Behavioral and preventative management of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in Maine wild blueberry (*Vaccinium angustifolium* Aiton) through attract and kill trapping and insect exclusion-netting

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The management of spotted wing drosophila (SWD) (*Drosophila suzukii* Matsumura) in small fruit agriculture continues to receive significant attention. While complementary monitoring and insecticidal application procedures can provide effective protection against this insect pest in some cropping systems, more sustainable alternatives are currently under development. In this investigation, we explored two methods of managing *D. suzukii* in Maine wild blueberry (*Vaccinium angustifolium* Aiton): 1) the efficacy of attract and kill trapping with volatile semiochemicals and 2) the use of insect exclusion netting. We found that exclusion netting provided a high degree of protection against SWD larval infestations. Consistent and significant reductions in larval infestations were observed during field trials conducted in 2014, 2015, and 2016. The second method, attract and kill trapping, appeared to reduce infestations in a preliminary trial in 2013. However, this result was not observed in a more thorough, replicated study in 2014. The capture rates of traps increased with trap deployment density in blueberry plots, suggesting that more traps per unit area concentrate flies in the local trapped area. As a result, a correlation existed between adult captures and larval infestation despite a contact poison (boric acid) coating the outside surface of the traps.

**Keywords:** Lowbush blueberry, wild blueberry, North America, *Drosophila suzukii*, Spotted Wing Drosophila, mass trapping, attract and kill, insect exclusion netting

**INTRODUCTION**

*Drosophila suzukii* Matsumura, commonly referred to as the spotted wing drosophila (SWD), is a recently established invasive insect pest of continental North American and European small fruit cultivation. The main damage caused by this pest is due to feeding of larvae inside the fruits. However, ovipositor insertions can also provide routes for secondary microbial invasion. The damage potential of this exotic pest in crop systems of foreign regions drastically exceeds the criterion for quality fruit marketability. Revenue losses attributed to SWD contamination in North America have been estimated in various Western Pacific U.S. regions, and amounted to roughly $US 42.9 million in three California counties alone (Bolda et al., 2011).

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Prior to the 2011 establishment of SWD in Maine, over 24,300 ha of wild (lowbush) blueberry (Vaccinium angustifolium Aiton) farmland generated roughly $250 million of farm gate revenue annually between 2007 and 2012 (Yarborough, 2013). Currently, no estimate of monetary loss in wild blueberry has been developed to include the direct SWD infestation of fruit, secondary microbial contamination, or the compounding costs of implementing monitoring and treatment protocols; although, in 2012 an estimate of 25% crop loss due to SWD is suspected (Drummond, pers. comm.). A widely adopted response to male detection during adult monitoring surveys entails the targeting of adults through application of pyrethroid, organophosphate or spinosyn class insecticides to suppress population growth before significant crop damage is inflicted (Bruick et al., 2011; Collins and Drummond, 2015 a;b). Unfortunately, many of these insecticides threaten the health of wild and managed pollinator species, insect natural enemies, and persist in surrounding landscapes and watersheds after runoff or drift dispersal (Hester et al., 2001; Beketov et al., 2013). The development of environmentally and economically sustainable SWD integrated pest management (IPM) programs is currently an ongoing process in North American and European small fruit agriculture. Ideally, findings will be translatable across a wide spectrum of agro-ecosystems given the species' generalist phytophagy, high generational turnover and reproduction rates that aid in its rapid spread and alarming crop injury potential (Asplen et al., 2015).

The use of capture-and-kill traps emitting volatile semio-chemicals constitutes an effective chemical pre-treatment monitoring tool of early stage SWD invasions throughout the fruit ripening and harvest periods. Currently, exploiting the positive chemotactic response of SWD adults to ethanol molecules emitted by yeast-containing baits is a widely implemented bait attraction protocol (Walsh et al., 2011; Burrack et al., 2015). The utilization of physiologically attractive compounds in mass capture and kill of SWD was first proposed by Kanzawa (1934). Since that time, results produced by two recently conducted mass-trapping efficacy evaluations have yielded conflicting results (Wu et al., 2007; Hampton et al., 2014). Thus, the implementation of this approach in the absence of complimentary control measures is widely regarded as a high-risk option for reducing the abundance of SWD larvae in fruit.

By exploiting the phago-stimulatory reflex of insects to desirable food (Cevik and Erden, 2012), the infusion of boric acid with aqueous sucrose solutions has provided an effective oral exposure mechanism for toxin delivery in both urban and disease vector associated pests. Insecticidal treatments in environments in and around homes have received scrutiny due to the high degree of direct spatial overlap between humans and target insects. Research has shown that boric acid ingestion by the German cockroach (Blattella germanica L.) and the Asian tiger mosquito, Aedes albopictus Skuse, can suppress population growth through a combination of chronic and acute toxicity. Naranjo et al. (2013) also described a reduction in the post-treatment oviposition rate of A. albopictus. According to Markow and Grady (2008), reproductive maturation of Drosophila melanogaster females progresses more rapidly when nutritional resources are available for immature fly consumption. Given that recent laboratory observations on SWD ovarian development (Alnajjar, 2016) provides evidence for a 5-6 day reproductive immaturity in general female flies, the ingestion of boric acid by foraging SWD females during this critical developmental stage might physiologically disrupt or delay the progression of reproductive maturity. Hampton et al. (2014) found that a majority of flies in close proximity to trapping grids exclusively contact the external surface of traps and remain free to propagate. Considering this, the incorporation of a boric acid and sucrose solution on the exterior surface of baited traps could expose a proportion of these non-captured SWD to the toxin and ultimately enhance the control efficacy of attract and kill trapping. In the absence of a gustatory rejection by SWD upon the ingestion of toxins in a food source, this strategy might provide a mechanism for increasing the proportion of flies treated upon contact with a trap surface.

Another preventative approach involves the deployment of fine mesh netting at ripening and pre-harvest crop intervals as a physical barrier to ovipositing SWD contacting potential host fruits. This technique has been effectively implemented in IPM programs for a variety of insect pests (Lloyd et al., 2005; Dib et al., 2010; Sauphanor et al., 2012), and has received attention in the ongoing efforts to provide organic growers with chemical management alternatives for SWD in a variety of agricultural systems (Cormier et al., 2015; Schattman, 2015). The work of Cormier et al. (2015) also addressed the significance of net shading on plant photosynthetic activity by quantifying the chlorophyll and sugar composition of blueberry fruits upon concluding the study. They found no apparent reduction in fruit quality from berries sampled in protected vs. unprotected crops. However, given the spatial limitations of the field and greenhouse evaluations conducted thus far, it is unknown whether exclusion netting can meet the criterion for economic cost effectiveness of SWD management in large-scale crop systems. Neither of the proposed management approaches has yet been demonstrated to provide a level of efficacy against SWD infestations.
needed in large-scale crop systems.

**Research objectives**

We conducted two independent field studies with the intention of further exploring the SWD control efficacy of these techniques in Maine wild blueberry, specifically for small-scale growers. The first study conducted in 2013 and 2014 involved quantifying the severity of fruit infestations in response to boric acid applications on ethanol emitting traps deployed at varying densities within trapping grids (attract and kill tactic). The second investigation conducted in 2014, 2015, and 2016 examined the efficacy of insect exclusion netting in wild blueberry fruits against SWD larval infestation.

**MATERIALS AND METHODS**

**Attract and kill trapping**

Field studies were designed to test the potential of an "attract and kill" trapping tactic for wild blueberry. The studies were conducted in wild blueberry crop fields at the University of Maine Blueberry Hill Experimental Farm in Jonesboro, Maine USA in 2013 and 2014. The traps utilized in both years consisted of red Solo® cups with seven, 3.2 mm-diameter holes punched about 2.5 cm below the cup’s upper rim. Within experimental grids, individual traps were positioned on a green, metallic 76 cm plant support post and baited with approximately 5 cm of a mixture containing 15 ml yeast granules, 60 ml white granulated sugar, and 0.35 L H₂O. Control traps were filled with about 5 cm of water only. We used sugar syrup/yeast bait since it has proven to be a superior attractant in wild blueberry (Burack et al., 2015). Each red Solo® cup was covered with a red foam photo-absorption cap to seal the trap and prevent the interference of light with fermentation reactions. The external surface of each experimental trap was then uniformly sprayed with a mixture of 1% borate L⁻¹ 25% (w/w) sucrose/water solution. Traps intended for monitoring purposes during these assessments did not have boric acid solution sprayed on the external surfaces. Fruit samples were collected to assess larval infestation. Each fruit sample occupied a volume of about 236 ml, and was gathered from random wild blueberry clones (genets) within each treatment plot. All fruit samples were processed within one week after collection. Each sample was gently crushed in a plastic bag and suspended in 10% saline solution for 30 min in order to induce disassociation of SWD larvae from fruit pulp. The contents were then filtered through a fine mesh sieve and suspended in a black bottom metallic tray filled with water. A lamp was utilized to illuminate the contents of samples so the abundance of SWD larvae inhabiting fruit samples could be determined (Drummond et al. 2016).

Red cup traps were gathered at different intervals depending on the experiment, and analyzed within one week of being collected. The contents of each trap were rinsed with H₂O and filtered through a sieve in the laboratory. The remaining trap contents were emptied into a white metal tray filled halfway with about 5 cm water. Male and female SWD counts were then obtained from each trap sample.

On 25 July 2013, a single 7.6 m x 4.9 m study area was set up at the University of Maine Blueberry Hill Experiment Station in Jonesboro, ME, for a preliminary investigation of the potential of mass trapping for suppression of SWD in pre-harvest fruits. Twelve traps were deployed in a 3 x 4 grid with approximately 1.8 m of spacing between traps. Traps were collected and replaced with freshly baited traps every seven days. Those containing flies were taken back to the laboratory where the abundance of male and female SWD was determined.

On the final collection date, 14 September 2013, two samples of blueberries were gathered from random areas inside and outside the grid to determine female SWD egg laying activity. Externally sampled plant clones were located a minimum of 9.1 m from the peripheral border of the trapping grid. Each blueberry sample was processed in the laboratory by using the methods described previously. A replicated field study was completed in 2014. On 27 August 2014, prior to the detection of juvenile SWD in preliminary fruit monitoring bouts, twelve, 9.1 m x 9.1 m study grids were established in wild blueberry. Experimental traps were deployed and spaced at 0.9 m (low density), 1.8 m (medium density) or 2.7 m (high density) in order to determine the impact of varying trap density on SWD abundance. Control traps were spaced 1.8 m apart. All treatment grids were replicated three times, in replicate blocks in the same 16.2 ha field at the University of Maine Blueberry Hill Experiment Station in Jonesboro, ME. Trapping grids within a block were positioned a minimum distance of 9 m from one another. For collection of adult abundance data, three randomly chosen monitoring traps were taken from each trapping grid. During weekly trap collections, all traps were cleaned and recharged with freshly prepared bait and boric acid solution. In addition, three blueberry samples (236 mL each) of random clones located within each study area were collected weekly so that larval infestations could be quantified.

Since treatments were not replicated in the preliminary attract and kill trapping study, assessment in...
2013, only the 2014 study results were statistically analyzed. Repeated measures analyses of variance (ANOVA, RCB, repeated measures are 4 sampling times) were utilized to examine the impact of treatment and block on the observed variation in adult and larval SWD abundance within trapping grids. Analysis was conducted on square root transformed dependent variables. Analyses of variance (ANOVA, RCB) were utilized to examine the impact of treatment and block on the observed variation in adult and larval SWD abundance within trapping grids. An initial test entailed two separately conducted ANOVAs to assess how varying trap density impacted SWD adult and larval abundances within study plots. A third ANOVA examined the relative abundance of SWD larvae found infesting fruit samples collected in control compared to medium density experimental plots. Mean separation was performed using a Turkey’s post-hoc test (JMP®, 2015).

Insect exclusion netting

Studies conducted in 2014, 2015, and 2016 were designed to test the effectiveness of minimizing SWD larval infestation of fruit by preventing female SWD adults access by covering the crop with netting. Anti-Insect Netting® (#25 Mesh) with mesh size meeting the pre-determined dimensions for effective exclusion of SWD adults (Caprile et al., 2013) was tested at Blueberry Hill Farm Experiment Station in Jonesboro, ME during the summers 2014 and 2015. In 2016, a finer, but less expensive mesh netting, AgriBon® + AG-19 Row Cover (commonly referred to as “Reemay”, a spun polyester material) was used to protect fruit from SWD attack.

In 2014 there were two replicated netting enclosures (each 3.96 m x 15.24 m), one replication was initiated on 9 July and a second on 18 July; in 2015, two netting enclosures (each 3.96 m x 15.24 m) were set up on 29 July, with a third (3.96 m x 30.48 m) initiated on 30 July. In 2016, two netting enclosures (each covering an area of 15.25 m x 45.7 m) were set up on 14 July. All of these replicates were deployed prior to capture of any adult SWD and prior to 0-5% of fruit being susceptible to attack (ripe fruit). An adjacent non-protected plot served as a control treatment for each replicate. In addition, ethanol baited, red cup, monitoring traps were deployed in close proximity to study areas to determine the onset of invasions. Upon initial capture of adult SWD, blueberries were periodically sampled from the non-protected study plots in order to track fruit infestation. After fruit was naturally infested by SWD, exclusion nets were removed and fruit samples were collected to determine the abundance of SWD larvae. In 2014 and 2015, five fruit samples were taken from areas protected by the netting and paired areas not protected by any netting (sample size was 472 and 157 ml in 2014 and 2015, respectively). In 2016, 10 samples (236 ml each) were obtained from each study plot. Samples were assessed utilizing the previously described salt extraction method for fruit samples. In addition, in 2016 fruit ripeness and yield were qualitatively assessed between the non-netted control and the net enclosure treatments. A randomized block design model, with year as a blocking factor, was utilized for analyzing the larval fruit infestation (adjusted to larvae per 236 ml of fruit) over the three years (JMP®, 2015).

RESULTS

Attract and kill trapping

In 2013, there was a considerable increase in the abundance of SWD flies captured in baited traps toward the end of the preliminary mass trapping experiment (Fig. 1), with 373.4 ± 188.2 (SD) flies/ trap on 14 September 2013. The majority of SWD flies were females. Fruit collected within the trapping grid contained 68.3 SWD larvae per sample in comparison to 125.4 larvae found infesting fruits obtained from the paired plot that was not trapped (Fig. 2). This corresponds to a 46% reduction in larval abundance, with a negligible 0.8 g difference in fruit sample weights of berries obtained from blueberry clones in trapped compared to non-trapped treatments. Despite the lack of replication, this preliminary experiment suggested that a high density of traps has the potential to reduce the infestation of berries by SWD.

In the 2014 replicated trapping out study, comparing control grids (1.8 m trap spacing) with the baited trapping grids of medium density spatial arrangement (1.8 m trap spacing), there was no statistically significant difference in larval abundance over time in blueberry samples ($F_{(1,19)}= 0.12, P = 0.97$). No adult SWD were captured in the non-baited control traps compared to 17.2 ± 21.1 (SD) SWD per trap captured in the medium density treatment. Figure 3 shows that varying trap spacing of experimental grids did have a significant effect on the larval fruit infestation in blueberry samples ($F_{(1,30)} = 15.00, P = 0.001$).

Larval infestation increased with increasing trapping intensity suggesting that female SWD flies were attracted into the plots at a higher frequency. While there was no significant difference detected in the mean abundance of adults captured in traps across the range of trap-densities ($F_{(1,30)} = 0.74, P = 0.40$), a trend of increasing fly trap captures with increasing trapping intensity can be seen in Figure 3. This trend in adults captures with trapping intensity is correlated with the abundance of larvae in the trapped plots ($r = +0.631, P < 0.0001$).
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**Figure 1.** Abundance of SWD flies captured in each of twelve traps constituting a single trapping grid located in Jonesboro, ME during the summer of 2013.

**Figure 2.** Abundance of SWD larvae infesting fruit samples gathered within a trapping grid plot or in a control plot, experimental site located in Jonesboro, ME during the summer of 2013.

**Insect exclusion netting**

There was no difference in mean larval infestation of 9.7 ± 7.7 (se), 2.2 ± 0.4, and 5.9 ± 2.1 per fruit sample (236 ml) from non-protected blueberry plots among the 2014, 2015, and 2016 trials (*P* = 0.734), nor was there any difference between mean counts of 0.29 ± 0.29, 0.13 ± 0.07, 0.20 ± 0.10 larvae obtained from net protected plots.
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**Figure 3.** Mean abundance of individual *D. suzukii* captured in control, low density (2.7 m), medium density (1.8 m) and high density (0.9 m) experiment trapping grids. Each mean represents the average of three replicates over four weeks of trapping. Letters above larval abundance columns represent Tukey post-hoc results, with bars displaying dissimilar letters signifying statistical significance.

**Figure 4.** Mean abundance of *D. suzukii* larvae inhabiting five blueberry samples (adjusted to 236 ml each) from uncovered control plots or crop plots protected with exclusion netting. The measurements presented here represent the combined data from three consecutive trials conducted in 2014, 2015, and 2016. Error bars represent the standard error of the mean.
between 2014, 2015, and 2016; respectively \( (P = 0.891) \). Over all three years the exclusion netting provided a high degree of protection from larval infestation in comparison to non-netted plots \( (F_{1,8} = 19.958, P = 0.002, \text{Fig. 4}) \).

In 2016 we also made qualitative observations the quantity and quality of fruit under the Agribon® row cover (Reemay). Although this less expensive cover proved highly effective against SWD fruit infestation, it was difficult to deploy as the cloth fabric was easily torn. In addition, fruit appeared to have been knocked off stems possibly due to excessive movement of the fabric in high winds, and some fruit was delayed in maturation (red not blue), but there was no evidence of diseased fruit (Fig. 5).

### DISCUSSION

After experimentation with attract and kill trapping and insect netting in wild blueberry, only preventative exclusion of adult SWD with netting demonstrated effective management potential. Over all three years, netting greatly suppressed infestation of wild blueberry fruit. These results complement the findings of Cormier et al. (2015) who reported zero SWD adult emergences from high bush blueberry fruits grown under net-protected plots. Furthermore, this approach is thought to entail a high degree of SWD exclusion efficacy in other cultivated, small-fruit producing plants such as raspberry (Schattman, 2015). To date, all positive results with this technique have been obtained from studies conducted under limited space where the area of the study plots represents only a fraction of crop coverage necessary in large-scale production operations. Increasing the land area for protection would substantially increase the amount of netting product, labor and maintenance inputs required.

Excluding labor requirements for seasonal installation and maintenance of netting, the deployment of the most inexpensive exclusion netting (Agribon®) on a single hectare would cost roughly US $4,600 according to product pricing by Gardeners Supply Company®. Therefore, relative to Anti-insect Netting® at US $6.09 / 13 ft² or US $49,680 per hectare, Agribon® appears to be a cost effective alternative. According to Chen et al. (2015), from 2010 to 2014 organic wild blueberry cultivation in Maine generated an annual net revenue of US $3,724 per hectare. In a single year, therefore, the financial inputs of purchasing netting units alone would exceed the total amount of generated revenue if the Agribon® netting was used. However, more economically justifiable cost estimates are derived when considering the repeated use of netting. Assuming a degree of material durability and longevity spanning 2, 3, 4 and 5 years, the respective proportional cost decreases to roughly 62%, 41%, 31%, and 25% of total revenue generated from organically produced wild blueberry. However, our experience with the Agribon® material suggests only a one year use for a two month duration during the growing season. Given the high degree of infestation mitigation obtained with exclusion netting reported in this and other studies, it might be more feasible to deploy this management tactic if the yield damage caused by SWD exceeds the depreciated annual cost estimate of using a single netting unit repeatedly over time. Presumably, this disparity will increase over longer re-use time periods. Unfortunately, monetary losses due to SWD infestation have not yet been...
quantified in the wild blueberry crop system; although, some farms have experienced up to 25% crop loss due to SWD in some years (Drummond, pers. comm.). Therefore, this technique has great promise only if multiple-year use of netting is possible.

Insect exclusion netting has also demonstrated considerable success against plant pathogen vectors, preventing their physical contact with host plants in order to suppress insect-mediated disease transmission. Stanley et al. (2004) demonstrated the potential for using this technique to limit the spread of tomato yellow leafcurl virus (TYLCV) among greenhouse tomato plants. Plants displayed TYLCV symptoms less frequently when protected with netting for exclusion of the pathogen’s insect vector, the whitefly *Bemisia tabaci* (Gennadius). Another relevant phenomenon has been described by Louise et al. (1996) for *Drosophila melanogaster* and the dispersal of its microbial symbiont *Botrytis cinerea*, a necrotrophic fungus responsible for the occurrence of grape rot in vineyards. External localization of fungal spores on fly integuments, in conjunction with internal occupancy of the flies’ alimentary canal is thought to facilitate passive spore dispersal throughout vineyards harboring *D. melanogaster* populations (Louise et al., 1996).

The symbiotic association of SWD with *Botrytis cinerea* or alternative pestiferous microbes has not been documented, nor have the risks of introducing microbial symbionts of SWD into recently colonized agro-ecosystems been addressed in the literature. However, considering the described mechanisms of passive grape rot transmission by *Drosophila melanogaster*, it is possible that symbiotic associations within SWD may diversify the pest pressures imposed on certain plant species that further reduce fruit marketability, perhaps even leading to the inadvertent introduction of additional pestiferous microbes into an agro-ecosystem.

The exclusion efficacy of netting in preventing SWD flies from contacting viable fruit hosts has been demonstrated in multiple crop systems. Coupled with the theoretical cost-efficiency in the context of Maine wild blueberry production, it is justifiable to consider exclusion netting as a viable avenue for further evaluation as a component of developing IPM programs for SWD. However, one potential limitation to this method of SWD prevention includes the alteration of microclimates located within netting units. If the accumulation and retention of moisture within the space encapsulated by the netting material effectively alters crop and pest growth conditions in relation to ambient environmental conditions, the consequent humidification could create a habitat in which plant pathogenic fungi flourish (Ciancio and Mukerji, 2008). Although not yet tested for, this is a necessary consideration to address should the technique be considered for SWD management in large-scale crop systems where fungal pests may become problematic and require damage control. We have not observed any increased fungal disease in wild blueberry due to the use of netting, but particularly wet summers could result in an increase in disease incidence.

We also evaluated attract and kill trapping as a tactic for reducing the quantity of SWD larvae infesting berries. In comparison to control study grids with traps containing water, deployment of traps containing an ethanol-emitting bait and topical boric acid application failed to reduce the abundance of larvae infesting blueberry samples. Our study provided evidence that increasing trap density in wild blueberry plots attracted a concomitant increasing number of flies to a study area. This aggregation effect, whereby the number of flies in the proximity of trapping grids increases proportionally with trap density, would have consequently increased the frequency of contact between egg laying females and healthy blueberry fruits. Alnajjar (2016) found that these same traps baited with sugar syrup and yeast only catch approximately 16% of flies restricted to a localized area of wild blueberry with field cages. The capture efficacy of a comparable trap design was assessed by Hampton et al. (2014) who found variable capture rates ranging from 10% - 30% in traps baited with a combination of acetic acid and ethanol. Although the impact of boric acid was not quantified in this study and may have negated the fitness of some non-captured individuals, it is also possible that the presence of boric acid acted as a repellent. Regardless of this, our “attract and kill” study did not achieve satisfactory control. Hampton et al. (2014) suggested the incorporation of insecticides directly on the external surface of traps, and/or on proximal plants as exposure reservoirs. Doing so might eliminate the attracted, non-captured adults given the high efficacy of various insecticides against SWD (Bruck et al., 2011; Collins and Drummond, 2016a; 2016b). The results of Hampton et al. (2014) also imply an inverse relationship between distance from trap and the probability of SWD infestation. It has therefore been proposed that trapping grids be deployed on the periphery of managed fields in order to confine invasions within trap cropping reservoirs in these areas so they can be effectively treated and prevented from further dispersal into the fields during pre-harvest intervals.

Additional evaluations on SWD bait preference and the chromatic attractiveness of traps have provided insights for the development of trap designs that enhance SWD fly attraction and capture rates. The physical localization of SWD flies on the exterior portion of traps is believed to occur more frequently on dark pigmented surfaces. Basoalto et al. (2013) reported double the observed fly capture rates in traps wielding a black

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