BIOAG PROJECT FINAL REPORT TEMPLATE

TITLE:

"Integrating manure-based amendments with pest control: Potential of predatory flies (Scathophagidae) as a secondary benefit of manure amendments, for early season control of spotted wing drosophila in red raspberry"

PRINCIPAL INVESTIGATOR(S) AND COOPERATOR(S):

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KEY WORDS

Spottted wing drosophila, *Drosophila suzukii*, dung flies, Scathophagidae, Red Raspberry, Biological Control

ABSTRACT

Yellow dung flies, *Scathophaga stercoraria* are associated with dung of large mammals. They are predatory on other flies particularly drosophila and are attracted to red raspberry fields following manure applications in early spring. We investigated their potential to provide season-long impact on spotted wing drosophila populations (*Drosophila suzukii*, SWD) in red raspberry by feeding on returning founder females in early spring, coinciding with the manure treatments. Due to the risk for human pathogens associated with raw manure, their attraction to anaerobic digestion by-products was also evaluated. Weekly timed counts were taken for each of the treatments. Dung flies exhibited lekking behavior and were only attracted to fresh raw manure. Despite high numbers of eggs laid on the manure slurry, no flies emerged. Laboratory studies included dissections of the dung flies, which confirmed the presence of highly sclerotized prestomal teeth, capable of predation on SWD. Presence of thoracic holes and total loss of body fluids and red eye pigmentation in SWD housed with the dung flies in cage trials provided forensic evidence of predation. Molecular analyses detected presence of SWD DNA in field-captured dung flies, suggesting SWD are a regular component in their diet. While the anaerobic digester by-products were not attractive to the dung flies in their current state, additional research may identify ways to increase attractivity of these less-risky soil amendments, enabling growers to utilize these predators to help control SWD in red raspberry.

PROJECT DESCRIPTION

Spotted wing drosophila has become the #1 pest of soft fruit in the state and listed as a top research priority for all small fruit commissions. Weekly spray programs are unsustainable and growers and researchers are investigating ways to reduce residue levels but attempts to reduce insecticide applications are risky due to unprotected fruit. Biological control can suppress SWD and natural enemies have been investigated. Parasitoid wasps are of particular interest due to their high mobility and native PNW larval and pupal parasitoids have been reared from infested fruit but not all have high fecundity and their ecological impact remains unknown. Also, *D. suzukii* is significantly more resistant to wasp parasitism than its close relatives, casting doubt on potential by local parasitoids to impact SWD populations. Large populations of predatory yellow dung flies (Diptera: Scathophagidae) were observed perched on red raspberry vegetation at a grower cooperator's field in Lynden, Washington in early May and could be associated with recent manure applications. Manure is plentiful in Whatcom County, home to the majority of the state's dairy farms, providing a rich source of manure for agricultural use and likely an equally rich population of dung flies. To increase soil fertility, the grower carefully applies two liquid manure/slurry applications in a narrow 20" band at the base of the canes on each side of the row, at the rate of 1600 gallons/acre/treatment/year, in February and early March, meeting the 90-day preharvest interval for raw manure treatments as required by NOP (National Organic Program). Scathophagids require manure for breeding and development and local populations are attracted to the manure application. Scathophagid adults are predators and capture and feed on other flies, particularly drosophila.

SWD leave their overwintering sites and return to fields in the spring. Once SWD invades fields and begins infesting the fruit, populations are more difficult to control because they become self-generating within the field. Keeping them out as long as possible is the challenge. The returning females, the manure application and appearance of the dung flies appear to coincide. Controlling founder-female SWD entering fields in early spring could be a "game-changer", potentially affecting populations during harvest and the late summer peak. This study looks at the potential for dung flies to impact SWD populations by predation on early season SWD founder-females.

OUTPUTS

Work Completed:

Objectives include 1. the ability of dung flies to feed on spotted wing drosophila; 2. to determine if dung flies exhibit attractivity to any of the manure-derived treatments including: phosphorus-rich solids, ammonium sulfate liquid, nutrient-reduced wastewater and raw undigested manure, and 3. investigate potential for integrating dung flies into an SWD control program for red raspberries.

1. Determine the ability of dung flies to feed on spotted wing drosophila

The mouthparts of the dung flies were dissected and slide-mounted in Strandtmann's. The mouthparts revealed highly sclerotized teeth, which could readily break through insect cuticle (Fig. 1). Yellow dung flies possess highly sclerotized prestomal teeth along the midline of the labellum surrounded by the pseudotracheae (Fig.1). The presence of these teeth, provide morphological evidence that they are capable of feeding on spotted wing drosophila. Literature also supports predation ability of dung flies to attack other flies including drosophila (Gibbons 1987).

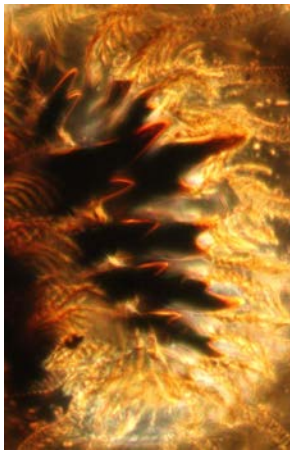

Fig. 1. Mouthparts of *Scathophaga stercoraria*. Photograph by B. Gerdeman. 2016. Nikon Labophot. 400X darkfield.

Forensic evidence of predation by dung flies on SWD

Laboratory studies were performed to verify predation by the dung flies. Dung flies were collected from the red raspberry field and placed into arenas, Bugdorms®, along with a potted wheat plant, to serve as a perch (Fig. 2). In a single bugdorm, dung flies and other small flies were placed including SWD. Dung flies were observed catching and feeding on smaller flies. In a second series of laboratory studies, dung flies were placed into a bugdorm with SWD and in a second arena only SWD were placed. After 7 days, cadavers from the both arenas were inspected and compared to provide forensic evidence that dung flies feed on SWD.

Fig. 2. Fly arenas for observation and forensic studies.

In the arena *without* dung flies (Table 1) 13% of the SWD cadavers were headless and recovered heads and bodies were still filled with contents but in the arena *with* dung flies, 46% of the SWD cadavers were headless and recovered heads and bodies were empty and shriveled (Figs. 3 + 5).

Table 1. Forensic evidence of dung fly predation on SWD

Forensic Evidence	7 days	7 days	1 day
	Dung flies	Without Dung flies	pesticide
% Headless	46 empty heads	13 heads with contents	0
% Empty thorax and/or heads	46	0	0
% With heads but holes in thorax	22	0	0

Holes were observed in the thoraxes of 22 % of the SWD cadavers in the arena *with* dung flies but none were observed in the arena *without* dung flies suggesting predation. To prove the effects were not the result of time and drying, SWD were placed in a bugdorm and treated with an insecticide and collected after 1DAT to provide a reference for fresh killed SWD (Fig. 4). The fresh SWD were not empty and their heads and eyes were fully pigmented.

Fig. 3. Lack of eye pigment, shriveled cadavers and holes in the bodies are Evidence of dung fly predation of SWD.

Fig. 4. Freshly killed SWD. Bright pigmented eyes indicate a healthy SWD.

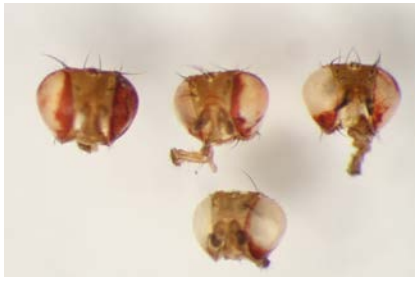

Fig. 5. Lack of eye pigment suggests that dung flies suck out the pigmentation and body fluids as they feed on SWD.

Molecular evidence of predation by dung flies on SWD

Molecular testing was an unplanned additional activity but was not included in the budget. Dr. Lydia Tyman (WSU NWREC) a molecular specialist performed all of the molecular tests. A standard PCR was run using only SWD or dung fly DNA and *confirmed no cross amplification*. A real time PCR

was then set up, using the published protocol by Dhami et al (2014). The primer pair amplifies a 186 bp region in the cytochrome oxidase subunit 1 gene. A sensitivity test found it to be effective to 0.25 picograms (1 trillionith of a gram) (Fig. 6). Locally collected flies (from a Skagit County dairy) were either placed in bugdorms and fed on SWD or immediately frozen after collecting, for testing. Results indicated a portion of SWD DNA was detected in the non-SWD fed, wild collected flies. It is highly likely that they had fed on SWD prior to their collection and that SWD is part of their normal diet, therefore dung flies in raspberry fields can and will feed on SWD.

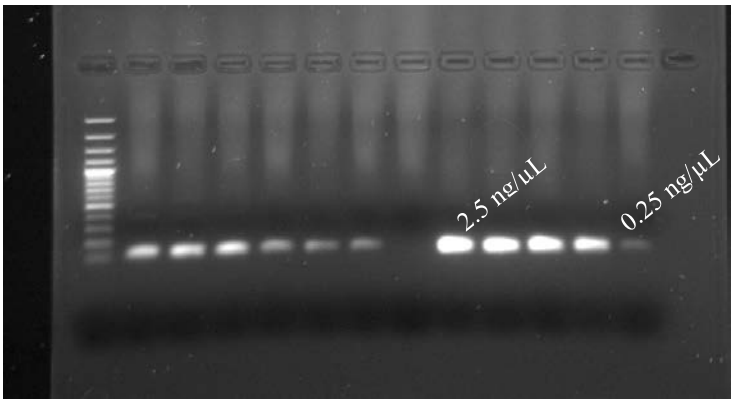

Fig. 6. Primers detected DNA in SWD Sensitivity test – effective to 0.25 pg.

2). Determine if dung flies exhibit attractivity to any of the manure-derived treatments including: phosphorus-rich solids, ammonium sulfate liquid, nutrient-reduced wastewater and raw undigested manure.

The grower cooperator made applications of straight run manure to all raspberry field areas except for the non-manure treatments on 26 Feb 2016 as part of his regular program, later a second application was made on the evening of 4 April. On 26 Feb we counted flies in the treatment areas at I HAT. We also recorded positions of flies on the raw manure in the remainder of the field to investigate distribution since basically all the manure treated areas were equally attractive to flies and did not differ. Weekly timed counts were taken at approximately the same time each day for each of the 5 treatments replicated 4X (including a UTC). Results were clear. Dung flies were only attracted to *fresh* raw manure treatments but not distributed evenly, suggesting dung flies exhibit lekking behavior. A lek is an aggregation of males gathered to engage in competitive displays, lekking, that may entice visiting females who are surveying prospective partners for copulation (Wickipedia 2016). In the literature lekking by dung flies has been argued. Aggregating flies were observed at various locations on the manure replicates but not all the manure replicates had lekking flies, indicated by the high variability among the replicated manure treatments (Tables 2 + 3). Dung fly distribution is random. This supports the notion that these flies are actively engaging in lekking. Flies appeared the day of the application, lekked, mated, dumped thousands of eggs and were gone by 2-3 DAT. Our counts for the 2nd application on 4 April were not made until nearly 20 hours later because the grower put the application on near dusk the night before. The counts however proved that the flies were still present and still attracted to the wet manure at 1 DAT.

Table 2. 26 Feb first raw manure application

#	Treat ID	Primary Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Means	sem
1	AS	Ammonium Sulfate (AS) slurry	0	0	0	0	0	0.00
2	PS	Phosphorous Solid (PS)	0	0	0	0	0	0.00
3	DLE	Digested Liquid Effluent (DLE)	0	0	0	0	0	0.00
4	MA1	Straight Lagoon Run (MA) Feb 26	6	0	8	83	24.25	19.66
5	MA2	Straight Lagoon Run (MA)	0	0	0	0	0	0.00
6	COM	Compost (COM)	0	0	0	0	0	0.00
7	CON	Standard Fertilization (CON)	0	0	0	0	0	0.00

Tabe 3. 4 April second raw manure application

#	Treat ID	Primary Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Means	sem
1	AS	Ammonium Sulfate (AS) slurry	0	0	0	0	0	0
2	PS	Phosphorous Solid (PS)	0	0	0	0	0	0
3	DLE	Digested Liquid Effluent (DLE)	0	0	0	0	0	0
4	MA1	Straight Lagoon Run (MA)	0	0	0	0	0	0
5	MA2	Straight Lagoon Run (MA) 4 April	1	6	0	37	11	8.77
6	COM	Compost (COM)	0	0	0	0	0	0
7	CON	Standard Fertilization (CON)	0	0	0	0	0	0

The large numbers of eggs laid in the field during lekking suggested the potential for in-field generation of the predatory dung flies, which would be ideal for season-long SWD control. To determine if flies emerged from the eggs, 12 emergence traps were positioned in recorded lekking locations and checked weekly until the end of the harvest season in August. No emergence occurred. The raw manure slurry is thin compared to a normal dung pat and it could be that the slurry is unsuitable for development. The failure of the biodigester-derived treatments to attract dung flies is a serious impediment to utilizing dung flies to control SWD since the biodigester byproducts would pose a method to attract the flies in to the field to provide control without the risk of human pathogens, unlike raw manure.

3. Investigate potential for integrating dung flies into an SWD control program for red raspberries.

The following points summarize complications of integrating dung flies into red raspberry production to control SWD:

- The flies are only attracted to the fresh raw manure treatment (1-2 days).
- PHI for raw manure applications to crops with berries not touching the ground is 90 days.

- Occurrence of food-borne illnesses is too risky for most berry growers to utilize raw manure.
- The current weekly SWD control program would eliminate the possibility of integrating dung flies throughout the season.

There are potentially 2 strategies for timing of integrating dung flies into an SWD control program: 1) integrating during the season and 2) integrating prior to the season. Integrating them during the season remains possible if biodigester solids can be tweaked (e.g. eau de dung) or bait (e.g. dung pats) could be positioned in the field at select locations that would attract flies into the field for hunting SWD without adding risk of pathogens.

Integrating them into the control program prior to the season when current raw manure applications coincide with the return of the overwintering founder females represents an opportunity for those growers who use raw manure. Additionally the grower cooperator has had no significant SWD populations in his raspberry field however he is a good berryman and utilizes weekly insecticide applications. Do dung flies play a role in this? Unless flies are swept from his field and tested using molecular techniques it remains unknown but arguable that it does.

- **Publications, Handouts, Other Text & Web Products:**

In prep - Morphological, forensic and molecular evidence of dung fly predation on Spotted wing drosophila, *Drosophila suzukii*

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- **Outreach & Education Activities:**

1) Anaerobic Digestion Systems Field Day, 9 June 2016, Lynden, WA
2) Trinity Western University, Invertebrate Zoo, Guest Speaker, 25 Oct 2016, Langley, BC, Canada
3) WSU Entomology Colloquium, Pullman, WA, 17 Oct 2016
4) ANBP Conference (Association of Natural Biocontrol Producers), 25 Oct 2016, Abbotsford, BC, Canada

IMPACTS

- **Short-Term:**

The expected short-term impacts are *Awareness by farmers of the presence of these fly predators in their fields that can provide an unexpected valuable service by reducing SWD populations*. Growers and dairymen attending the above outreach activities received firsthand information on the study.

- **Intermediate-Term:**

Intermediate-term impacts include grower awareness of these beneficial flies and *efforts to maintain their populations on their red raspberry farms through timely applications of selected insecticides that may reduce negative impacts on the population.* Growers are concerned about the overuse of insecticides and we are currently working on ways to reduce insecticide applications but thus far nothing is as effective as the broadspectrum insecticide sprays and so these intermediate goals have not yet been met.

- **Long-Term:**

Long-term impacts include the *potential for active management of dung flies using manure-based amendments as attractants, coinciding with early founder-female SWD and further potential for season-long control of subsequent generations of SWD.* The biodigester by-products were not attractive to the dung flies, only the raw manure slurry was attractive. Growers may not want to take the risk of applying raw manure to their berry fields in light of the new FSMA restrictions however there remains potential for dung flies to play a role in SWD control programs (see *Recommendations for future research*).

ADDITIONAL FUNDING APPLIED FOR / SECURED

No additional funds have been applied for or secured.

Graduate students funded

No graduate students were funded.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future research could investigate:

- Enhance attractivity of dung flies to the biodigester by-products, which do not result in increased numbers of human pathogen.
- Methods of attracting flies into fields through use of point-source, manure-bait systems without applying it directly to field crops.

References:

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