spray per season and this was dependant on starting the program with a low pest population density and having sufficient isolation from sources of immigrating females. But the cost of placing multiple traps (5–44 ha^{-1}) and the frequent lure and liner replacements required during the season did not allow this approach to be competitive with standard spray programs in the 1970's. Nevertheless, the drivers to develop new integrated pest management technologies, i.e., threat of expanding insecticide resistances and disruption of secondary pests, such as mites and aphids, continued to worsen and the call for alternative, more-selective programs remained clear (Croft & Hoyt 1983).

It took nearly two decades for a second major initiative to develop what was called attract-and-kill technology, this time driven by industry, consisting of proprietary hand-applied technologies for codling moth males using mixed

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formulations of sex pheromone and insecticides (Charmillot et al. 2000; Lösel et al. 2000; Krupke et al. 2002). The push for these technologies came from both the continued worldwide spread of resistance to various classes of insecticides, concerns with disruption of natural control of a suite of secondary pests, and the enactments of new national and global restrictions making entire classes of insecticides unavailable for codling moth management (Kogan 1998). Attract-and-kill showed some efficacy in field trials, two products were registered, and these continued to be investigated and slowly adopted for more than a decade as an alternative to sex pheromone-based mating disruption (MD) (Stará et al. 2008; Somsai et al. 2010; Mansour 2010). Key factors driving continued interest in this approach were the beliefs that attract-and-kill would be more useful than MD in smaller and irregular shaped orchards, and especially in these blocks near interfaces with urban development to minimize environmental externalities associated with spray runoff and drift (Lösel et al. 2002; Ioriatti & Angeli 2002). However, the labour involved with 2–3 specialized applications of 1,200–4,500 drops ha^{-1} during the season was costly and attract-and-kill did not displace the use of MD for codling moth (Witzgall et al. 2008; Witzgall et al. 2010; Miller & Gut 2015; Gregg et al. 2016; Ioriatti et al. 2016). More recently, other approaches combining insecticides with semiochemical attractants have been proposed, such as treated pouches (Huang et al. 2013) or nets (Knight & Mujica 2019), but these have not yet been registered for grower use.

Models clearly show that female removal requires a much lower efficiency than male-targeted approaches and should be more effective against a multi-voltine, polygamous pest, such as codling moth (Knight et al. 2001a; Gregg et al. 2016). Thus, the third wave of trying to manipulate insect behaviour to directly manage codling moth began with the discovery of the bisexual attractancy of (*E,Z*)-2,4-ethyl decadienoate (pear ester, PE) (Light et al. 2001). Unfortunately, PE could not be used in 'lure and kill' pastes as it was shown that females did not closely approach or touch the laced drops or PE lures (Knight et al. 2001b; Knight & Light 2005).

Over the past two decades various experimental efforts, primarily reported at scientific conferences, have been conducted to explore how PE could be used to manage codling moth effectively through trapping or killing female codling moths (Knight 2003, Il'ichev & Williams 2016). Traps alone, waxed cards, plastic panes, screens and netting coated with insecticides and baited with PE have all been investigated. A microencapsulated formulation (MEC) of PE was registered and shown to improve various larvicides (Light & Knight 2011; Knight & Light 2013). Nevertheless, the lack of effective adulticides with registrations for organic use precluded the combination of the MEC formulation with insecticides for this key group of growers in need of new tools to manage codling moth (Knight 2010). Also, the difficulty and costs in obtaining registrations for attract-and-kill formulations with non-sex pheromone actives is a key factor limiting this approach (Gregg et al. 2018). Instead, the potential of developing female removal (FR) as an effective component using lure-baited traps was left as the more viable option.

Fortunately, a series of discoveries of new increasingly more effective lures for female codling moth led from pear ester to a binary lure with acetic acid (AA) (Landolt et al. 2007), a ternary lure with (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) added (Knight et al. 2011; Knight & Light 2012), and most recently with a quaternary lure blend with the addition of 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX) (Knight et al. 2019a; 2019b). However, the first peer-reviewed report of testing FR as an effective component for codling moth management used a different three-component lure with pear ester released from a septum and acetic acid and the bacterial volatile *n*-butyl-sulfide released separately from polyethylene vials (Landolt et al. 2014; Jaffe & Landolt 2018). Mass trapping trials (4 to 11 weeks in length) were conducted in paired 1.6 ha apple plots not treated with MD over four seasons using white delta traps (Jaffe et al. 2018). The protocol used a trap density of 120 ha^{-1} and lures and trap liners were replaced every 14 days. Moth catches were recorded determining the sex but not the mating status of the female moths. Levels of fruit injury were reduced overall by 57% with the addition of traps. A second study was conducted by placing individual traps in extra-orchard settings of apple and crab apple, *Malus* spp. Traps were serviced weekly and fruit injury was only marginally reduced at the end of the 90-day trial (Jaffe & Landolt 2019).

Coinciding with these studies the quaternary blend of PE/DMNT/LOX/AA was found to outperform the blend of PE/AA with *n*-butyl sulfide by 3.7-fold (Knight et al. 2019a). A binary lure was developed with PE/DMNT/LOX in a black PVC matrix and AA released from a white closed membrane loaded and proved to be effective for > 8-weeks (Preti et al. 2021a). A second binary lure with PH/PE/DMNT/LOX in the PVC matrix and the same AA co-lure was tested, but the addition of PH in this lure was found to reduce female catches (Preti et al. 2021a).

We hypothesized that the use of a more attractive, and long-lasting lure placed in low-maintenance bucket traps could significantly improve the efficacy and/or reduce the cost of grower implementation of FR. Studies were conducted in both Italy and USA with FR in 17 organic pear, *Pyrus communis* L., blocks treated with or without MD during 2019-2020 (Preti et al. 2021b). Fruit injury from codling moth was significantly reduced with the implementation of FR in both pear production regions. Here we report apple trials conducted with a similar protocol in 17 apple orchards in Washington State from 2018-2020 in both organic and conventional blocks treated with sex pheromone dispensers for MD. Supplemental studies also compared several trap-lures and trap densities to optimize FR in apple.

2 Materials and methods

2.1 Lure and trap evaluations

Trécé Inc. (Adair, Oklahoma, USA) provided all proprietary lures. A black PVC matrix was loaded with (*E,Z*)-2,4-ethyl decadienoate (pear ester, PE), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX) or with a quaternary blend including the addition of (*E,E*)-8,10-dodecadien-1-ol (sex pheromone, PH). The active ingredient loadings of these lures were the same as reported in Knight et al. (2019a; 2019b). Two other lures were included in our research including PH/PE loaded into a grey halobutyl elastomer septum or into a black PVC matrix. All of these lures were used in combination with a white plastic membrane cup co-lure loaded with acetic acid (AA) (Knight & Light 2012).

Several types of traps were used in our studies, including orange delta traps Pherocon® VI (Trécé Inc.), all clear, green lid/yellow top/white bottom, all green, and green lid/green top/clear bottom bucket traps Unitrap® (Great Lakes IPM, Vestaburg, MI, USA), and the green lid/green top/white bottom Multipher® I and III traps (Services Bio-Contrôle, Ste-Foy, Quebec, Canada). Bucket trap types were partially filled with 250 ml of propylene glycol (Duda Energy, Decatur, AL) or a 20:80 mix of propylene glycol and mineral oil (HI Supreme Spray Oil-NW®, Integrated Agribusiness Professionals, Fresno, CA) to retain moths. Delta traps used removable sticky inserts (Clean-Brake®, Trécé Inc.). Traps were attached to PVC or bamboo poles and placed in the upper third of the canopy in all studies.

A first study was conducted in 2019 to compare moth catches in orange delta traps baited with four different lures that were used in the FR trials conducted in apple and in pear. These included PH/PE lure (either loaded in a septum or a PVC matrix), the PE/DMNT/LOX and the PH/PE/DMNT/LOX lure. All four lures were used in traps with the AA co-lure. Studies with 4 replicates were established in three apple orchards cv Delicious on 17 July near Tieton (46°41’44.18”N 120°45’39.57”W), Yakima (46°32’38.15”N 120°31’59.84”W), and Wapato (46°24’33.08”N 120°29’0.55”W), in Washington State. Orchards size was 5.5 ha, 6.0 ha and 4.0 ha, respectively. Traps were spaced 30–50 m apart and checked weekly until 11 September, liners were replaced each week, and traps were not rotated.

A second trial was conducted in a mixed-cultivar apple orchard (Golden Delicious, Honeycrisp, Delicious, and Ambrosia) situated near Tieton, WA (46°42’49.90”N 120°45’55.28”W), from 21 July to 15 September in 2020 to evaluate trap types used in the apple MD-FR trials. Traps included the orange delta and three bucket traps of different color (all clear, all green, and green lid/green top/clear bottom). Moths catch among these four traps (N = 7 to 64 replicates) were compared with the use of two lures: PH/PE + AA and PE/DMNT/LOX + AA. Trap-lure combinations were randomized within a 10 ha apple orchard and spaced 20–30 m apart. Also in this trial liners were replaced weekly and traps were not rotated.

A third study was conducted to evaluate for potential trap interference with three densities of traps. Three 0.25 ha plot replicates of three densities, 37, 62, and 99 traps ha^{-1} were randomly placed in an apple block cv Fuji near Wapato, WA (46°24’48.99”N 120°28’30.12”W). This study used the green/white Multipher® trap baited with the PE/DMNT/LOX + AA binary lure set. Traps were placed on 19 May and retrieved on 16 June 2019, then the moth catches were sorted and counted in laboratory at the end of the study.

2.2 MD-FR field trials

Seventeen studies were conducted in apple orchards situated near Tieton, Yakima, and Wapato, WA during 2018–2020. Orchard locations were chosen based on grower cooperation and the presence of moderate to high population densities of codling moth. Orchards were evenly subdivided into two halves treated with MD-FR or MD-only (1.0–2.0 ha each). It was typical that the paired plots were adjacent to orchards treated with or without MD. All paired blocks in the four 2018 studies were treated with Cidetrak® CMDA Combo™ PP (Trécé Inc.) at the maximum label rate of 1,000 dispensers ha^{-1}. Four sites were established in 2019 and treated with Cidetrak® CMDA Combo™ PP at the minimum label rate of 500 dispensers ha^{-1}. All paired blocks in 2020 were treated on 18–20 June with the minimum label rate, 45 ha^{-1} of Cidetrak® CMDA Combo™ Meso™-A dispensers (Trécé Inc.). Cidetrak® CMDA Combo™ PP dispensers were loaded with 90 and 60 mg of PH and PE; and Cidetrak® CMDA Combo™ Meso™-A dispensers were loaded with 850 and 500 mg of PH and PE, respectively.

A green/white Multipher® trap was used in all orchards in 2018. Several trap types were used during 2019 including Multipher® traps and three types of bucket traps: all green, all clear, and a green lid/yellow top/white bucket. An orange delta trap Pherocon® VI was used in all blocks in 2020. Traps were evenly spaced at density of 60 ha^{-1} beginning 5 m from the physical edges of the plot. Traps in 2018 and 2019 were placed in orchards in late April. Traps were emptied and new lures and oil were added at mid-season (end of June-beginning of July. Studies in 2020 were initiated in late June with orange delta traps. Liners in these traps were replaced every 1–4 weeks depending on the density of the combined target and non-target catch. Lures were not replaced over the 8 to 11-week trials.

All traps in the MD-FR blocks in 2018 were initially baited with the PH/PE (septum) + AA binary lure. These lures were replaced with the same but new lures after eight weeks. All traps in the MD-FR blocks in 2019 were initially baited with the PH/PE (PVC) + AA binary lure. These lures were replaced at mid-season with the PE/DMNT/LOX + AA binary lure. Similarly, in 2020 delta traps used in the MD-FR blocks were baited with PE/DMNT/LOX + AA.

All paired blocks were treated with the same growers' spray program. All sites in 2018 and 2019 were certified organic and received one or more applications of granulosis virus (Virosoft CP4®, BioTEPP Inc., Lévis, Quebec, Canada) with 1.0% mineral oil. Study sites in 2020 included both organic and conventional orchards. The organic blocks were treated with several virus/oil applications and borders were sprayed with spinosyn (Entrust SC®, Dow) plus a microencapsulated pear ester formulation, Cidetrak® CMDA MEC (Trécé Inc.). The paired conventional blocks received the same but variable numbers of diamide (Altacor®, FMC Corporation, Philadelphia), spinosyn (Success®, Corteva Agriscience), and neonicotinyl (Assail®, UPI Inc., King of Prussia, PA) insecticides for codling moth during the season.

Levels of fruit injury were sampled in each of the paired plots at mid-season in July (not in 2020) and prior to harvest from mid-August to mid-September by inspecting 1,500–2,400 fruits on trees (30–40 fruits per tree from the mid- to upper canopy) in the central area of each plot. All moths were sieved out of the bucket traps in 2018–2019 at mid-season and traps were replenished with new liquid. Sticky liners used in delta traps in 2020 were either not replaced over the entire 11-week study or replaced up to 3 times depending on the magnitude of codling moth and the non-target catch in each trap. Non-targets were not prevalent on sticky liners and included muscid flies, vespid wasps, and an occasional noctuid or pyralid moth. Codling moths were counted and sexed following each trap check. A sample of pooled females (≤ 100) on each trap check date from each block were dissected to determine their mating status. Females were scored as unmated, single mated, or multiple mated which included those with 2–4 spermatophores in their *bursa copulatrix*. Additional apple orchards which were not treated with MD were monitored each year with 6–10 traps baited with PE/DMNT/LOX + AA lure. Samples of females from each flight were dissected to determine their mating status.

2.3 Statistical analyses

Male, female, and total moth catches, and the proportions of females, mated females, and fruit injury were transformed with sqrt(x + 0.05) and arcsin(sqrt(x + 0.05)), respectively, to stabilize variances prior to analysis. All data were successfully normalized (Shapiro-Wilks test) and tested with analysis of variance (ANOVA) (Analytical Software, Statistix 9, Tallahassee, FL, USA). A completely randomized ANOVA was used to compare female mating status between blocks treated with MD-FR and those not treated with MD in 2018–2019. A complete randomized block design was used for the lure comparison trial conducted in three orchards in 2019. A two-way ANOVA including the interaction term was used with the trap and lure trial in 2020. Tukey's test was used to designate significant differences between means following ANOVA, $P < 0.05$. Linear regression was used to estimate the moth catches per trap as a function of trap density. The adjusted R^2 was calculated and the test of the regression slopes was conducted for female, and total moth catch. A paired t-test was used to compare levels of fruit injury in the paired MD versus MD-FR plots.

3 Results

3.1 Lure and trap evaluations

Significant differences were found for male, female, and total codling moth catches among the four lures tested in delta traps in 2019 (Table 1). Traps baited with the PH/PE (PVC) + AA lure caught significantly more male and total moths than the PH/PE (septum) + AA lure and the PE/DMNT/LOX + AA lure, and similar numbers to the PH/PE/DMNT/LOX + AA lure set. However, traps with the PE/DMNT/LOX + AA lure caught significantly more (2–4-fold) female codling moth than traps with the other three lures.

Table 1. Comparison of codling moth catches in orange delta traps with four multi-component lures comprised of (*E,E*)-8,10-dodecadien-1-ol (sex pheromone, PH), (*E,Z*)-2,4-ethyl decadienoate (pear ester, PE), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX) loaded into different matrixes. Two lures with PH/PE were loaded into either a grey halobutyl septum or PVC, while two lures with PE/DMNT/LOX or PH/PE/DMNT/LOX were loaded into PVC; all lure treatments included an acetic acid (AA) membrane co-lure, N = 4 in three apple orchards in Washington State, USA in 2019.

		Mean (SE) moth catch per trap		
Lure substrate	**Lure loading + co-lure**	**Male**	**Female**	**Total**
Septum	PH/PE + AA	12.3 (2.7)c	5.3 (1.1)c	17.7 (3.2)c
PVC	PH/PE + AA	63.0 (9.6)a	8.2 (1.6)bc	71.2 (9.9)a
PVC	PE/DMNT/LOX + AA	18.6 (2.5)bc	23.0 (2.2)a	41.6 (3.8)b
PVC	PH/PE/DMNT/LOX + AA	40.0 (8.3)ab	12.8 (2.0)b	52.8 (7.8)ab
RCB ANOVA: df = 3, 42		$F = 16.63$ $P < 0.0001$	$F = 24.77$ $P < 0.0001$	$F = 15.60$ $P < 0.0001$

Column means followed by different letters were significantly different, $P < 0.05$, Tukey's test.

Two studies were conducted to evaluate the effects of trap density and trap type on moth catches. In the 2019 trial there was a slight but non-significant drop in male, female, and total moth catch per trap as a function of the increase in density of Multipher® traps baited with the PE/DMNT/LOX + AA lure across the range of 37–99 traps ha^{-1} (Fig. 1). Significant differences in moth catches were found among the four traps tested in 2020 with two different lures (Table 2). Both the orange delta and the green/clear bucket trap caught significantly more total moths than all clear or all green bucket traps. The green/clear bucket caught significantly more female moths than the other three trap types. No difference was found among the four traps for male capture. The PE/DMNT/LOX + AA lure caught significantly more females and the PH/PE + AA lure caught significantly more total moths than the competing lure, respectively. The PH/PE + AA lure caught significantly more male moths in all of the traps tested except for the green bucket.

3.2 MD-FR field trials

Seventeen paired trials were conducted over the three years of the FR study (Table 3). Significant reductions of codling moth fruit injury were found at mid-season and/or at pre-harvest in all years of the study (Fig. 2). Despite the different MD treatments and trap types used across the trials, the number of moths caught per trap and the proportion of females caught were similar across the three years (Table 3). Traps were baited with the PH/PE + AA lure for the first moth flight in both 2018 and 2019 and females constituted 20–25% of the total catch. However, traps in the second moth flight in all three years caught 42–45% females when baited with either PH/PE + AA or PE/DMNT/LOX + AA lures.

The mean proportion of females trapped in the MD-FR plots which were mated ranged from 56–71% (Table 4). The proportion of mated females was significantly lower in both moth flights in 2018 when the maximum rate of the Cidetrak® CMDA Combo™ PP dispensers was deployed. But blocks in 2019 were treated with the minimum-rate of MD dispensers and there was not a significant difference in the proportion of mated females when compared to blocks not treated with MD during the first flight. The proportion of mated females was significantly lower in the MD-FR versus the untreated blocks during the second flight in both 2018 and 2019. During 2020 there was no comparison between FR-MD and outside untreated blocks during the second flight. However, in the first flight the proportion of mated females in the outside block was similar to the data from the two previous years. The data from the nine MD-FR blocks treated with the minimum rate of Cidetrak® CMDA Combo™ Meso™-A dispensers in the second flight in 2020 was low and similar to the full rate of MD used in 2018. Significant differences were found in the proportion of females that were mated

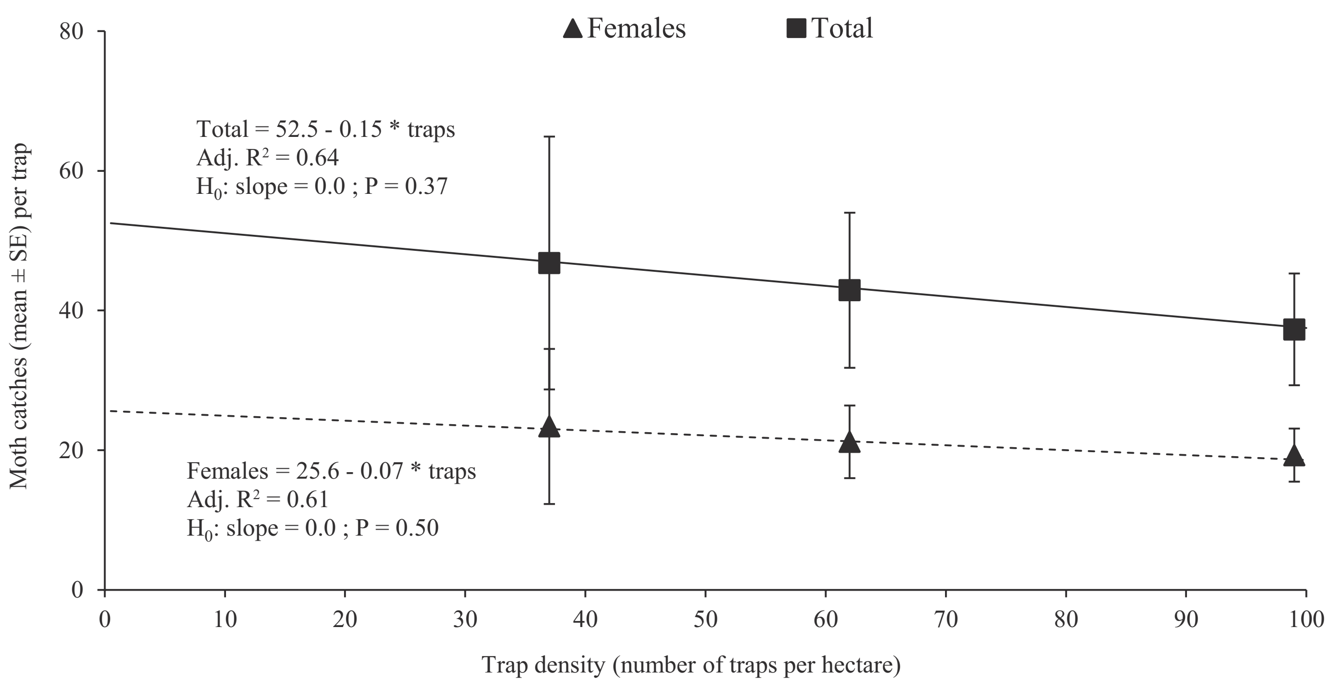

Fig. 1. Captures of codling moth in traps baited with the binary lure comprised of a PVC matrix loaded with (*E,Z*)-2,4-ethyl decadienoate, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and 6-ethenyl-2,2,6-trimethyloxan-3-ol plus a closed plastic membrane lure loaded with acetic acid, in replicated (N = 3) 0.4 ha-plots treated with three density of traps in 2019 in Washington State, USA.

Table 2. Summary of codling moth counts in trap studies conducted during 2020 to compare several trap types baited with (*E,E*)-8,10-dodecadien-1-ol (sex pheromone, PH) and (*E,Z*)-2,4-ethyl decadienoate (pear ester, PE) loaded together in a PVC matrix or a PVC lure loaded with PE, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX); all traps included a membrane lure loaded with acetic acid (AA).

Trap type and color	Trap #	Lure	Mean (SE) catch per trap		
			Males	Females	Total
Orange delta	64	PE/DMNT/LOX + AA	3.5 (0.4)b	3.9 (0.3)Ba	7.3 (0.6)Ab
	47	PH/PE + AA	14.0 (1.3)a	1.6 (0.2)Bb	15.6 (1.4)Aa
All clear bucket	51	PE/DMNT/LOX + AA	1.6 (0.2)c	3.2 (0.4)Ba	4.9 (0.6)Bb
	35	PH/PE + AA	6.5 (1.5)b	3.7 (0.8)Bb	10.2 (2.4)Ba
All green bucket	7	PE/DMNT/LOX + AA	0.9 (0.3)bc	1.7 (0.5)Ca	2.6 (0.6)Bb
	15	PH/PE + AA	3.7 (1.2)bc	0.6 (0.3)Cb	4.3 (1.5)Ba
Green top/clear bucket	25	PE/DMNT/LOX + AA	3.2 (0.6)bc	6.9 (1.4)Aa	10.1 (1.9)Ab
	14	PH/PE + AA	10.0 (1.4)a	5.2 (1.0)Ab	15.2 (2.3)Aa
ANOVA: Trap:			$F_{3, 250} = 20.13$, $P < 0.0001$	$F_{3, 250} = 12.66$, $P < 0.0001$	$F_{3, 250} = 16.73$, $P < 0.0001$
Lure:			$F_{1, 250} = 50.97$, $P < 0.0001$	$F_{1, 250} = 12.58$, $P = 0.0005$	$F_{1, 250} = 10.87$, $P = 0.0011$
Trap*Lure:			$F_{3, 250} = 2.82$, $P = 0.0393$	$F_{3, 250} = 2.55$, $P = 0.0563$	$F_{3, 250} = 1.35$, $P = 0.2602$

Column means followed by a different letter were significantly different, $P < 0.05$, Tukey's test. Significant differences for females and total moth catches were designated with uppercase letters for traps and lowercase letters for lures. All pair-wise comparisons for male catch are shown with lowercase letters due to a significant trap-lure interaction.

Table 3. Summary of trials conducted from 2018-2020 in seventeen paired plots both treated with mating disruption (MD) and either treated or not with female removal (FR) for codling moth using several different lure/trap combinations, including (*E,E*)-8,10-dodecadien-1-ol (sex pheromone, PH), (*E,Z*)-2,4-ethyl decadienoate (pear ester PE), (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX), and acetic acid (AA).

Mean values (SE)								
	1st flight				2nd flight			
Year No. trials	Lure	No. moths per trap	Proportion females	Proportion fruit injury	Lure	No. moths per trap	Proportion females	Proportion fruit injury
2018 N = 4	PH/PE + AA	18.9 (3.7)	0.21 (0.07)	MD-FR: 0.020 (0.002)b MD: 0.045 (0.005)a $t_3 = 10.80$, $P = 0.002$	PH/PE + AA	20.2 (3.9)	0.42 (0.04)	MD-FR: 0.016 (0.002)b MD: 0.055 (0.010)a $t_3 = 6.88$, $P = 0.006$
2019 N = 4	PH/PE + AA	22.8 (3.9)	0.25 (0.05)	MD-FR: 0.012 (0.005)b MD: 0.023 (0.007)a $t_3 = 18.95$, $P = 0.0003$	PE/DMNT/ LOX + AA	21.8 (8.4)	0.42 (0.02)	MD-FR: 0.014 (0.002)b MD: 0.040 (0.011)a $t_3 = 3.70$, $P = 0.034$
2020 N = 9	–	–	–	–	PE/DMNT/ LOX + AA	19.9 (6.5)	0.45 (0.02)	MD-FR: 0.015 (0.006)b MD: 0.071 (0.036)a $t_8 = 3.69$, $P = 0.006$

All traps in 2018 were green/white bucket traps (Multipher) baited with the PH/PE loaded into a septum lure and AA membrane co-lure. Several color bucket traps (Unitrap) were used in 2019 and were all baited with the PH/PE loaded into a PVC matrix and AA membrane co-lure in the first generation and replaced with PE/DMNT/LOX + AA lures for the second half of the season. Orange delta traps (Pherocon) baited with PE/DMNT/LOX + AA lures were used in all plots for the second half of the season in 2020.

at least twice between the MD-FR and untreated blocks in both flights during 2018 and 2019 (Table 4). Levels of multiple mated females were low in 2020 in both the blocks not treated with MD during the first flight and in the MD-FR blocks during the second flight.

4 Discussion

The use of MD-FR was shown over three years and across 17 paired apple trials to be a consistently effective tactic to reduce levels of codling moth injury. These results were sim-

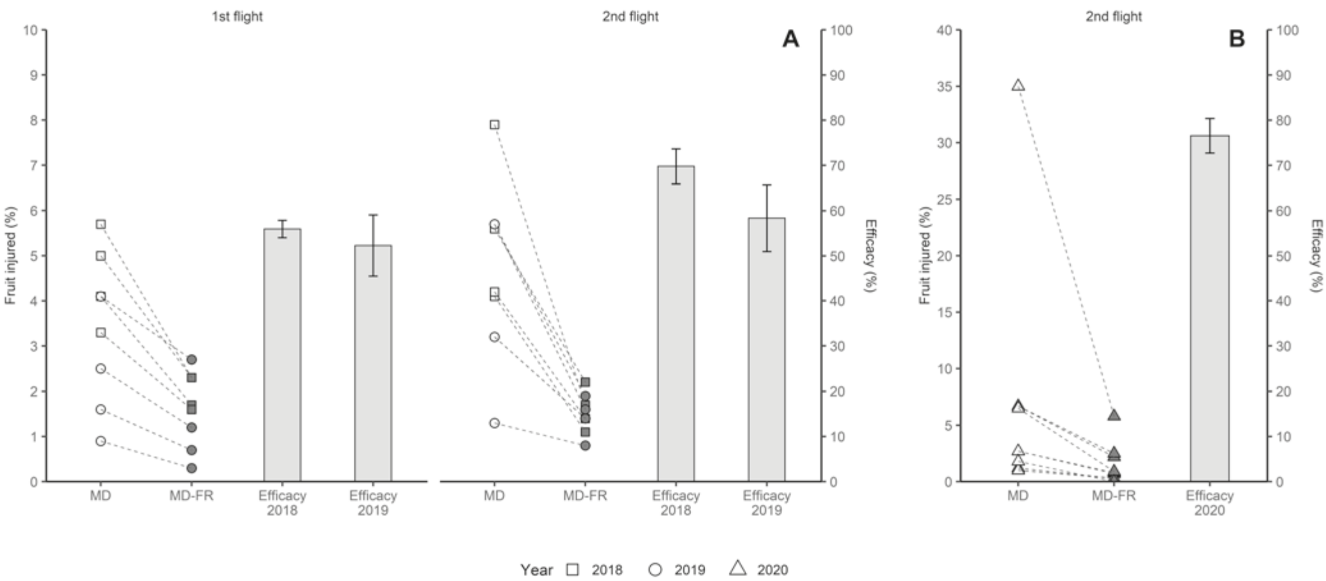

Fig. 2. Summary of codling moth apple injury in seventeen MD-FR trials conducted during 2018–2020 in paired plots treated with mating disruption dispensers (MD) and with or without 60 traps ha^{-1} (female removal, FR) baited with bisexual lures, Washington State, USA.

Table 4. Summary of the mating success of female codling moth collected from apple orchards either untreated or treated with dispensers loaded with sex pheromone (*E,E*)-8,10-dodecadien-1-ol and pear ester (*E,Z*)-2,4-ethyl decadienoate for mating disruption (MD), 2018–2020.

Year	MD, period of treatment [a]	Number of blocks	Mean (SE) proportion 1st generation		Mean (SE) proportion 2nd generation	
			Mated females	Multiple-mated females	Mated females	Multiple-mated females
2018	None	8	0.70 (0.02)a	0.18 (0.01)a	0.82 (0.02)a	0.32 (0.03)a
	Full rate, all season	4	0.56 (0.04)b	0.03 (0.01)b	0.64 (0.04)b	0.10 (0.03)b
	ANOVA df = 1, 10		$F = 16.88$ $P = 0.0021$	$F = 81.47$ $P < 0.0001$	$F = 18.64$ $P = 0.0015$	$F = 20.87$ $P = 0.0010$
2019	None	8	0.77 (0.02)	0.17 (0.04)a	0.90 (0.03)a	0.42 (0.09)a
	½ rate, all season	4	0.69 (0.08)	0.03 (0.01)b	0.71 (0.03)b	0.07 (0.01)b
	ANOVA df = 1, 10		$F = 1.26$ $P = 0.2886$	$F = 11.49$ $P = 0.0069$	$F = 11.60$ $P = 0.0067$	$F = 9.85$ $P = 0.0105$
2020	None	5	0.73 (0.01)	0.04 (0.01)	–	–
	½ rate, 2nd half of season	9	–	–	0.61 (0.05)	0.03 (0.01)

Column means within each year followed by a different letter were significantly different, P < 0.05, Tukey's test.
[a] Orchards were treated with 1,000 and 500 Cidetrak® CMDA Combo™ PP dispensers ha^{-1} in 2018 and 2019, respectively; and 40 Cidetrak® CMDA Combo™ Meso™-A dispensers ha^{-1} in 2020 (Trécé Inc.).

ilar to studies conducted in pear in California, Oregon, and Washington with clear and coloured bucket traps (Preti et al. 2021b). However, levels of reduction in fruit injury in the USA apple trials were nearly double the results from FR pear trials conducted in Italy. This significant difference is thought to be due to the lower attractancy of the PE/DMNTLOX + AA lure demonstrated in Italy compared to the western USA (Preti et al. 2021a).

Many factors were varied in our studies developing MD-FR, including the types of lures, traps, and MD deployed over the three years. No trial likely used an optimal set of these factors to achieve maximum crop protection. For example, all trials in apple and pear were conducted with 60 traps ha^{-1}, but we found that trap density could be increased to at least 99 ha^{-1} without creating trap-to-trap interference (Suckling et al. 2014). The PE/DMNT/

LOX + AA lure can catch 3-fold more females than the PH/PE + AA lure and has not yet been used for apple MD-FR over an entire season in the USA. Also, the use of orange delta traps with removable liners in 2020 was a less effective trap than if the bucket traps with a green top and clear bottom had been deployed.

The earlier FR study by Jaffe et al. (2018) used 120 white delta traps ha^{-1} in apple blocks without MD. The three-part lure of PE + *n*-butyl-sulfide + AA was previously shown to be equivalent in attractancy to the PH/PE + AA lure used in our 2018 study and for the first flight in 2019 (Knight et al. 2019a; 2019b). A white delta trap was previously shown to be less effective than an orange delta in catching female codling moths due a visual repellence (Knight & Fisher 2006). The experimental protocol of Jaffe et al. (2018) required trap liners and lures to be replaced every two weeks, which likely improved the effectiveness of capturing and retaining moths compared to our 2020 study, with 8-week lure replacement schedule and less frequent liner replacements (Knight et al. 2002). The previous work in apple did not combine MD with FR and unfortunately the authors did not report the mating status of female moths (Jaffe et al. 2018). Our results suggests that without MD fewer unmated females would be removed through trapping. In Jaffe et al. (2018) the trapped and untrapped paired plots were 200 m apart and it is not clear if study sites were sprayed with insecticides or were in proximity to other orchards with codling moth infestations. In our study the paired blocks were adjacent, the organic blocks were managed with a minimal set of sprays, and largely surrounded by orchards with similarly high levels of infestation. Another important difference between the two studies may be due to sampling protocols. Jaffe et al. (2018) sampled fruits for injury from each row of the plots, and it is not clear if this included trees along the border. In our study fruits were sampled only from the center of each block to minimize effects from higher infestations along physical edges of the plots and to minimize the effects from significant gradients of injury from borders to the center of blocks. Finally, the Jaffe et al. (2018) studies were conducted only during the 1st flight (11 weeks) or over a small portion of either the 1st or 2nd flight (4-5 weeks). These considerable differences in these two studies are significant; yet the proportion reduction in fruit injury achieved by Jaffe et al. (2018) (57%) was close to our results in apple (55–75%) and previously in pear (65%) (Preti et al. 2021b). These similar outcomes despite broad differences in protocols suggests that future optimization of MD-FR with the implementation of best-practices in terms of each of these factors could achieve an even higher impact on codling moth management.

Implementation of FR with MD appears to be a complementary approach to manage codling moth. The use of sex pheromones to disrupt mating of codling moth has been widely adopted as a key component of integrated management programs in Washington State and worldwide (Brunner et al. 2002; Witzgall et al. 2008). Today, growers can choose to use this behavioral modification approach with a variety of hand applied dispensers, sprayables, or aerosol devices (Miller & Gut 2015). However, preventing mating by female codling moth is difficult. A relatively high proportion (> 60%) of female codling moth captured in our traps placed in blocks treated with MD were mated. These data are consistent with previous studies that found 60–85% of females are mated within MD orchards compared with 84–88% in untreated orchards (Knight 2000; Knight & Light 2005; Knight 2006). A series of studies demonstrated that levels of mating in wild codling moth populations and male catch in virgin female-baited or female equivalent synthetic lures were significantly decreased when PH/PE dispensers versus PH dispensers were used (summarized in Knight & Light 2014). In addition, sprays of the PE MEC formulation also further decreased levels of fruit injury when applied in PH/PE dispenser-treated blocks.

In our current study we did not see a clear pattern in the levels of mating based on the density of PH/PE dispensers. While the proportion of mated females was higher in 2019 with the reduction in the density of Cidetrak® CMDA Combo™ PP dispensers it was lower in 2020 in blocks treated with a reduced rate of the Cidetrak® CMDA Combo™ Meso™-A dispensers. Clearly, more data should be collected to assess the importance of MD in the MD-FR approach, including assessments of the mating status of wild female codling moths in orchards treated with sprayable and aerosol applications of sex pheromone alone and in combination with MEC PE (Knight & Larsen 2004; Kovanci 2015; McGhee et al. 2016).

Reductions in the proportion of female codling moth that had mated more than once was significant in both moth flights in both years between blocks treated with and without MD. The effectiveness of sex pheromone dispensers in reducing the incidence of multiple mating has been noted before and occurs more strongly in the second moth flight (Knight 2007a). Multiple mating of females was shown in this work to significantly increase their fecundity under laboratory conditions. Also, serial male copulations produced smaller spermatophores and resulted in reduced fecundity and increased egg infertility in laboratory assays. Interestingly, females caught in PE-baited traps in MD-treated blocks had on average smaller spermatophores than in untreated blocks, suggesting that a restricted proportion of males are successfully mating. Thus, the full effect of MD on codling moth population dynamics includes four mechanisms: (1) disruption of mating; (2) mating delay and a reduced fecundity / lower egg fertility (Vickers 1997; Knight 1997; Jones & Wiman 2012); (3) lower rate of multiple mating and associated reduced fecundity; in females and (4) increase in multiple mating by presumed older males and a reduced fecundity and increased egg infertility in their partners (Knight 2007a). The overlay and integration of FR with MD should be compatible, and removal of male moths should likely impact each of these mechanisms favourably.

MD works best with low populations of adult codling moth and obviously cannot be an effective tactic if mated females immigrate into the treated orchard (Knight 2007b). Our 17 trials were all conducted in small, paired blocks and typically surrounded on several sides by other pome fruit production and in some areas with high levels of codling moth fruit injury. The use of small plots in field trials can be an important constraint in fully evaluating management programs impacted by either dispersal of semiochemicals or dispersal of pests. Data were not collected from the physical borders of the paired blocks or the internal borders either adjacent to the untreated pair or surrounding orchard. However, it was apparent in the high pest pressure sites that a much higher level of fruit injury was present in trees along the edges of these plots. In some cases, the physical edges of blocks along roadways were at least 10-fold higher. These qualitative observations suggest that FR would need to be used with a higher trap density along borders to intercept female immigration. Future studies should treat larger and more isolated blocks to establish the full efficacy of MD-FR.

The economics of MD-FR must compete with effective programs based on MD, cultural practices, and insecticides (Judd et al. 1997; Jones et al. 2009; Weddle et al. 2009). Traps in our studies removed a mean of 10 females per trap per moth flight or 600 females per hectare for each generation. Of these approximately 30% were unmated. Fecundity of female codling moth in Washington State has been reported to be 25–30 eggs for the spring and 50 eggs for the summer generations, respectively (Ferro et al. 1975; Brown et al. 1978). Dissections of field-collected mated codling moth females found 72% fewer ovarioles than in unmated moths (Knight 2000). Thus, it is possible to roughly estimate the reduction of egg laying that was achieved with this approach (see supplementary material, Table S1). Estimates based on reasonable values for key population parameters and crop production figures (2,000 fruit per bin and 148 bins ha^{-1}) in Washington State (Taylor & Granatstein 2013) suggest that on average about 1.0–5.0% of the crop at mid-season and pre-harvest could escape larval attack in blocks under MD-FR. The mean reductions in levels of fruit injury sampled with MD-FR in each generation of codling moth were nearly identical 1.0–5.5% (Table 3). The value of different cultivars within organic versus conventional markets creates a wide disparity in growers' incentive to invest in multi-faceted integrated crop protection programs (Orpet et al. 2020). Further calculations will need to produce a benefit-cost analysis of MD-FR within this broad market to establish whether it makes financial sense to growers to implement this technology.

MD-FR studies in pear in Italy with the PE/DMNT/LOX + AA lure in all clear bucket traps significantly reduced injury by only 25% (Preti et al. 2021b). Parallel studies demonstrated that this lure and other lures containing pear ester are not as effective in Italy when compared with orchards in the western USA (Preti et al. 2021a). Alternative multi-component lures with several different terpenes or sesquiterpenes replacing LOX were found to be as attractive as the quaternary lure used in our studies (Preti et al. 2021c). Obviously, studies are needed to assess the effectiveness of different kairomone blends for female codling moth in various geographical pome fruit production areas and in other hosts such as walnut, *Juglans regia* L. Companion analytical studies are needed to clarify key physical and chemical attributes of these blends, including lure's release rate, chemical stability and longevity.

Finally, new complex bisexual lures have been developed for other important tortricid species, including oriental fruit moth, *Grapholita molesta* Busck (Padilha et al. 2018; Mujica et al. 2018; Preti et al. 2020), grapevine moth, *Lobesia botrana* Denis & Schiffermüller (El-Sayed et al. 2019; Herrera et al. 2020), and various tortricid leafrollers (Giacomuzzi et al. 2016; Knight et al. 2017). Currently, MD is an important tactic used in management of tortricid pests with moderate to good success (early review by Cardé & Minks 1995). However, the combination of extremely low pest tolerance and female dispersal capabilities creates the potential for MD-FR to be expanded to include these pests in a variety of crops.

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Supplementary table

Table S1. Estimating the impact of MD-FR on reductions in realized oviposition by female codling moth on apple crop in Washington State, USA.

Equation Variable [a]	Variable factor in model	1st flight		2nd flight		
		Max MD 2018	Min MD 2019	Max MD 2018	Min MD 2019	Min MD 2020
A	Mean moth catch per trap	18.9	22.8	20.2	21.8	19.9
B	Proportion of females	0.21	0.25	0.42	0.42	0.45
C	Number of traps per hectare	60	60	60	60	60
D	Estimated fecundity	25	25	50	50	50
E	Proportion of fecundity, unmated females	1.0	1.0	1.0	1.0	1.0
F	Proportion of fecundity, mated females	0.28	0.28	0.28	0.28	0.28
G	Proportion of unmated females	0.44	0.31	0.36	0.29	0.39
H	Proportion of mated females	0.56	0.69	0.64	0.71	0.61
Eggs not laid ha^{-1}	ENL = (A*B*C)*((D*E*G)+(D*F*H))	3,552	4,309	13,743	13,405	15,044
I	Proportion of success (egg to larval entry)	0.90	0.90	0.90	0.90	0.90
J	Apples per bin	2,000	2,000	2,000	2,000	2,000
K	Bins of fruit per hectare	148.2	148.2	148.2	148.2	148.2
Estimated reduction in injury = (I*ENL)/(J*K)		0.011	0.013	0.042	0.041	0.046
Actual reduction in codling moth injury sampled		0.025	0.011	0.039	0.026	0.046

[a] Equation variables 'A–C' and 'G–H' were data from this study. Variables 'D' and 'I' were from Ferro et al. (1975) and Brown et al. (1978). Variables 'E–F' were from Knight (2000). Production variables 'J–K' were from Taylor & Granatstein (2013).