Multi-Species Mating Disruption in Cranberries (Ericales: Ericaceae): Early Evidence Using a Flowable Emulsion

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Abstract

Pheromone-based mating disruption has proven to be a powerful pest management tactic in many cropping systems. However, in the cranberry system, a viable mating disruption program does not yet exist. There are commercially available pheromones for several of the major pests of cranberries, including the cranberry fruitworm, Acrobasis vaccinii Riley (Lepidoptera: Pyralidae) and blackheaded fireworm, Rhopobota naevana (Hübner) (Lepidoptera: Tortricidae). Previous studies have shown that mating disruption represents a promising approach for R. naevana management although carrier and delivery technologies have remained unresolved. The present study examined the suitability of Specialized Pheromone & Lure Application Technology (SPLAT; ISCA Technologies, Inc., Riverside, CA), a proprietary wax and oil blend, to serve as a pheromone carrier in the cranberry system. In 2013 and 2014, we tested a blend of pheromones targeting A. vaccinii and R. naevana in field-scale, replicated trials. Pheromones were loaded into SPLAT and the resulting “SPLAT BFW CFW” formulation was deployed in commercial cranberry marshes. We compared moth trap-catch counts within SPLAT-treated blocks to those of conventionally managed blocks. In 2013, applications of SPLAT BFW CFW resulted in highly successful disruption of R. naevana and promising, though inconsistent, disruption of A. vaccinii. To improve disruption of A. vaccinii, the pheromone load was increased in 2014, providing 92% and 74% reductions in trap-catch for R. naevana and A. vaccinii, respectively. Importantly, larval infestation rates in SPLAT-treated blocks were lower than those of conventionally managed blocks. These results suggest that a multispecies mating disruption system (SPLAT BFW CFW) may represent an effective pesticide-alternative for serious pests of cranberries.

Key words: blackheaded fireworm, cranberry fruitworm, pheromone, SPLAT

Mating disruption is a pest management technology in which the sex pheromones of insect species are released within a cropping system, limiting the capacity of the targeted species to find mates and reproduce (Miller and Gut 2015). The proposed mechanisms by which mating disruption operates include camouflage of the female’s pheromone signal (such that mating is significantly delayed or preempted altogether), sensory habituation, and blocking of the receptor sites on the male’s antennae (Minks and Cardé 1988, Cardé and Minks 1995, Sanders 1997, Miller and Gut 2015). Given its unique mode of action and reliable efficacy, mating disruption can be incorporated into existing pest management programs without interfering with other pest management strategies, such as biological control (Brunner et al. 2002, Jones et al. 2010). Use of mating disruption can lead to a reduction in the number of conventional chemical insecticide applications needed to prevent economic damage to the crop, while helping to forestall resistance to insecticides (Suckling et al. 1990).

A critical element in the success of any mating disruption system is the carrier/dispenser. Such carrier technologies must be suitable for the cropping system and easily incorporated into management practices. This often represents a significant obstacle, but in the cranberry system, it appears to have been resolved using a proprietary wax and oil emulsion, referred to as SPLAT (Specialized Pheromone & Lure Application Technology; ISCA Technologies, Inc., Riverside, CA). SPLAT is a food-grade wax and oil emulsion into which synthetic pheromones can be impregnated (Mafra-Neto et al. 2013). The SPLAT matrix serves as a slow-release carrier of pheromones (Stelinski et al. 2010, Deutsch 2014) and protects the...
pheromones from premature degradation due to rain or ultraviolet radiation, allowing the pheromones to persist within its matrix for months. Additionally, the SPLAT matrix is biodegradable, so it does not interfere with harvest and can be applied mechanically (Teixeira et al. 2010). Mixed with insect sex pheromones, SPLAT formulations have been shown to successfully disrupt mating of insect species in a variety of agricultural systems, including the oriental fruit moth (Grapholita molesta Busck) (Stelinski et al. 2007), grape berry moth (Paralobesia viteana Clemens) (Jenkins and Issacs 2008, Teixeira et al. 2010), citrus leaffminer (Phyllocnistis citrella Stanton) (Stelinski et al. 2010), and gypsy moth (Onufrieva et al. 2010, Miller and Gut 2015). Further, the per-acre costs of SPLAT for growers appear to be comparable to those of conventional pest control technologies, as evidenced by the worldwide use of SPLAT and continued commercial availability of this and similar pheromone-based technologies (e.g., see online catalogs of ISCA Technologies, Inc.).

Typically, mating disruption in a given cropping system focuses on a single pest species. Multi-species mating disruption systems must contend with a variety of additional issues, such as asynchrony in the timing of pest flights, unavailability of pheromones from multiple pest species, and interference among pheromone components (Mafra-Neto et al. 2013). Further, any multispecies mating disruption system will require the integration of multiple pheromone blends, necessitating a greater volume of semiochemicals (pheromones) needed for effective disruption, which can become cost-prohibitive. Semiochemicals, per unit mass, tend to be much more expensive than insecticides (Miller and Gut 2015), so the cost-benefit tradeoff can impose significant constraints on the amount of semiochemicals that can be deployed to achieve pest suppression. Thus, in crops with multiple significant pests, an effective multispecies mating disruption system could be highly advantageous if it could preclude the expense of “clean-up” sprays, which are sometimes needed when single-species mating disruption systems allow secondary pests to proliferate.

In U.S. cranberries, there are multiple significant insect pests that are perennial problems for the industry. The cranberry plant (Vaccinium macrocarpon Aiton) is native to North America and is attacked by many native herbivores, notably three lepidopteran species: cranberry fruitworm (Acrobasis vaccinii Riley), blackheaded fireworm (Kropotobata naevana Hübner), and Sparganothis sulfureana Clemens (Eck 1990). The cranberry fruitworm, in particular, is generally considered the top pest threat for Wisconsin growers (Chasen and Steffen 2016). Fortuitously, the pheromones of these moth pests have been isolated, characterized, and tested for use within lures or mating disruption programs (McDonough et al. 1987; Fitzpatrick et al. 1993, 2004; Polavarapu et al. 2001). In addition, the spring flights of these three moth species are synchronous in Wisconsin (Steffan et al. 2017), which means that a single application of a pheromone-loaded carrier should be able to simultaneously provide mating disruption of all three species.

Previous work in cranberries has targeted either R. naevana, the blackheaded fireworm (Fitzpatrick et al. 1995, 2004; Baker et al. 1997; Fadamiro et al. 1998), or S. sulfureana (Polavarapu et al. 2001). These studies reported very promising evidence that mating disruption represents an effective, viable approach to cranberry pest management. Despite the early successes with blackheaded fireworm and sparganothis fruitworm disruption, currently there is no mating disruption system commercially available for cranberry growers. This is likely the result of many factors, including logistical and economic issues associated with the carrier system, regional differences in pest complexes (Deutsch 2014), and the lack of MD efficacy data for what is arguably the most significant pest of the U.S. cranberry crop: A. vaccinii, the cranberry fruitworm.

The objective of the research presented here was to test the capacity of a flowable emulsion (SPLAT) to provide effective mating disruption of the blackheaded fireworm and cranberry fruitworm by volatilizing sex pheromones within the cranberry canopy during the spring mating flights of these species. This work spanned 2 yr, focusing on two of the most consistent pests of cranberries in the upper Midwest, USA. The sparganothis fruitworm was not included in the current study because preliminary testing had indicated this moth’s response to the SPLAT formulation was highly variable, which appeared to be too time-intensive and expensive to resolve at the time. Given funding constraints, the near-term solution to the issue was to increase the pheromone load of the cranberry fruitworm rather than spend inordinate amounts of time and resources investigating the appropriate sparganothis pheromone blend.

Previous studies have shown that the SPLAT matrix can hold and slowly volatilize pheromones for 8+ wk, even when exposed to direct sunlight, heat, rain, and wind (Deutsch 2014). Efficacy data for these two species will serve as the basis for future work in which additional pest species (e.g., sparganothis fruitworm) and new, mechanized deployment systems are investigated as candidates for an area-wide, multi-species mating disruption system for cranberries.

Materials and Methods

“SPLAT BFW CFW” Formulation

The molecular identities and loading concentrations of the sex pheromones for the targeted species (A. vaccinii and R. naevana) were based on previous studies (McDonough et al. 1994, Fitzpatrick et al. 1995). The active ingredients (AIs) for the cranberry fruitworm (A. vaccinii) pheromone blend were E8,Z10-15:Ac and E9-15:Ac, combined at a 100:4 ratio (McDonough et al. 1994). These compounds were blended and applied at 25.7 g Al/ha in 2013. The rate for the A. vaccinii pheromone blend was increased 40% to 36.0 g Al/ha in 2014. The AIs of the blackheaded fireworm (R. naevana) pheromone were 69.2% Z11-14:Ac, 23.1% Z11-14:OH, and 7.7% Z9-12:Ac, applied at 74.1 g Al/ha in both 2013 and 2014. All sex pheromone components were incorporated into SPLAT, and the final formulation (SPLAT BFW CFW) was applied at 2.47 kg/ha, in “dollops” of 1 g each. Respectively, 20.2 and 20.0 ha were treated with SPLAT BFW CFW in 2013 and 2014.

Site Selection

This research project was performed on commercial cranberry marshes in central Wisconsin. Given that the project was conducted at large spatial scales on commercial acreage, there was the potential that completely untreated controls would impose undue risk on grower revenue; thus, it was necessary to consider standard grower practice vs. standard grower practice

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Marshes were selected based on similarity in historical pest pressure, bed size, and total marsh size. All beds selected were of the...
“Stevens” variety, and each tended to be 1.5–2 ha. In 2013, SPLAT BFW CFW was applied at six marshes, three in Wood County and three in Monroe County. In 2014, SPLAT BFW CFW was applied at five marshes (three of which were repeated from the previous year). At each marsh, 2–5 contiguous beds were treated, and a buffer distance of approximately 460 m (ranging from 130 to 730 m) was maintained between the treated beds and the corresponding conventionally managed (control) beds. The SPLAT BFW CFW beds were located near a marsh edge to prevent excessive moth immigration into the treated beds from neighboring untreated beds. Both years, the SPLAT BFW CFW beds and conventional beds were managed for insect pests (via an insecticide application or spring flood) before SPLAT BFW CFW was applied. In 2013, the SPLAT-treated beds were managed with insecticides only if the moth populations were above acceptable levels, as determined by crop consultants and growers, but the conventional beds were treated with insecticides at the growers’ discretion. However, because this created irregular, asymmetric pest management effort within and between treatments, the treatment regimen was refined in 2014 such that the SPLAT BFW CFW beds were treated with insecticides whenever the conventional beds were treated with insecticides. Thus, in 2014, the effect of SPLAT BFW CFW represented the added marginal benefit of having mating disruption operating across insecticide-managed acreage. All beds had been treated similarly with regard to fertilizer, fungicide, or herbicide.

**SPLAT BFW CFW Application**

SPLAT BFW CFW was applied in 1 g dollops using 18-V grease guns (Lincoln; Gempler’s, Janesville, WI) and deposited on the woody runners (stolons) of the cranberry canopy, forming a grid of dollops comprised of 16 rows running length-wise down each cranberry bed. SPLAT rows were evenly spaced across the beds, and given that bed dimensions varied somewhat, the spacing between treated rows ranged from 3 to 4 m. Distance between dollops within a row were approximately 2 m. Both years a total of 1 kg of SPLAT BFW CFW was applied per 0.4 ha, resulting in approximately 1,000 point sources per 0.4 ha. Each dollop was dispensed directly onto the woody “runners” (stolons) of the cranberry vines (Fig. 1). SPLAT BFW CFW was applied just before the beginning of adult flight for the two species. The initiation of their spring flights was predicted using DD benchmarks determined in previous phenology studies (Deutsch et al. 2014, Steffan et al. 2017). In 2013, SPLAT BFW CFW was applied in the week of June 10. In 2014, it was applied the week of May 19, approximately 2 wk before flight began.

**Sampling**

Mating disruption efficacy was based on male moth counts in pheromone-baited traps, as well as berry infestation rates. Trap-catch data were collected in both 2013 and 2014; berry infestation data were collected in 2014.

**Pheromone Trapping.** Two sets of Pherocon II traps (Great Lakes IPM, Vestaburg, MI), each containing lures for both species (2 traps/species × 2 species = 4 traps in a given block) were staked along the edge of the innermost bed for each marsh-treatment. Trap sets were placed on either end of the bed to ensure adequate representation for each marsh-treatment. Traps were checked and emptied weekly, and replaced as needed. Lures were replaced every 3 wk per manufacturers’ recommendations. Trap counts were averaged per species per marsh-treatment.

In 2013, *A. vaccinii* lures were purchased from Great Lakes IPM, and *R. naevana* lures were purchased from Scentry Biologicals, Inc. (Billings, MT). However, 5 wk after the SPLAT BFW CFW application, all of the CFW traps (including the conventionally managed control blocks) produced no moths. At this point, the Great Lakes IPM cranberry fruitworm lures were replaced by lures made by ISCA Technologies. In 2014, all lures were supplied by ISCA Technologies, and trapping continued for the subsequent 5 wk.

**Damaged Berry Assessment.** In 2014, to assess damaged berries, prematurely red berries were collected in treated and conventional beds. Damaged cranberries turn red as a stress response, so they are readily visible upon inspection (Franklin 1948, Neunzig 1972). Berries were collected by walking the edge of the innermost bed for each marsh-treatment, randomly stopping 40 times along this transect and visually scanning for 10 s at each stop. We repeated this sampling protocol three separate times over the course of 3 wk. Damaged berries were reported as number of insect-damaged berries per sampling effort. In the second week of berry sampling, only four of the five sites could be sampled because a recent insecticide application precluded entry at one of the marshes.

**Statistical Analysis**

*Pheromone Trapping.* Because pheromone loading in SPLAT BFW CFW differed between years, data were analyzed separately for each year. All replicates were analyzed within a randomized complete block design. Trap catches for both species were analyzed as mixed-effects models, in which the random effect was treatment nested within marsh and included an autocorrelation to account for repeated measures (Pinheiro and Bates 2000). The response variable was the *A. vaccinii* or *R. naevana* moth-counts, square-root transformed in order to best meet model assumptions. Fixed effects were the main effects of treatment and week and their interaction. While analyses were performed on transformed data, untransformed data are represented in figures.

*Damaged Berry Assessment.* Damaged berry counts were analyzed via one-way repeated-measures analysis of variance (ANOVA). After determining that Marsh was not a significant predictor of damaged berry response, independent variables were confined to treatment and week (and their interactions) on the number of damaged berries. To account for non-normality of data, rank
transformations were performed. Data points with standardized residuals > 3.0 were considered outliers and removed from the data set because damaged berries often occur in hotspots and we wanted the sample to be representative; this resulted in the removal of two data points. All analyses were conducted in R version 3.2.2 (R Core Team 2015) with the package nlme (Pinheiro et al. 2014). Figures were created with package ggplot2 (Wickham 2009).

Results

2013

**Rhopobota naevana**

Significantly more *R. naevana* moths were caught in conventionally treated beds than in SPLAT-treated beds during the 2013 flight (June 24–July 16) ($F_{1,5} = 8.19$, $P = 0.04$) (Fig. 2a). Mean trap-catch in the conventional beds was 1.17 ± 0.42 (± 1 SE) moths, while in the SPLAT BFW CFW beds mean trap-catch was 0.25 ± 0.10 moths, representing a 79% reduction in the number of moths caught in disrupted beds. Across all traps, populations of *R. naevana* were low throughout the season, regardless of treatment. Difference in trap catches did not vary by week ($F_{3,10} = 1.27$, $P = 0.30$) (Fig. 2a).

**Acrobasis vaccinii**

During the first 5 wk in 2013, the commercial pheromone lures (manufactured by Great Lakes IPM, Inc.) that had been deployed for *A. vaccinii* did not attract moths at any site. At week-6 of trap deployment, lures were replaced with those of a new supplier (ISCA Technologies, Inc.), at which point moths were immediately caught. Thus, during the first month of the *A. vaccinii* flight, it was not possible to compare SPLAT BFW CFW beds to conventional beds, which essentially truncated the duration of treatment effects by approximately one-half. Following the lure-swap, data were available to assess the impacts of SPLAT BFW CFW (July 15 to August 13). There was no significant difference in cranberry fruitworm trap-catch between the SPLAT BFW CFW and conventional beds ($F_{1,5} = 3.30$, $P = 0.13$) and this trend did not vary significantly across time ($F_{4,38} = 0.65$, $P = 0.63$) (Fig. 2b). Mean trap-catch in conventional beds was 25.1 ± 5.2, while in SPLAT BFW CFW beds, mean trap-catch was 11.8 ± 3.5, representing a 53% reduction.

2014

**Rhopobota naevana**

Populations of *R. naevana* populations were more abundant in 2014 than in 2013. Based on trap counts, the male flight lasted 8 wk, from June 9 to July 28. Male numbers in the conventional beds were significantly higher than those in the SPLAT BFW CFW beds ($F_{1,4} = 8.78$, $P = 0.04$) (Fig. 3a). Across all sites and sample dates, mean trap-catch in SPLAT BFW CFW beds was 0.15 ± 0.07 (± 1 SE) moths while mean trap-catch in the conventional beds was 1.80 ± 0.58 moths, which represented a 92% reduction in the number of moths caught in SPLAT BFW CFW beds. Difference in trap catches did not vary as a function of time ($F_{7,56} = 1.42$, $P = 0.22$).

**Acrobasis vaccinii**

Male *A. vaccinii* moths were caught in traps for six weeks, from June 9 to July 30. There were significantly more moths caught in conventional blocks than in SPLAT BFW CFW blocks ($F_{1,4} = 37.71$, $P < 0.01$), and the degree of this effect varied significantly over time ($F_{6,48} = 2.59$, $P = 0.03$) (Fig. 3b). Across the summer and sites, mean moth trap-catch in SPLAT BFW CFW beds was 9.2 ± 2.0 (± 1 SE) while mean trap-catch in conventional beds was 35.3 ± 4.6. Disruption across the season averaged 74%.

**Damaged Berries**

Initial analysis showed that marsh was not a significant predictor of the number of damaged berries ($F_{4,14} = 1.90$, $P = 0.17$) so analysis proceeded with a repeated measures ANOVA. The number of damaged berries found in the conventional beds was significantly greater
than in beds treated with SPLAT BFW CFW ($F_{1,20} = 5.78, P = 0.03$). The mean number of damaged berries found in SPLAT BFW CFW beds over the three sample weeks was $12.6 \pm 2.6$ (± 1 SE), while the mean number found in conventional beds was $25.4 \pm 4.8$. This represented a 50% reduction in damaged berries. Neither week ($F_{2,20} = 3.00, P = 0.07$) nor the interaction of treatment x week ($F_{2,20} = 1.64, P = 0.22$) was significant. It is important to note that cranberry fruits are damaged directly by both $A. vaccinii$ and another pest ($S. sulfureana$). To distinguish between $A. vaccinii$ and $S. sulfureana$ damage in those instances where the larva was no longer present within the berry, we relied on a distinctive behavioral attribute of $A. vaccinii$ larvae: the presence of frass within the berry (Neunzig 1972). Based on the frequency of frass observed in damaged berries, the vast majority of the berry damage in our study was due to $A. vaccinii$.

Discussion

This study represents the first field deployment of a multi-species mating disruption system in cranberries, and the first time that a mating disruption system targeting the cranberry fruitworm has been shown to be successful. Further, it is the first reported use of SPLAT as a pheromone carrier in cranberries. Our data indicate that the multi-species pheromone blend within the SPLAT BFW CFW formulation provided significant evidence of mating disruption for two major cranberry pests. For both $R. naevana$ and $A. vaccinii$, reduced numbers of moths were caught in SPLAT BFW CFW beds in each year of the study, suggesting that males of each species suffered reduced mate-finding capacities. As further evidence of mating disruption, in 2014, we observed a significantly decreased number of damaged berries in the beds treated with SPLAT BFW CFW.

Across all beds and years, populations of $R. naevana$ were relatively low at our study sites. Nonetheless, significantly fewer moths were caught in the SPLAT BFW CFW beds than in the conventionally managed beds. Based on these results, future mating disruption work with $R. naevana$ should maintain the respective pheromone concentrations used in the present study. Indeed, the average level of disruption found in our study was similar to that of previous studies (Fitzpatrick et al. 1995, 2004; Baker et al. 1997), demonstrating that $R. naevana$ populations can be readily suppressed using mating disruption. $R. naevana$ is a serious pest in most North American cranberry growing regions, so mating disruption using SPLAT BFW CFW has the potential to be a valuable resource for the entire cranberry industry.

Similarly, $A. vaccinii$ trap-counts in 2014 indicated that $A. vaccinii$ can be significantly disrupted using SPLAT BFW CFW. In 2013, the pheromone load was lower, and while it did reduce trap-catch by 53% compared to that of conventional blocks, this level of disruption was not high enough to be considered adequate. To address this, in 2014, the pheromone concentrations were increased, and this increase led to significant disruption (74%). Additionally, in 2014 we found significantly fewer damaged berries in SPLAT-treated beds than in conventional beds (a 50% reduction in berry infestation). This reduction represented a substantial improvement in the level of protection conferred by conventional cranberry pest management.

Future work will incorporate three refinements. First, the particular blend of $S. sulfureana$ sex pheromones, and ratios thereof, will need to be explored further, hopefully converging on a recipe that is more alluring for the male moths. Such work will be based on various preliminary investigations in 2012 and 2013 (Deutsch 2014). This pheromone blend will be added to the current mix of $A. vaccinii$ and $R. naevana$, making the SPLAT “cocktail” a three-species blend. Doing so will allow a single SPLAT treatment to address three major insect pests of cranberries. The second refinement to the current mating disruption system will be an increase in the total area treated. Whole-marsh treatments, and eventually area-wide applications will serve to eliminate breeding hotspots within cranberry growing regions. Mating disruption is most effective when implemented at very large, area-wide spatial scales because it limits the possibility that mated females can emigrate from untreated areas into treated areas (Welter et al. 2005). Therefore, increasing the area treated has the potential to not only cast a broader

Fig. 3. (a) 2014 adult male blackheaded fireworm ($R. naevana$) (mean ± 1 SE) caught in pheromone traps. Trap-catch in conventionally managed (i.e., insecticide-only) control beds (solid line) and SPLAT-treated beds (broken line) were compared over time. (b) 2014 adult male cranberry fireworm ($A. vaccinii$) (mean ± 1 SE) caught in pheromone traps. Trap-catch in conventionally managed control (solid line) and SPLAT-treated (broken line) beds were compared over time.
“net,” but also close gaps in this net. Finally, future work will include testing of methods to mechanize the deployment of SPLAT, including via unmanned aerial vehicles and retrofitting of boom sprayers. Such trials will make the deployment of mating disruption systems more efficient, thereby facilitating integration into commercial-scale IPM programs.

Acknowledgments

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