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Evaluating the use of phenylacetonitrile plus acetic acid to monitor *Pandemis pyrusana* and *Cydia pomonella* (Lepidoptera: Tortricidae) in apple

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Abstract

Recent studies have demonstrated that key herbivore-induced foliage volatiles from apple, *Malus domestica* Borkhausen (Rosaceae), can be attractive to conspecific adult male and female tortricid leafrollers when used together with acetic acid lures. Our study reported here was conducted in Washington State during 2013–2014 and assessed the attractiveness of both sexes of the leafroller, *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae), to blends of acetic acid with phenylacetonitrile. Interestingly, traps baited with phenylacetonitrile plus acetic acid caught significantly more males, females, and total moths than the number of males caught in traps baited with a commercial sex pheromone lure. The evaporation rate of the acetic acid co-lure was shown to be an important factor affecting catches of *P. pyrusana* with phenylacetonitrile. Adding phenylacetonitrile to traps baited with pear ester, ethyl (E, Z)-2,4-decadienoate and acetic acid significantly reduced both total and female moth catches of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). However, neither *C. pomonella* nor *P. pyrusana* catch were impacted when phenylacetonitrile and acetic acid were added to traps baited with pear ester plus (E, E)-8,10-dodecadien-1-ol, the sex pheromone of codling moth. These results support our broader work to develop lures to improve monitoring and management of a number of tortricid pests attacking horticultural crops worldwide.

Key Words: kairomones; host plant volatiles; codling moth; leafroller; trapping; sex pheromone

Resumen

Estudios recientes han demostrado que volátiles foliares claves inducidos por herbívoros en manzano, *Malus domestica* Borkhausen (Rosaceae), pueden ser atractivos para hembras y machos adultos conspecificos de enrolladores tortricidos cuando se usan junto con cebos de ácido acético. Nuestro estudio, descrito aquí, se llevó a cabo en el estado de Washington durante 2013-2014 evaluando la atracción de ambos sexos del enrollador, *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae), a mezclas de ácido acético con fenilacetonitrilo. Curiosamente, las trampas cebadas con fenilacetonitrilo y ácido acético capturaron significativamente más machos, hembras y polillas totales que el número de machos atrapados en trampas provistas de un cebo comercial de feromonas sexuales. Demostrándose que la tasa de evaporación del co-atractante de ácido acético era un factor importante que afectaba las capturas de *P. pyrusana* con fenilacetonitrilo. La adición de fenilacetonitrilo a las trampas cebadas con éster de pera, etil (E, Z)-2,4-decadienoato y ácido acético redujo significativamente tanto la captura total como la de hembras de polilla de la manzana, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Sin embargo, ni la captura de *C. pomonella* ni la de *P. pyrusana* se vieron afectadas cuando se añadieron fenilacetonitrilo y ácido acético a las trampas cebadas con éster de pera y (E, E)-8,10-dodecadien-1-ol, feromona sexual de polilla de la manzana. Estos resultados respaldan nuestro trabajo más amplio para desarrollar cebos que mejoren el monitoreo y manejo de varias especies de plagas de la familia Torticididae, que atacan cultivos hortícolas en todo el mundo.

Palabras Clave: kairomonas; volátiles de plantas hospederas; polilla de la manzana; enrollador de hojas; trampeo; feromona sexual

*Pandemis* spp. leafrollers (Lepidoptera: Tortricidae) are an important group of pests attacking pome fruits (apple, *Malus domestica* Borkhausen [Rosaceae], and pear, *Pyrus communis* (L.) [Rosaceae], in North America (Chapman 1973; Dombroskie & Sperling 2012), Europe, and Asia (Dickler 1991). Both species are bivoltine, and overwintering larvae in the fall through spring and summer generations feed on the skin of developing fruits adjacent to leaves, thereby creating culls (Brunner 1996). In the western United States, *Pandemis pyrusana* (Kearfott) (Lepidoptera: Tortricidae), *Pandemis limitata* (Robinson) (Lepidoptera: Tortricidae), and *Charistoneura rosacea* Harris (Lepi-
The key leafroller pest species (Madsen & Madsen 1980; Brunner & Beers 1990) are Spilonota ocellana (Lepidoptera: Tortricidae), are the key leafroller pest species (Madsen & Turner 1999; Judd & Gardiner 2004). Sex pheromone-baited traps are used to monitor adult populations to assess density and phenology of these pests, but pest managers feel these traps are insensitive to local population densities within the orchards (Brunner 1996). Alternative lures, including acetic acid, have been evaluated to monitor both sexes of P. pyrusana (Knight 2001), but acetic acid alone was found to be a relatively weak lure and was not adopted by Washington State growers (Alway 2003). In addition, acetic acid added to traps with pome fruit and green leaf volatiles, or terpenes (substances typically released by healthy or insect-infested fruit or foliage) as attractants for leafrollers did not catch significantly more moths than traps baited with acetic acid alone (Knight et al. 2014). Yet, traps baited with a mixture of (E, E)-8,10-dodecadien-1-ol (the sex pheromone of codling moth, Cydia pomonella [L.] [Lepidoptera: Tortricidae]), pear ester, ethyl (E, Z)-2,4-decadienioate, and acetic acid were evaluated in commercial orchards as a promising new tool (Knight et al. 2014). This multispecies approach to simultaneously monitor coding moth and leafrollers was found to be more effective in characterizing local population densities of leafroller immature stages compared with sex pheromone-baited traps (Knight et al. 2014). Because moth catches were generally low across the orchards monitored in that study, we presumed that an improved monitoring approach could be developed with the identification of more effective attractants for leafrollers.

Several recent studies have identified that the combination of acetic acid with either phenylacetonitrile or 2-phenylethanol produced effective bisexual lures for a number of tortricid pests, including Pandemis heparana (Denis and Schiffermüller) (Lepidoptera: Tortricidae) in Sweden, P. limitata in Canada, and P. pyrusana in Washington State (Giacomuzzi et al. 2013; Giacomuzzi et al. 2016; Judd et al. 2017a; Giacomuzzi et al. 2017). However, the purported pheromone potency of these binary lures has perhaps been overstated (El-Sayed et al. 2016). For example, traps baited with the sex pheromone of C. rosaceana caught significantly more moths (3.5×) than traps with 2-phenylethanol and acetic acid (Knight et al. 2017). Similarly, traps baited with the sex pheromone of P. limitata caught significantly more moths (1.5×) than traps baited with the same binary lure (Judd et al. 2017a). Previously, moth catches of the eye-spotted bud moth, S. ocellana (Knight et al. 2017a). Previously, moth catches of the eye-spotted bud moth, S. ocellana (Judd et al. 2017b).

A second useful attribute of these lures is their attraction to female moths. The sex ratio of moths caught in baited traps has been close to 1:1 in most studies. As previously shown with Cydia pomonella (Lepidoptera: Tortricidae), the catch of female moths can allow pest managers to develop more accurate phenological models and action thresholds (Knight & Light 2005a; Knight & Light 2005b).

Codling moth is generally the key tortricid pest of pome fruit orchards, and growers use a greater density of traps for this species than other tortricid leafrollers (Knight 2008). Some efforts to reduce monitoring costs have previously considered combining sex pheromone lures of C. pomonella and leafrollers in a single trap (Knight & Christianson 1999). Several studies have already examined the possible interaction of these new attractants with sex pheromones and other plant volatiles. For example, traps baited with leafroller sex pheromone caught significantly fewer males when lures loaded with either 2-phenylethanone or phenylacetoni-trile alone (C. rosaceana) or acetic acid plus 2-phenylethanol (P. limitata) were added (Knight et al. 2017; Judd et al. 2017a). The addition of (E)-4,8-dimethyl-1,3,7-nonatriene to traps to enhance the catch of C. pomonella has previously been reported and may be most useful in orchards treated with mating disruption technologies incorporating pear ester (Knight et al. 2015). However, co-monitoring C. pomonella and C. rosaceana in traps including (E)-4, 8-dimethyl-1,3,7-nonatriene significantly reduced the catch of latter species (Knight et al. 2017). These studies suggest that the optimal development of a multi-species trapping approach combining sex pheromones with plant and food volatiles will likely require extensive testing of blend combinations. Furthermore, the correlation of moth catch and lure with pest pressure within orchards remains to be established (Knight et al. 2014). Herein, we report studies conducted from 2012–2014 to measure the response of adult P. pyrusana to acetic acid and phenylacetonitrile compared with sex pheromone baited traps, and to assess the utility of combining lures for P. pyrusana and C. pomonella in the same trap.

Materials and Methods

CHEMICALS AND LURES

Sachet lures loaded with 100 mg phenylacetonitrile (> 99% purity, Sigma Aldrich, St. Louis, Missouri, USA) consisted of a heat-sealed, semi-permeable polyethylene bag (45 × 50 mm, 150 μm wall thickness), with a piece of cellulose acetate filter (15 × 45 mm, Moss Packaging Co. Ltd., Wellington, New Zealand) inserted as the carrier substrate. Three acetic acid lures were used in our studies. Two lures were made by drilling either 1.0 or 3.2 mm holes in the cap of 8 ml Nalgene vials (Nalge Nunc International, Rochester, New York, USA) and loading each vial with 2 small cotton balls and 5 ml of glacial acetic acid (99% purity, Sigma Aldrich). A 3rd acetic acid lure consisted of a plastic membrane cup (3.4 cm diam, Pherocon AA, Trécé Inc, Adair, Oklahoma, USA) loaded with 0.72 ml of this acid. Also, halobutyl grey septa lures consisting of Pherocon CM-DA loaded with 3.5 mg pear ester, Pherocon CM-DA combo loaded with 3.5 mg of both the coding moth sex pheromone (E, E)-8,10-dodecadien-1-ol and pear ester (E, Z)-2,4-decadienioate, and Pherocon PLR (#3147) loaded with 1.0 mg of the P. pyrusana sex pheromone (94:6 blend of (Z)-11-tetradecenyl acetate and (Z)-9-tetradecenyl acetate) were obtained from Trécé Inc.

FIELD EXPERIMENT PROTOCOLS

Studies were conducted in 2 apple orchards: the USDA research farm situated east of Moxee, Washington, USA (46.5061°N,
120.1675°W, and in a commercial orchard located southwest of Naches, Washington, USA (46.7228°N, 120.7009°W) in 2012, 2013, and 2014. Sex pheromone dispensers for mating disruption of *P. pyrusana* and *C. pomonella* were not used in either orchard. Orange delta traps with polybutane-coated sticky inserts (Pherocon VI, Trécé Inc.) were used in all studies. Five replicates of each lure treatment were randomized and spaced 20 to 30 m apart. Traps were placed at a 2 m height in the 4 m canopy. All lures were placed on the liner. Acetic acid vials with 3.2 mm holes were used in all studies unless specified otherwise. Traps baited with a blank sachet lure were used as a negative control in each study. Moths were sexed in all studies.

**MULTI-SPECIES LURE COMBINATIONS**

Studies were conducted to compare moth catches of *P. pyrusana* and *C. pomonella* in blank traps and in traps baited with phenylacetonitrile, pear ester, and acetic acid lures either alone or in binary or ternary lure combinations. The 2012 study included 6 lure treatments: blank, phenylacetonitrile alone, pear ester plus phenylacetonitrile, pear ester plus acetic acid, phenylacetonitrile plus acetic acid, and phenylacetonitrile plus pear ester plus acetic acid. Two additional lure treatments were included in the 2013 study: acetic acid alone and pear ester alone. Traps in 2012 were initially placed in the Moxee orchard on 1 Aug to monitor *C. pomonella*, and traps were checked and re-randomized on 8 Aug. This study was terminated on 13 Aug and traps were moved to the Naches orchard to monitor *P. pyrusana*. Traps were checked and re-randomized in this orchard on 22 and 30 Aug, and the study was terminated on 12 Sep. The 2013 study was conducted from 20–27 Jun at both the Moxee and Naches orchards and from 5–12 and 17–27 Aug in the Moxee orchard.

A 3rd study was conducted from 7–21 Jul 2014 in the Moxee orchard to evaluate moth catches of *C. pomonella* in traps baited with or without phenylacetonitrile added to the sex pheromone-pear ester combinational lure plus the acetic acid cup lure. A 4th study was conducted in the Naches orchard from 30 Aug to 10 Sep 2014, to compare the magnitude of the total bisexual moth catches of *P. pyrusana* in traps with phenylacetonitrile plus the 3.2 mm hole acetic acid vial lure with male catches in traps baited with *P. pyrusana* sex pheromone.

**ACETIC ACID LURE PERFORMANCE**

The mean weekly weight loss (evaporation rate) of acetic acid from the 3 lures was recorded from 30 Aug to 26 Sep 2013. Individual lures (N = 10) were placed in red Pherocon VI delta traps (Trécé Inc.) without sticky liners that were hung in the canopy of 5 linden trees, *Tilia cordata* Miller (Malvaceae), at the Yakima Agricultural Research Laboratory, Wapato, Washington, USA. Similarly, the weight loss of sachet lures loaded with phenylacetonitrile (N = 5) was recorded from 28 Aug to 19 Sep 2013.

A field study was conducted in the Moxee orchard in 2013 to compare moth catches in delta traps baited with sachet lures loaded with phenylacetonitrile and 1 of 3 different acetic acid lures: the membrane cup lure and vials with 1.0-mm and 3.2-mm apertures. Experiments were run for 1 wk beginning on 3 dates: 28 Aug, 4 Sep, and 12 Sep.

**STATISTICAL ANALYSES**

Count data were subjected to a square root transformation prior to analysis of variance (ANOVA) to normalize the variances. A randomized, complete block design was used in most studies with date and orchard as the blocking variable. Following a significant F-test in the ANOVA, means were separated with Tukey’s HSD test with an alpha value of 0.05. A completely randomized design was used to test the effect of adding phenylacetonitrile to traps baited with the sex pheromone, pear ester, and acetic acid for *C. pomonella*. These data could not be normalized and a Kruskal-Wallis nonparametric test was used instead. Treatments with zero catches or only incidental catch (mean total catch < 0.2 moths) were not included in these analyses.

**RESULTS**

**MULTI-SPECIES LURE COMBINATIONS**

Consistent data were collected in 2012 and 2013 when comparing catches of *C. pomonella* with phenylacetonitrile alone and with binary and ternary blends including pear ester and acetic acid (Table 1). Pear ester in combination with acetic acid caught significantly more total and female *C. pomonella* than other lures. Adding phenylacetonitrile to pear ester and acetic acid significantly reduced total and female moth collections. Moth abundance was low in traps baited with phenylacetonitrile only and in binary blends with either acetic acid or pear ester but did not differ significantly from each another. In 2013, *C. pomonella* abundance from traps baited with only phenylacetonitrile was similar to that from traps with acetic acid or pear ester alone. The addition of phenylacetonitrile to traps baited with the sex pheromone-pear ester combination lure (Pherocon CM-DA) plus acetic acid did not significantly reduce male (*K* = 0.10; *P* = 0.75), female (*K* = 2.47; *P* = 0.12), or total (*K* = 0.40; *P* = 0.53) moth catches in 2014 (Fig. 1).

Counts of *P. pyrusana* were more varied in traps than those of codling moth during both years of the study (Table 1). During 2013, traps baited with phenylacetonitrile plus acetic acid, or in combination with pear ester, caught significantly more total and female moths compared with the rest of the treatments and did not differ among themselves. However, moth catches in 2012 were lower than in 2013 and no significant difference among treatments was found for female moth collections. Also, during that period total moth catch, in traps baited with pear ester plus acetic acid, did not differ significantly from the 4 treatments that included phenylacetonitrile.

**Fig. 1.** Mean (SE) number of male, female, and total *Cydia pomonella* caught in traps with a sex pheromone plus pear ester combo lure and an acetic acid (AA) co-lure versus in traps with these same lures with the addition of a phenylacetonitrile (PAN) sachet lure, 7–21 Jul 2014, Moxee, Washington, USA. ‘N.S.’ denotes a nonsignificant difference in moth catches between the 2 types of lures, *P* > 0.05.
Comparison of Acetic Acid and Phenylacetonitrile
With Sex Pheromone

Pandemis pyrusana from traps placed in the Naches orchard were considerably greater in 2014 compared with 2013 (Fig. 2). Total moth catch was significantly different and nearly 6-fold higher in traps baited with phenylacetonitrile and acetic acid than the sex pheromone ($F_{1,8} = 54.64; P < 0.001$). Mean catch of male ($F_{1,8} = 9.30; P < 0.05$) and female ($F_{1,8} = 20.01; P < 0.01$) moths were significantly greater with this binary lure compared with male collections.

**LURE PERFORMANCE**

The average evaporation rate of acetic acid from the co-lures used with phenylacetonitrile was a significant factor affecting moth catches of *P. pyrusana* (Table 2). Evaporation rates ranged 18-fold across the 3 dispensers tested. Moth catches were significantly lower in traps containing acetic acid lures with the lowest evaporation rate (plastic cup lure) and did not differ between traps with different hole sizes (Table 2). Mean evaporation rate of phenylacetonitrile from the sachet lure was $6.3 \pm 0.2$ mg per d.

**Discussion**

Our results show that the use of a binary lure of phenylacetonitrile plus acetic acid has potential use in developing improved pest management of *P. pyrusana*. Traps baited with phenylacetonitrile and acetic acid caught greater numbers of moths than traps baited with sex pheromone in an orchard not treated with leafroller sex pheromones. In comparison, sex pheromone lures were consistently more attractive for males and caught significantly more total numbers of moths than the use of acetic acid with either phenylacetonitrile or 2-phenylethanol when tested with *C. rosaceana*, *S. ocellana*, or *P. limitata* (Knight et al. 2017; Judd et al. 2017a; b). These data may suggest that the commercial lures for *P. pyrusana* are not optimized, or that other species in the *P. limitata* (Robinson) group with different sex pheromone blends are more dominant in the 2 orchards studied (Dombroskie & Sperling 2012). The sex pheromones of *P. limitata* and *P. pyrusana* have been identified as 91:9 and 94:6 blends of (Z)-11-tetradecenyl acetate and (Z)-9-tetradecenyl acetate, respectively (Roelofs et al. 1976, 1977). However, previous studies conducted in 1996 with these 2 blend ratios found that *P. pyrusana* caught similar numbers of *Pandemis* adults in a different Washington apple orchard (Knight unpublished). Curiously, at least 1 commercial lure has previously been reported to be relatively ineffective for *P. pyrusana* due to contamination with < 1.0% of (Z)-9-dodeceny acetate (Brunner & Fisher 1998). Because of the uncertainty in comparative performance of sex pheromone lures, it is likely that further studies are needed to validate our findings.

Apple orchards are commonly attacked by more than 3 species of leafroller, so this group of pests must be carefully monitored...
during the season (Chapman 1973). Studies have found either that acetic acid plus 2-phenylethanol performs better than phenylacetonitrile and acetic acid (Knight et al. 2017), similarly (Giacomuzzi et al. 2013, 2016; Giacomuzzi et al. 2017), or worse (El-Sayed et al. 2016) with different tortricid species. A ternary blend of acetic acid and both compounds also can significantly increase moth catches compared with either binary blend (Judd et al. 2017b). Clearly, additional studies are needed to optimize these aromatic plus acetic acid lures for various geographical regions attacked by different suites of tortricid leafrollers. In addition, more studies will be needed to assess their utility in monitoring tortricids in orchards treated with their respective sex pheromones. Ultimately, we expect that developing improved timing models and pest thresholds based on these lures can significantly improve management of orchards with reduced insecticide inputs.

The ability of pest managers to monitor multiple species within single traps could fuel the adoption of labor-saving, remote electronic traps (Guarnieri et al. 2011; Kim et al. 2011). Our study found that adding pear ester to traps baited with phenylacetonitrile and acetic acid did not impact the catch of *P. pyrusana*. Nor did it affect the catch of *C. pomonella* when phenylacetonitrile and acetic acid were added to traps baited with the combination sex pheromone plus pear ester lure. This latter combination lure is considered to be the industry standard for orchards using mating disruption on codling moth (Knight et al. 2005). Future studies should consider the creation of bait stations for even more co-occurring tortricid pests within orchards, such as the oriental fruit moth *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae). Development of binary pheromone lures combining the sex pheromone of *C. pomonella* and *G. molesta* already has been developed (Knight et al. 2015), and testing of various host plant volatiles for this latter species have been reported (Lu et al. 2014; Yu et al. 2015). Further testing and refinement of this multi-species approach could lead to the development of both lower-cost monitoring programs and perhaps multi-species management tools, i.e., lure and kill strategies.

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