RESEARCH

Relationship Between *Drosophila suzukii* and Postharvest Disorders of Sweet Cherry (*Prunus avium*)

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ABSTRACT

Spotted wing drosophila, *Drosophila suzukii*, utilizes intact ripe fruits for oviposition and larval development. Sweet cherry (*Prunus avium*) and *D. suzukii* share a saprophytic microbial community, or microbiome, that colonizes the interior and exterior of the fruit, which benefits the nutrition and development of the flies. Some of the microbes, specifically yeast species, are also reportedly associated with a newly described slip-skin-like disorder of sweet cherries. In British Columbia (BC), Canada, contact-based insecticides and fungicides are applied to sweet cherry to suppress *D. suzukii* populations and cherry diseases, respectively. To date, no resistance to the organophosphate insecticide, malathion, in *D. suzukii* field or laboratory populations has been reported. Laboratory bioassays with malathion-incorporated diet determined that when microorganisms associated with the *D. suzukii*

microbiome were sterilized with potassium metabisulfite (KMS), survival of the flies was significantly affected. These findings led to speculation that malathion residues on cherry fruit may be degraded due to the greater presence of yeast species that are spared as a result of selective fungicide use patterns in cherry orchards. In orchard trials, KMS was shown to be effective in suppressing the surface yeast counts on cherry, but this did not impact symptoms of slip-skin-like disorder. Based on these findings, it is recommended that other products functioning as systemic biocides need to be investigated to address these two microbial-connected pest management concerns in sweet cherries.

Additional keywords: host–microbe–pest interaction, microbiome-induced degradation, postharvest disorder associations.

A potential focus for management of spotted wing drosophila, *Drosophila suzukii* (Matsumura), is the manipulation of its microbiome, which plays a key role in metabolism and detoxification of ingested food (Broderick and Lemaitre 2012; Starmer and Fogleman 1986). A recent drosophilid dietary study suggested that frequent replenishment of bacterial influx is mandatory for the maintenance of the common fruit fly, *D. melanogaster* gut microbiome (Blum et al. 2013), and that male drosophilids harbor approximately double the bacterial population of females 2 to 3 days post-eclosion (Blum et al.

2013). Microbial associations with drosophila are beneficial and crucial in several other aspects, including detoxification of toxins (Starmer and Fogleman 1986; Trinder et al. 2016). This includes increased metabolism of malathion residues on cherry by *D. suzukii* as demonstrated in artificial diet bioassays in the laboratory (Smirle et al. 2017). The microbial degradation of malathion has been observed within soil and water environments (Bhagobaty and Malik 2008; Bourquin 1977; Kim et al. 2005; Sharma et al. 2005; Singh and Walker 2006; Yang et al. 2011), and inside the insect gut (Trinder et al. 2016), but little is known about malathion biodegradation on plant surfaces. Previous studies do support this possibility, as biodegradation of a plant-produced toxin, phloridzin, was facilitated by the bacteria *Enterobacter agglomerans* (now *Pantoea agglomerans*) associated with the microbiome found on the phylloplane or leaves of apples (Rezzonico et al. 2009), which can in turn be transported by the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Lauzon et al. 2003).

Many microbes colonize plant surfaces (Leff and Fierer 2013), and alterations in pesticide type and use patterns can affect species abundance and ecology (Calhella et al. 2006). For example, fungicides

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to control *Botrytis cinerea* Pers., the causal agent of bunch rot of grapes, might have eliminated the competition for yeasts. This in turn could have resulted in a modified role for yeast in the microbial complex of vineyards and triggered a higher incidence of grape sour rot (Guerzoni and Marchetti 1987). Similarly, fungicide residues in wine grapes can affect fermentation such that fermentation-friendly practices are now used close to harvest (Boudreau et al. 2017; Calhella et al. 2006). In the case of sour rot, the major symptoms include browning and disaggregation of the internal tissues and replacement of fluids with strong volatile acids resulting from ethyl acetate accumulation (McFadden-Smith 2012; Wilcox 2015).

Another recently discovered malady of tree fruit, predominantly found in late maturing sweet cherry cultivars, is called cherry slip-skin-like disorder (see Chong 2011 for images) and has been reported to occur as a postharvest rot (Kim 2014). The major symptoms of this new disorder appear shortly after harvest as the fruit develop a soft area on the shoulder where the subepidermal tissue has become macerated and the skin easily slips off with only minor pressure. The increased incidence of slip-skin-like disorder may be associated with recent changes in fungicide use patterns, as the altered microbiome includes biocontrol agents that become pathogenic, in part due to a biphasic dose response of the microbiome (Partida-Martínez and Heil 2011). *Aureobasidium pullulans* (de Bary) G. Arnaud, a yeast species associated with grape sour rot, has also been attributed to be the causative agent for this new postharvest rot in sweet cherries (Kim 2014). Since the spread of sour rot is also associated with *D. melanogaster* (Lam and Howell 2015) and facilitated by *D. suzukii* (Rombaut et al. 2017), we speculate that a microbial complex may also include a drosophilid-microbiome association and abiotic factors that favor microbial proliferation.

The events (*D. suzukii* incidence, slip-skin-like disorder, and sour rot) appear to be independent but may share a similar microbiome in varied proportions due to the overlapping time period (the preharvest, harvest, and postharvest) in which these organisms occur in sweet cherry (Fig. 1 adapted from Marshall and Walkley 1951). A portion of the microbial complex introduced by the fly also exists in and on the fruit surface (Chandler et al. 2011, 2012, 2014). For example, *Pantoea punctata* (now *Tatumella punctata*) exists on *D. suzukii* (Chandler et al. 2014) and fruit surfaces such as strawberries (de Melo Pereira et al. 2012). Removal of the microbes could possibly delay insecticide degradation and allow for longer residual activity with efficient control of *D. suzukii* in cherries and, in turn, help reduce postharvest rots (temporally separate events). An alternative to fungicides is the use of a sterilizing agent, for example potassium metabisulfite (KMS) used by the wine industry (Settanni et al. 2012) against *Botrytis* bunch rot (Balasubramaniam and Poole 1995) in the field and in postharvest treatment facilities (Adiyaman et al. 2013; Sivakumar et al. 2002, 2005).

In accordance to the above-mentioned microbial associations, we hypothesize that KMS may have an important role to play in managing both *D. suzukii* and cherry slip-skin-like disorder. The objectives of this study were to examine the effect of KMS on (i) *D. suzukii* survival and development parameters (larval development time and percent eclosion or a combination) in a choice and no-choice scenario when malathion was incorporated into the diet; (ii) insecticide degradation under laboratory conditions by observing relative *D. suzukii* percent eclosion; and (iii) both the surface yeast counts and slip-skin-like symptom development on sweet cherry under field conditions. The findings of the study will be important for understanding the possible analogous associations

Fig. 1. Schematic depiction of microbial trends during the late fruit growing season. The figure was adapted from Marshall and Walkley (1951) and is solely a hypothetical model demonstrating changes in the plant dwelling microbiome and interplay with *Drosophila suzukii*.

between known factors (microbes and the fruit) shared in different proportions between *D. suzukii* and slip-skin-like disorder and the development of common management strategies for respective periods of susceptibility.

MATERIALS AND METHODS

Chemicals. Products used included the insecticide Malathion 85 EC (Loveland Products Canada, Inc., Dorchester, ON), the fungicide propiconazole (Tilt 250E) (Syngenta Canada, Guelph, ON, Canada), potassium metabisulfite (KMS), 98%, and L-2,3-dihydroxybutanedioic (L(+)) tartaric acid (Sigma-Aldrich, Oakville, ON, Canada). All standard reagents used in buffer preparations were obtained from Sigma-Aldrich. Solutions of 0.1× KMS were prepared in distilled water by a 10-fold dilution of freshly prepared KMS (5,000 mg of potassium metabisulfite and 1,000 mg of L-2,3-dihydroxybutanedioic acid in 1 liter of distilled water). A working solution of 50 µg of malathion a.i. liter⁻¹ was prepared by diluting the stock solution (1,000 mg of malathion a.i. liter⁻¹ in acetone) in distilled water.

Insects. *D. suzukii* were collected from blueberries (*Vaccinium corymbosum* L.) at Pitt Meadows, BC (49°33'40''N, 122°43'50''W) in 2013 and reared at the Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, BC. The *D. suzukii* colony was maintained as previously described by Smirle et al. (2017). Briefly, the *D. suzukii* colony was held in a Conviron growth chamber (Controlled Environments Ltd., Manitoba, MB) at 16-h light/8-h dark and 25 ± 2°C with external humidifiers achieving 50 ± 5% relative humidity. Cohorts of adults were contained in a Bugdorm-1 Collapsible Insect Rearing Cage (MegaView Science Co. Ltd., Taichung, Taiwan) and fed a 10% sugar solution contained in a sealed 29.5-ml Solo cup (Solo Cup Company, Lake Forest, IL) with a wick. Adults were provided an oviposition site prepared from an artificial diet, Equation 4-24 Instant *Drosophila* Medium (Carolina, Burlington, NC), as per manufacturer's instructions and held in a 350-ml Snaptops container (Accent Home Products, Saint-Laurent, QC, Canada). The diet container, sugar solution, and cage were changed every 4 days, 10 days, and 1 month, respectively. After removal from the adult cage, the diet container was covered with a modified top, labeled, and held in a separate growth chamber under similar environmental conditions (as described above) until adults eclosed.

Bioassays. *Effect of KMS and malathion on D. suzukii survival and development—no-choice bioassay.* Diet vials (GEN32-121, Diamed Lab Supplies Inc., Mississauga, ON, Canada) were prepared by pouring 5 ± 0.1 g of prepared diet into a fresh vial and adding 15 ml of solution, immediately churned between palms for even distribution and making a slant while the diet set. Solutions used in the experiments were either water (control), 0.1× KMS, 50 µg of malathion a.i. liter⁻¹, or a combination of 0.1× KMS and 50 µg of malathion a.i. liter⁻¹. Newly eclosed flies were transferred into a new cage and held for 3 days, after which either 10 females or 20 adults (10 of each sex) were aspirated into the test vials (12 replicates of each) that were secured with flugs (GEN49-101, Diamed Lab Supplies Inc., Mississauga, ON, Canada). Flies were chilled for approximately 5 min in a refrigerator prior to handling. In this no-choice set-up, adults (parental generation) were exposed in vials to the treatments for 3 days in a separate growth chamber with similar environmental conditions as described above. Parental adults were then discarded. The flugged vials were held until no further eclosion of new flies (F1 generation) occurred. Total numbers of adults (F1) eclosed per vial were recorded daily as they were removed. The experiment was repeated three times. The choice of KMS concentration used in the experiments was based on preliminary trials where 0.1× KMS produced a statistically different response than the control yet was not detrimental to *D. suzukii*

adults that were exposed for 3 days or to the number of eclosed adults at the end of the experiment. Hydrogen peroxide has also been used to suppress microbes in a *D. melanogaster* model (Blum et al. 2013), but we preferred to use KMS instead for the sole reason of its availability to growers in other countries but not in Canada (Balasubramaniam and Poole 1995), in addition to the literature supporting its efficacy in fruit disease management.

Effect of KMS and malathion on D. suzukii survival and development—choice bioassay. Newly eclosed adults were transferred into new cages, 50 of each sex, and held in the growth chamber for 3 days under the conditions described above before being aspirated. Unlike the no-choice bioassays, these bioassays were conducted with mixed sexes only. Three replicate cages were set up with each containing a 10% sugar solution in a sealed Solo cup with a wick. Diet vials were prepared with distilled water, 0.1× KMS, 50 µg of malathion a.i. liter⁻¹, or a combination of 0.1× KMS and 50 µg of malathion a.i. liter⁻¹, as described above. In every cage, a set of four vials representing each treatment were supported in a 350-ml Snaptops container in random order. After 3 days of exposure to adult flies (Parental generation), diet vials were removed, flugged, and held in the same growth chamber until all the adults (F1 generation) eclosed. The number of adult flies eclosed per vial was recorded each day and the flies were removed and discarded. The experiment was repeated three times.

Effect of KMS on malathion degradation. The diet vial preparation and adult cohort collections were completed as per the procedures described above. Ten female and 10 male *D. suzukii* were transferred to the treated diet vials for 3 days in a growth chamber with conditions as described above, after which they were removed and the vials held for an additional 10 days. There were three replicates each consisting of five vials per treatment of which four vials were randomly selected and pooled (active area scraped to weigh approximately 5 g per vial), frozen, and held at -80°C for later residue analyses. The experiment was repeated three times as a 2 (KMS-present/absent) × 2 (malathion-present/absent) × 2 (flies-present/absent) factorial design. The first repetition had three additional replicates to be held until all the adults eclosed (termed as malathion degradation follow-up: *D. suzukii* survival and development—no-choice bioassay).

The frozen samples were code-labeled and shipped on dry ice to SGS Canada Inc., Vancouver, BC, for analyses of malathion concentrations as per proprietary quality control guidelines. In brief, the protocol included (i) a calibration curve constructed by spiking the blank diet with known amounts of malathion; (ii) a Carbofuran-d₃ internal standard (2 µg/ml); (iii) acetonitrile as the solvent and sodium chloride to separate the inorganic components; and (iv) QuEChERS (quick, easy, cheap, effective, rugged, safe) powder mixture to concentrate the sample as it provides high recovery for the target compound and removes water alongside undesired coextracts. The final extraction involved dissolving the samples in a mixture of ammonium acetate and acetonitrile for analysis by liquid chromatography/tandem mass spectrometry. Malathion residues are reported as the percent recovered.

Field trials. *Efficacy of KMS and propiconazole on yeasts of cherries.* In 2015, a field trial was designed to examine the efficacy of KMS for control of common yeast species identified from an earlier field survey (data not shown) that colonize the surface of cherry fruit and potentially influence the postharvest quality of the fruit. Five cherry trees were used in the trial, with seven treatments per tree (treatments are listed in Table 1). Each replicate was represented by a single cluster of cherry fruit (five replicates per treatment). Clusters hanging approximately 0.5 to 2.0 m from ground level were randomly selected and flagged for application of the various treatments. Ten fruits from each cluster were sampled for downstream processing. Additionally, the fungicide propiconazole (Tilt 250E) was applied as the commercial standard.

Using a spray bottle, all spray applications including yeast solutions used for inoculations were applied to the cherry clusters until run off. A solution of sterile deionized water, 0.01% Tween 80, and a mix of four yeast species (*Aureobasidium pullulans* [3161-1A]; *Candida railenensis* C. Ramírez & A.E. González [3157-2B]; *Cryptococcus victoriae* M.J. Montes, Belloch, Galiana, M.D. García, C. Andrés, S. Ferrer, Torr.-Rodr. & J. Guinea [3157-1]; and *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff [3160]) was used as the inoculation mixture and the final concentration consisted of 10^6 CFU ml⁻¹ of each yeast species. After inoculation,

the clusters were allowed to dry for approximately 2 h before the treatments were applied.

Fruit quality and microbial isolation. Ten cherries from each replicate were collected and visually inspected for a postharvest assessment of slip-skin-like disorder. A subsample of five cherries from each replicate were then selected and washed in 200 ml of phosphate buffer (0.1 M), pH 7.0, 0.01% Tween 80 on a shaker at 200 rpm for 20 min. Yeast malt dextrose agar (YMD) plus tetracycline was prepared at 1/3 normal concentration (0.8 g of peptone [HiMedia, Mumbai, India]; 1.7 g of dextrose [JT Baker, Center Valley, PA]; 0.5 g of yeast extract, 0.5 g of malt extract, 9.0 g of agar [Amresco, Solon, OH]; and tetracycline at 7.5 mg/ml [Alfa Aesar, Tewksbury, MA] per 500 ml of water) similar to protocols described elsewhere (Chand-Goyal and Spotts 1996; Sholberg et al. 2006). The wash buffer was subsequently used to make 10-fold serial dilutions down to 10⁻³ and 100 µl of each dilution was used for spread-plate on 1/3-strength YMD. Plates were incubated at room temperature for 2 days prior to enumeration and reisolation of yeast species.

Statistical analyses. All experiments were a randomized complete block design, with treatments being the main factors of interest. Sex (females only versus both the sexes combined) for no-choice bioassay was considered as a covariate. Each bioassay was considered an experimental block, and was conducted three times. Data were analyzed using Proc Mixed in SAS, ver.6 (SAS Institute, Cary, NC) unless otherwise stated. For the number of eclosed adults on a given day (endpoint) data, repeated measures analyses were conducted. Compound symmetry represented the appropriate type of dependence for covariance structure. Residuals were used to verify the assumptions of normal error distribution and constant variance. Data were transformed (sqrt + 0.5) as needed to meet the assumptions. If means were significantly different, they were separated using Tukey test ($\alpha = 0.05$). Back-transformed means (tabular or graphical) are reported as required. Percent malathion degradation data were analyzed after the data were checked for normality assumptions.

TABLE 1
Spray treatments to assess the effect of potassium metabisulfite (KMS) and the fungicide Tilt 250E on slip-skin-like symptoms and total yeast counts on sweet cherry cultivar Staccato

Treatment	Fruit inoculated ^x	Application timing (days preharvest) ^y	Chemical ^z
T1	Yes	7	KMS
T2	Yes	7, 3	KMS
T3	Yes	7, 3, 1	KMS
T4	Yes	7	Tilt
T5	Yes	7	Water
T6	No	7	KMS
T7	No	7	Water

^x Cherries inoculated or not with yeasts (text provides information for species and methodology under the heading “efficacy of KMS and propiconazole on yeasts of cherries”).
^y Fruit clusters were inoculated only once at 7 days preharvest with four yeast species, allowed to dry for approximately 2 h, and sprayed with respective chemicals and schedules.
^z KMS = KMS + 0.01% Tween 80; Tilt = Tilt 250E (propiconazole at 125 mg liter⁻¹); and water = sterile deionized water + 0.01% Tween 80.

Fig. 2. Average number of eclosed *Drosophila suzukii* first generation adults per day after rearing the parental generation in control diet compared with diet with treatments of potassium metabisulfite (KMS), malathion, or combination in the presence or absence of male flies. Bars with different letters indicate statistically significant differences.

When the interactions between malathion, KMS and flies were statistically significant, least square (LS) means were separated using Tukey test. The data from presence or absence of slip-skin-like symptoms was assumed to be in binomial distribution and hence were analyzed using Proc Glimmix and total yeast counts (CFU ml⁻¹) were analyzed after the data were checked for normality assumptions. Statistically significant contrasts, where LS means were separated using a Tukey test, among the treatments were reported for presence or absence of the slip-skin-like symptoms. For the total yeast counts data, if the means were significantly different, they were separated using a Tukey test.

RESULTS

Effect of KMS and malathion on *D. suzukii* survival and development—no-choice bioassay. Among the treatments, the combination of malathion and KMS resulted in the least number of eclosed adults when males were absent, while the control treatment had the highest average number of eclosed adults in the presence of males (Fig. 2). Similarly, the combination of KMS and malathion appeared to affect development time by 1 day, but there was no significant difference (treatment–sex–date, $P > 0.05$). *D. suzukii* survival to the adult stage was affected by the contribution of males as the interaction between treatments and the presence or absence of males (sex) was statistically significant (Table 2). Interestingly, in the absence of males there was no difference between KMS and malathion treatments, however when males were added, there were a higher number of eclosed adults in the KMS treatment than in the malathion treatment. Although temporal interaction with treatments and sex (treatment–sex–date) did not have a significant effect ($P > 0.05$), there were differences in total number of eclosed adults per day among the biological replicates that might reflect a block effect. The daily number of adults for control and malathion treatments appeared to be similar as the highest numbers were observed on day 3 (Fig. 3A).

Effect of KMS and malathion on *D. suzukii* survival and development—choice bioassay. When *D. suzukii* flies were provided with a choice, there were statistically significant treatment differences (Table 2). Among treatments, controls had the highest number of eclosed *D. suzukii* while the combination of malathion and KMS had the least number of flies eclose. The similarity between the no-choice (Fig. 3A) and choice bioassays (Fig. 3B) was the delay observed when the first adults eclosed in the combined KMS and malathion treatment. Since the peak number of eclosed adults for the control and KMS treatments in the choice bioassay (Fig. 3B) was similar to the peak observed for the control and malathion treatments in the no-choice bioassay (Fig. 3A), it may be that the flies avoid malathion if given a choice, but KMS alone does not affect their behavior. The plateau in the number of eclosed adults that occurred on days 3 and 4 in the choice bioassay (Fig. 3B) and on days 4 and 5 in the no-choice bioassay (Fig. 3A) was in part due to the delay caused by initial combination of KMS and malathion. As the efficacy of KMS diminished and malathion was degraded, survival of flies increased with time.

Effect of KMS on malathion degradation. The interaction of KMS, malathion, and flies had a significant effect on the residual amounts of malathion recovered from the diet after a 10-day incubation period (Table 2). The least amount was recovered when there was no KMS and the maximum amount recovered was when there were no flies present (Fig. 4). For treatments with introduced flies, the decrease in residual malathion due to the addition of KMS was 21% and without KMS it was 38%.

Efficacy of KMS to yeasts on cherries. Spray treatments had a significant effect on slip-skin-like symptoms and yeast populations

(Table 2). KMS applied at 7 and 3 days (treatment 2 or T2) and at 7, 3, and 1 days (T3) preharvest were shown to be effective treatments at reducing yeast populations on the surface of the inoculated cherry fruit when compared with the uninoculated water control (T7) (Table 3). Applications of KMS 7 days preharvest (T1) and the commercial fungicide Tilt 250E (T4) were shown to be similar in efficacy and reduced populations by 33 and 48%, respectively. However, the reduction of yeast populations was not always associated with the reduction in slip-skin-like symptom development. For example, although KMS reduced yeast on the surface of the fruit, none of the three KMS treatments (T1 to T3) were effective at reducing symptom development ($P > 0.05$).

TABLE 2
Analysis of covariance for potassium metabisulfite (KMS) on *Drosophila suzukii* survival and development, the degradation of malathion, and the incidence of slip-skin-like symptoms and yeast populations on sweet cherries

Experiment	Source of variance	df	F	P
<i>D. suzukii</i> survival and development—no choice				
	Treatment ^w	3,128	38.33	<0.0001
	Sex ^x	1,128	168.84	<0.0001
	Treatment–sex	3,128	2.87	0.0388
	Day ^y	7,128	123.42	<0.0001
	Treatment–day	21,128	9.09	<0.0001
	Sex–day	7,128	8.7	<0.0001
	Treatment–sex–day	21,128	1	0.4683
Malathion degradation				
	KMS	162	10.08	0.0023
	Malathion	162	2,142.52	<0.0001
	KMS–malathion	162	10.08	0.0023
	Flies ^z	162	63.24	<0.0001
	KMS–flies	162	4.28	0.0428
	Malathion–flies	162	33.24	<0.0001
	KMS–malathion–flies	162	4.28	0.0428
Malathion degradation follow-up: <i>D. suzukii</i> survival and development—no choice				
	Treatment	3,120	1.11	0.3484
	Day	14,120	15.98	<0.0001
	Treatment–day	42,120	12.14	<0.0001
<i>D. suzukii</i> survival and development from an oviposition choice test				
	Treatment	3,88	11.39	<0.0001
	Day	10,88	13.17	<0.0001
	Treatment–day	30,88	11.14	<0.0001
KMS dose-response effect on <i>D. suzukii</i> survival and development				
	Treatment	4,60	78.83	<0.0001
	Day	5,60	500.19	<0.0001
	Treatment–day	20,60	32.86	<0.0001
Slip-skin-like symptoms and yeast populations				
	Yeast populations	6,28	4.17	0.0004
	Symptom development	6,343	2.5	0.0250

^w Water, KMS, malathion, or both.

^x Females only versus males and females.

^y Day F1 *D. suzukii* adults eclosed.

^z Presence or absence of flies.

DISCUSSION

This study was developed from our interest in explaining why selection for malathion resistance did not occur in our field-collected *D. suzukii* populations. Others speculated that it could be due to a genetic bottleneck of these migratory populations (Bahder et al. 2015) while we thought microbial degradation of the insecticide could reduce the exposure to the flies (Smirle et al. 2017). To address this question, a malathion–KMS–*D. suzukii* model was applied. What appeared at first as a coincidence was that the microorganisms present in and on *D. suzukii* that contribute to its nutritional and other vital requirements (Chandler et al. 2011, 2012, 2014; Hamby et al. 2012; Hoang et al. 2015) are closely related to the microorganisms associated with, but in dissimilar proportions to slip-skin-like disorder (O’Gorman et al. 2016). The novel interplay of biotic, viz., microbial association of fruit and insect, with abiotic, viz., humidity and temperature (Brilli et al. 2014; Hamby et al. 2016; Tochen et al. 2014; Wiman et al. 2014), are factors now recognized as a temporally independent complex (Fig. 1).

When incorporated into *D. suzukii* diet, KMS (1×) functioned to dehydrate and sterilize the diet leading to either reduced larval hatch from the deposited eggs, reduced larval development and/or the number of adults that eclosed (Table 4), possibly suggesting an obligate relationship for *D. suzukii* with the microbiome (Koch 1960). Follow-up trials with 0.1× KMS diet incorporation also indicated significant differences in total numbers of adults eclosed, but

not to the same extent as 1× KMS. A previous study with a 3-day diet exposure of 3-day-old *D. melanogaster* flies showed they obtained adequate microbial deposits necessary to replenish the gut microbial community and the inclusion of males doubled the microbial deposits compared with females only (Blum et al. 2013). It was speculated that the addition of the male-transferred microbes was potentially responsible for creating an improved niche that allowed for higher fecundity (Grosjean et al. 2011; Keesey et al. 2016).

Our experiments have shown a pronounced delay in time to peak numbers of eclosed adults from diet with KMS compared with the control diet, and that malathion degradation was reduced when KMS was present in the diet. A similar relationship was observed in the *E. agglomerans*–apple–apple maggot fly model where control of microbial degradation caused a plant-derived toxin to have greater effects against the apple maggot fly (Lauzon et al. 2003). The presence of the microbiome on leaf and fruit surfaces would be a greater source of these microbes under field conditions (Sipiczki 2016) where microbial degradation could definitely occur. Similar bacterial-driven degradation of toxins is noted for various model systems other than plants. For example, salt marsh bacterial isolate not only degraded malathion, but also used it as a sole carbon source (Bourquin 1977), and *Flavobacterium* sp. isolated from paddy water rapidly hydrolyzed diazinon to carbon dioxide and parathion to *p*-nitrophenol, also used as a carbon source (Sethunathan and Yoshida 1973). When *D. suzukii* were given a choice of

Fig. 3. A, Average number of eclosed *Drosophila suzukii* first generation (F1) adults when parental generation (P) adults were exposed to control, potassium metabisulfite (KMS), malathion or both, combination of KMS and malathion, in a diet no-choice bioassay and **B**, average number of eclosed *D. suzukii* F1 adults when P adults were exposed to control, KMS, malathion or both, combination of KMS and malathion, in a diet choice bioassay. § represents significant statistical difference due to incorporation of KMS in diet versus control on a given day; * represents significant statistical difference due to incorporation of malathion in diet versus control on a given day; † represents significant statistical difference due to incorporation of both, combination of KMS and malathion, in diet versus control on a given day.

treatments (Fig. 3B), their response was different compared with the no-choice bioassay (Fig. 3A). One explanation for the difference is that the flies delayed ovipositing on diet with malathion relative to the control diet, which led to the lower adult eclosion observed in the malathion-treated diet. When the oviposition of *D. melanogaster* was studied, females avoided the insecticide-treated dish and deposited more eggs on the control dishes, and postponed oviposition in treated dishes and deposited higher numbers of eggs the following day (Renou et al. 1997). Another explanation is that larvae did not hatch until the malathion residue degraded, possibly via microbes deposited when the number of flies visiting the diet increased (Kim et al. 2005; Regueiro et al. 2015; Sharma et al. 2005). Chemical cues normally perceived by the flies via microbial associations may also have been absent or altered if the microbes allocated their resources to metabolize malathion (Sinsabaugh and Moorhead 1994).

The combination of KMS and malathion in the diet had a greater effect on the *D. suzukii* population, likely because malathion was degraded more slowly due to the negative effect of KMS on the microbial population. Yeast populations on the surface of sweet cherries were affected by KMS when it was applied frequently and closer to harvest (Table 3). However, there was no strong association between slip-skin-like symptom development and the reduction of surface yeast populations on KMS-treated cherries. Surface sterilization is temporary and the microbes carried by the flies also exist as an endophytic population inside the undamaged fruit (Chandler et al. 2011, 2012, 2014; O’Gorman et al. 2016) where KMS does not penetrate. KMS, likely due to its nonsystemic mode of action, was ineffective for the control of slip-skin-like symptoms. However, the application of propiconazole resulted in a moderate but not statistically significant reduction of both yeast counts and slip-skin-like symptoms (Table 3). Many species of yeasts and bacteria reside inside and on the surfaces of plant tissues, especially fruits (Dudley 2004), and propiconazole can penetrate further in to the fruit than KMS. Late varieties of cherries often meet

the appropriate temperature and humidity conditions for maximum yeast abundance, as seen in wine grapes with *H. uvarum* (Brilli et al. 2014) or in apples with total yeasts (Marshall and Walkley 1951). Sour rot in grapes is assumed to be initiated internally in tight clusters and spreads outwards (McFadden-Smith 2012), and is speculated to be triggered by post-powdery mildew infestation. As in grape sour rot, we hypothesize that the symptoms in sweet cherry may occur during ripening with the rapid cell expansion and the creation of micro fissures, as the fruit press tightly together in large, heavy clusters (Beyer et al. 2005) and/or undetectable damage by powdery mildew. High populations of surface microbes would then be able to gain additional access to internal tissue via these minute openings. The seasonal increase and prevalence of yeast on cherry (Fig. 1), as with other fruit such as wine grapes could be due to their starch capsules assisting in clumping and adherence to the fruit surfaces (Clark et al. 1954). This could promote fermentation and attract drosophilids, thus aggravating microbial deposition and vectoring. Additionally, events such as the incidence of *D. suzukii* in cherry orchards and or fungicide use patterns as discussed above (Yang et al. 2011) could change the balance between resident and external microbiota, possibly by quorum sensing (Sprague and Winans 2006), as noted in the sour rot–*D. melanogaster* model where the fly is considered as a vector (Lam and Howell 2015).

Observations of sound cherry fruit left to hang slightly past the optimal harvest dates showed they were prone to increased insect damage leading to numerous different fungal rots and noticeably greater volatile acids (higher proportions of decay bacteria assimilating trehalose produced and accumulated by yeasts) (O’Gorman et al. 2016) (Fig. 1). Other postharvest disorders such as melting decay in table grapes (Morgan and Michailides 2004), sour rot in peaches and nectarines (Michailides et al. 2004), and box rot of prune plums (Sholberg and Ogawa 1983), may have a similar association with the vectored microbiota of *Drosophila* spp. or other insects, especially if a harvest-delay (normally harvested at a lower brix than wine grapes) occurs. Additionally, in wounding experiments with

Fig. 4. Percent residual malathion recovered (± SE) from diet exposed to *Drosophila suzukii* adults, with and without potassium metabisulfite (KMS).

sweet cherries where *H. uvarum*-fed *D. suzukii* were used, volatile acids were noted in the fruits exposed to natural inoculums (data not shown). It is interesting to note similar associations of acetic acid bacteria with *D. suzukii* (Mazzetto et al. 2016) have been observed.

To summarize, a probable imbalance created between interior and exterior resident microbes occurs at the time when saturation of the fruits with sugars permits minimal penetration of pesticides yet promotes microbial activity. Combinations of higher temperatures and humidity commonly reached during the late growing season are among conditions known to promote fermentation. Under these conditions the exchange of metabolites produced by the microbiome may provide cues that promote *D. suzukii* incidence and behavior, as noted previously for *D. melanogaster* (Fischer et al. 2017), and possibly cause increased pesticide degradation as noted by others (Lauzon et al. 2003). Similar conditions could also promote a classical postharvest problem termed slip-skin-like disorder where the yeast density is high. Temporally separated events, beginning with increased *D. suzukii* incidence (2 to 3 weeks preharvest) may lead into slip-skin-like disorder incidences (1 week

preharvest) due to changing microbial densities (Fig. 1). This could be controlled by continuous nonselective suppression of the microbes using commercially available systemic biocides. Since such products currently are not registered in Canada, further studies may support the information required to decide which biocides would be the most appropriate to manage these overlapping, microbial-driven conditions.

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TABLE 3

Effect of potassium metabisulfite (KMS) spray applications on populations of yeast (CFU ml⁻¹ × 1,000) on the fruit surface of sweet cherry cultivar Staccato

Treatment	Fruit inoculated ^w	Application timing (days preharvest) ^x	Chemical ^y	Total yeast counts ^z
T1	Yes	7	KMS	116.6 ± 27.0 abc
T2	Yes	7, 3	KMS	46.8 ± 27.0 c
T3	Yes	7, 3, 1	KMS	64.2 ± 27.0 bc
T4	Yes	7	Tilt 250E	105.4 ± 27.0 abc
T5	Yes	7	Water	122.0 ± 27.0 abc
T6	No	7	KMS	172.8 ± 27.0 ab
T7	No	7	Water	202.4 ± 27.0 a

^w Cherries inoculated or not inoculated with yeasts (text provides information for species and methodology under the heading "efficacy of KMS and propiconazole on yeasts of cherries").

^x Fruit clusters were inoculated only once at 7 days preharvest with four yeast species, allowed to dry for approximately 2 h, and sprayed with respective chemicals and schedules.

^y KMS = KMS + 0.01% Tween 80; Tilt = Tilt 250E (propiconazole at 125 mg liter⁻¹); and water = sterile deionized water + 0.01% Tween 80.

^z Numbers followed by same letter are not statistically significant ($P > 0.05$) based on Tukey's test.

TABLE 4

The dose-response of potassium metabisulfite (KMS) incorporated into artificial diet on the eclosion of first generation *Drosophila suzukii* that developed from parent generation adults transferred for 3 days to treatment vials^z

KMS (mg liter ⁻¹)	Mean eclosed flies ± SE
0	17.7 ± 0.9 a
5	19.3 ± 0.9 a
50	18.3 ± 0.9 a
500	13.9 ± 0.9 b
5,000	0.0 ± 0.9 c

^z Numbers followed by the same letter are not significantly different ($P > 0.05$) based on Tukey's test ($N = 10$ adults of each sex).

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