In search of a better fly trap:

Chemical and visual ecology of *Drosophila suzukii*

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A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Biology / Faculty of Science

Memorial University of Newfoundland

Submitted December 2020

St. John’s, Newfoundland and Labrador

Abstract

Drosophila suzukii is an invasive species of concern to fruit growers throughout temperate regions worldwide. Unlike most *Drosophila* species, *D. suzukii* has an enlarged and heavily sclerotized ovipositor that allows female flies to lay eggs in fruits before they are fully ripened and, in most cases, before fruits are harvestable. Initial efforts at mitigating damage have relied on chemical pesticides to reduce *D. suzukii* populations in crop areas; however, on-going research efforts have focused on more environmentally sustainable integrated pest management alternatives.

This thesis investigates aspects of *D. suzukii* behaviour and physiology that promoted its successful global invasion. Chapter one discusses the role of behavioural and physiological plasticity in giving *D. suzukii* an ecological edge during introduction and successful invasion. Chapter two investigates *D. suzukii* host selection behaviour and preference among commercial fruits and novel native fruits in a boreal environment. I investigated the fruit characters thought to play a role in host choice, including fruit sweetness (brix), fruit acidity (pH), and fruit firmness (penetration force [gfmm^2]). Based on *D. suzukii* behaviour observed in field settings, the investigation was expanded to include the role of fruit and foliage colour in host selection. Additionally, we beta-tested a citizen science initiative to identify native fruit species at risk and to confirm the range limits of *D. suzukii* in Atlantic Canada. Chapter three further explores colour preference and use of colour by *D. suzukii* as attraction cues, first as cues to differentiate among fruits of different ripeness stages, and second as visual targets for potential use in monitoring traps. Chapter four investigates *D. suzukii* physiological sensitivity and behavioural activity to odorants associated with fruits and foliage, and odorants known to be important to

other *Drosophila* species. An iterative process of laboratory and field trials was used to test individual odorant compounds and odorant blends in combination with results of colour preference testing to improve trapping efficacy. Given the behavioural and physiological plasticity of *D. suzukii*, trials were conducted among different fruit crops and growing environments. Chapter five synthesizes lessons learned about *D. suzuki*i behaviour and preferences to make recommendations for effective monitoring traps for blueberry and raspberry crop systems.

Acknowledgements

I respectfully acknowledge that this research was conducted on the ancestral homelands of the Mi’kmaq and Beothuk peoples. I would like to thank my supervisory committee: Kirk Hillier, Tom Chapman, Deb Moreau, Peggy Dixon, and Brian Stavely for their ongoing guidance and support. I would like to thank my lab mates at Acadia University and Memorial University, including Lara Thomas, Rebecca Rizzato, Logan Gray, Heather Crozier, Simon Pawlowski, Emma Rand, Rylee Isett, Megan MacIsaac, Lise Charbonneau, Colin MacKay, Michael Light, Joel Goodwin, Jesse Saroli, Adam Discher, Ersa Gjelaj, Kate Carson, Chris Hammill, Todd Power, Andrew Chaulk, and Holly Caravan. I would also like to thank Brent Robichaud, Marlene Snyder, Dawn Miner, Tanya Morse-Outhouse, Michael Robertson, Justin Renkema, David Kristie, Melanie Prieztniz, Meghan Swanburg, Ian Fleming, Carolyn Parsons, Madonna Bishop, Shelley Vaughan, Nathan Patstone, Katrin Sommerfeld, and Susan Carbyn. This research would not have been possible without the generous support of Nova Agri Inc. (Blueberry Acres and Vital Berry Farm), MacBerry Farm, Lester’s Farm, Rodrigues Winery, Wright's Family Farm, Campbellton Family Farm, and Mark’s Market. A special thanks goes to my family and friends for their love and support.

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Chapter 1

General introduction

1.1 Introduction

Invasive species are an increasingly common problem for agricultural producers. *Drosophila suzukii* Matsumara (Diptera: Drosophilidae) was first recognized as a potential invasive pest of soft fruits and berries outside of southeast Asia in 2008 (Hauser 2011, Walsh et al. 2011). Since that first identification, *D. suzukii* has become an economically-significant pest throughout much of North America, South America, and Europe (Calabria et al. 2012, Deprá et al. 2014, Asplen et al. 2015).

A wide variety of soft fruits, stone fruits, and berries are susceptible to damage from *D. suzukii* (Lee et al. 2011, 2015, 2016). Damage is due to feeding by adults and, more often, from oviposition damage and larval feeding. Unlike most *Drosophila* species, female *D. suzukii* are able to oviposit in fruits before they are fully ripe (Burrack et al. 2013). By virtue of its wide host range and short generation period, *D. suzukii* is capable of multiple generations each year throughout most temperature regions (Hamby et al. 2016). Even a small population of cold-adapted mated female flies can survive through winter to founder the next year's population, which can grow exponentially by late summer or early fall.

Recognizing when *D. suzukii* populations have grown to potentially damaging levels is vital for fruit growers. Costs associated with pesticides and other control efforts must be balanced against potential losses due to damaged crops. Effective monitoring traps are an integral part of developing an efficacious integrated pest management system to reduce control costs and mitigate crop damage.

Drosophila suzukii uses a combination of sensory systems to identify potential host fruits for feeding and oviposition. We investigated the visual, olfactory, and tactile cues which act as signals of fruit suitability for *D. suzukii*. The results of this work can inform growers of potential risks for commercial fruit crops. Visual and olfactory cues also offer potential for improving efficacy of monitoring traps.

1.2 Thesis outline

Chapter 1. "Plasticity is key to success of *Drosophila suzukii* (Diptera: Drosophilidae) invasion" discusses the sudden and rapid expansion of *D. suzukii* internationally by examining this species adaptability to diverse environmental conditions and changeable host availability. *Drosophila suzukii* has expanded across most temperate regions, establishing enduring populations capable of causing significant crop losses for local fruit growers (Hauser 2011, Walsh et al. 2011, Asplen et al. 2015). Recent evidence suggests that the ongoing climate crisis will exacerbate environmental changes in Atlantic Canada, allowing *D. suzukii* to further increase its distribution (Langille et al. 2017, Taylor et al. 2017, Reyes and Lira-Noriega 2020).

The ability to successfully identify and utilize a wide range of potentially novel host fruits for feeding and reproduction relies in large part to the use of a combination of environmental cues, including olfactory and visual signals. This flexibility underlies both the potential risk to commercial fruit production and the potential for identifying methods of monitoring and mediating infestation in specific fruit crops.

Chapter 2. As an invasive species in boreal regions of Canada, *D. suzukii* will encounter a wide range of novel native and commercially grown fruit species. "Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii* (Diptera: Drosophilidae)" investigates characters which make fruits more or less attractive and suitable for reproduction for *D. suzukii*. We explore potential susceptibility of common native and crop fruit species growing in Atlantic Canada.

“Role of fruit characters and colour on host selection of boreal fruits and berries by *Drosophila suzukii* (Diptera: Drosophilidae)” expands the investigation of attractive fruit characters to include an objective measure of fruit colour as a visual cue for *D. suzukii*. We further expand our understanding of which native and crop fruit species are susceptible to infestation by *D. suzukii*.

“FlySpotter: using citizen science to identify range expansion and fruit at risk from *Drosophila suzukii* in Nova Scotia and Newfoundland and Labrador” explores the potential for citizen science as a means to confirm the extent of *D. suzukii* expansion across a broad geographic area.

Chapter 3. “Effect of colour and contrast of highbush blueberries to host-finding behavior by *Drosophila suzukii* (Diptera: Drosophilidae)” further explores the role of colour as a visual cue for identification of fruits suitable for reproduction.

“Colour preference of the spotted wing Drosophila, *Drosophila suzukii*” investigates *D. suzukii* colour preferences independent of fruit cues, as a potential means of improving trap design efficacy.

Chapter 4. “Assessment of attractant lures and monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) using electrophysiology, laboratory choice assays, and field trials” investigates odorants and odorant blends for sensitivity and attractiveness for *D. suzukii*. These olfactory cues are field tested as probative attractant lures in combination with refinements in trap design and trap colour to improve trap efficacy and specificity.

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1.4 Co-authorship statement

This research was conducted under the co-supervision of Dr. N. Kirk Hillier and Dr. Thomas W. Chapman. I wrote the original drafts of all manuscripts included in this thesis.

Financial support for research conducted for this thesis was provided by Memorial University of Newfoundland and Labrador, Acadia University, Atlantic Canada Opportunities Agency Atlantic Innovation Fund, Canada Foundation for Innovation, Natural Sciences and Engineering Research Council of Canada, and Growing Forward 2 in Newfoundland and Labrador. Additional support for field research was provided by Agriculture and Agri-Food Canada and Nova Agri Inc.

Published and anticipated publications resulting from this thesis are:

Chapter 1

Little, C.M., Chapman, T.W., and Hillier, N.K. 2020. Plasticity is key to success of *Drosophila suzukii* (Diptera: Drosophilidae) invasion. J Insect Sci **20**(3): 1–8. doi:10.1093/jisesa/ieaa034.

Chapter 2

Little, C.M., Chapman, T.W., Moreau, D.L., and Hillier, N.K. 2017. Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii* (Diptera: Drosophilidae). Pest Manag Sci **73**(1): 160–166. doi:10.1002/ps.4366.

Little, C.M., Dixon, P.L., Chapman, T.W., and Hillier, N.K. 2020. Role of fruit characters and colour on host selection of boreal fruits and berries by *Drosophila suzukii* (Diptera: Drosophilidae). Can Entomol: 1–17. doi:10.4039/tce.2020.1.

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Chapter 3

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Chapter 4

Little, C.M., Dixon, P.L., Moreau, D.L., Chapman, T.W., and Hillier, N.K. 2021. Assessment of attractant lures and monitoring traps for *Drosophila suzukii* (Diptera: Drosophidae) using electrophysiology, laboratory choice assays, and field trials. J Econ Entomol: toab006. doi:10.1093/jee/toab006.

1.5 Plasticity is key to success of *Drosophila suzukii* (Diptera: Drosophilidae) invasion.

A version of this chapter section has been published in Journal of Insect Science:
Little, C.M., Chapman, T.W., and Hillier, N.K. 2020. Plasticity is key to success of *Drosophila suzukii* (Diptera: Drosophilidae) invasion. J Insect Sci **20**(3): 1–8. doi:10.1093/jisesa/ieaa034.

Author Contribution Statement
CML proposed and researched the subject of this review. NKH and TWC advised on presentation and scope. All authors read, edited, and approved manuscript.

1.5.1 Abstract

After its initial discovery in California in 2008, *Drosophila suzukii* Matsumura has become one of the most important invasive agricultural pest insects across climate zones in much of Asia, Europe, North America, and South America. Populations of *D. suzukii* have demonstrated notable behavioral and physiological plasticity, adapting to diverse environmental and climatic conditions, interspecific competition, novel food sources, and potential predators. This adaptability and plasticity have enabled rapid range expansion and diversified niche use by *D. suzukii*, making it a species particularly suited to changing habitats and conditions. This article reviews factors and evidence that influence plasticity in *D. suzukii* and promotes this species' invasiveness.

1.5.2 Introduction

Plasticity is a driving force behind the spread of numerous invasive species. Plasticity refers to the degree to which traits of individuals or populations can rapidly adapt to new or changing environmental conditions (Sgrò et al. 2016). Adaptations can be in the form of phenotypic, behavioural, developmental, or physiological traits.

Resistance to or tolerance of stressful conditions and a short generation time increase the risk of unintentional transportation and facilitate introduction (Gippet et al. 2019). Newly introduced species often suffer from genetic bottlenecks, which can be offset by plasticity. Once introduced into a new environment, such phenotypic or behavioural plasticity can promote an exotic species to becoming established in its new environment by exploiting vulnerable niches in these habitats (Sakai et al. 2001; Engel et al. 2011; Garnas et al. 2016). Introduced species with limited plasticity in key traits would be compromised in their ability to adapt to new environments, including novel biotic and abiotic factors (Chown et al. 2007; Engel et al. 2011). Plasticity can further promote the continued spread of newly established species beyond its point of introduction (Sakai et al. 2001).

Physiological or behavioural plasticity can result from differences in environmental conditions (e.g., temperature, humidity, photoperiod), available diet, or pressure from predators or competitors (Hamby et al. 2016; Wallingford et al. 2016; Guédot et al. 2018). This plasticity can take many forms, including the ability to exploit novel food resources, as in the Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) which is able to use many apple varieties as oviposition substrates, or the ability to outcompete local species, as in the Asian ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) which develop more quickly than the North American native ladybird *Adalia bipunctata* L. (Coleoptera: Coccinellidae) (Beukeboom 2018).

Drosophila suzukii Matsumura (Diptera: Drosophilidae) was first described in Japan (Matsumura 1931) but is believed to have originated in mainland Asia. Shortly thereafter, it was identified as the source of damage in fruit crops in Japan (Kanzawa 1935, 1939). By 1980, *D. suzukii* had been confirmed in Hawai'i (Kaneshiro 1983; O'Grady et al. 2002; Leblanc et al.

2009). *Drosophila suzukii* was first reported in North America in California in 2008 and has since spread across the continental US, north into Canada, and south into Mexico (Hauser 2011; Walsh et al. 2011). Since 2008, *D. suzukii* has also invaded most of Europe and South America (Calabria et al. 2012; Cini et al. 2014; Deprá et al. 2014; Benito et al. 2016; Andreazza et al. 2017). *Drosophila suzukii* is now well established throughout most sub-tropical, temperate, and boreal regions (Andreazza et al. 2017; dos Santos et al. 2017; Little et al. 2017; Manduric 2017). *Drosophila suzukii* is now considered one of the most important agricultural pest species throughout most of its invasive range (Benito et al. 2016; Gutierrez et al. 2016).

Drosophila suzukii possess a number of traits which predispose it to dispersal and unintentional introductions to new environments (Gippet et al. 2019). For example, the species is closely associated with small fruit species that are routinely transported internationally. All life stages of *D. suzukii* are small and inconspicuous. Additionally, eggs and larvae are usually located within the fruits and are not visible during external examination. Thus, *D. suzukii* is less likely to be detected during transportation of these fruits or upon arrival in new geographic regions (Gippet et al. 2019). Since 2008, *D. suzukii* has been highly successful at moving from unintentional introductions to become invasive species due in large part to notable plasticity in development, adult phenotype, and behaviour (Jakobs et al. 2015, 2017; Hamby et al. 2016; Shearer et al. 2016; Fraimout et al. 2018; Stockton et al. 2018).

1.5.3 Phenotypic plasticity

Morphological plasticity

At its most basic level, phenotypic plasticity refers to differences in individual morphological traits in response to environmental conditions, diet, or other factors (Moczek 2010). Colder temperatures during slow larval development resulting in adult *D. suzukii* with larger wings, which allows cold-reared flies to accelerate and fly faster, but not for greater duration, than flies reared in warmer temperatures (Shearer et al. 2016; Fraimout et al. 2018). In contrast, warmer temperatures during development result in smaller wings and smaller wing spots in male flies, which fly less quickly (Fraimout et al. 2018; Varón-González et al. 2020). Thus, cold-reared *D. suzukii* could be able to disperse faster and further during flights of the same duration compared to flies reared under more moderate temperatures, potentially further increasing the risk of increased range expansion in temperate and boreal regions. In mark-recapture trials in Trentino, Italy winter morph *D. suzukii* were recovered more than 9000 m from a point source (Tait et al. 2018). Larval diet can induce differences in adult morphology, including wing size and shape (Pajač Živković et al. 2018). Furthermore, differences in wing morphology as populations of *D. suzukii* experience seasonal and regional differences in fruit availability can influence flight ability and potential dispersal distances. Diet also affects mating behaviour of adult *D. suzukii*, which when reared on suboptimal diet are both less selective of and less successful at attracting potential mates (Young et al. 2017). Therefore, such environmental drivers which subsequently alter wing morphology of *D. suzukii* may result in positive, negative, or neutral impacts on fitness.

At its most extreme, morphological plasticity is revealed as polyphenism, where distinct phenotypes are expressed in response to different conditions (Moczek 2010). In subtropical regions, where environmental conditions are suitable year-round, *D. suzukii* are active year-round (Harris et al. 2014; Andreazza et al. 2017; dos Santos et al. 2017). In temperate regions, mated adult females overwinter as reproductively-quiescent winter morphs rather than experiencing true reproductive diapause (Dalton et al. 2011; Shearer et al. 2016; Toxopeus et al.

2016; Guédot et al. 2018). Acclimation to cold temperatures and shorter photoperiods promotes cold tolerance in both adult and pupal *D. suzukii*, improving survival of the induced winter morph adults (Wallingford et al. 2016; Stockton et al. 2018). Winter morph flies are more cold-tolerant and usually larger than summer morphs (Stephens et al. 2015; Shearer et al. 2016; Wallingford and Loeb 2016). Given favourable diet, post-overwintering female winter morph *D. suzukii* are longer lived and have greater fecundity than summer morph flies; however, when diet is suboptimal, fecundity and longevity of summer morph flies are less restricted by low temperatures (Rendon et al. 2018, 2019).

1.5.4 Developmental plasticity

Temperature and desiccation tolerance

A species which demonstrates an ability to adapt to a range of temperature and humidity conditions can more readily become established in novel habitats. Individuals or populations can acquire increased tolerance to temperature extremes through hardening (short term exposure), acclimation (long term exposure in a laboratory setting), or acclimatization (long term exposure in a natural setting) (Sinclair et al. 2015).

Generation time of *D. suzukii* is approximately 12-15 days; however, development time is dependent on temperature and larval diet (Stockton et al. 2019a). Larvae emerge from eggs within 12-72 h of oviposition and progress through three instars. Larvae develop most quickly at 26-28^{o}C (Kinjo et al. 2014; Tochen et al. 2014; Asplen et al. 2015). Temperatures below this range or fluctuating temperatures can slow larval development to as much as 64 d (Jakobs et al. 2017).

Exposure to fluctuating temperatures during development induces greater cold tolerance in adult flies (Stephens et al. 2015; Stockton et al. 2018). This cold tolerance is due in part to increased accumulation of cryoprotectant compounds (Enriquez et al. 2018). Despite this, freezing will kill larvae and adults, and both third instar larvae and adults are chill susceptible (Jakobs et al. 2015, 2017; Enriquez and Colinet 2017; Stockton et al. 2018). Temperatures between 22.6 °C and 28.2 °C are optimal for *D. suzukii* development; however, larval development and adult emergence can occur within a wider range of 8.1°C to 30.9°C (Tochen et al. 2014; Ryan et al. 2016). Adult activity, including oviposition behaviour, is limited below 10°C (Wallingford et al. 2016; Zerulla et al. 2017; Leach et al. 2019). Complete development is most reliable at constant temperatures of 20-26°C (Kinjo et al. 2014; Tochen et al. 2014; Asplen et al. 2015).

Pupae are more tolerant of extreme heat than adult *D. suzukii*, provided heat stress is not compounded with low humidity (Enriquez and Colinet 2017). Fifty percent of pupae can survive temperatures as high as 37°C for up to 4 hours (Enriquez and Colinet 2017). Temperatures during development also affects adult morphology, particularly wing size and shape, which in turn affects flight ability (Fraimout et al. 2018). Heat stress reduces adult life span, fecundity, and reproductive activity, as evidenced by a lack of oviposition behaviour at temperatures of 33°C and above (Enriquez and Colinet 2017; Evans et al. 2018; Kirk Green et al. 2019). Male *D. suzukii* are more susceptible to effects of heat stress than are female flies (Kirk Green et al. 2019). Egg viability, pupal development, and adult eclosion were also compromised above 28°C (Evans et al. 2018; Kirk Green et al. 2019).

Oviposition and successful larval development can occur at temperatures as low as 11.1°C (Tonina et al. 2016). While larvae and pupae are not able to survive prolonged temperatures below 5^{o}C, adult winter morph *D. suzukii* survival has been reported to survive continuous six-week exposure at temperatures as low as 1^{o}C (Ryan et al. 2016; Stockton et al. 2018; Stockton et al. 2019a). Survival of adult *D. suzukii* at colder temperatures could be possible when temperatures fluctuate, allowing for repair of cold damage during warmer periods in a mechanism similar to that observed in cold-acclimated *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) (Renault et al. 2004). Adult *D. suzukii* can survive 1h exposure to temperatures as low as -7.5^{o}C (Jakobs et al. 2015; Stockton et al. 2018). Acclimation to cold temperatures improves both survival during short term exposure and duration of survivable exposure (Jakobs et al. 2015). Acclimation to cold temperatures induces upregulation of up to 1583 genes, including genes for ion transport, cellular signalling, and carbohydrate metabolism, while also inducing down-regulation of an additional 1325 genes, including genes for oogenesis (Shearer et al. 2016; Enriquez and Colinet 2019). Thus, exposure to cold temperatures can result in epigenetic changes in physiology that promote metabolic homeostasis and enable increased tolerance to more extreme environmental conditions (Enriquez et al. 2018). Five days after cold shock exposure, fecundity of female *D. suzukii* returns to pre-exposure levels (Plantamp et al. 2016).

Gradual acclimation to cold temperatures and low humidity may interact to further facilitate cold tolerance (Guédot et al. 2018; Stockton et al. 2018). Acclimation to cold temperatures allows adult *D. suzukii* to survive temperatures below 0^{o}C for longer periods, withstand chill coma symptoms at lower temperatures, and recover from cold exposure more quickly (Jakobs et al. 2015). Acclimation to cold temperatures at both developmental and adult stages infers chill protection in adult *D. suzukii* through greater homeostatic stability due to accumulated cryoprotectant amino acids and carbohydrates (Enriquez et al. 2018). Female winter morph *D.*

suzukii that survive initial exposure to low humidity conditions are able to withstand continued dry conditions for longer periods than summer morph flies (Fanning et al. 2019).

Low humidity levels limit survival of all life stages of *D. suzukii* (Tochen et al. 2014, 2016a; Gutierrez et al. 2016). Low relative humidity decreases both fecundity and longevity in *D. suzukii* (Tochen et al. 2014, 2016a; Guédot et al. 2018; Fanning et al. 2019). Attempts to limit ambient humidity in crop areas through irrigation practices or crop system management can be of limited efficacy, because low relative humidity does not limit *D. suzukii* flight distance or duration (Wong et al. 2018; Rendon and Walton 2019). Additionally, humidity levels in and below organic mulches, such as sawdust or woodchip, can be higher than above the mulch surface, potentially providing suitable conditions for pupal development (Rendon and Walton 2019). However, effects of mulch on *D. suzukii* adult emergence during field trials are inconclusive (Rendon et al. 2020). Natural refuges, including accumulations of leaf litter, as well as microclimates in and around built-structures permit increased survival during extreme winter weather conditions (Zerulla et al. 2015; Gutierrez et al. 2016, Wallingford et al. 2018; Stockton et al. 2019a). Overwintered female *D. suzukii* have been found bearing mature eggs as early in spring as at 7 degree-days and begin ovipositioning in the first available fruits of spring at only 87 degree-days (Grassi et al. 2018; Panel et al. 2018). Therefore, adult *D. suzukii* can survive and reproduce in less favourable environments through behavioural plasticity, by making short-distance movements in and out of nearby more favourable microclimates (Klick et al. 2016; Tochen et al. 2016a).

It is apparent that *D. suzukii* can adapt physiologically and behaviourally to tolerate a wide range of temperature and humidity conditions, particularly if those conditions are localized or transient. As the effects of climate change become more pronounced, fluctuations in both temperature and humidity will be more prevalent over a wider geographic area, promoting further opportunities for *D. suzukii* to find favourable habitats. *Drosophila suzukii* could also use marginally suitable habitats, provided more favourable microclimate areas are accessible within their flight range.

1.5.5 Behavioural plasticity

Plasticity in circadian activity

Locomotor activity of *D. suzukii* is mediated by light conditions, ambient temperatures, and relative humidity, with flies most active at dawn/dusk during summer conditions and at the warmest portion of the day during winter conditions (Hamby et al. 2013; Evans et al. 2017; Hansen et al. 2019; Shaw et al. 2019). Social interactions within groups of flies increases synchronicity of activity among individuals and reinforces locomotor activity patterns, particularly crepuscular activity and movements among localized microclimates, which would further promote increased grouping of flies (Hansen et al. 2019; Shaw et al. 2019). Localized populations exposed to different microclimate conditions could experience shifts in gene allele frequencies and differences in behaviour that could ultimately lead to microevolutionary changes among populations. Upregulation of detoxification transcription factors also fluctuates in response to an endogenous circadian clock, which results in daily periods of increased and decreased pesticide susceptibility (Hamby et al. 2013). Preliminary research suggests that *D. suzukii* is not at peak insecticide susceptibility during peak periods of activity in crop areas (Hamby et al. 2013).

Activity levels of female *D. suzukii* varies with their mating status. Virgin flies of both sexes are quiescent in mid-afternoon to reduce exposure to sun and heat (Ferguson et al. 2015).

However, mated female flies remain active throughout this period, tolerating both heat and lower humidity (Ferguson et al. 2015). Gravid female *D. suzukii* oviposit greater numbers of eggs when temperatures are between 25-28°C and will shift timing of oviposition behaviour based on daily temperature fluctuations (Kinjo et at. 2014; Evans et al. 2017). Fly activity on fruit and in flight near fruit plants is flexible depending on temperature and humidity levels, but *D. suzukii* behaviour is largely unaffected by irrigation and insecticide application procedures (Van Timmeren et al. 2017).

Olfactory plasticity

While *D. suzukii* is more sensitive than *D. melanogaster* Meigan (Diptera: Drosophilidae) to volatiles produced by ripening fruit, there is evidence to suggest *D. suzukii* also uses differences in leaf tissue volatiles produced during fruit development as a supplemental means to locate potential feeding and oviposition sites (Keesey et al. 2015; Bolton et al. 2019). In laboratory studies, *D. suzukii* demonstrates unique antennal response profiles to fruit and yeast associated odorants that enable identification of ripening fruits and sugar receptors that enable identification of floral nectars (Scheidler et al. 2015; Hickner et al. 2016). Preference for volatile odours differ dependent on the sex and physiological state (mated or unmated females) of adult *D. suzukii*, and on environmental states (temperature and ambient temperatures (Wong et al. 2018; Clymans et al. 2019)). Gravid female *D. suzukii* select oviposition sites using a combination of chemosensory cues, including olfactory, tactile, and potential gustatory signals (Karageorgi et al. 2017). Environmental odours can reduce *D. suzukii* attraction to otherwise desirable olfactory cues, which can further hamper the effort to identify effective olfactory attractant lures for monitoring purposes (Cloonan et al. 2019). Male and unmated female *D. suzukii* prefer volatiles associated with fermentation and indicative of high protein food sources; however, mated female flies prefer fruit odours indicative of substrates more suitable for oviposition sites (Karageorgi et al. 2017; Wong et al. 2018; Clymans et al. 2019). However, as with other phytophagous insects, previous experience can induce host acceptance or alter host preference hierarchies (Jaenike 1990; Anderson and Anton 2014). While *D. suzukii* larvae reared on blackberry (*Rubus* L. subgenus *rubus* Watson (Rosales: Rosaceae)) had no oviposition preference as adults between blackberry and American pokeweed (*Phytolacca Americana* L. (Caryophyllales: Phytolaccaceae)), flies reared on American pokeweed preferred to oviposit on blackberry (Diepenbrock et al. 2016; Stockton et al. 2019a).

Differences between summer and winter morph flies also extends to physiological and behavioural responses to odorant stimuli. Summer morph *D. suzukii* are more responsive to ecologically relevant volatile odorants, including both potential attractant and deterrent compounds (Kirkpatrick et al. 2018). This responsiveness in summer morph individuals is reflective of greater activity for host-finding for adult feeding and also of host-finding for oviposition sites. Winter morph *D. suzukii* undergo a reproductive diapause and search out food sources during periods when ambient temperatures make volatiles are less prevalent. Winter morph flies prefer shelter sites containing food sources; either for winter feeding or as a protein source for egg development as they emerge from reproductive quiescence (Wallingford et al. 2018).

Dietary plasticity

Drosophila suzukii are polyphagous and highly adaptable. Beukeboom (2018) identified the propensity of a species to identify and use alternative hosts as oviposit sites as a critical determinant of its invasiveness. Availability (and/or apparency) of suitable host plants and breadth of diet usually have an inverse relationship (see Jaenike 1990). Plant species differ in terms of chemistry, physical characteristics, and phenology. To-date, infestation by *D. suzukii* has been confirmed in 198 plant species representing 73 genera in 39 angiosperm families and two genera in one gymnosperm family (Supp. Table S1 & S2). In addition, in 41 instances, host fruits had been identified to genus rather than species level (Supp. Table S1). Preferences among fruit species have been documented; however, it is evident that host choice is to some extent opportunistic (Lee et al. 2011; Burrack et al. 2013; Poyet et al. 2015; Little et al. 2017; Stockton et al. 2019a). Seasonal availability due to plant phenology is a key factor in risk of damage from *D. suzukii* (Wiman et al. 2014; Haviland et al. 2016; Kenis et al. 2016). Plants which produce fruit in spring or early summer, such as gooseberries, and early-season strawberries and cherries, are less vulnerable than are plants which fruit in late summer or autumn in temperate regions when *D. suzukii* populations are larger (Wiman et al. 2014; Kenis et al. 2016). Recent evidence suggests that *D. suzukii* prefer wounded fruits for adult feeding and prefer healthy undamaged fruits for oviposition sites, but when availability of healthy fruits is limited, *D. suzukii* will accept damaged fruits as acceptable egg-laying site substitutes (Kienzle et al. 2020). Selecting for less susceptible varieties of a fruit species can help growers mitigate crop damage (Sward et al. 2016). Early ripening varieties of blueberries and raspberries could be harvested before *D. suzukii* populations reach threshold densities. Soft fruits and berries with firmer flesh or tougher skins could withstand oviposition efforts. Fruit varieties which do not change colour until late stages of ripeness or that complete the ripening process after harvest would be less conspicuous and potentially limit attraction cues. For example, early harvesting of wild blueberries can limit fruit infestation, but incurs additional costs due to lost immature fruit (Drummond et al. 2019).

Given the extreme plasticity in host-use by *D. suzukii*, growers need to assess fruit breeding and cultivation practices to limit risk of infestation. Removal of windfall and damaged fruits could reduce access by *D. suzukii* to potential reproductive sites post-harvest (Bal et al. 2017; Kienzle et al. 2020). In semi-tropical regions, warm temperatures early in the calendar year permit more rapid increases in *D. suzukii* populations and putting early season fruit crops at greater risk (Wiman et al. 2014).

Characters associated with fruit species have been correlated with suitability for feeding and oviposition behaviour of *D. suzukii.* Sweetness (Brix), skin toughness, and acidity (pH) have been investigated most commonly (Lee et al. 2011, 2016; Burrack et al. 2013; Little et al. 2017). Other factors have been explored, including fruit size, fruit shape, fruit texture (peach indumenta or strawberry accessions), fruit ripeness stage, fruit odours, fruit colour, damage to fruit, fruit phenology, location of fruit relative to rest of plant (height, outer vs. inner part of plant), leaf coverage (obscured vs exposed), hanging vs. fallen fruit, competition (previous oviposition by conspecifics or heterospecifics) (Stewart et al. 2014; Gong et al. 2016; Haviland et al. 2016; Sward et al. 2016; Rice et al. 2017; Cha et al. 2018; Little et al. 2018; Thistlewood et al. 2018). *Drosophila suzukii* are able to use a wide variety of host fruits by selecting for characteristics which signal health and ripeness of host fruits and a lack of potential competitors, pathogens, or predators, Other plant characters, such as leaf odours, leaf colour, and overall health of plant, have received less attention, but are important supplemental cues used to locate host fruits of healthy plants (Keesey et al. 2015; Little et al. 2018; Bolton et al. 2019). Foraging behaviour and responses to food cues can be modified by the composition of the fly's own gut microbiota (Wong et al. 2017).

Larval development time varies depending upon larval diet. Larvae feeding on fruits such as cherry, blueberry, or raspberry, develop more quickly than those that feed on standard diet media (Jaramillo et al. 2015; Hamby et al. 2016). Larvae reared on raspberry and blackberry are better able to withstand competitive pressures than larvae reared on other fruits (Olazcuaga et al. 2019). Naturally occurring yeasts and fungi associated with fruit provide developing *D. suzukii* larvae with critical nutrients, including protein, vitamins, and minerals (Hamby and Becher 2016; Bellutti et al. 2018; Lewis et al. 2018). As with other *Drosophila* species, yeast quality impacts development, fecundity, and lifespan of *D. suzukii* (Hamby and Becher 2016; Bellutti et al. 2018; Grangeteau et al. 2018). However, unlike many other Drosophila species, *D. suzukii* larvae develop most successfully on foods containing lower ratios of protein to carbohydrates, developing more quickly into larger adults with greater potential fecundity (Jaramillo et al. 2015; Silva-Soares et al. 2017; Rendon et al. 2018, 2019; Young et al. 2018).

In temperate regions, fruit can be of limited availability or quality as a food source for adult flies and as an oviposition site. In laboratory studies, adult *D. suzukii* have been documented feeding on floral nectar, tree sap, and honeydew when other food sources were unavailable (Kanzawa 1939; Lee et al. 2015; Tochen et al. 2016b; Wong et al. 2018; Stockton et al. 2019b). In the absence of suitable fruits, female *D. suzukii* will also oviposit and larvae can successfully develop on less ideal materials, including mushrooms and chicken manure (Stockton et al. 2019b). Thus, the adaptability of *D. suzukii* to novel dietary choices, for both the adult and larval stages, contributes to its invasion success and interact with other aspects of its plasticity.

Plasticity in community interactions

Interspecific and intraspecific competition can induce changes in oviposition behaviour of female adult *D. suzukii* and in behaviour of larvae. Chemical cues or signs of previous oviposition by heterospecifics such as *D. melanogaster*, can deter female *D. suzukii* from ovipositing in the same fruit (Shaw et al. 2018; Kidera and Takahashi 2020). This may be due to repellent olfactory cues such as trace amounts of the male *D. melanogaster* sex pheromone, *cis*-vaccenyl acetate (*c*VA), transferred to fruit during oviposition. In direct interspecific competition situations, *D. melanogaster* larvae have greater survival than *D. suzukii* larvae (Gao et al. 2018). This is due in part to higher tolerance to ethanol produced through decay and fermentation of fruit damaged by larval feeding and that higher levels of ethanol are produced in fruit containing *D. melanogaster* larvae than fruit containing *D. suzukii* larvae (Sampson et al. 2016; Gao et al. 2018). Although *D. suzukii* females prefer to oviposit in ripe fruit, they are able to shift ecological niches and use ripening fruit to avoid competitive pressures and reduce potential ethanol exposure to their larvae.

In contrast, female *D. suzukii* were not deterred from ovipositing in fruit containing eggs from other *D. suzukii* females (Shaw et al. 2018). In response to intraspecific competition, *D. suzukii* larvae are less likely to remain within their original host fruits throughout their development. In the absence of competition, larvae remain on or in host fruits throughout pupation. However, as the number of intraspecific competitors within a fruit host increases, larvae move greater distances away from the fruit to pupate (Bezerra Da Silva et al. 2019).

In the presence of parasitoid populations, *D. suzukii* select fruit for oviposition that contain atropine compounds to infer prophylactic protection to the next generation (Poyet et al. 2017). This is similar behaviour to *D. melanogaster*'s use of ethanol laden oviposition sites following detection of parasites and parasitoids (Milan et al. 2012; Kacsoh et al. 2013).

1.5.6 Discussion

Success of an introduced species in a novel environment depends primarily on its ability to adapt and explore its surroundings (Fordyce 2006). Species introduced into locations with changeable environmental conditions, such as temperate regions, are especially reliant on plastic morphological, behavioral and physiological characteristics for survival (Fordyce 2006). Novel environments expose species to different potential food sources, competitors, and predators.

The relatively small size and inconspicuous nature of *D. suzukii* makes it easily overlooked or misidentified in field settings. Without close inspection, it is easily mistaken for native Drosophilids in each of its invasive regions. Although no voucher specimens exist in mainland American entomological collections prior 2008, it is highly likely that *D. suzukii* was introduced to North America or Europe long before its first recognized detection in California, but it was not present in sufficient numbers to cause significant crop damage or invite notice (Hauser 2011). Effects of escalating climate change and extinction of native insect species could be contributing factors in promoting ecological niche availability beneficial to *D. suzukii* invasion (Ward and Masters 2007; Rhodes 2019).

Drosophila suzukii has demonstrated a notable ability to adapt behaviourally, physiologically, and morphologically to new environments. While each of these responses are of themselves modest in scope, the nature of its adaptive responses is arguably more important to its success as an invasive species than is the extent of any one variable response (Chown et al. 2007). *Drosophila suzukii* has shown itself to be highly opportunistic and it has been able to adapt to a wide range of host plant fruit for rearing its larvae and for adult feeding. As a result of its willingness to explore and test novel fruit species, it has become extremely polyphagous. In addition, *D. suzukii* has adapted to a wide range of temperature and humidity conditions, through its behaviour and through physiological, developmental, and morphological plasticity. As *D. suzukii* has expanded its range into temperate regions, its ability to adapt to changing environmental conditions and then reverse those adaptations as conditions change through successive seasons as been vital to its success. Plasticity within multiple aspects of behaviour, physiology, and morphology has allowed *D. suzukii* to move from a localized introduced species to an established invasive species over a global range.

1.5.7 Acknowledgements

We gratefully acknowledge funding provided by Atlantic Canada Opportunities Agency Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21516-519, GF21617-773, and GF21718-956).

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1.5.9 Supplementary Information

Supplementary Table S1.5.1. Confirmed host plants for *D. suzukii*.

Family	Genus	Species	Location	Reference
Actinidiaceae	*Actinidia*	*Actinidia arguta*	OR USA	Lee et al. 2015
			NS Canada	Little et al. 2019
		Actinidia chinensis	Italy	Kenis et al. 2016
Adoxaceae	*Sambucus*	*Sambucus canadensis*	NS Canada	Little et al. 2019
			MN USA	Sward (Thesis) 2017
			MI USA	Leach et al. 2019
		Sambucus ebulus	Italy	Kenis et al. 2016
			France	Poyet et al. 2015
		Sambucus nigra	Italy	Grassi et al. 2011
			OR USA	Lee et al. 2015
			France	Poyet et al. 2015
			Slovenia	Seljak et al. 2015
			Italy	Grassi et al. 2011
			Spain	Arnó et al. 2016
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			Italy	Tonina et al. 2016
			BC Canada	Thistlewood et al. 2019
		Sambucus nigra cerulea	BC Canada	Thistlewood et al. 2019
		Sambucus racemosa	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			Italy	Tonina et al. 2016
		Sambucus racemosa melanocarpa	BC Canada	Thistlewood et al. 2019
		Sambucus spp	AR USA	Herrera (Thesis) 2017

Family	Genus	Species	Location	Reference
			Sweden	Manduric 2017
			MI USA	Lee et al. 2015
	Viburnum	*Viburnum cassinoides*	NS Canada	Little et al. 2019
		Viburnum dilatatum	Japan	Mitsui et al. 2010
		Viburnum lantana	Italy	Kenis et al. 2016
		Viburnum opulus	Slovenia	Seljak et al. 2015
		Viburnum rhytidophyllum	Netherlands	Kenis et al. 2016
Aquifoliaceae	*Ilex*	*Ilex mucronata*	ME USA	Ballman and Drummond 2017
Araceae	*Arum*	*Arum italicum*	Netherlands	Kenis et al. 2016
		Arum maculatum	France	Poyet et al. 2015
Araliaceae	*Hedera*	*Hedera helix*	Italy	Grassi et al. 2018
			Netherlands	Panel et al. 2018
Arecaceae	*Butia*	*Butia eriospatha*	Brazil	Andreazza et al. 2015
Asparagaceae	*Polygonatum*	*Polygonatum multiflorum*	Netherlands	Kenis et al. 2016
Basellaceae	*Basella*	*Basella alba*	NC USA	Diepenbrock and McPhie 2018
Berberidaceae	*Berberis*	*Berberis aquifolium* (=*Mahonia aquifolium*)	OR USA	Lee et al. 2015
			Netherlands	Kenis et al. 2016
			BC Canada	Thistlewood et al. 2019
			France	Poyet et al. 2015
		Berberis thunbergii (=*Mahonia thunbergii*)	MI USA	Leach et al. 2019
		Berberis hortensis (=*Mahonia x media*)	France	Poyet et al. 2015
		Berberis spp. (=*Mahonia* spp.)	Italy	Kenis et al. 2016
			BC Canada	Thistlewood et al. 2019
Buxaceae	*Sarcococca*	*Sarcococca confusa*	OR USA	Lee et al. 2015
Caprifoliaceae	*Lonicera*	*Lonicera alpigena*	Italy	Kenis et al. 2016
			Italy	Tonina et al. 2016
		Lonicera caerulea	Italy	Kenis et al. 2016
			OR USA	Lee et al. 2015

Family	**Genus**	**Species**	**Location**	**Reference**
			NS Canada	Little et al. 2020
			Italy	Tonina et al. 2016
		Lonicera caprifolium	Netherlands	Kenis et al. 2016
		Lonicera ferdinandii	Netherlands	Kenis et al. 2016
		Lonicera japonica	ME USA	Ballman and Drummond 2017
		Lonicera maackii	AR USA	Herrera (Thesis) 2017
			AR USA	Knipp (Thesis) 2018
			MI USA	Leach et al. 2019
		Lonicera morrowii	MA USA	Elsensohn and Loeb 2018
			MN USA	Sward (Thesis) 2017
			MI USA	Leach et al. 2019
		Lonicera nigra	Italy	Kenis et al. 2016
			Italy	Tonina et al. 2016
		Lonicera nitida	Netherlands	Kenis et al. 2016
			France	Poyet et al. 2015
		Lonicera sempervirens	AR USA	Knipp (Thesis) 2018
		Lonicera spp.	Italy	Grassi et al. 2011
			AR USA	Herrera (Thesis) 2017
			Switzerland	Kenis et al. 2016
			MI USA	Lee et al. 2015
		Lonicera tatarica	MN USA	Sward (Thesis) 2017
			MI USA	Leach et al. 2019
			BC Canada	Thistlewood et al. 2019
		Lonicera xylosteum	France	Poyet et al. 2015
			Italy	Tonina et al. 2016
			Italy	Kenis et al. 2016
	Symphoricarpos	*Symphoricarpos albus*	Netherlands	Kenis et al. 2016
			OR USA	Lee et al. 2015
			France	Poyet et al. 2015
			BC Canada	Thistlewood et al. 2019

Family	**Genus**	**Species**	**Location**	**Reference**
Cornaceae	*Alangium*	*Alangium platanifolium*	Japan	Mitsui et al. 2010
	Cornus	*Cornus alba*	Netherlands	Kenis et al. 2016
		Cornus amomum	MI USA	Lee et al. 2015
			MA USA	Elsensohn and Loeb 2018
			MI USA	Leach et al. 2019
		Cornus canadensis	ME USA	Ballman and Drummond 2017
		Cornus controversa	Japan	Mitsui et al. 2010
		Cornus foemina	MI USA	Lee et al. 2015
		Cornus kousa	Netherlands	Kenis et al. 2016
			OR USA	Lee et al. 2015
			Japan	Mitsui et al. 2010
		Cornus mas	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
		Cornus racemosa	MN USA	Sward (Thesis) 2017
		Cornus sanguinea	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
		Cornus sericea	OR USA	Lee et al. 2015
			France	Poyet et al. 2015
		Cornus spp.	ME USA	Ballman and Drummond 2017
Cucurbitaceae	*Bryonia*	*Bryonia cretica*	Spain	Arnó et al. 2016
	Cucurbita	*Cucurbita pepo*	MI USA	Bal et al. 2017
Dioscoreaceae	*Dioscorea*	*Dioscorea communis (=Tamus communis)*	Italy	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
Ebenaceae	*Diospyros*	*Diospyros kaki*	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
			Japan	Mitsui et al. 2010
			Slovenia	Seljak et al. 2015
Elaeagnaceae	*Elaeagnus*	*Elaeagnus multiflora*	Japan	Kanzawa 1939
			Japan	Sasaki and Sato 1995

Family	**Genus**	**Species**	**Location**	**Reference**
		Elaeagnus x ebbingei	Netherlands	Panel et al. 2018
			France	Poyet et al. 2015
		Elaeagnus spp.	AR USA	Herrera (Thesis) 2017
		Elaeagnus umbellata	MI USA	Lee et al. 2015
			MI USA	Leach et al. 2019
	Hippophae	*Hippophae rhamnoides*	Switzerland	Kenis et al. 2016
			NS Canada	Little et al. 2017
			France	Poyet et al. 2015
Ericaceae	*Arbutus*	*Arbutus unedo*	Spain	Arnó et al. 2012
			OR USA	Lee et al. 2015
			Italy	Kenis et al. 2016
	Empetrum	*Empetrum nigrum*	NS Canada	Little et al. 2020
	Vaccinium	*Vaccinium angustifolium*	ME USA	Ballman and Drummond 2017
			NS Canada	Little et al. 2020
		Vaccinium ashei	Uruguay	González et al. 2015
		Vaccinium corymbosum	Italy	Grassi et al. 2011
			Japan	Kinjo et al. 2013
			Slovenia	Seljak et al. 2015
			NS Canada	Little et al. 2017
			MN USA	Sward (Thesis) 2017
			NS Canada	Little et al. 2018
			NS Canada	Little et al. 2019
			NS Canada	Little et al. 2020
		Vaccinium macrocarpon	WI USA	Steffan et al. 2013
			NS Canada	Little et al. 2017
		Vaccinium myrtilloides	Netherlands	Kenis et al. 2016
		Vaccinium myrtillus	Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
		Vaccinium oldhamii	Netherlands	Kenis et al. 2016

Family	**Genus**	**Species**	**Location**	**Reference**
		Vaccinium ovatum	OR USA	Lee et al. 2015
		Vaccinium praestans	Netherlands	Kenis et al. 2016
		Vaccinium spp.	Japan	Mitsui et al. 2010
			CA USA	Haviland et al. 2016
			Sweden	Manduric 2017
			Netherlands	Panel et al. 2018
		Vaccinium uliginosum	France	Poyet et al. 2015
		Vaccinium virgatum	Japan	Kinjo et al. 2013
		Vaccinium vitis-idaea	OR USA	Lee et al. 2015
			Netherlands	Kenis et al. 2016
			NS Canada	Little et al. 2017
	Gaultheria	*Gaultheria adenothrix*	Japan	Mitsui et al. 2010
		Gaultheria shallon	OR USA	Lee et al. 2015
		Gaultheria x wisleyensis	Netherlands	Kenis et al. 2016
Garryaceae	*Aucuba*	*Aucuba japonica*	Japan	Mitsui et al. 2010
			Netherlands	Panel et al. 2018
			France	Poyet et al. 2015
Grossulariaceae	*Ribes*	*Ribes aureum*	BC Canada	Thistlewood et al. 2019
		Ribes hudsonianum var. petiolare	BC Canada	Thistlewood et al. 2019
		Ribes nigrum	MI USA	Leach et al. 2019
		Ribes rubrum	Netherlands	Kenis et al. 2016
			Sweden	Manduric 2017
			France	Poyet et al. 2015
		Ribes sanguineum	France	Poyet et al. 2015
		Ribes uva-crispa	OR USA	Lee et al. 2015
Iridaceae	*Iris*	*Iris* spp.	France	Poyet et al. 2015
Lamiaceae	*Callicarpa*	*Callicarpa americana*	AR USA	Knipp (Thesis) 2018
Lauraceae	*Lindera*	*Lindera benzoin*	MI USA	Lee et al. 2015
Melanthiaceae	*Paris*	*Paris quadrifolia*	Switzerland	Kenis et al. 2016

Family	Genus	Species	Location	Reference
Menispermaceae	*Cocculus*	*Cocculus carolinus*	AR USA	Herrera (Thesis) 2017
Moraceae	*Ficus*	*Ficus carica*	Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			CA USA	Yu et al. 2013
		Ficus spp.	Italy	Grassi et al. 2011
	Morus	*Morus alba*	Japan	Kanzawa 1939
		Morus alba x rubra	CA USA	Yu et al. 2013
		Morus australis (=*bombycis*)	Japan	Mitsui et al. 2010
		Morus nigra	OR USA	Lee et al. 2015
			Slovenia	Seljak et al. 2015
		Morus rubra	AR USA	Knipp (Thesis) 2018
			FL USA	Plant Inspection Advisory 2010
		Morus spp.	Japan	Kanzawa 1935
			Japan	Sasaki and Sato 1995
			France	Poyet et al. 2015
			AR USA	Herrera (Thesis) 2017
			Argentina	Lavagnino et al. 2018
Myricaceae	*Myrica*	*Myrica rubra* (=*Morella rubra*)	Japan	Yukinari 1988
Myrtaceae	*Acca*	*Acca sellowiana*	Brazil	Souza et al. 2017
	Psidium	*Psidium cattleyanum*	Brazil	Andreazza et al. 2015
			Brazil	Andreazza et al. 2017
		Psidium guajava	Mexico	Lasa et al. 2017
			Brazil	Andreazza et al. 2015
	Eugenia	*Eugenia involucrata*	Brazil	Andreazza et al. 2017
		Eugenia uniflora	FL USA	Plant Inspection Advisory 2010
			Brazil	Andreazza et al. 2015
			Brazil	Andreazza et al. 2017
Oleaceae	*Ligustrum*	*Ligustrum lucidum*	Slovenia	Seljak et al. 2015
		Ligustrum vulgare	Slovenia	Seljak et al. 2015

Family	**Genus**	**Species**	**Location**	**Reference**
Onagraceae	*Fuchsia*	*Fuchsia spp.*	France	Poyet et al. 2015
Phytolaccaceae	*Phytolacca*	*Phytolacca americana*	Japan	Sasaki and Sato 1995
			France	Poyet et al. 2015
			MI USA	Lee et al. 2015
			Italy	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			AR USA	Herrera (Thesis) 2017
			AR USA	Knipp (Thesis) 2018
			MI USA	Leach et al. 2019
			MA USA	Elsensohn and Loeb 2018
		Phytolacca esculenta	Netherlands	Kenis et al. 2016
Punicaceae	*Punica*	*Punica granatum*	Slovenia	Seljak et al. 2015
Rhamnaceae	*Frangula*	*Frangula alnus*	Italy	Grassi et al. 2011
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			France	Poyet et al. 2015
		Frangula caroliniana	AR USA	Herrera (Thesis) 2017
		Frangula purshiana	OR USA	Lee et al. 2015
	Rhamnus	*Rhamnus caroliniana*	AR USA	Knipp (Thesis) 2018
		Rhamnus cathartica	MA USA	Elsensohn and Loeb 2018
			Netherlands	Kenis et al. 2016
			MN USA	Sward (Thesis) 2017
		Rhamnus fallax	Slovenia	Seljak et al. 2015
Rosaceae	*Amelanchier*	*Amelanchier alnifolia*	NS Canada	Little et al. 2020
		Amelanchier lamarckii	Netherlands	Kenis et al. 2016
		Amelanchier ovalis	Italy	Kenis et al. 2016
	Aronia	*Aronia melanocarpa*	KS USA	Hietala-Henschell et al. 2017
	Cotoneaster	*Cotoneaster apiculatus*	MI USA	Leach et al. 2019
		Cotoneaster bullatus	France	Poyet et al. 2015

Family	**Genus**	**Species**	**Location**	**Reference**
		Cotoneaster franchetii	Netherlands	Kenis et al. 2016
		Cotoneaster lacteus	Italy	Kenis et al. 2016
			OR USA	Lee et al. 2015
		Cotoneaster rehderi	Netherlands	Kenis et al. 2016
		Cotoneaster watereri	France	Poyet et al. 2015
	Duchesnea	*Duchesnea indica*	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			France	Poyet et al. 2015
			OR USA	Lee et al. 2015
	Eriobotrya	*Eriobotrya japonica*	Japan	Kanzawa 1939
			FL USA	Plant Inspection Advisory 2010
			Italy	Kenis et al. 2016
			Brazil	Andreazza et al. 2017
	Fragaria x Ananassa hybrid	*Fragaria × Ananassa*	Italy	Grassi et al. 2011
			CA USA	Goodhue et al. 2012
			Slovenia	Seljak et al. 2015
			NS Canada	Little et al. 2017
			NS Canada	Little et al. 2020
			Brazil	Andreazza et al. 2017
	Fragaria	*Fragaria vesca*	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			France	Poyet et al. 2015
		Fragaria spp.	Sweden	Manduric 2017
	Malus	*Malus baccata*	Netherlands	Kenis et al. 2016
		Malus domestica	NS Canada	Little et al. 2019
		Malus pumila	Japan	Kanzawa 1939

Family	Genus	Species	Location	Reference
			NS Canada	Little et al. 2019
		Malus sylvestris	France	Poyet et al. 2015
		Malus spp.	MI USA	Bal et al. 2017
			MI USA	Leach et al. 2019
	Photinia	*Photinia beauverdiana*	Netherlands	Kenis et al. 2016
		Photinia prunifolia	Netherlands	Kenis et al. 2016
		Photinia villosa	Netherlands	Kenis et al. 2016
	Prunus	*Prunus armeniaca*	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
			Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
		Prunus avium	Japan	Kanzawa 1939
			Italy	Grassi et al. 2011
			France	Poyet et al. 2015
			OR USA	Lee et al. 2015
			Slovenia	Seljak et al. 2015
			CA USA	Haviland et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			Italy	Grassi et al. 2018
			NS Canada	Little et al. 2017
			NS Canada	Little et al. 2020
		Prunus buergeriana	Japan	Sasaki and Sato 1995
		Prunus cerasifera	Italy	Kenis et al. 2016
		Prunus cerasus	Japan	Kanzawa 1939
			Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
		Prunus domestica	Slovenia	Seljak et al. 2015
			Switzerland	Kenis et al. 2016
		Prunus donarium	Japan	Kanzawa 1939

Family	**Genus**	**Species**	**Location**	**Reference**
			Japan	Mitsui et al. 2006
		Prunus japonica	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
		Prunus laurocerasus	Sweden	Manduric 2017
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			OR USA	Lee et al. 2015
		Prunus lusitanica	Italy	Kenis et al. 2016
			OR USA	Lee et al. 2015
			France	Poyet et al. 2015
		Prunus mahaleb	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
			France	Poyet et al. 2015
			Spain	Arnó et al. 2016
			Italy	Kenis et al. 2016
			BC Canada	Thistlewood et al. 2019
		Prunus nipponica	Japan	Mitsui et al. 2010
		Prunus padus	Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
		Prunus persica	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
			Japan	Sasaki and Sato 1995
			CA USA	Stewart et al. 2014
			Slovenia	Seljak et al. 2015
			Brazil	Andreazza et al. 2017
			MI USA	Bal et al. 2017
		Prunus persica nucipersica	Slovenia	Seljak et al. 2015
		Prunus pensylvanica	NS Canada	Little et al. 2020
		Prunus salicina (=triflora)	Japan	Kanzawa 1935

Family	**Genus**	**Species**	**Location**	**Reference**
			Japan	Kanzawa 1939
		Prunus sargentii	Japan	Kanzawa 1935
		Prunus serotina	PA USA	Turcotte et al. 2018
			AR USA	Herrera (Thesis) 2017
			Netherlands	Kenis et al. 2016
			France	Poyet et al. 2014
			France	Poyet et al. 2015
		Prunus spinosa	France	Poyet et al. 2015
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Sweden	Manduric 2017
		Prunus spp.	Japan	Mitsui et al. 2010
			Italy	Grassi et al. 2011
			MI USA	Bal et al. 2017
			ME USA	Ballman and Drummond 2017
		Prunus virginiana	NS Canada	Little et al. 2017
			MI USA	Leach et al. 2019
			BC Canada	Thistlewood et al. 2019
		Prunus yedoensis	Japan	Kanzawa 1935
			Japan	Kanzawa 1939
			Japan	Sasaki and Sato 1995
	Pyracantha	*Pyracantha spp.*	Netherlands	Kenis et al. 2016
	Pyrus	*Pyrus calleryana*	France	Poyet et al. 2015
		Pyrus communis	NS Canada	Little et al. 2019
		Pyrus sinensis	Japan	Kanzawa 1939
		Pyrus spp.	MI USA	Bal et al. 2017
	Rosa	*Rosa acicularis*	Netherlands	Kenis et al. 2016
		Rosa canina	Spain	Arnó et al. 2016
			Netherlands	Kenis et al. 2016
		Rosa glauca	Netherlands	Kenis et al. 2016

Family	Genus	Species	Location	Reference
		Rosa pimpinellifolia	Netherlands	Kenis et al. 2016
		Rosa rugose	Netherlands	Kenis et al. 2016
	Rubus	*Rubus allegheniensis*	MA USA	Elsensohn and Loeb 2018
			NS Canada	Little et al. 2019
		Rubus armeniacus	OR USA	Lee et al. 2015
			MI USA	Leach et al. 2019
		Rubus caesius	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Italy	Tonina et al. 2016
		Rubus chamaemorus	NS Canada	Little et al. 2020
		Rubus crataegifolius	Japan	Mitsui et al. 2010
		Rubus fruticosus	France	Poyet et al. 2015
			Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
		Rubus idaeus	Argentina	Andreazza et al. 2017
			Italy	Grassi et al. 2011
			CA USA	Goodhue et al. 2012
			France	Poyet et al. 2015
			Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			NS Canada	Little et al. 2017
			Argentina	Andreazza et al. 2017
			MN USA	Sward (Thesis) 2017
			MI USA	Leach et al. 2019
			NS Canada	Little et al. 2019
			NS Canada	Little et al. 2020

Family	**Genus**	**Species**	**Location**	**Reference**
		Rubus laciniata	NS Canada	Little et al. 2017
		Rubus microphyllus	Japan	Kanzawa 1939
			Japan	Mitsui et al. 2010
		Rubus occidentalis	MN USA	Sward (Thesis) 2017
			MA USA	Elsensohn and Loeb 2018
		Rubus parvifolius (=triphyllus)	Japan	Kanzawa 1939
			Japan	Sasaki and Sato 1995
		Rubus phoenicolasius	Switzerland	Kenis et al. 2016
		Rubus saxatilis	Italy	Kenis et al. 2016
			Italy	Tonina et al. 2016
		Rubus spectabilis	OR USA	Lee et al. 2015
		Rubus strigosus	MI USA	Leach et al. 2019
		Rubus spp.	Italy	Grassi et al. 2011
			Slovenia	Seljak et al. 2015
			MI USA	Bal et al. 2017
			ME USA	Ballman and Drummond 2017
			AR USA	Herrera (Thesis) 2017
			Sweden	Manduric 2017
		Rubus ulmifolius	Spain	Arnó et al. 2016
	Sorbus	*Sorbus aria*	Italy	Kenis et al. 2016
		Sorbus aucuparia	Netherlands	Kenis et al. 2016
		Sorbus sitchensis	OR USA	Lee et al. 2015
	Crataegus	*Crataegus chrysocarpa*	Netherlands	Kenis et al. 2016
		Crataegus monogyna	Netherlands	Kenis et al. 2016
Rubiaceae	*Rubia*	*Rubia peregrina*	France	Poyet et al. 2015
Rutaceae	*Murraya*	*Murraya paniculata*	FL USA	Plant Inspection Advisory 2010
	Skimmia	*Skimmia japonica*	France	Poyet et al. 2015
			Netherlands	Panel et al. 2018
	Citrus	*Citrus sinensis*	Argentina	Lavagnino et al. 2018
		Citrus X sinensis	CA USA	Haviland et al. 2016

Family	Genus	Species	Location	Reference
Santalaceae	*Viscum*	*Viscum album*	France	Poyet et al. 2015
			Germany	Briem et al. 2016
			Netherlands	Panel et al. 2018
Solanaceae	*Atropa*	*Atropa belladonna*	France	Poyet et al. 2015
	Lycium	*Lycium barbarum*	Italy	Kenis et al. 2016
	Physalis	*Physalis alkekengi*	France	Poyet et al. 2015
	Solanum	*Solanum carolinense*	MI USA	Leach et al. 2019
		Solanum chenopodioides	Spain	Arnó et al. 2016
		Solanum dulcamara	MI USA	Lee et al. 2015
			OR USA	Lee et al. 2015
			France	Poyet et al. 2015
			Spain	Arnó et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			ME USA	Ballman and Drummond 2017
			MA USA	Elsensohn and Loeb 2018
			MI USA	Leach et al. 2019
		Solanum luteum	Spain	Arnó et al. 2012
		Solanum lycopersicum	Japan	Kanzawa 1935
			FL USA	Plant Inspection Advisory 2010
			OR USA	Lee et al. 2015
		Solanum nigrum	Spain	Arnó et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016
			France	Poyet et al. 2015
		Solanum tuberosum	France	Poyet et al. 2015
		Solanum villosum	Spain	Arnó et al. 2012
Taxaceae	*Taxus*	*Taxus baccata*	Italy	Kenis et al. 2016
			Netherlands	Kenis et al. 2016
			Switzerland	Kenis et al. 2016

Family	**Genus**	**Species**	**Location**	**Reference**
			France	Poyet et al. 2015
	Torreya	*Torreya nucifera*	Japan	Mitsui et al. 2010
Thymelaeaceae	*Daphne*	*Daphne mezereum*	Italy	Kenis et al. 2016
			Italy	Tonina et al. 2016
Vitaceae	*Ampelopsis*	*Ampelopsis glandulosa* brevipedunculata	AR USA	Herrera (Thesis) 2017
	Parthenocissus	*Parthenocissus quinquefolia*	Switzerland	Kenis et al. 2016
			MI USA	Leach et al. 2019
	Vitis	*Vitis labrusca*	Slovenia	Seljak et al. 2015
		Vitis rotundifolia	GA USA	Grant and Sial 2016
		Vitis spp.	MI USA	Bal et al. 2017
			Sweden	Manduric 2017
			MI USA	Leach et al. 2019
		Vitis vinifera	Italy	Grassi et al. 2011
			Slovenia	Seljak et al. 2015
			Italy	Kenis et al. 2016
			NS Canada	Little et al. 2019

Supplementary Table S1.5.2 Taxonomic classification of *D. suzukii* host plants using APG IV system (The Angiosperm Phylogeny Group 2016).

Division	**Class**	**Subclass**	**Superclade**	**Clade**	**Subclade**	**Order**	**Family**	**Genus**	**# Species**
Gymnospermae (Pinophyta)	Pinopsida					Pinales	Taxaceae	*Taxus*	1
								Torreya	1
Angiospermae	Mesangiospermae	Magnoliids				Laurales	Lauraceae	*Lindera*	1
		Monocots	Alismatids			Alismatales	Araceae	*Arum*	2
			Liliods			Dioscoreales	Dioscoreaceae	*Dioscorea*	1
						Liliales	Melanthiaceae	*Paris*	1
						Asparagales	Asparagaceae	*Polygonatum*	1
							Iridaceae	*Iris*	1
			Commelinids			Arecales	Arecaceae	*Butia*	1
		Eudicots				Buxales	Buxaceae	*Sarcococca*	1
						Ranunculales	Berberidaceae	*Berberis*	3
							Menispermaceae	*Cocculus*	1
			Superrosids			Saxifragales	Grossulariaceae	*Ribes*	6
				Rosids		Vitales	Vitaceae	*Ampelopsis*	1
								Parthenocissus	1
								Vitis	3
					Fabids	Cucurbitales	Cucurbitaceae	*Bryonia*	1
								Cucurbita	1
						Fagales	Myricaceae	*Myrica*	1
						Rosales	Rosaceae	*Amelanchier*	3
								Aronia	1
								Cotoneaster	6
								Duchesnea	1
								Eriobotrya	1
								Fragaria x ananassa hybrid	1
								Fragaria	1

							Malus	4
							Photinia	3
							Prunus	21
							Pyracantha	1
							Pyrus	3
							Rosa	5
							Rubus	16
							Sorbus	3
							Crataegus	2
						Elaeagnaceae	*Elaeagnus*	3
							Hippophae	1
						Rhamnaceae	*Frangula*	3
							Rhamnus	3
						Moraceae	*Ficus*	1
							Morus	5
				Malvids	Malvales	Thymelaeaceae	*Daphne*	1
					Myrtales	Myrtaceae	*Acca*	1
							Psidium	2
							Punica	1
							Eugenia	2
						Onagraceae	*Fuchsia*	1
					Sapindales	Rutaceae	*Murraya*	1
							Skimmia	1
							Citrus	2
		Superastrids			Caryophyllales	Phytolaccaceae	*Phytolacca*	2
						Basellaceae	*Basella*	1
					Santalales	Santalaceae	*Viscum*	1
			Asterids		Cornales	Cornaceae	*Alangium*	1
							Cornus	10
					Ericales	Ebenaceae	*Diospyros*	1

						Actinidiaceae	*Actinidia*	2
						Ericaceae	*Arbutus*	1
							Empetrum	1
							Vaccinium	12
							Gaultheria	3
				Lamiids	Gentianales	Rubiaceae	*Rubia*	1
					Garryales	Garryaceae	*Aucuba*	1
					Solanales	Solanaceae	*Atropa*	1
							Lycium	1
							Physalis	1
							Solanum	8
					Lamiales	Lamiaceae	*Callicarpa*	1
						Oleaceae	*Ligustrum*	2
				Campanulids	Apiales	Araliaceae	*Hedera*	1
					Aquifoliales	Aquifoliaceae	*Ilex*	1
					Dipsacales	Adoxaceae	*Sambucus*	4
							Viburnum	5
						Caprifoliaceae	*Lonicera*	12
							Symphoricarpos	1

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Chapter 2

Host preference and suitability

2.1 Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii*

A version of this chapter section has been published in Pest Management Science:
Little, C.M., Chapman, T.W., Moreau, D.L., and Hillier, N.K. 2017. Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii* (Diptera: Drosophilidae). Pest Manag Sci **73**(1): 160–166. doi:10.1002/ps.4366.

Author Contribution Statement

CML and NKH designed research. DLM and TWC advised on suitable fruits for testing and methodology. CML conducted research, analyzed data, and prepared manuscript. NKH, DLM, and TWC edited manuscript. All authors read and approved manuscript.

2.1.1 Abstract

Drosophila suzukii Matsumara has recently emerged as a major invasive pest species in soft-skinned fruits in berries throughout North America and Europe. Its distribution has spread so rapidly that little is known of the extent of fruit susceptibility, particularly in boreal regions. Populations of *D. suzukii* increase dramatically in late summer in boreal regions, concurrent with fruiting seasons for commercially and culturally significant fruits and berries. We tested fruit preference and susceptibility of lingonberry, blueberry, chokecherry, sea buckthorn and raspberry fruits to *D. suzukii*. Female *D. suzukii* attempted to oviposit on all fruit types tested. Fruits with lower brix and lower pH levels were preferred in choice tests. Undamaged lingonberries were relatively safe from infestation; however, bruised or frost-damaged fruits were easily penetrated. Sea buckthorn and raspberry fruits were highly preferred. Although blueberry growers have experienced severe economic crop losses due to *D. suzukii*, we have

found that blueberries were the least preferred of the fruits tested. This suggests that *D. suzukii* are largely opportunistic and highlights the importance of fruit phenology in fruit susceptibility.

2.1.2 Introduction

Having only recently expanded from its East Asian origins, the fruit damaging spotted wing drosophila, *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), now has the attention of farmers in North America and Europe. The first North American observation of *D. suzukii* occurred in California in 2008 (Walsh et al. 2011). By 2012 *D. suzukii* had been confirmed in 28 states with single year (2013) US crop losses estimated in excess of US$27 million (Burrack HJ (http://swd.ces.ncsu.edu/working-group-activities/swd-impacts-2013/). By 2014, *D. suzukii* had spread to 8 Canadian provinces, including Nova Scotia and Newfoundland (Hauser 2011; Moreau and Foster 2012; Moreau et al. 2013). Male flies bear a dark wing spot that gives this species its common name, but it is the female *D. suzukii* that worry agriculturists. Most Drosophilids lay their eggs in over-ripe or rotting fruits; however, female *D. suzukii* use their heavily sclerotised, serrated ovipositors to lay eggs in ripening, pre-harvest soft skinned fruits and berries (Walsh et al. 2011; Kinjo et al. 2013; Atallah et al. 2014; Calabria et al. 2014; Jaramillo et al. 2014; Cha et al. 2015). As *D. suzukii* spreads rapidly across new regions, an increasing broad range of potential fruit hosts may be at risk.

Oviposition success has been confirmed for *D. suzukii* on a wide variety of fruits, including raspberry (*Rubus idaeus* L.), strawberry (*Fragaria* × *ananassa* Duchesne), blackberry *(Rubus laciniata* L.), cherry (*Prunus avium* L.), blueberry (*Vaccinium corymbosum* L. and *Vaccinium virgatum* Aiton), French prune (*Prunus domestica* L.), mulberry (*Morus* spp. L.), fig (*Ficus carica* L.), and under limited conditions, cranberries (*Vaccinium macrocarpon* Aiton) and grapes

(*Vitis* spp. L.) (Lee et al. 2011, 2015; Yu et al. 2012; Bellamy et al. 2013; Burrack et al. 2013; Kinjo et al. 2013; Steffan et al. 2013; Ioriatti et al. 2015; Poyet et al. 2015; Kenis et al. 2016). *Drosophila suzukii* also infest a wide variety of wild and ornamental non-crop fruits, effectively expanding its reproductive season well in excess of ripening periods of commercial fruit crops (Lee et al. 2016, 2015). As *D. suzukii* continues its spread North to subarctic regions, additional soft fruits and berries are potentially at risk. Here we attempt to add to this species long list of potentially attractive fruits. Lingonberries (*Vaccinium vitis-idaea* L.) are prized for preserves and sauces across Europe and are known locally in Newfoundland as patridgeberries. Chokecherries (*Prunus virginiana* L.) are common in hedgerows across much of N. America. Sea buckthorn (*Hippophae rhamnoides* L.) is a nutraceutical crop grown throughout northern Europe and Asia, and is growing in popularity in Canada (Tiitinen et al. 2005; Christaki 2012; Socaci et al. 2013). We tested these fruits for susceptibility to oviposition by and larval viability of *D. suzukii*. We hypothesized that *D. suzukii* will demonstrate distinct preferences among fruit hosts and, when choices are limited, *D. suzukii* would opportunistically make use of any fruit available.

2.1.3 Materials and Methods

Source materials

We collected raspberries from commercial ‘Heritage’ and ‘Caroline’ varieties, and high-bush blueberries from a mixed field of Jersey and Colville varieties (*V. corymbosum*) from commercial growers in Nova Scotia. Blueberry cultivars were not readily discernable from each other. Chokecherries were picked from hedgerows beside an unmanaged blueberry field. Lingonberries were collected from coastal headlands on the Avalon Peninsula, Newfoundland (NL), and two hybrid cultivars of sea buckthorn fruits (hereafter light and dark sea buckthorn) were obtained from a commercial grower in Whitbourne, NL. Since lingonberries are typically collected throughout the fall and early winter in Newfoundland, lingonberries were also picked post-frost and retested. Fruits were examined microscopically for signs of infestation and stored at 4° C and used within 21 d. 'Caroline' raspberries and high-bush blueberries were picked 5-6 days after last application of pesticides to minimize potential residues. All other fruits were unsprayed. Fruits were collected simultaneously for all experiments.

Drosophila suzukii were obtained from colonies initiated in February 2013 at the Kentville Research and Development Centre (Agriculture and Agri-Food Canada, Kentville, NS) and subsequently maintained in the laboratory at Acadia University (Wolfville, NS). Colonies were reared in 250-ml *Drosophila* flasks (Genesee Scientific, San Diego, CA, USA) containing 50 ml Formula 4-24 Instant Drosophila medium (Merlan Scientific Ltd., Mississauga, ON, Canada) mixed with 50 ml dH_2O and 2-3 granules of yeast. Reproductively mature females, aged up to approximately two weeks, were removed from colonies started one month previously at approximately 2 h before the start of each assay.

Host selection – 2-choice assays

Choice assays were conducted within a sealed airtight 600 ml arena (hereafter airtight arena; 18 x 12 x 6.5 cm) and/or with constant flow of humidified air into 750 ml arena (hereafter airflow arena; 15.5 x 7.5 x 9 cm). One replicate of airflow trials and a minimum of three airtight trials were performed for each choice. For each trial, approximately 10 ml of each fruit were placed within clear plastic traps constructed by inserting a truncated 200 µl pipette tip through the lid of a 30 ml portion cup. Fifteen mature female flies were placed in the centre of each arena and checked at 24 h, 48 h, and 72 h. Fruits were tested versus one other and against empty traps (control). Frosted lingonberry was tested only against empty traps because no other fruits were locally available at that time. Preference was measured using a response index (RI) based on Dweck et al. (2013) calculated as *(A-B)/T*, where A and B are the number of flies in each trap and T is the total number of flies in the trial. Resulting scores ranged from -1 to 1, with positive values representing preference for fruits in trap A.

Fruit characters

We tested twenty-five individual fruits of each species or variety for firmness (penetration force), brix (sugar content), and acidity (pH) to identify characters that could affect susceptibility to *D. suzukii* (Lee et al. 2011; Wilson et al. 2013). Fruits were tested individually for firmness measured in gram force (gf) using a Wagner Fruit Penetrometer FT02 gram force gage (Wagner Instruments, Greenwich, CT) fitted with a 3 mm tip (Christaki 2012). A 6 mm tip was used to test firmness of Heritage raspberries. Penetration force is hereafter stated in gf/mm^2 to correct for tip surface area of the penetrometer. Depending on size and juiciness of each fruit, the same fruits were macerated individually or in groups of 3-4 for testing for brix and pH. Brix was measured with a Westover^{TM} Model RHR – 32ATC handheld brix refractometer with automatic temperature compensation (Cole-Parmer, Montreal, PQ) and pH was measured using a Fisher Scientific Accumet® Basic AB15 pH meter (Fisher Scientific, Toronto, ON).

Oviposition and larval success – No-choice assays

Approximately 10 ml of each fruit were sealed in 30 ml plastic cups with two sexually mature female *D. suzukii*. Female flies were removed after 72 h and fruit examined for eggs or newly hatched larvae. Fruit was checked weekly for 5 weeks for developing larvae and pupae, and emerging adult flies (Burrack et al. 2013). Non-destructive sampling methods were employed to minimize risk of damaging developing larvae. Fruits were visually examined under a dissecting microscope at 120-250X magnification. Egg, larvae, and pupae counts were completed primarily to confirm that oviposition had occurred and that larvae were developing. Statistical analyses were done using counts of emerged adult flies only. Adult flies were removed for sexing and counting purposes. Numbers for eggs, larvae, and pupae represent maximum counts during the experimental period, whereas, numbers for emerging adult flies were cumulative. Frost-exposed lingonberry were replicated 8 times with each cup arena as a replicate, Caroline raspberry were replicated 24 times, and other fruits were replicated 16 times.

Statistical analysis

Welch's 2-sample t-tests were used to assess potential differences between assays conducted with and without airflow. Repeated measures analyses were used to account for effect of treatment, time and time*treatment. Three-way factorial ANOVAs were used to assess relationships between host selection and interactions among fruit characters. One-way ANOVAs and Tukey post-hoc tests used to analyze all other data were performed using RStudio (Version 0.98.1091 © 2009-2014, http://www.rstudio.org/).

2.1.4 Results

Host selection

In choice tests, female *D. suzukii* preferences were consistent between airtight arenas and arenas with airflow (Welch 2-sample t-test; *p-values*>0.05) so combined results are shown. After 24 h, only 20.8% of female flies had made a choice. However, after 48 h, 60.0% of flies had made a choice and by 72 h, this figure had increased to 76.2%. Repeated measures ANOVA confirms that differences between fruit host selection are significant, accounting for within-trial variability in choice decisions between days (ANOVA; treatment: $F_{7,201}$=8.63, p<0.0001; time: $F_{2,416}$=4.89, p<0.01; treatment*time: $F_{14,402}$=3.94, p<0.0001). Order of fruit preferences was consistent among 24-, 48-, and 72-hour counts (Fig. 2.1.1).

Significant differences in preferences were observed among some fruits after 24 h (ANOVA; $F_{7,226}$=11.3, p<0.0001), 48 h ($F_{7,226}$=13.81, p<0.0001), and 72 h ($F_{7,226}$=13.63, p<0.0001)(Table. 2.1.1). Sea buckthorn fruits were chosen most frequently over all other fruits and differences were significant between sea buckthorn fruits and all other fruits tested (Table 2.1.1). In choice tests against blueberry, *D. suzukii* consistently chose the other fruits (ANOVA; after 24 h: $F_{6,20}$=2.1, p=0.10; 48 h: $F_{6,20}$=4.4, p<0.01; 72 h: $F_{6,20}$=5.5, p<0.01), suggesting that when viable alternative fruits are available, blueberries are less desirable hosts. Chokecherries were comparable in preference to lingonberries but not preferred to commercial raspberries and sea buckthorn fruits. Lingonberries were comparable in preference to commercial raspberries. Fresh and frost-exposed lingonberries were comparable in attraction (Tukey HSD; after 24 h: p=0.18; 48 h: p<0.05; 72 h: p=1.00). All fruits were more attractive to female *D. suzukii* when a blank control trap was the alternative (ANOVA; *p-values*<0.01), although differences in mean response indices between controls and blueberries were not significant at any time period tested and differences between controls and both lingonberries were not significant at 24- or 72-h (Table 2.1.1).

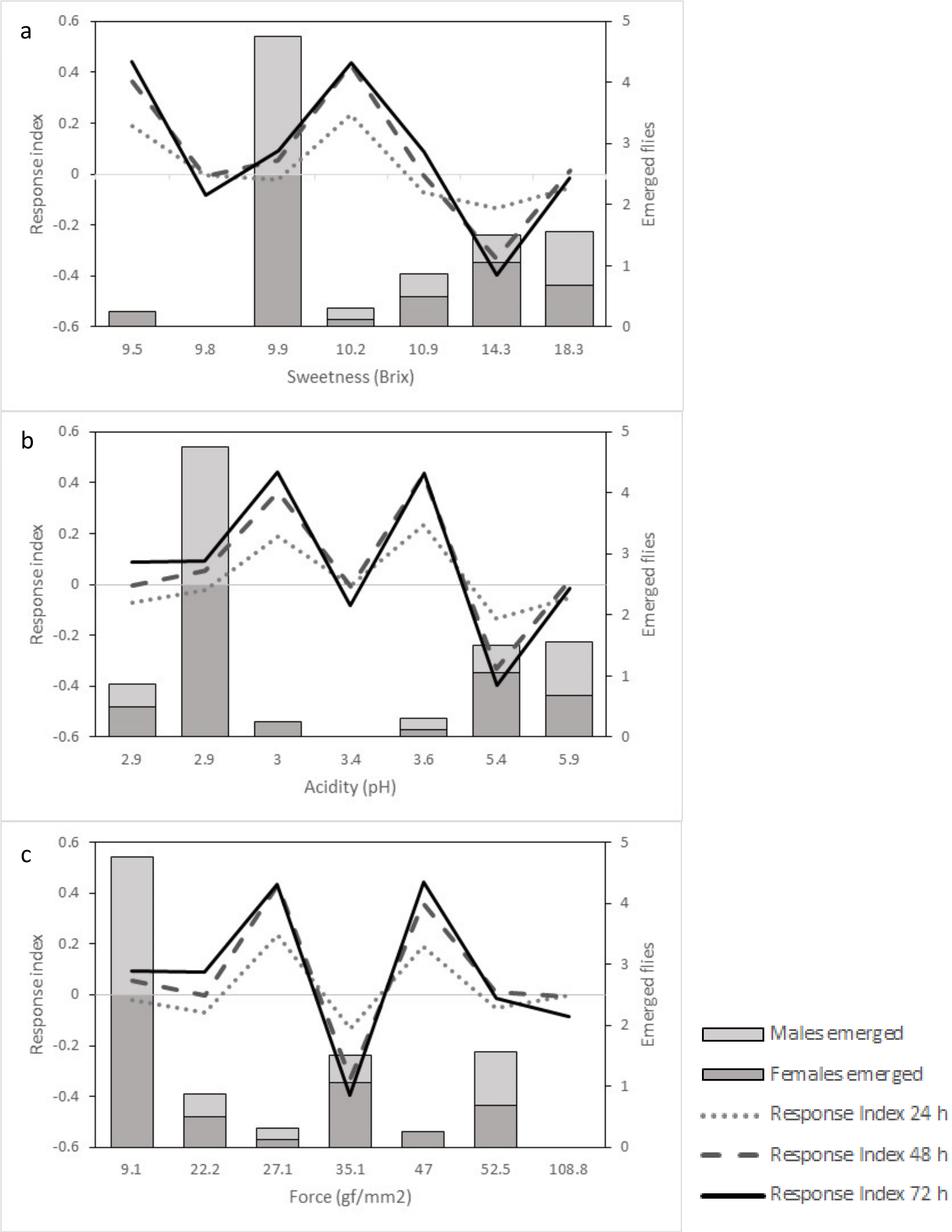

Figure 2.1.1. Mean response indices and mean count of emerged flies for each fruit type contrasted with a) sweetness, and b) acidity, and c) fruit firmness. Lines represent response indices at 24, 48, and 72 h. Mean counts for emerged female and male *D. suzukii* are represented by shaded bars. Standard errors are omitted for clarity (refer Tables 2.1.1 and 2.1.2). “One replicate of airflow trials and a minimum of three airtight trials were performed for each choice.” Sweeter fruits that were less preferred in choice assays (a); however, more adult flies emerged from sweeter fruits during no-choice assays. Fruits pH (b) were more attractive during choice assays, but no relationship was found between acidity and larval success. No linear relationship

between fruit firmness (c) and host selection by female *D. suzukii* was observed; however, no flies emerged from lingonberries (108.8 gf/mm^2).

Fruit characters

Lingonberry skins require significantly more force to penetrate than other fruits tested when undamaged (Tukey HSD; *p's*<0.0001; Table 2.1.2). However, exposure to frost softens fruits to a point comparable with other fruits tested. Heritage raspberries were least firm (Table 2.1.2). Sea buckthorn fruits were comparable in skin toughness to other fruits tested. Chokecherries and highbush blueberries were significantly sweeter (higher Brix) than other fruits tested (Table 2.1.2). Lingonberries and sea buckthorn fruits were comparable in sweetness to commercial raspberries. Chokecherries and highbush blueberries were least acidic (Table 2.1.2). Lingonberries and sea buckthorn fruits were comparable in acidity to commercial raspberries, although frost exposure reduces lingonberry acidity (Table 2.1.2).

Among the fruits tested, there was no correlation between sweetness and acidity (Pearson's correlation; r_{188}=0.03, p=0.70); however, fruit firmness was weakly correlated with both sweetness (r_{188}=0.26, p<0.001) and acidity (r_{188}=0.25, p<0.001). The relative weights model suggested that sweetness accounts for the highest proportion of the variance in host selection (at 24 h: 71.7%, R^2=0.09; 48 h: 57.0%, R^2=0.06, at 72 h: 49.4%, R^2=0.08).[25] All-subsets regression analysis suggested that a model including all three characters and their interactions was the best predictor for host selection ($RI \sim Brix*pH*gf/mm^2$; 24 h: adjusted R^2=0.21; 48 h: adjusted R^2=0.16; 72 h: adjusted R^2=0.19). Correlation coefficients were relatively low in all analyses, suggesting that other factors such as fruit odour or colour, likely have a stronger influence on host choice. Although fruit sweetness was the most important measurable fruit character in host selection (Fig. 2.1.1), the interaction of *sweetness:acidity:fruit firmness* was the primary factor in host selection by female *D. suzukii* (Table 2.1.3). No direct relationships were found between host selection and in fruit firmness (Table 2.1.3). Overall, *D. suzukii* preferred fruits that were less sweet, more acidic, and that were less firm when given the choice between two different fruit types (ANOVA; sweetness: $F_{1,524}$=21.8, p<0.0001; acidity: $F_{1,524}$=23.5, , p<0.0001; firmness: $F_{1,524}$=7.5, p<0.01).

Table 2.1.1. Mean response index scores (± standard error) in 2-choice tests (each fruit as choice A against all other choices, including control) for each fruit type after: (A) 24 h , (B) 48 h, and (C) 72 h. Positive RI scores reflect attraction to fruit listed beside of figure. Significant differences were found between attraction to sea buckthorn fruits and all other fruits tested at 24 h. After 48 h and 72 h, sea buckthorn fruits were still preferred to most fruits. At 48 h and 72 h, significant differences were observed between attractions for a variety of fruits versus control traps. At 72 h, preferences for raspberry varieties over blueberry were significant .Negative RI scores reflect attraction to other fruits. Different capital letters on indicate significant differences between fruits tested (Tukey post-hoc test, *p*<0.05).

	24 h		48 h		72 h	
fruit	response index	differences	response index	differences	response index	differences
blueberry	-0.13 ± 0.05	A	-0.33 ± 0.08	A B	-0.40 ± 0.10	A B
chokecherry	-0.05 ± 0.03	A	0.01 ± 0.09	B C	-0.02 ± 0.12	B C
lingonberry	0.00 ± 0.05	A	-0.01 ± 0.10	B C	-0.08 ± 0.11	A C
raspberry - Caroline	-0.07 ± 0.05	A	0.00 ± 0.12	B C	0.09 ± 0.12	C D
raspberry - Heritage	-0.02 ± 0.04	A	0.06 ± 0.08	B D	0.09 ± 0.10	C D
seabuckthorn - dark	0.24 ± 0.05	B	0.43 ± 0.08	D	0.44 ± 0.09	D
seabuckthorn - light	0.19 ± 0.05	B	0.36 ± 0.08	C D	0.44 ± 0.08	D
control	-0.17 ± 0.04	A	-0.57 ± 0.06	A	-0.62 ± 0.06	A

Oviposition and larval success

Eggs were observed on or in all fruit types (Table 2.1.2). However, eggs were difficult to see, particularly in raspberry varieties and sea buckthorn. When eggs were oviposited into fruits, only the egg filaments remained on the surface and are easily obscured by fruit juices or, in the case of raspberries, the hair-like styles. Therefore, egg counts presented are conservative estimates used to confirm that egg-laying had occurred and are excluded from further analysis. Larvae were found on or in all fruit types (Table 2.1.2) with highest numbers in raspberries, sea buckthorn fruits, and chokecherries. Highest numbers of pupae (Table 2.1.2) were found in raspberry fruits and chokecherries. No pupae were found in lingonberries.

Adult *D. suzukii* flies emerged from all fruit types except lingonberries. Highest numbers of female flies emerged from raspberries and highbush blueberries (Table 2.1.2). Highest numbers of male flies emerged from raspberries and chokecherries (Table 2.1.2).

The number of pupae increased with sweetness (ANOVA; $F_{1,122}=13.4$, $p<0.001$). Although a linear relationship between sweetness and emerging adult flies was not observed, in general terms, fruits with higher Brix produced more flies (Fig. 2.1.1a). The number of pupae increased with pH ($F_{1,122}=4.9$, $p<0.05$)(Table 2.1.2) and adult flies successfully emerged from fruits across the full range of pH values (Fig. 2.1.1b). Numbers of pupae (ANOVA; $F_{1,122}=4.8$, $p<0.05$) and adult female *D. suzukii* ($F_{1,122}=7.9$, $p<0.01$) to emerge from fruits decreased with fruit firmness (Fig. 2.1.1c). Fruits requiring the greatest force for penetration (lingonberries) had the lowest counts for all life stages (Table 2.1.2).

Table 2.1.2. Fruits attributes were measured for sweetness (ANOVA; $F_{7,179}=138$, $p<0.0001$), acidity ($F_{7,179}=368.2$, $p<0001$) and firmness (ANOVA, $F_{7,182}=107.9$, $p<0.0001$). Different capital letters indicate significant differences among fruit character measures (Tukey post-hoc, $p<0.05$). Whole fruits were exposed to 2 sexually mature female *D. suzukii* for 72 h, checked immediately thereafter for eggs, and were checked weekly for larvae, pupae, and emerging flies. Emerged flies were counted and sexed. (Mean ± SE).

	sweetness (Brix)		acidity (pH)		firmness (gf/mm^2)						
Fruit	mean ± SE	differences	mean ± SE	differences	mean ± SE	differences	eggs	larvae	pupae	female flies	male flies
blueberry	14.3 ± 0.4	C	5.4 ± 0.1	C D	35.1 ± 5.5	E	5.5 ± 1.3	0.5 ± 0.2	1.5 ± 0.5	1.1 ± 0.3	0.4 ± 0.2
chokecherry	18.3 ± 0.3	D	5.9 ± 0.1	E	52.5 ± 14.0	F	5.6 ± 0.7	3.4 ± 0.7	2.8 ± 0.8	0.7 ± 0.3	0.9 ± 0.4
lingonberry	9.8 ± 0.2	A	3.4 ± 0.1	F	108.8 ± 11.4	B	3.1 ± 0.5	0.9 ± 0.4	0	0	0
lingonberry - frosted	9.6 ± 0.6	A	4.2 ± 0.1	B C	30.4 ± 19.2	D	5.0 ± 0.9	0.6 ± 0.3	0	0	0
raspberry - Caroline	10.9 ± 0.2	B	2.9 ± 0.0	B	22.2 ± 5.9	A	3.0 ± 0.7	1.5 ± 0.5	1.1 ± 0.3	0.5 ± 0.2	0.4 ± 0.1
raspberry - Heritage	9.9 ± 0.2	A B	2.9 ± 0.0	A	9.1 ± 1.1	A	1.3 ± 0.7	2.6 ± 1.5	2.2 ± 0.6	2.5 ± 0.6	2.3 ± 0.6
sea buckthorn - dark	10.2 ± 0.1	A B	3.6 ± 0.0	B C	27.1 ± 4.9	C	2.1 ± 0.6	2.3 ± 0.7	0.6 ± 0.3	0.1 ± 0.1	0.2 ± 0.1
sea buckthorn – light	9.5 ± 0.1	A	3.0 ± 0.0	D E	47.0 ± 13.0	A	2.2 ± 0.9	2.5 ± 0.6	0.6 ± 0.3	0.3 ± 0.1	0

Table 2.1.3. Interactions among fruit characters were important factors in host selection for female *D. suzukii* over 24 h, 48 h, and 72 h in 2-choice assays (three-way factorial ANOVA). Significant results are shown in bold.

	24 h		48 h		72 h	
	$F_{1,201}$	p	$F_{1,201}$	p	$F_{1,201}$	p
Brix	**20.16**	**<0.0001**	**11.74**	**<0.001**	**14.54**	**<0.001**
pH	0.39	0.54	0.47	0.50	1.20	0.27
gfmm^2	2.19	0.14	1.53	0.22	4.64	0.03
Brix : pH	**10.28**	**<0.01**	**12.71**	**<0.001**	**15.10**	**<0.001**
Brix : gfmm^2	0.36	0.55	2.44	0.12	2.82	0.09
pH : gfmm^2	1.11	0.29	3.32	0.07	**10.93**	**0.001**
Brix : pH: gfmm^2	**28.21**	**<0.0001**	**14.53**	**<0.001**	**6.10**	**0.01**

2.1.5 Discussion

Novel host information

The protein-rich microbial communities associated with over-ripe and rotting fruits are crucial nutrient sources for larval development in most Drosophilid species (Becher et al. 2012; Jaramillo et al. 2014). Thus, most female Drosophilids are attracted to volatiles associated with fermentation and yeasts (Becher et al. 2012). In contrast, *D. suzukii* larvae develop on sound, healthy fruits, and adult *D. suzukii* are attracted to volatiles associated with fruit ripening (Keesey et al. 2015; Revadi et al. 2015). The role of visual cues is less understood in *D. suzukii* host selection. Therefore, assays were designed so that host fruit olfactory cues were the primary mechanism for choice without excluding potential visual cues associated with fruit colour. Potential olfactory or visual cues from host plant foliage were not considered in this study.

In all cases, more female *D. suzukii* were attracted to traps baited with fruits than to empty control traps, suggesting that fruit availability is a major determinant of potential fruit susceptibility and confirming that associated fruit volatiles, such as ethyl acetate, play a key role in *D. suzukii* locating fruit hosts. Although *D. suzukii* is known to be a serious pest in commercial highbush blueberry crops, in choice tests of blueberries paired against other fruits, all other fruits tested were preferred over blueberries in choice assays. This is consistent with recent studies that found that blueberries are less attractive than raspberries and less effective host fruits than raspberries (Lee et al. 2011; Bellamy et al. 2013; Abraham et al. 2015). Highbush blueberries are the least fragrant of the fruits tested, which may account for its limited attractiveness when other fruits are available (Forney 2001). High infestation rates in commercial blueberry crops may be an unfortunate intersection of fruit phenology and peak *D. suzukii* intensities. *Drosophila suzukii* have been documented ovipositing in a wide variety of fruits and have observed breeding on *Camellia* spp. L. and *Styrax japonicus* Sieb. et Zucc. flowers when suitable fruit hosts were not available (Mitsui et al. 2010; Walsh et al. 2011). In the absence of other food sources, *D. suzukii* have been observed feeding on tree sap and honeydew (Moreau DL, unpublished; Walsh et al. 2011). Thus, in the absence of more suitable host fruits, *D. suzukii* would readily use abundant blueberry crops. Chokecherries were preferred over highbush blueberries and lingonberries. Where chokecherry bushes occur in hedgerows near blueberry fields, chokecherry fruits were more heavily infested than blueberries (Moreau DL, unpublished). In choice assays, lingonberries were comparable in attraction to raspberry varieties. Sea buckthorn varieties were preferred over all other fruits in choice assays. Sea buckthorn fruits have a strong odor described as fruity, berry-like, or citrus-like (Tiitinen et al. 2005). Attraction to citrus volatiles is thought to be an ancestral trait of Drosophilids and may account for the attractiveness of sea buckthorn berries (Dweck et al. 2013). Lingonberries are slightly smaller than cranberries (*Vaccinium macrocarpon* Aiton), but comparable in other respects. As was the case with cranberries, no larvae developed to maturity on unwounded lingonberries (Steffan et al. 2013). However, bruised or frost-damaged lingonberries are susceptible to oviposition and larval damage. Although no adult flies emerged from fruits tested, this may have been due to laboratory conditions (sealed container at room temperature during trials) and results may differ in field conditions. Once cranberries are wounded and begin to decay, they become suitable hosts to support *D. suzukii* larvae to maturity, suggesting that the difference in larval success may be attributable to the microbial community associated with decay (Steffan et al. 2013). In a field setting, frost-damaged lingonberries may be as suitable a host as decaying cranberries and augment *D. suzukii* populations through the winter and early spring.

Host preference

Relatively few female flies made a choice for any of the fruits within the first 24 h; however, by 48 h, the majority of flies had made a choice decision. Fly behavior adhered to predictable activity patterns including an initial burst of exploratory behavior, a decline in activity as flies habituated to their new environment, and a final period of spontaneous activity during which they responded to olfactory cues (Soibam et al. 2013). Female *D. suzukii* were starved no more than 2 h before the start of each choice assay, thus hunger was presumed not to motivate choices between potential food sources during the first 24 h. Additionally, because choice assays were maintained at room temperature, responses may have increased as the fruit odours intensified over time as the assay progressed. Because assays were conducted with 15 flies in each trial, there was the potential that flies could have been attracted to visual or auditory cues from other flies within the same arena. Arenas were isolated from each other to prevent further bias.

When *D. suzukii* were offered a choice between fruits, those fruits with lower pH and that were less sweet were preferred. Although most fruits increase in brix and pH as they ripen, in early ripening stages these values may decrease in some fruits, as in some *Rubus* spp (Tosun et al. 2008). Thus, identifying lower pH and brix levels associated with early ripening may give *D. suzukii* a competitive advantage by permitting female *D. suzukii* to identify host fruits in which to oviposit eggs at much earlier fruit development stages than possible for other. In contrast, pH levels remain consistently low and brix levels decrease throughout the ripening process in sea buckthorn fruits (Raffo et al. 2004). These may account for *D. suzukii*'s strong attraction to sea buckthorn fruits in choice assays. Skin toughness is a major determinant of suitability of a fruit host for *D. suzukii* (Lee et al. 2011). Among blueberry cultivars, *D. suzukii* oviposition is negatively correlated with fruit firmness; however, this correlation did not hold over a broader range of fruit species and fruit firmness (Kinjo et al. 2013). Furthermore, when more suitable, softer-skinned fruits are not available, female *D. suzukii* will search out soft spots on otherwise firm fruits or lay eggs on the surface of fruits. Thus *D. suzukii* is less constrained by fruit skin toughness than many other *Drosophila* spp. and can readily oviposit on relatively wide range of fruit firmness (Walsh et al. 2011; Calabria et al. 2012). This suggests that beyond a threshold firmness of approximately 55-60 gf/mm^2, larval success is limited.

Larval development

Eggs or larvae were found on all fruit types tested for oviposition success. Highest numbers of adult flies emerged from raspberry varieties, highbush blueberries, and chokecherries. These fruits have relatively soft flesh and thin skins, easily punctured by *D. suzukii* ovipositors. Within fruit types, *D. suzukii* are known to prefer higher Brix levels; however, among fruit types in this study, less sweet fruit species were preferred in choice tests (Lee et al. 2011, 2015, 2016). Blueberries and chokecherries had the highest Brix levels and highest pH of fruits tested and although *D. suzukii* larvae could successfully develop in these fruits, they were the least preferred in choice tests. Raspberries were preferred in choice tests and intermediate in sweetness and among the most acidic of the fruits tested. This suggests that *D. suzukii* larvae can tolerate a wide range of sweetness and acidity, provided that the fruit host satisfies minimum nutritional needs, either through carbohydrates or proteins (Becher et al. 2012; Steffan et al. 2013; Wilson et al. 2013; Jaramillo et al. 2014).

Applications for fruit growers

Regardless of relative preference of one fruit species over another, *D. suzukii* is a generalist fruit pest that appears to be highly opportunistic. Fruit susceptibility may be primarily a factor of fruit phenology, with characters such as Brix, pH, and fruit firmness serving only as limiting factors in host choice. Although female *D. suzukii* showed a marked preference for sea buckthorn varieties in choice assays, few adult flies emerged from pupae that had developed in sea buckthorn fruits. This may have been due to the tendency of sea buckthorn fruits to decay fairly quickly in sealed containers at room temperature and larvae likely perished from ethanol poisoning. In a field setting, sea buckthorn fruits remain on-stem up to two years without decaying. Under these conditions, more larvae could be expected to reach maturity. This suggests that sea buckthorn fruits may be attractive hosts for *D. suzukii*.

The opportunistic nature of *D. suzukii* paired with its reproductive success on commercial raspberries and blueberries is of particular concern in fruit growing regions such as Nova Scotia. Raspberry, blueberry, and chokecherry fruits ripen at similar times, such that as burgeoning populations of *D. suzukii* emerge from one fruit, they can rapidly move from one crop to another. Fall-bearing highbush blueberry crops are potentially at greater risk when fruit from other, more preferred, hosts are no longer available later in the season.

2.1.6 Acknowledgements

We thank K. Bassett and P.L. Dixon for their assistance locating and collecting fruits and C. Hammill for statistical advice. We gratefully acknowledge Nova Agri Inc, MacBerry Farms, Rodrigues Winery, and Agriculture and Agri-Food Canada for their on-going support. Funding was provided by Atlantic Canada Opportunities Agency Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21516-519).

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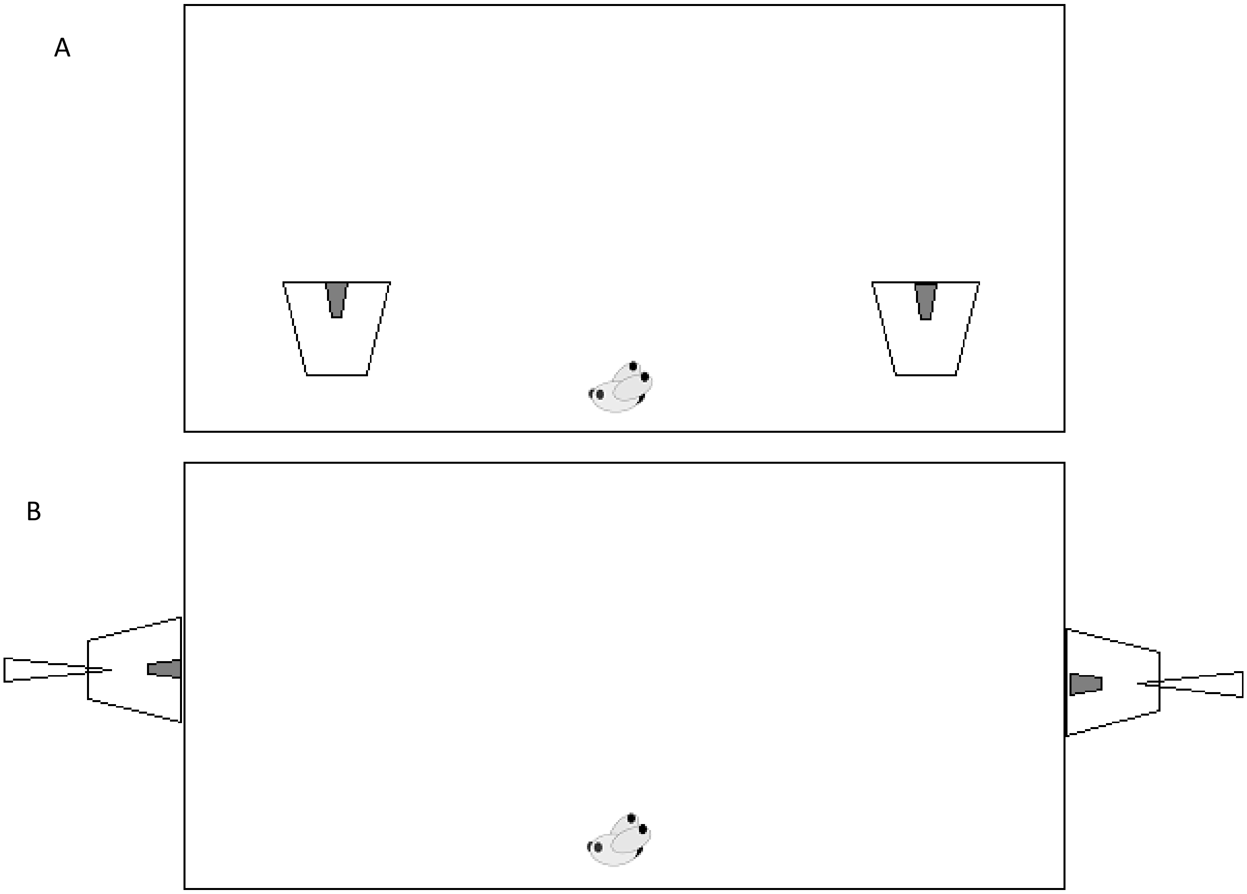
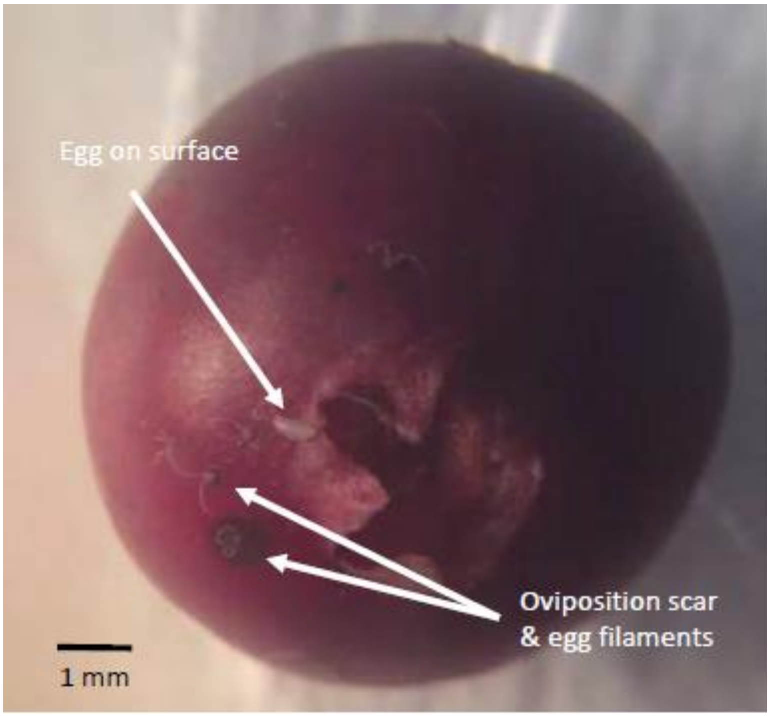
2.1.8 Supplementary information



Supplementary Figure S2.1.1. Side-view diagram of airtight arena (A) and airflow arena (B) showing location of traps within each arena and inputs for airflow through traps. Fifteen female *D. suzukii* were released at the point marked with an illustration of a fly (male fly shown for illustration purposes only).



Supplementary Figure S2.1.2. Eggs and oviposition scars are clearly visible on a lingonberry after 72 h exposure to sexually mature female *D. suzukii*.

2.2 Role of fruit characters and colour on host selection of boreal fruits and berries by *Drosophila suzukii*

A version of this chapter section has been published in The Canadian Entomologist:
Little, C.M., Dixon, P.L., Chapman, T.W., and Hillier, N.K. 2020. Role of fruit characters and colour on host selection of boreal fruits and berries by *Drosophila suzukii* (Diptera: Drosophilidae). Can Entomol: 1–17. doi:10.4039/tce.2020.1.

Author contribution statement

CML and NKH designed research. PLD and TWC advised on suitable fruits for testing and methodology. CML conducted research. CML analysed data and prepared manuscript. NKH, PLD, and TWC edited manuscript. All authors read and approved manuscript.

2.2.1 Abstract

Continued range expansion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is exposing new species of soft fruits and berries to potential infestation. Our understanding of cues that drive host finding and selection in this highly polyphagous pest insect is still incomplete. Fruit firmness influences host choice behaviour by limiting suitability for oviposition and larval development. Other factors such as fruit sweetness and acidity act as cues for fruit ripening. Here we assess the role of these cues and fruit colour on host selection. We demonstrate that the use of objective and non-anthropocentric methods of quantifying colour in studies of colour preference is critical to understanding the cues evoking responses from insects. Acidity but not sweetness increased *D. suzukii* attraction and larval success. Differences in *D. suzukii* attraction were most strongly correlated with short wavelength reflectance (blue, cyan, and green (470–560 nm)). Growers could select for fruit varieties with relatively higher reflectance values upon maturity to reduce susceptibility to *D. suzukii*.

2.2.2 Introduction

Since 2008, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) has spread across much of North America, South America, and Europe (Grassi *et al*. 2011; Hauser 2011; Walsh *et al*. 2011; Calabria *et al*. 2012; Cini *et al*. 2014; Deprá *et al*. 2014; Asplen *et al*. 2015; Funes *et al*. 2018). Unlike most Drosophilidae, *D. suzukii* is a serious pest of soft fruits and berries. Female flies use a serrated ovipositor to lay eggs in ripening rather than over-ripe fruits, causing the fruit to spoil and become unmarketable. Male flies are more readily recognised by their characteristic wing colourisation that give the species their common name, spotted-wing drosophila. Costs associated with crop losses and increased pesticide use worldwide exceed one billion USD annually (Bolda *et al*. 2010; Lee *et al*. 2011; Cini *et al*. 2014; Wiman *et al*. 2016). To date, studies have demonstrated that fruits of 151 plant species from 31 families are at least somewhat susceptible to infestation (Lee *et al*. 2015; Kenis *et al*. 2016; Lee and Sial 2016; Little *et al*. 2017; Elsensohn and Loeb 2018).

The full potential for continued range expansion by *D. suzukii* in North America and abroad has yet to be determined. Dynamic acclimation to cold conditions may allow *D. suzukii* to successfully overwinter in colder climates and further expand its geographic range (Stockton *et al*. 2018). Overwintering populations of *D. suzukii* have been found in numerous locations in Canada and northern Europe where winter temperatures drop well below -17 ^{o}C and laboratory studies confirm physiological adaptations suitable to cold hardiness (Hamby *et al*. 2016; Rossi Stacconi *et al*. 2016; Thistlewood *et al*. 2018). Fruits and berries grown commercially or that are endemic to boreal regions at the presumed limit of their current range may be at-risk as *D. suzukii* continues to extend its range northward.

Host choice by *D. suzukii* is generally thought to be a factor of changes in fruit characters associated with ripening, particularly firmness or skin resistance (penetration force), sweetness

(Brix), and acidity (pH) (Lee *et al*. 2011, 2015; Burrack *et al*. 2013). Fruits susceptible to *D. suzukii* tend to be limited to soft-skinned fruits and berries, with some exceptions, particularly when fruits are damaged or overripe (Lee *et al*. 2015; Kenis *et al*. 2016; Lee and Sial 2016; Little *et al*. 2017; Elsensohn and Loeb 2018). Other fruit characters thought to be important for host selection and suitability to *D. suzukii*, include size, shape, structure, volatile odour profile, and colour of fruits (Poyet *et al*. 2015). Fruit firmness is also a limiting factor in oviposition success by *D. suzukii* (Entling *et al*. 2018).

Most previous studies of fruit preference by *D. suzukii* that investigated fruit colour did not quantify colour parameters (Lee *et al*. 2011, 2016; Karageorgi *et al*. 2017; Jaffe *et al*. 2018). Other studies that investigated colour preference in *D. suzukii* used measures of perceived colour based on human vision (Kirkpatrick *et al*. 2016, 2018). Parameters such as hue, saturation, chroma, and brightness are comparative measures of colour appearance based on human perception (Fairchild 2005). Colour appearance models compare similarities or differences among perceived colours and assume identical environmental conditions (Fairchild 2005; Kelber and Osorio 2010; Lunau 2014; Cuthill *et al*. 2017). The colour of an object is a property of both the object being perceived and the perception of the animal that perceives it (Glover and Whitney 2010). *Drosophila* Fallén and human visual sensitivity differ; therefore, we chose a percentage reflectance across a range of wavelengths as an objective measure of fruit colour, independent of the observer (Paulk *et al*. 2013; Little *et al*. 2018).

Drosophila suzukii is highly polyphagous, infesting fruits of widely diverse characters and colours (Lee *et al*. 2015; Kenis *et al*. 2016; Lee and Sial 2016; Little *et al*. 2017; Elsensohn and Loeb 2018). We hypothesise that fruit reflectance may play an important role in host selection among diverse fruit species available in an area. Host choice among blueberries (*Vaccinium* *corymbosum* Linnaeus (Ericaceae)) by *D. suzukii* is partially dependent upon visual contrast cues or conspicuousness against the background of foliage (Little *et al*. 2018). Similar behaviour in female *Delia radicum* Linnaeus (Diptera: Anthomyiidae) flies has been described for host choice based on “appropriate-inappropriate landings”, with appropriate landings defined as being on host plants and inappropriate landings as being on non-host plants (Finch and Collier 2000).

We identified 11 fruit-producing plant species of interest that were found across Nova Scotia and Newfoundland and Labrador, Canada. Pin cherries, *Prunus pensylvanica* Linnaeus (Rosaceae), and bittersweet nightshade, *Solanum dulcamara* Linnaeus (Solanaceae) are common in hedgerows and wooded areas throughout much of Canada. Lowbush or wild blueberry, *Vaccinium angustifolium* Aiton (Ericaceae), is found in wooded areas and hedgerows and is grown commercially throughout much of Canada. Haskap, *Lonicera caerulea* Linnaeus (Caprifoliaceae), also known as blue honeysuckle and honeyberry, is a relatively new commercial and ornamental berry plant in North America but widely grown in northern Asia (Celli *et al*. 2014). *Amelanchier alnifolia* Nuttall (Rosaceae), commonly known as Saskatoon berry or service berry, is native to boreal regions of North America but is now grown in northern regions of Europe (Bakowska-Barczak and Kolodziejczyk 2008; Lavola *et al*. 2012). Crowberry, *Empetrum nigrum* Linnaeus (Ericaceae) is an ecologically and culturally important species in the northern heathlands of North America, Europe, and Asia (Koskela *et al*. 2010; Buizer *et al*. 2012). Cloudberry, *Rubus chamaemorus* Linnaeus (Rosaceae), also known as bakeapple in Newfoundland and Labrador, is a perennial herbaceous plant found in boreal regions of North America, Europe, and Asia (Thiem 2003). Fruit colour changes from red to orange-yellow as it ripens. Strawberries, *Fragaria × ananassa* Duchesne (Rosaceae); highbush blueberries, *Vaccinium corymbosum* Linnaeus (Ericaceae); raspberries, *Rubus idaeus* Linnaeus

(Rosaceae); and cherries, *Prunus avium* Linnaeus (Rosaceae), are commercially grown in boreal regions of Canada.

Here we tested whether fruit colour is a determinant of host-choice in the absence of background foliage. We also examined whether aspects of fruit colour can be used during host-search as a reliable proxy for other fruit characters. We quantified potential preference of fruits to *D. suzukii* based on host selection in choice assays and host suitability based on larval success in no-choice assays. Host preference and suitability measures were then compared to fruit characters including firmness, sweetness, acidity, and colour. We also assessed the susceptibility of a variety of fruits that are of commercial or cultural importance in boreal regions across Canada.

Key message

- Fruits growing in boreal regions are suitable hosts for *D. suzukii* and are at risk of infestation as its invasive range expands.
- How *D. suzukii* find and discriminate among diverse potential host fruits is poorly understood.
- Characters such as sweetness, acidity, fruit firmness, and colour can be signals for fruit ripeness and host suitability.
- This study gives first evidence that *D. suzukii* can use fruit colour reflectance as cues for host suitability across multiple fruit species.
- Selection for fruit varieties with greater reflectance across the visible spectrum may reduce attractiveness to *D. suzukii*.

2.2.3 Materials and methods

Fruit and insect sources

Ripe fruits from 11 plant species were collected and tested between 2014 and 2017. Pin cherry, bittersweet nightshade, and wild blueberry were picked from wild plants in the Annapolis Valley, Nova Scotia, Canada. Haskap and Saskatoon berry were picked at u-pick farms in Nova Scotia. Crowberry was picked by a colleague in Iqaluit, Nunavut, Canada. Fresh cloudberry was not available due to adverse weather conditions in 2015 and 2016 throughout much of Newfoundland and Nova Scotia. We obtained frozen cloudberry that had been picked in Newfoundland in 2014. Host selection assays and oviposition and larval development assays were also conducted using bittersweet nightshade (*Solanum dulcamara*) collected from wild plants in Nova Scotia after hearing anecdotal accounts of *D. suzukii* associated with these plants. Strawberry (*Fragaria* × *ananassa* varieties “Wendy”, “Honeyeye”, and “Cabot”), and cherry (*P. avium* variety “Stella”) were obtained from local growers at farm markets in the Annapolis Valley, Nova Scotia. Highbush blueberry (*V. corymbosum* varieties “Jersey” and “Coville”) and raspberries (*R. idaeus* variety “Caroline”) obtained from commercial growers in Nova Scotia were also used to determine relative preference in host selection assays.

Drosophila suzukii used in the experiments were obtained from colonies maintained since 2013 at Acadia University from stocks originally reared by the Kentville Research and Development Centre (Agriculture and Agri-Food Canada, Kentville, Nova Scotia) and replenished with wild flies reared from local fruit in 2016. Colonies were housed in 250-mL *Drosophila* flasks (Genesee Scientific, San Diego, California, United States of America) containing 50 mL Formula 4-24 Instant *Drosophila* Medium (Merlan Scientific, Mississauga, Ontario, Canada) mixed with 50 mL dH_2O. Approximately two hours prior to the start of each assay, reproductively mature females aged up to two weeks were removed from colonies created one month earlier and held in 30-mL vials without access to food or water. Vouchers are held on deposit at the Acadia University Wildlife Museum, Wolfville, Nova Scotia.

Fruit characters

Fruit were assessed for colour, firmness (gfmm^2), sweetness or sugar content (brix), and acidity (pH) to elucidate characters that could affect susceptibility and suitability to *D. suzukii* (Arnó *et al*. 2016; Little *et al*. 2017, 2018). Ten intact ripe fruits were selected randomly from each plant species. Mean values of each character for each fruit species were used for comparison and analysis. Due to limited quantities of available undamaged nightshade fruits, fruit characters were not measured for that species.

First, fruit colour was quantified. Reflectance spectra for each fruit were measured with an Alta II reflectance spectrometer (Vernier Software & Technology, Beaverton, Oregon, United States of America) to quantify colour. All reflectance values were measured in a dark room with the spectrometer providing the sole light source. Percentage reflectance measures were obtained for seven visible colour wavelengths (470–700 nm).

Fruits were then tested for firmness using a Wagner Fruit Penetrometer FT02 gram force gage (Wagner Instruments, Greenwich, Connecticut, United States of America), sweetness was measured with a Westover Model RHR – 32ATC handheld brix refractometer with automatic temperature compensation (Cole-Parmer, Montréal, Québec, Canada), and acidity was measured using a Fisher Scientific Accumet Basic AB15 pH meter (Fisher Scientific, Toronto, Ontario, Canada) (Lee *et al*. 2011; Little *et al*. 2017, 2018).

Fruit preference

We used two-choice trap assays to assess the relative preference of *D. suzukii* among fruit species (Abba *et al*. 2012; Brodie *et al*. 2015). Two-choice assays were conducted within a sealed 600-mL airtight arena (18 x 12 x 6.5 cm) (Little *et al*. 2017, 2018). Each arena contained two clear plastic traps, each consisting of a 30-mL portion cup with a truncate 200 µL pipette tip inserted through the lid (Little *et al*. 2017, 2018). Approximately 10 mL of fruit was placed in each trap (Little *et al*. 2017, 2018). Fifteen mated female *D. suzukii* were placed in each arena (Little *et al*. 2017, 2018). Host choices were checked after 24, 48, and 72 hours. Each fruit species was tested against each other and against a blank (control) trap. Three to five replicates were completed for each trial. Due to limited fruit availability and differences in fruit phenology, we were unable to conduct trials with all possible combinations of fruit. Response index (RI) scores ranging from -1 to +1 were calculated as a measure of host preference based on Dweck *et al*. (2013), where

$$\mathrm{RI} = (A-B)/T$$

A and B are the number of flies in each trap

T is the total number of flies per trial

Fruit suitability

Approximately 10 mL of each fruit and two sexually mature, mated female *D. suzukii* were placed in a 50-mL *Drosophila* vial (Genesee Scientific, San Diego, California, United States of America) capped with cellulose acetate plugs. Flies were removed after 72 hours and fruit visually inspected under a dissecting microscope at 120-250 times magnification for eggs and larvae. Fruits were visually inspected weekly for six weeks thereafter for emerging flies. Non-destructive examination methods were employed to reduce risk of damage to developing larvae. Adult flies were removed for counting and sexing. We assessed ten replicates for each fruit, with each vial representing one replicate.

Statistical analysis

We accounted for effect of treatment (fruit type), time and trial on response indices using repeated measures Friedman χ^2 analyses. We used Kruskal-Wallis χ^2 tests and Tukey and Kramer (Nemenyi) Post Hoc tests ($P < 0.05$) to assess differences in host selection among fruit types. Fractional analysis and multiple-factor analyses of variance were used to assess relationships between host selection and interactions among fruit characters. All other data were analysed with one-way analysis of variance and Tukey post-hoc tests performed using RStudio (Version 1.1.419, https://rstudio.com/products/rstudio/download; using R version 3.4.3 (www.r-project.org)).

2.2.4 Results

Fruit characters

Ripe fruits of all species tested had higher mean percentage reflectance values at longer wavelengths than at shorter wavelengths (Fig. 2.2.1A). Mean percentage reflectance values of crowberry and wild blueberry were relatively consistent across wavelengths tested, with no strong peak discernable for either fruit species (Fig. 2.2.1A). Pin cherry and Saskatoon berry were the sweetest, although differences in sweetness among pin cherry, Saskatoon berry, and cherry were not significant (Fig. 2.2.1B). Saskatoon berry and wild blueberry were the least acidic (Fig. 2.2.1C). Cloudberry was most difficult to puncture, whereas haskap was very easily punctured (Fig. 2.2.1D).

Among ripe fruits, percentage reflectance was strongly correlated across short wavelength colours (blue–orange (470–600 nm)) and across long wavelength colours (red–deep red (645–700 nm)) (Supp. Table S2.2.1). Fruits with low reflectance values for blue (470 nm), therefore also had proportionally low reflectance values for cyan (525 nm) and green (560 nm). However, reflectance was not correlated between short and long wavelength colours (Supp. Table S2.2.1). Therefore, reflectance values at short wavelengths such as blue (470 nm) are not predictive of reflectance values at longer wavelengths such as red (645 nm).

Fruit colour across most of the measured spectra was interrelated with other fruit attributes, irrespective of the fruit species (Table 2.2.2). Patterns of relative proportions of reflectance measures during ripening are fruit species-specific or variety-specific and can be a reliable proxy for fruit ripeness and quality (Li *et al*. 2014; Hu *et al*. 2018). Among the fruits tested, fruit sugar content or sweetness (brix) was positively correlated with cyan (525 nm) to orange (600 nm), which is well within the visual range of *Drosophila* species (Hernández de Salomon and Spatz 1983). In a separate study, we found that the visual range of *D. suzukii* is consistent with that of *D. melanogaster* (Little *et al*. 2019). Fruit firmness (gf/mm^2) is positively correlated with green (560 nm) to deep red (700 nm), thus is also detectable within the visual range of *Drosophila*. Fruit acidity is positively correlated with blue (470 nm). Further testing would be required to confirm if correlations also occur in the ultraviolet range.

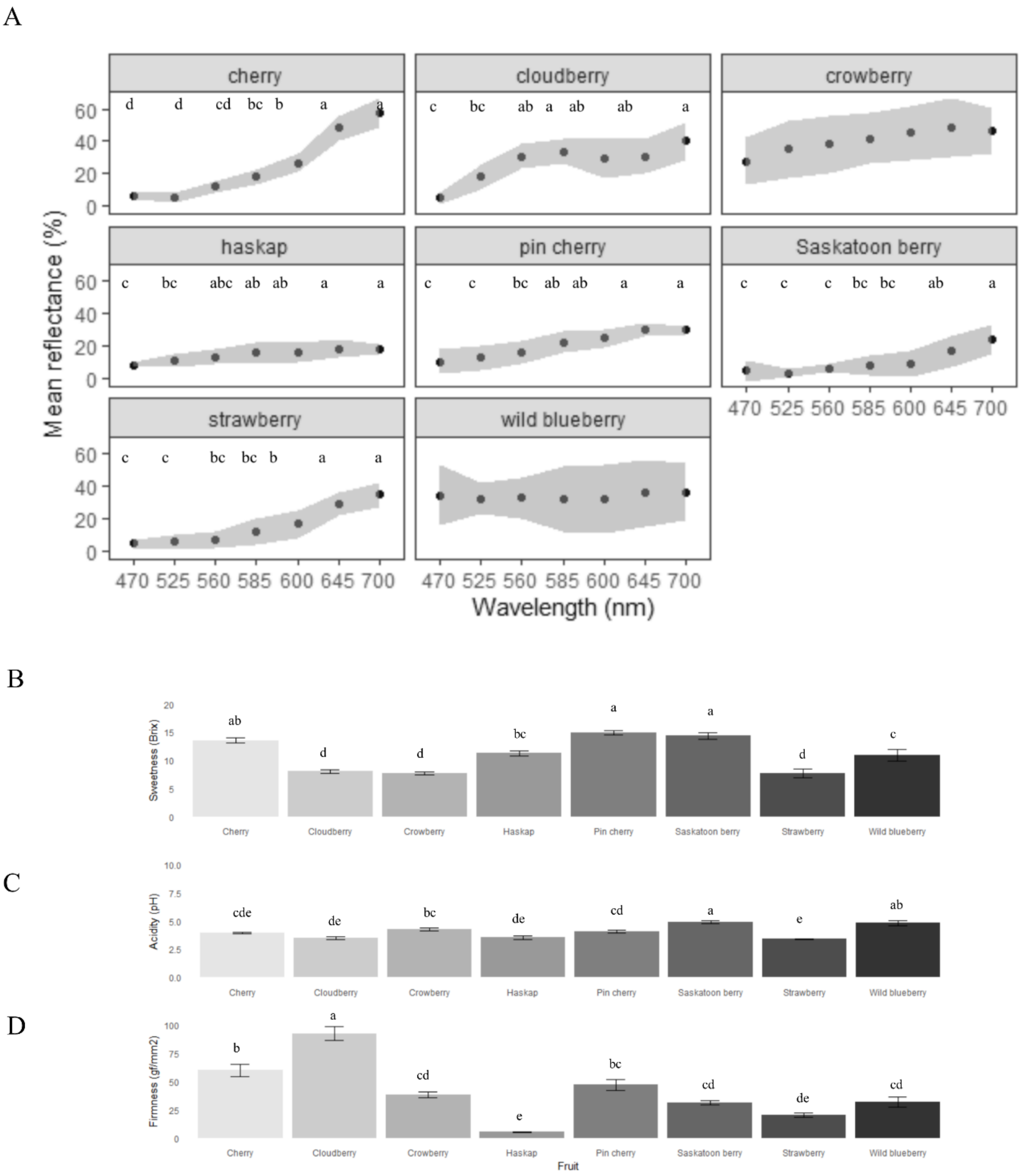


Figure 2.2.1. Comparison of fruit colour and characters. **A**. Spectra of mean percentage reflectance (± standard error) at each wavelength. Differences were observed in reflectance among wavelengths for each fruit and among fruits at each wavelength (see Table 2.2.3) (two-way analysis of variance, fruit: $F_{7,544} = 65.39$, $P < 0.0001$, wavelength: $F_{1,544} = 306.41$, $P < 0.0001$, interaction: $F_{7,544} = 16.81$, $P < 0.0001$). Differences were also observed among fruits sweetness, acidity, and firmness as follows. **B**. Brix levels (sweetness) (one-way analysis of variance $F_{7,72} = 30.31$, $P < 0.0001$); **C**. acidity (pH) (one-way analysis of variance $F_{7,72} = 19.09$,

$P < 0.0001$); **D**. firmness (resistance to puncture) (one-way analysis of variance $F_{7,72} = 46.82$, $P < 0.0001$). Whiskers denote minimum and maximum response index values. Different letter values above the figure denote significant differences among fruits (Tukey and Kramer (Nemenyi) post hoc, $P < 0.05$).

Fruit preference

Mean response indices for each two-choice fruit trial showed that preference for any given fruit was in part dependent upon the alternative available (Table 2.2.1). Mean response indices for each fruit type from two-choice assays were consistent across time periods measured (Friedman $\chi^2 = 0$, df = 2, $P = 1$); therefore, host selection results, hereafter, are presented for response indices at 72 hours. Fewest flies were found in highbush blueberry and pin cherry with mean response indices not statistically different than empty control traps (Fig. 2.2.2A). Numerical counts of *D. suzukii* were highest in strawberry, Saskatoon berry, raspberry, and bittersweet nightshade; however, differences were significant only between Saskatoon berry and highbush blueberry or pin cherry (Fig. 2.2.2A). Response indices were not correlated with fruit firmness overall (Spearman's rank correlation, Rs = -0.07, $P = 0.87$), fruit sweetness (Rs = -0.21, $P = 0.62$), or fruit acidity (Rs=-0.19, $P = 0.65$). However, in two-choice assays with fruits of differing characteristics, the relatively softer fruit was consistently preferred over firmer fruits (*t*-test, $t = 2.3$, df = 208.9, $P = 0.03$) and relatively more acidic fruits were preferred over those with higher pH ($t = -3.4$, df = 441.4, $P < 0.001$). No preference was observed between sweeter and less sweet fruits in two-choice tests ($t = -1.1$, df = 209.1, $P = 0.29$). Response indices were negatively correlated with percentage reflectance across most of the visible spectrum (Table 2.2.2). Percentage reflectance within short wavelength (470–560 nm) and long wavelength (575–700 nm) ranges were highly correlated for fruits tested (Supp. Table S2.2.1).

Fruit suitability

We were not able to accurately count eggs, larvae, and pupae in all fruit species using non-destructive methods, so fruit suitability was assessed based on emergence of adult flies. Adult *D. suzukii* that emerged from test fruits in no choice assays were removed for counting and sexing. Counts differed among fruits; however, emergence of female and male *D. suzukii* were consistent within fruit species (two-way analysis of variance, fruit: $F_{8,162} = 19.78$, $P < 0.0001$; sex: $F_{1,162} = 0.28$, $P = 0.60$; fruit:sex: $F_{8,162} = 0.53$, $P = 0.84$). We observed eggs, larvae, and emerging adults in all fruit species except bittersweet nightshade. Highest numbers of adult *D. suzukii* emerged from cloudberry, strawberry, and haskap; however, differences among strawberry, haskap, cherry, pin cherry, and Saskatoon berry were not significant (Fig. 2.2.2B**)**. Ten mL of cloudberry produced a maximum count of 41 emergent adult flies.

We found no correlation between number of adult flies emerged from fruits and fruit sweetness (Spearman's rank correlation, $R_s = -0.05$, $P = 0.67$) or fruit firmness ($R_s = -0.06$, $P = 0.60$). Number of emerged flies was negatively correlated with pH, with higher numbers of adult *D. suzukii* emerged from species with more acidic fruit ($R_s = -0.38$, $P < 0.001$). Numbers of emerged flies was also negative correlated with percentage reflectance across most of the visible spectrum (Table 2.2.2).

Table 2.2.1. Mean response index scores (± standard error) for two-choice trials of each potential fruit combination. Positive response index values represent net attraction (shown in bold) toward the fruit listed in the first column versus the fruit listed in the top row of the table.

	Control	Cherry	Cloudberry	Crowberry	Haskap	Highbush blueberry	Nightshade	Pin cherry	Raspberry	Saskatoon berry	Strawberry	Lowbush blueberry
Cherry					**0.57**			**0.09**		-0.73	-0.73	**0.78**
					0.17			**0.32**		0.04	0.10	**0.08**
Cloudberry	**0.87**			**0.47**	**0.12**	**0.32**			-0.55		-0.20	-0.41
	0.05			**0.19**	**0.31**	**0.28**			0.17		0.22	0.24
Crowberry	**0.82**		-0.47		-0.15	0.80			-0.60		-0.34	-0.36
	0.07		0.19		0.35	0.06			0.17		0.30	0.10
Haskap		-0.57	-0.12	**0.15**				**0.31**		-0.87	-0.74	**0.89**
		0.17	0.31	**0.35**				**0.26**		0.04	0.23	**0.04**
Highbush blueberry	**0.51**		-0.32	-0.80			-0.40		-0.36		-0.12	**0.07**
	0.08		0.28	0.06			0.10		0.29		0.20	**0.24**
Nightshade	**0.56**					**0.40**						
	0.35					**0.10**						
Pin cherry		-0.09			-0.31					-0.89	-0.82	0.09
		0.32			0.26					0.02	0.10	0.15
Raspberry	**0.77**		**0.55**	**0.60**		**0.36**					**0.59**	-0.22
	0.08		**0.17**	**0.17**		**0.29**					**0.25**	0.18
Saskatoon berry		0.73			**0.87**			**0.89**			-0.13	**0.78**
		0.04			**0.04**			**0.02**			0.35	**0.12**
Strawberry	**0.82**	**0.73**	**0.20**	**0.34**	**0.74**	**0.12**		**0.82**	-0.59	**0.13**		**0.82**
	0.07	**0.10**	**0.22**	**0.30**	**0.23**	**0.20**		**0.10**	0.25	**0.35**		**0.05**
Lowbush blueberry	**0.82**	-0.78	**0.41**	**0.36**	-0.89	-0.07		-0.09	**0.22**	-0.78	-0.82	
	0.05	0.08	**0.24**	**0.10**	0.04	0.24		0.15	**0.18**	0.12	0.05	

Table 2.2.2. Correlations among reflectance measures at each wavelength versus other fruit attributes, response index values during two-choice trials (see Fig. 2A, Table 1), and adult *D. suzukii* emergence during fruit suitability trials. Pearson's rank correlation. Significant results are shown in bold.

	Sweetness (brix)		**Acidity (pH)**		**Firmness (gf/mm^2)**		**Response index**		**Adults emerged**	
Wavelength	**R**	**P**	**R**	**P**	**R**	**P**	**R**	**P**	**R**	**P**
Blue (470 nm)	-0.14	0.23	**0.37**	**< 0.001**	-0.11	0.34	**-0.17**	**0.02**	**-0.41**	**< 0.0005**
Cyan (525 nm)	**-0.31**	**0.005**	0.19	0.08	0.08	0.47	**-0.20**	**0.005**	**-0.34**	**< 0.005**
Green (560 nm)	**-0.36**	**< 0.001**	0.13	0.24	**0.27**	**0.02**	**-0.20**	**< 0.005**	**-0.23**	**0.04**
Yellow (585 nm)	**-0.32**	**< 0.005**	0.12	0.30	**0.29**	**0.01**	**-0.22**	**< 0.005**	**-0.22**	**0.05**
Orange (600 nm)	**-0.24**	**0.03**	0.09	0.45	**0.24**	**0.03**	**-0.22**	**< 0.005**	**-0.30**	**< 0.01**
Red (645 nm)	-0.12	0.27	0.005	0.97	**0.27**	**0.02**	**-0.14**	**0.05**	**-0.31**	**< 0.005**
Deep red (700 nm)	-0.11	0.35	-0.08	0.48	**0.44**	**< 0.0001**	-0.04	0.54	-0.21	0.06

Table 2.2.3. Differences were observed in reflectance among fruits at each wavelength (2-way ANOVA, fruit: $F_{7,544}$=65.39, P<0.0001, wavelength: $F_{1,544}$=306.41, P<0.0001, interaction: $F_{7,544}$=16.81, P<0.0001).

	Wavelength						
	470 nm	**525 nm**	**560 nm**	**585 nm**	**600 nm**	**645 nm**	**700 nm**
$F_{7.72}$	15.81	20.44	19.27	12.40	9.29	10.28	13.78
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Fruit							
cherry	b	c	b	cd	b	a	a
cloudberry	b	b	a	ab	ab	bc	bc
crowberry	a	a	a	a	a	a	ab
haskap	b	bc	b	d	bc	c	e
pin cherry	b	bc	b	bcd	bc	bc	cde
Saskatoon berry	b	c	b	d	c	c	de
strawberry	b	c	b	d	bc	bc	bcd
wild blueberry	a	a	a	abc	ab	ab	bcd

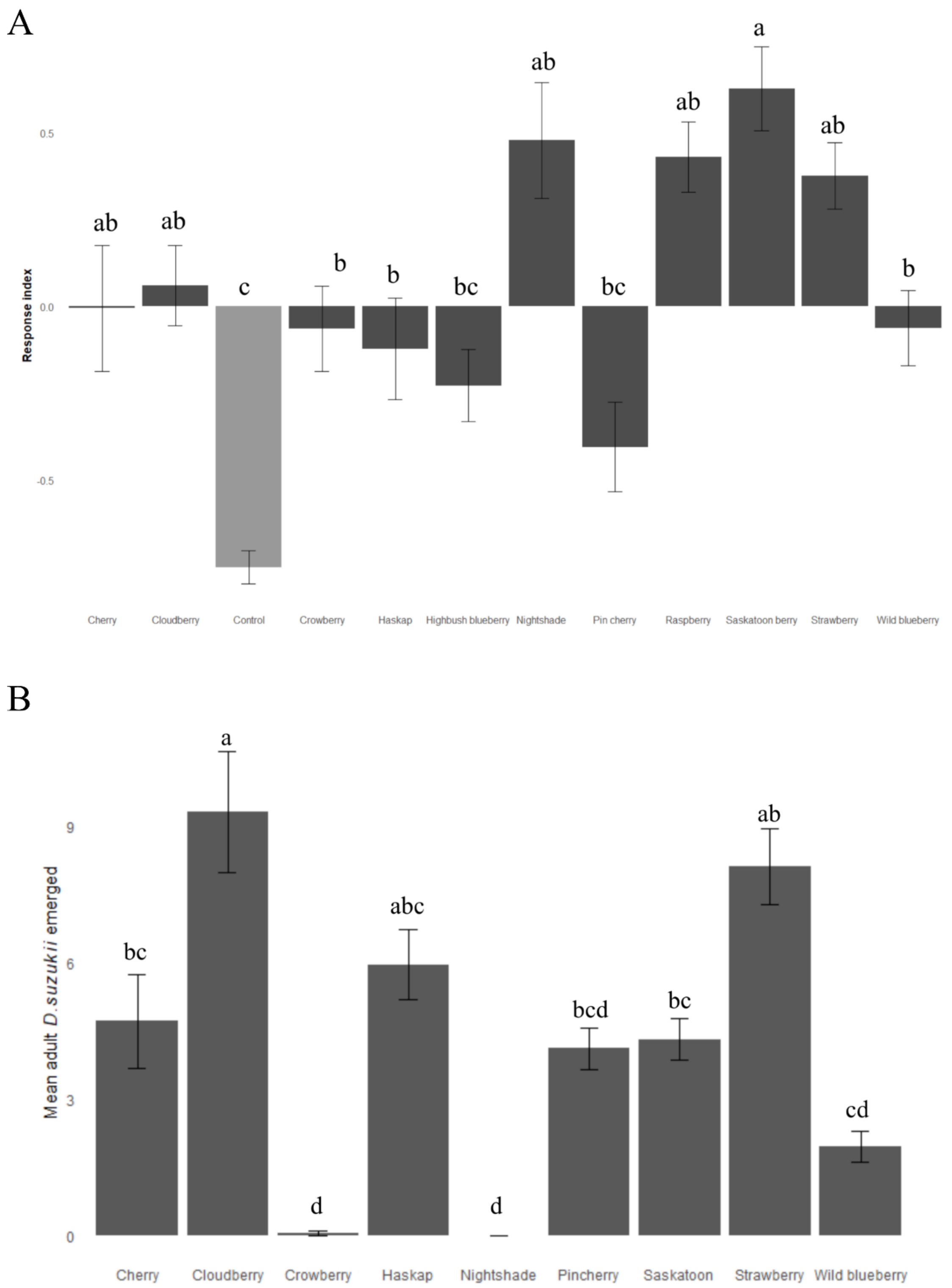


Figure 2.2.2. A. Mean response indices for fruits and controls in two-choice attraction assays with adult female unmated *Drosophila suzukii* (n = 10 per fruit species). A positive response index score indicates preference for that fruit. Different letter values above the figure denote significant differences among fruits (Kruskal-Wallis $\chi^2 = 79.29$, df = 11, $P < 0.0001$,Tukey and Kramer (Nemenyi) post hoc, $P < 0.05$). B. Mean adult *Drosophila suzukii* emerged from equal amounts of different fruits. Different letter values above the figure denote significant differences among fruits (analysis of variance; $F_{8,81} = 12.21$, $P < 0.0001$, Tukey and Kramer (Nemenyi) post hoc, $P < 0.05$).

2.2.5 Discussion

Given the rapid expansion of *D. suzukii* across Asia, Europe, North America, and South America, significant attention has been focused on risk to commercial fruit and berry crops. Studies addressing the potential attractiveness or host-preference of *D. suzukii* have suggested fruit characters, including fruit sweetness, acidity, and firmness, as important factors (Lee *et al*. 2011, 2016; Burrack *et al*. 2013). Volatile profiles of ripening fruit and fruit-associated yeasts have also been identified as potentially important olfactory cues for host-finding by *D. suzukii* (Cha *et al*. 2012, 2014; Hamby *et al*. 2012; Yu *et al*. 2013; Abraham *et al*. 2015; Keesey *et al*. 2015; Revadi *et al*. 2015; Hamby and Becher 2016; Hickner *et al*. 2016; Huang *et al*. 2017; Karageorgi *et al*. 2017; Cloonan *et al*. 2018; Liu *et al*. 2018). Changes in fruit profiles following damage due to injury or prior insect oviposition can alter attraction of *D. suzukii* (Yu *et al*. 2013; Lasa *et al*. 2017; Wang *et al*. 2019). Fruit phenology has also been identified as a risk factor (Sward *et al*. 2016; Little *et al*. 2017; Elsensohn and Loeb 2018). However, as *D. suzukii* continues to expand its geographic range, predicting which fruits are at risk is of increasing importance. We tested fruit susceptibility and preference among fruits at the northern range limit of *D. suzukii*.

In two-choice assays, numerical counts of *D. suzukii* were higher in Saskatoon berries, cloudberries, strawberries, raspberries, and bittersweet nightshade than in highbush blueberries and pin cherries. We note that in choice tests among these fruits, only Saskatoon berries had significantly higher mean response index values compared to the other fruits. This is consistent with previous findings that *D. suzukii* oviposits more eggs in raspberry than in many other commercially grown berries (Burrack *et al*. 2013). However, in two-choice assays of fruit versus a blank control, *D. suzukii* were attracted to all fruit species tested and differences in attraction among fruit species were significant only between Saskatoon berry and highbush blueberry or pin cherry. This is consistent with previous findings that *D. suzukii* is highly opportunistic and makes use of less preferred hosts when options are limited (Diepenbrock *et al*. 2016; Little *et al*. 2017).

In no-choice trials, eggs were observed in all fruit species except bittersweet nightshade. Lee *et al*. (2015) previously documented oviposition in this fruit but larvae did not develop. We observed eggs, larvae, and a single emergent adult in crowberry. Under laboratory conditions, crowberry fruit became increasingly desiccated. In a more natural environment, fruits attached to the plant may be more resistant to desiccation and therefore more suitable to larval development. For the purposes of this study, equal volumes of fruits were used for host preference two-choice assays and host suitability no-choice trials. This would not be the case in a field setting, where fruit size, proximity of fruits to each other, relative abundance of fruits, and foliage characteristics may play important roles in host fruit selection and/or suitability for larvae. Commercial berry crops are typically bred for large fruit size, high fruit abundance, and clustered fruit for ease of harvest. These characteristics which are beneficial for commercial growers also promote host fruit selection by pest insects.

Host selection by *D. suzukii* among fruits of a single species has previously been correlated with firmness, acidity, and sweetness in raspberries and blackberries but not grapes (*Vitis* Linnaeus; Vitaceae) (Burrack *et al*. 2013; Pelton *et al*. 2017). This study confirms that fruit firmness and acidity are factors in host selection among several host fruit species, but that fruit sweetness is less reliable as a host selection factor.

Fruit firmness or resistance to penetration is a limiting factor in host suitability among undamaged fruits for *D. suzukii* (Diepenbrock *et al*. 2016; Lee *et al*. 2016; Little *et al*. 2017).

Oviposition has previously been correlated with fruit firmness and we found that softer fruits were preferred in two-choice assays, but fruit firmness did not limit larval development (Lee *et al*. 2016; Little *et al*. 2017). Fruit sweetness was not a factor in either host selection or host suitability among the fruits tested. No more or fewer adult flies emerged relative to fruit sweetness levels. Among the fruits tested, more flies were attracted to and emerged from fruits with lower pH. This is contrary to results in previous studies, suggesting that *D. suzukii* may rely on a combination of factors for determining host selection and host suitability, even under controlled laboratory conditions (Lee *et al*. 2016). Little is yet known of the effects of different larval host fruits on adult *D. suzukii* fecundity and longevity. However, previous studies have demonstrated that *D. suzukii* maternal life history, particularly maternal diet, can significantly influence the viability of their offspring regardless of the fruit host of the larvae (Plantamp *et al*. 2017). Further research is needed to determine the role these fruits may play in the growth of local *D. suzukii* populations.

Within a fruit species or cultivar, fruit colour has been used as a proxy for assessing fruit ripeness (Li *et al*. 2014; Hu *et al*. 2018; Little *et al*. 2018). These relationships are more pronounced within a single fruit species or variety but can also be useful across diverse fruit species. Across the fruit species tested, mid-length wavelength reflectance values (cyan-orange (525–600 nm)) were characteristic of fruit sweetness and reflectance across most of the visible spectrum was representative of fruit firmness. Fruit acidity was not correlated with reflectance.

Previous studies that investigated relationships between host-choice and fruit colour used broad categories of colour based on what is perceptible to human vision, rather than *Drosophila* visual sensitivities (Lee *et al*. 2015). This research builds on our previous study of the role of fruit colour and contrast between fruit and foliage in host selection of ripening blueberries (Little *et al*. 2018). We observed a negative correlation between fruit preference and reflectance across most of the visible spectrum. Adult emergence was also negatively correlated with reflectance. Thus, fruits that were more reflective were less preferred by *D. suzukii* and potentially less suitable hosts. This is the first study to explore relationships between host-choice among different fruits and objective, quantified assessments of fruit colour, with recognition that the visual range of *Drosophila* differs from that of humans (Little *et al*. 2018).

This study is the first confirmation that Saskatoon berry, crowberry, and cloudberry are susceptible to infestation by *D. suzukii*. These fruits currently occur at the presumed northern range limit for *D. suzukii*; however, exposure of fruit to this invasive fly will most likely increase with continuing effects of climate change (Hamby *et al*. 2016; Rossi Stacconi *et al*. 2016; Langille *et al*. 2017; Stockton *et al*. 2018; Thistlewood *et al*. 2018). There may also be sufficient plasticity in *D. suzukii* cold tolerance that northern populations may slowly become more cold-adapted (Jakobs *et al*. 2015; Stockton *et al*. 2018).

Drosophila suzukii can assess relative risks and benefits of different fruit species and has demonstrated behavioural plasticity in host selection behaviour (Diepenbrock *et al*. 2016; Sward *et al*. 2016). This lack of host fidelity by *D. suzukii* may be key to its rapid near global expansion (Diepenbrock *et al*. 2016). Host selection by *D. suzukii* is subject to numerous factors, including fruit characteristics, fruit and foliage volatile odours, fruit abundance, availability of other fruit species, competition with other insect species, and risk of predation. Fruit colour, particularly reflectance of short to mid-length wavelengths (blue–orange (470–600 nm)) light, provides *D. suzukii* with a reliable proxy for fruit quality and ripeness, regardless of fruit species and may contribute to host selection. Growers of fruits at greatest risk from *D. suzukii* may benefit from selecting fruit varieties that retain high reflectance values, particularly at shorter wavelengths

(470–600 nm), as the fruit ripens to reduce the attractiveness of those fruit crops to *D. suzukii*. Future research is needed to determine how fruit colour and host volatile odours interact in host selection by *D. suzukii* and to determine the importance of fruit colour relative to other host cues. Commercial and native fruits in boreal and even Nearctic regions are suitable hosts for *D. suzukii* and with growing effects of climate change, are at increasing risk of infestation.

2.2.6 Acknowledgements

We are grateful for the assistance of Rebecca Rizzato, Aaron Spares, and Velma Dixon for collecting fruits for testing. We thank Heather Crozier for her assistance measuring fruit characters. We are grateful for the generosity of Nova Agri Group and MacBerry Farms for their continuing support and access to their blueberry fields. We gratefully acknowledge the financial support of Atlantic Canada Opportunities Agency Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21415-309, GF21516-519, GF21617-773, and GF21718-956).

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2.2.8. Supplementary information

Supplementary Table S2.2.1. Mean reflectance values of ripe fruits were tested for correlation among different wavelengths. Pearson's correlation coefficient; R is on 1st line and P is on 2nd line. Significant results are shown in bold.

Wavelength	**Cyan 525 nm**	**Green 560 nm**	**Yellow 585 nm**	**Orange 600 nm**	**Red 645 nm**	**Deep red 700 nm**
Blue 470 nm	**0.91** **<0.005**	**0.76** **0.03**	**0.70** **0.05**	**0.71** **0.05**	0.46 0.25	0.11 0.80
Cyan 525 nm		**0.95** **<0.0005**	**0.91** **<0.005**	**0.86** **<0.01**	0.49 0.22	0.23 0.58
Green 560 nm			**0.98** **<0.0001**	**0.90** **<0.005**	0.53 0.18	0.35 0.40
Yellow 585 nm				**0.95** **<0.0005**	0.62 0.10	0.44 0.27
Orange 600 nm					**0.83** **0.01**	0.63 0.10
Red 645 nm						**0.92** **0.001**

2.3 *FlySpotter*: using citizen science to identify range expansion and fruit at risk from *Drosophila suzukii* in Nova Scotia & Newfoundland and Labrador

A version of this chapter section has been published in The Journal of the Acadian Entomological Society:

Little, C.M., Rand, E., MacIsaac, M., Charbonneau, L., and Hillier, N.K. 2019. FlySpotter: using citizen science to identify range expansion and fruit at risk from *Drosophila suzukii* in Nova Scotia and Newfoundland and Labrador. J Acadian Entomol Soc **15**: 27–39. Available from http://acadianes.org/journal/papers/little_19-4abs.pdf [accessed 19 October 2019].

2.3.1 Abstract

Monitoring the spread of invasive insects across broad geographic regions and into remote areas can impose considerable financial and time costs. Volunteer citizen scientists can impart people power, local knowledge, and enthusiasm to research endeavours while also reducing time requirements and costs to principal investigators. Through our volunteers and research partners, we identified new records of alternative host plants of *Drosophila suzukii* in Atlantic Canada and collected fruit samples from across Nova Scotia and Newfoundland and Labrador.

2.3.2 Introduction

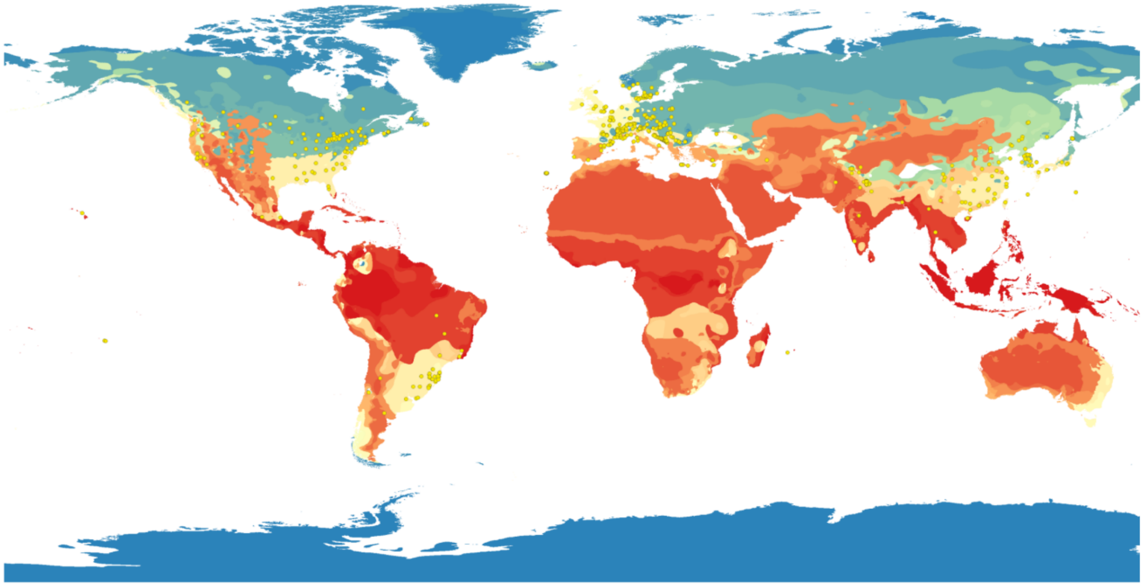
Since 2008, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) has expanded its geographic range across much of Europe, Asia, North America, and South America (Hauser 2011, Walsh et al. 2011, Cini et al. 2014, Andreazza et al. 2017, dos Santos et al. 2017, Fraimout et al. 2017, Lavagnino et al. 2018, Ørsted and Ørsted 2018). Human-mediated transport of fresh fruits, including both international trade and transport by private citizens, has been implicated in the global spread of *D. suzukii*, with the majority of 'first records' of this invasive species near ports or major trade routes (Hauser 2011; Calabria et al. 2012, Kiss et al. 2013, Rota-Stabelli et al. 2013, Cini et al. 2014, Deprá et al. 2014, Lavrinienko et al. 2016). The first identification of

D. suzukii in Canada occurred in 2009 in the Okanagan Basin of British Columbia (Thistlewood et al. 2012). In 2010, populations of *D. suzukii* had been identified in Alberta, Manitoba, Ontario, and Quebec in 2010 (Hauser 2011, Fisher 2012, Saguez et al. 2013, Asplen et al. 2015, Jakobs et al. 2015). Populations of *D. suzukii* were identified in Nova Scotia in 2011 and New Brunswick in 2012 (Agriculture and Agri-Food Canada [AAFC] Pest Management Centre 2013). *Drosophila suzukii* has since been identified in all provinces except Saskatchewan (CABI/EPPO 2016). Although *D. suzukii* has been detected every year since 2013 in Newfoundland, monitoring and mitigation programs through both federal and provincial agencies have as yet been unable to confirm if *D. suzukii* populations have been overwintering in the region or have been reintroduced each year (AAFC Pest Management Centre 2013).

Comparing distribution records for *D. suzukii* (Hauser 2011, Burrack et al. 2012, Calabria et al. 2012, Fisher 2012, Thistlewood et al. 2012, AAFC 2015, Asplen et al. 2015, Jakobs et al. 2015, Bombin & Reed 2016, CABI/EPPO 2016, Gibert et al. 2016, Shearer et al. 2016, Andreazza et al. 2017, Fraimout et al. 2017, Ögür et al. 2018, Orlova-Bienkowskaja et al. 2018, Ørsted & Ørsted 2018) against global climate records (Peel et al. 2007a, b), it is evident that *D. suzukii* can withstand a broad range of environments in terms of temperature and humidity (Fig. 2.3.1). *Drosophila suzukii* has been confirmed within 17 of 29 climate regions (58.6%) and has been recorded at the transition (edge) of 7 (21.4%) additional climate regions, suggesting that local populations may move between regions when weather is suitable. No record of *D. suzukii* has yet been found in the remaining 5 (17.2%) climate regions. This invasive pest is anticipated to continue to expand its range in coming years as climate change progresses and new habitats become suitable (Walsh et al. 2011, dos Santos et al. 2017, Langille et al. 2017). Most models estimating *D. suzukii* range expansion in the advent of climate change are incomplete, limited to the contiguous United States of America and central Canada, neotropical South America, and temperate Europe (Benito et al. 2016, Gutierrez et al. 2016, Andreazza et al. 2017, Langille et al. 2017). Models developed by dos Santos et al. (2017) are more inclusive and suggest that the entire Atlantic Canada region is at risk of greatest potential expanded *D. suzukii* distribution in North America.

Drosophila suzukii is highly polyphagous and can lay its eggs in a wide variety of fruit species (Lee et al. 2011, 2015, 2016, Poyet et al. 2015). Host use by female flies is opportunistic, limited primarily by fruit firmness (Burrack et al. 2013; Lee et al. 2016; Little et al. 2017). Most efforts for monitoring and mediation of *D. suzukii* in Canada have focused on protection of commercially grown tender fruits (cane berries – raspberries [*Rubus idaeus* Linnaeus (Rosaceae)] and blackberries [*Rubus* spp. (Rosaceae)], and blueberries [*Vaccinium* spp. Rydberg (Ericaceae)], grapes [*Vitis vinifera* Linnaeus (Vitaceae)], and cherries [*Prunus avium* Linnaeus (Rosaceae) and *Prunus cerasus* Linnaeus (Rosaceae)]) in response to commercial fruit growers' concerns (AAFC Pest Management Centre 2013). Additional commercially grown soft fruits such as strawberries (*Fragaria* × *ananassa* Duchesne (Rosaceae)) and currants (*Ribes rubrum* Linnaeus (Grossulariaceae) and *Ribes nigrum* Linnaeus (Grossulariaceae)), are also susceptible to damage (Lee et al. 2011, Lee & Sial 2016, Little et al. 2017). It is anticipated that climate change will result in the geographic ranges of invasive *D. suzukii* and temperate zone plant species to converge with boreal plant species (Gauthier et al. 2015). Additionally, *D. suzukii* has demonstrated a high degree of adaptability, not just in terms of host selection, but also in terms of phenotypic plasticity or genetic adaptation to diverse temperature and humidity conditions (Gibert et al. 2016, Gutierrez et al. 2016, Kenis et al. 2016, Langille et al. 2017, Clemente et al. 2018, Fraimont et al. 2018, Guédot et al. 2018). Due to its short generation time, *D. suzukii* is multivoltine throughout most of its invasive range which allows successive generations to adapt to diverse seasonal environmental conditions and could allow it to undergo rapid evolutionary change (Gibert et al. 2016, Gutierrez et al. 2016). ‘Winter morph’ *D. suzukii* have demonstrated increased cold tolerance due to developmental plasticity (Jakobs et al. 2015, Shearer et al. 2016, Toxopeus et al. 2016). Previous research on effects of exposure of *D. suzukii* and related *Drosophila* spp. Fallén (Diptera: Drosophilidae) to non-lethal temperature changes have shown that cold- and heat-hardening or long-term acclimation can occur (Langille et al. 2017).

Discerning the invasive spread of an alien species across a broad geographic area and across diverse taxa of potential hosts poses unique challenges. Accessing remote regions, curating samples, and identifying relevant species requires considerable time and people power. Non-scientist volunteers are increasingly stepping in to fill this need in cooperation with scientific research teams through citizen science programs (Acorn 2017). Citizen scientists, whether motivated by environmental activism, public engagement, education experience, or scientific curiosity can be a valuable resource to a research program (Newman et al. 2012). The rise of the citizen science movement pairs a centuries-long history of amateur naturalist contributions to science with emerging technologies. Amateur birdwatchers and butterfly enthusiasts are now able to contribute their expertise and passion using mobile apps and online networks (i.e. eBird, NestWatch, [http://www.birds.cornell.edu and http://ebird.org/canada/home], Budworm Tracker [http://budwormtracker.ca/#/], and eButterfly [http://www.e-butterfly.org/]). Volunteers become de facto stakeholders, contributing time, local knowledge, direction for future research, and community support for environmental protection (Newman et al. 2012). Perhaps the greatest benefits of citizen science are advancing scientific knowledge and promoting public education about local environmental issues (Bonney et al. 2009). Programs range in complexity and scope, some focusing on long-term changes in a single species, while others monitor overall biodiversity across a geographic region (Devictor et al. 2010; Dickinson et al. 2010).



				D. suzukii
🟥	tropical	rainforest		absent
🟥		monsoon		transitional
🟥		savannah		present
🟥	arid	hot	desert	transitional
🟧		cold	desert	present
🟧		hot	steppe	transitional
🟧		cold	steppe	present
🟧	temperate	dry summer	hot summer	present
🟧		dry summer	warm summer	present
🟧		dry winter	hot summer	present
🟨		dry winter	warm summer	present
🟨		dry winter	cold summer	present
🟨		no dry season	hot summer	present
🟨		no dry season	warm summer	present
🟨		no dry season	cold summer	present
🟩	boreal	dry summer	hot summer	transitional
🟩		dry summer	warm summer	transitional
🟩		dry summer	cold summer	transitional
🟩		dry summer	very cold winter	absent
🟩		dry winter	hot summer	present
🟩		dry winter	warm summer	present
🟩		dry winter	cold summer	present
🟩		dry winter	very cold winter	absent
🟩		no dry season	hot summer	transitional
🟦		no dry season	warm summer	present
🟦		no dry season	cold summer	present
🟦		no dry season	very cold winter	present
🟦	polar	tundra		absent
🟦		permafrost		absent

Figure 2.3.1. Map of reported *D. suzukii* collection records shown against Köppen climate classification scale (Peel et al. 2007a, b). Presence or absence of *D. suzukii* within each climate zone classification is shown within the legend. Zones labelled as transitional indicate that *D. suzukii* has been reported at the margins between that zone and an adjacent climate zone generally thought to be more suitable to *D. suzukii*.

Citizen science programs are not without their challenges (Dickinson et al. 2010). Non-scientist volunteers can be less rigorous about data collection and potentially more prone to errors (Dickinson et al. 2010). To combat this, many citizen science programs rely on a select group of volunteers with a pre-existing skill set (Bonney et al. 2009; Burrack et al. 2012). However, the consensus is that the benefits of citizen science outweigh the challenges (Bonney et al. 2009; Devictor et al. 2010; Dickinson et al. 2010; Newman et al. 2012; Acorn 2017). We evaluated citizen science as an effective tool to understand potential range expansion and host use across Nova Scotia and Newfoundland and Labrador. The *FlySpotter* project was beta-tested with the aim of surveying areas in Atlantic Canada for *D. suzukii* that are of limited accessibility or that would be physically or financially infeasible to include in standard monitoring efforts. With the assistance of partner organizations in Nova Scotia and Newfoundland and Labrador, we enlisted the cooperation of members of the public to collect fruit samples throughout Atlantic Canada.

We beta-tested a citizen science initiative in Nova Scotia and Newfoundland and Labrador to identify wild and ornamental fruits used as hosts by *D. suzukii*. We anticipated collecting fruit samples from geographic areas not otherwise easily accessible for study. In a novel approach to citizen science, participants are not looking for species of interest, but instead are collecting samples of potential host plants including non-crop fruits. Volunteers are a diverse cohort of entomologists, botanists, and members of the general public. Through this pilot project, we assessed the feasibility of using a citizen science model for determining host use and range expansion or previously unidentified populations of *D. suzukii* at the presumed northern limit of its geographic range in N. America.

2.3.3 Materials and methods

We distributed citizen science participant kits at Acadia University in Wolfville NS and Memorial University of Newfoundland and Labrador (MUN) in St. John's, NL, and through a number of partner sites, including Agriculture and Agri-Food Canada (AAFC) in Kentville, NS (display at Open House day) and St. John's, NL (display at Farm and Field day), MUN Botanical Gardens in St. John's, NL, K.C. Irving Environmental Science Centre and the Harriet Irving Botanical Gardens in Wolfville, NS, and the Acadian Entomological Society Annual General Meeting in Charlottetown, PEI. With the support of Acadia University Technology Services, we developed the *FlySpotter* website (http://flyspotter.acadiau.ca/home.html) to share information on *D. suzukii* and the citizen science initiative with members of the public.

Citizen scientists were provided with *FlySpotter* participant kits containing everything required to collect and submit four fruit samples. Instructions included in each kit provided examples of how to use each of the four 50-ml Falcon^{TM} tubes (Thermo Fisher Scientific, Mississauga, ON) with labels for use as sample collection vials, record sheets, and prepaid return envelopes. Participants were also encouraged to send pictures of fruit samples or collection sites to our email address flyspotter@acadiau.ca. Both the website and starter kits provided participants with suggestions of useful botanical field guides (Roland & Zinck 1998, Scott 2010, Boland 2011, Fernald & Kinsey 2012, Munro et al. 2014) and links to plant identification websites (vtree [http://dendro.cnre.vt.edu/dendrology/factsheets.cfm]). Links to mobile apps were also provided, including Leafsnap: An Electronic Field Guide (http://leafsnap.com/), MyTree (available at iTunes Store or Google Play), Pl@ntNet (http://m.plantnet-project.org/), and Useful Nova Scotia Plants (https://www.usefulnovascotiaplants.com/). Participants were asked to label vials with fruit species and variety (when possible), collection date, and collection location.

All fruit samples were returned to Acadia University for processing. From 20 June to 5 November 2017, distributed collection tubes were delivered or mailed to Acadia University from regions across the Atlantic provinces. Upon receipt, we curated all samples, confirming fruit identification and cataloguing each sample. We replaced the Falcon^{TM} tube lids with a bonded cellulose acetate plug (Genesee Scientific Corporation, El Cajon, CA). Tubes were stored at room temperature (approximately 20ºC and 50-60% RH) and examined twice weekly for emerging insects until fruit degraded and no further insects emerged. Each emerging insect was removed from the tube using an aspirator and placed into 1.5 mL microcentrifuge tubes containing 70% ethanol. Collected insects were identified under a dissection microscope (Markow & O’Grady 2005, Thistlewood & DeLury 2010, Marshall 2012, 2017, Van Timmeren et al. 2012, Martínez et al. 2017).

Beginning November 2017, vials containing fruit judged as still potentially viable but that were no longer producing new *Drosophila* spp. emergences were refrigerated at 4 ºC for one week, moved to a freezer for two weeks at -4ºC, and then refrigerated an additional week to simulate an overwintering period and stimulate potential new insect emergence. Following chilling, fruit was kept at room temperature for two weeks. If nothing emerged after two weeks, the samples were thoroughly examined and discarded. Fruits with excessive mould or that liquified were also discarded since such conditions inhibited the rearing of *Drosophila* spp.

A sub-sample of emergent insects (10 insects) were processed with Lifescanner© kits (http://lifescanner.net/) per package directions and sent to the Centre for Biodiversity Genomics (University of Guelph, Guelph ON) for DNA barcoding to obtain conclusive identification.

Genetic data collected using multiple animal specific primers from DNA Genotek Inc. (https://www.dnagenotek.com/ROW/index.html) were compared and contributed to Barcode of Life Data Systems (http://v4.boldsystems.org/) and the International Barcode of Life Project (http://ibol.org/). Insect pupae still present in fruit in late November 2018 were chilled for four weeks as above to simulate winter conditions to promote pupal development and subsequent adult emergence.

2.3.4 Results

We were pleased to have students, researchers, and members of the public from both Nova Scotia and Newfoundland participate in the *FlySpotter* project. Response from partner sites was enthusiastic and was key to a successful pilot project. We distributed 125 FlySpotter kits (4 sample collection vials per kit) directly to participants and through our partner sites from June to October 2017.

We received 344 fruit samples primarily from participants in Nova Scotia and Newfoundland (Fig. 2.3.2, Table 2.3.1). Fruits from 107 species representing 61 genera from 29 plant families were monitored daily for emerging insects (Table 2.3.1). *Drosophila suzukii* emerged from 20 fruit samples (5.8% of fruit samples), representing 11 species (10.3% of species sampled) from 6 plant families (Table 2.3.1). Previous observations of host-plant use were confirmed through these samples. Adult *Drosophila suzukii* emerged from fruits grown commercially in Nova Scotia, including Arctic kiwi fruit (*Actinidia arguta* Siebold and Zuccarini (Actinidiaceae)), wine grapes (*Vitis vinifera* Linnaeus (Vitaceae)), apples (*Malus* spp. Miller (Rosaceae), pears (*Pyrus communis* Linnaeus (Rosaceae)), highbush blueberries, (*Vaccinium corymbosum* Linnaeus (Ericaceae)), blackberries (*Rubus* spp. (Rosaceae)), and raspberries (*Rubus idaeus* (Rosaceae)).

Adult *Drosophila suzukii* also emerged from introduced species Tatarian honeysuckle (*Lonicera tatarica* Linnaeus (Caprifoliaceae)) and crab-apple (*Malus* spp. Miller (Rosaceae). Endemic plants were also suitable hosts for *Drosophila suzukii* in Nova Scotia. Adult flies emerged from wild blackberry (*Rubus allegheniensis* Porter (Rosaceae)), wild raisin (*Viburnum nudum cassinoides* Linnaeus (Adoxaceae)), and common elderberry (*Sambucus canadensis* Linnaeus (Adoxaceae)). An expanded geographical range of *D. suzukii* was observed for crop and non-crop plants as well as condition and stage of ripeness of fruit at time of infestation. For example, Arctic kiwi fruit (*Actinidia arguta*) can be a suitable host following even slight damage and need not be fully ripe as was found in previous studies (Lee et al. 2015). This study is the first record for natural infestations of *D. suzukii* in Nova Scotia for *A. arguta*, *Lonicera* spp., *Malus* spp., *Pyrus* spp., *S. canadensis*, *V. nudum cassinoides*, and *Vitis vinifera*. The sole previous record of *Lonicera tatarica* as a host was recorded in British Columbia (Thistlewood et al. 2018). Natural *D. suzukii* infestations for *Vitis vinifera*, *Lonicera tatarica*, *Sambucus* spp., and *Vibernum* spp. have been described in elsewhere in Canada, primarily in British Columbia, Ontario, and Quebec (Cormier et al. 2015, Pelton et al. 2017, Thistlewood et al. 2018). *Vaccinium* spp. and *Rubus* spp. have been previously described as hosts in Nova Scotia and Newfoundland as well as elsewhere in Canada (AAFC 2013, Little et al. 2017, Thistlewood et al. 2018). We obtained new reports of expanded range which might have been difficult or costly to obtain via other means. Fruit phenology patterns and fruit availability differ across geographic regions and result in differences in relative importance of plant species as alternative hosts (Haviland et al. 2016, Thistlewood et al. 2018).

The earliest *D. suzukii* emergence occurred 1 September 2017 and the latest emergence occurred 15 January 2018. All fruits from which *D. suzukii* emerged were collected between 21

August 2017 and 2 November 2017. Multiple species of *Drosophila* (Diptera: Drosophilidae), including *D. simulans* Sturtevant, *D. melanogaster* Meigen, *D. affinis* Sturtevant, *Chymomyza fuscimana* Zetterstedt, and *C. amoena* Loew, emerged from 18 fruit samples, representing 13 plant species from 5 families, beginning 5 September 2017 and ending 1 February 2018 (Table 2.3.1). Other insects emerged from 69 fruit samples, representing 41 plant species from 12 families between 4 July 2017 and 19 December 2017 (Table 2.3.1). Other species of flies (Diptera), hymenopterans (Hymenoptera), lepidopteran caterpillars (Lepidoptera), and weevils (Coleoptera) were also common emergent insects. Non-*Drosophila* insect species were identified to at least order for general information only. Many of the fruit samples gave rise to multiple insect species. In some cases, a single fruit or berry produced parasitoid wasps and one or more *Drosophila* species. Earliest insect emergence, across all groups, occurred 4 July 2017and some fruits were still producing insects until 1 February 2018. 39.2% (135/344) of fruit samples were exposed to simulated overwinter conditions. Seven fruit samples (5.2%) produced other *Drosophila* species after chill treatment. No other insects emerged post simulated winter treatment. The remainder of the fruit samples were discarded after fruit had degraded, insect emergence had ceased, and no further signs of invertebrate life were observed. Results of DNA barcoding of a subset of 10 emergent insects revealed that we collected a variety of plant- and insect-feeding insects, including two parasitoid wasps (Table 2.3.2).

Figure 2.3.2. Map of collection sites for fruit samples submitted by *FlySpotter* participants in Atlantic Canada.

Results of this citizen science initiative were promising but highlighted opportunities for improvement. Participants were able to collect fruit samples across a wide geographic area, but definitive identification of fruit samples was a challenge. Participants varied in their botanical knowledge and most participants did not submit photos of fruit plants which would have helped us confirm plant species identification. All fruit samples were identified to genus; however, we were not able to confirm species for 49 (14.2%) fruit samples.

Table 2.3.1. Fruits collected by citizen science participants and identified to genus and species. We have differentiated between commercially-grown crops (agricultural), plants which were grown in gardens (cultivated), and plants growing wild (not cultivated). Drosophila and other insect emergences recorded for each plant species.

			Collection site			Insects emerged		
Source / Use	Plant family	Plant Species	NB	NL	NS	*D. suzukii*	Other *Drosophila*	Other insects
Introduced / agricultural	Actinidiaceae	*Actinidia arguta* ((Siebold & Zuccarini) Planchon ex Miquel)			x	x	x	
	Elaeagnaceae	*Hippophae rhamnoides* (Linneaus)			x			
	Rosaceae	*F*ragaria hybrid (Linneaus)			x			x
	Rosaceae	*Malus domestica* (Borkhausen)	x		x	x	x	x
	Rosaceae	*Malus pumila* (Borkhausen)		x	x	x	x	x
	Rosaceae	*Prunus avium* (Linneaus)			x			
	Rosaceae	*Prunus domestica* (Linneaus)		x				
	Rosaceae	*Pyrus communis* (Linneaus)			x	x	x	x
	Rosaceae	*Rubus idaeus* (Linneaus)		x	x	x	x	
	Solanaceae	*Solanum lycopersicum* (Linneaus)			x			x
	Vitaceae	*Vitis vinifera* (Linneaus)			x	x	x	x
Introduced / cultivated	Apiaceae	*Coriandrum sativum* (Linneaus)			x			
	Aquifoliaceae	*Ilex x meserveae* (Meserve)		x	x			
	Asparagaceae	*Convallaria majalis* (Linneaus)			x			
	Berberidaceae	*Berberis thunbergii* (de Candolle)			x			
	Grossulariaceae	*Ribes nigrum* (Linneaus)		x				
	Grossulariaceae	*Ribes rubrum* (Linneaus)		x				
	Grossulariaceae	*Ribes uva-crispa* (Linneaus)			x			
	Oleaceae	*Ligustrum vulgare* (Linneaus)			x			
	Rosaceae	*Chaenomeles japonica* ((Thunberg) Lindley ex Spach)			x			
	Rosaceae	*Cotoneaster horizontalis* (Dacaisne)			x			

			Collection site			Insects emerged		
Source / Use	Plant family	Plant Species	NB	NL	NS	*D. suzukii*	Other *Drosophila*	Other insects
	Rosaceae	*Cydonia oblonga* (Miller)			x			
	Rosaceae	*Malus sargentii* (Rehder)		x				
	Rosaceae	*Malus sylvestris* ((Linnaeus) Miller)			x			x
	Rosaceae	*Rosa rubiginosa* (Linnaeus)		x	x			x
	Rosaceae	*Sorbus aucuparia* (Linnaeus)		x	x			
	Sapindaceae	*Aesculus hippocastanum* (Linnaeus)			x			
	Solanaceae	*Physalis pruinose* (Linnaeus)			x			
	Taxaceae	*Taxus baccata* (Linnaeus)			x			
	Thymelaeaceae	Daphne *mezereum* (Linnaeus)			x			
Introduced / not cultivated	Caprifoliaceae	*Lonicera tatarica* (Linnaeus)			x		x	x
	Rhamnaceae	*Frangula alnus* (Miller)			x			x
	Rosaceae	*Crataegus mollis* ((Torrey & Gray) Scheele)	x		x			x
Endemic / agricultural	Ericaceae	*Vaccinium angustifolium* (Aiton)		x	x			x
	Ericaceae	*Vaccinium corymbosum* (Linnaeus)		x	x	x		x
	Ericaceae	*Vaccinium macrocarpon* (Aiton)		x				
	Ericaceae	*Vaccinium myrtilloides* (Michaux)			x			
	Ericaceae	*Vaccinium vitis-idaea* (Linnaeus)		x	x			
	Rosaceae	*Rubus allegheniensis* (Porter)		x	x	x	x	x
Endemic / cultivated	Aquifoliaceae	*Ilex verticillate* ((Linnaeus) Gray)			x			
	Caprifoliaceae	*Symphoricarpus albus* ((Linnaeus) Blake)		x	x			
	Cornaceae	*Cornus alternifolia* (Linnaeus filius)			x			x
	Cornaceae	*Cornus sericea* (Linnaeus)		x	x		x	x
	Cornaceae	*Cornus stolonifera* (Linnaeus)		x	x			x
	Cupressaceae	*Juniperus communis* (Linnaeus)		x				

				Collection site			Insects emerged	
Source / Use	Plant family	Plant Species	NB	NL	NS	*D. suzukii*	Other *Drosophila*	Other insects
	Ericaceae	*Arctostaphylos uva-ursi* ((Linnaeus) Sprengel)			x			
	Grossulariaceae	*Ribes hirtellum* (Michaux)		x	x			x
	Iridaceae	*Ilex verticillate* ((Linnaeus) Gray)			x			
	Ranunculaceae	*Anemone canadensis* (Linnaeus)		x	x			x
	Rosaceae	*Amelanchier alnifolia* (Nuttall)			x			
	Rosaceae	*Aronia* (Medikus) *x Sorbus* (Linnaeus) *hybrid*		x				
	Rosaceae	*Prunus nigra* (Aiton)			x			
	Rosaceae	*Prunus pensylvanica* (Linnaeus filius)		x	x			x
	Rosaceae	*Prunus serotine* (Ehrhart)			x			x
	Rosaceae	*Sorbus americana* (Marshall)		x	x			x
	Rosaceae	*Sorbus decora* (Schneider)		x				
	Rubiaceae	*Mitchella repens* (Linnaeus)			x			
	Tiliaceae	*Tilia Americana* (Linnaeus)			x			
	Violaceae	*Viola labradorica* (Schrank)			x			
	Vitaceae	*Parthenocissus quinquefolia* ((Linnaeus) Planchon)			x			
Endemic / not cultivated	Adoxaceae	*Sambucus canadensis* (Linnaeus)			x	x	x	
	Adoxaceae	*Sambucus pubens* (Michaux)		x				
	Adoxaceae	*Viburnum cassinoides* (Linnaeus)			x	x		x
	Adoxaceae	*Viburnum trilobum* (Marshall)			x			
	Adoxaceae	*Viburnum lantanoides* (Michaux)			x			
	Aquifoliaceae	*Ilex mucronate* ((Linnaeus) Powell, Savolainen, & Andrews)		x	x			
	Asparagaceae	*Maianthemum canadensis* (Desfontaines)			x			

			Collection site			Insects emerged		
Source / Use	Plant family	Plant Species	NB	NL	NS	*D. suzukii*	Other *Drosophila*	Other insects
	Asparagaceae	*Maianthemum trifolium* ((Linnaeus) Sloboda)			x			
	Caprifoliaceae	*Lonicera canadensis* (Bartram)		x	x			
	Cornaceae	*Cornus canadensis* (Linnaeus)		x	x			x
	Cornaceae	*Cornus rugosa* (Lamarck)			x			
	Ericaceae	*Empetrum nigrum* (Linnaeus)		x				
	Ericaceae	*Gaultheria hispidula* ((Linnaeus) Muhlenberg ex Bigelow)		x				
	Ericaceae	*Gaultheria procumbens* (Linnaeus)			x			
	Ericaceae	*Gaylussacia baccata* ((Wangenheim) Koch)		x				
	Ericaceae	*Kalmia angustifolia* (Linnaeus)			x			
	Ericaceae	*Pyrola elliptica* (Nuttall)			x			x
	Ericaceae	*Vaccinium boreale* (Hall & Aalders)		x				
	Ericaceae	*Vaccinium boreale* (Hall & Aalders) *x V. myrtilloides* (Michaux)			x			
	Ericaceae	*Vaccinium oxycoccus* (Linnaeus)			x			
	Fagaceae	*Fagus grandifolia* (Ehrhart)			x			
	Geraniaceae	*Geranium robertianum* (Linnaeus)			x			
	Iridaceae	*Iris versicolor* (Linnaeus)			x			
	Liliaceae	*Clintonia borealis* ((Aiton) Rafinesque-Schmaltz)		x	x			x
	Myricaceae	*Comptonia peregrina* ((Linnaeus) Coulter)			x			
	Myricaceae	*Morella pensylvanica* (Mirbel)			x			
	Myricaceae	*Myrica pensylvanica* (Mirbel)			x			

			Collection site			Insects emerged		
Source / Use	Plant family	Plant Species	NB	NL	NS	*D. suzukii*	Other *Drosophila*	Other insects
	Ranunculaceae	*Actaea pachypoda* (Elliott)		x				
	Ranunculaceae	*Actaea rubra* ((Aiton) Willdenow)		x				
	Rosaceae	*Amelanchier bartramiana* ((Tausch) Roemer)		x				x
	Rosaceae	*Amelanchier canadensis* ((Linnaeus) Medikus)		x	x			x
	Rosaceae	*Amelanchier laevis* (Wiegand)		x				
	Rosaceae	*Aronia melanocarpa* ((Michaux) Elliott)		x				
	Rosaceae	*Aronia prunifolia* ((Marshall) Rehder)		x				
	Rosaceae	*Crataegus douglasii* ((Loudon) Eggleston ex Rehder)		x				
	Rosaceae	*Crataegus flabellate* ((Bosc ex Spach) Rydberg)			x			
	Rosaceae	*Crataegus brainerdii* (Sargent)			x			
	Rosaceae	*Fragaria vesca* (Linnaeus)			x			
	Rosaceae	*Fragaria virginiana* (Duchesne)		x	x		x	
	Rosaceae	*Geum rivale* (Linnaeus)			x			x
	Rosaceae	*Prunus virginiana* (Linnaeus)	x	x	x			x
	Rosaceae	*Rosa canina* (Linnaeus)			x			x
	Rosaceae	*Rosa Carolina* (Linnaeus)			x			
	Rosaceae	*Rosa palustris* (Marshall)			x			x
	Rosaceae	*Rosa virginiana* (Miller)		x	x			x
	Rosaceae	*Rubus eubatos* (Focke)	x					
	Rosaceae	*Rubus strigosus* (Michaux)		x				
	Solanaceae	*Solanum dulcamara* (Linnaeus)			x			

Table 2.3.2. Emergent insects identified through DNA barcoding.

Fruit species	Insect species	Insect family	Description
Malus domestica (Borkhausen)	*Chymomyza fuscimana* (Zetterstedt, 1838)	Drosophilidae	vinegar fly
Rosa palustris (Marshall)		Torymidae	gall-forming wasp
Rosa virginiana (Miller)		Torymidae	gall-forming wasp
Prunus virginiana (Linnaeus)	*Pseudanthonomus crataegi* (Walsh, 1867)	Curculionidae	hawthorn weevil
Malus sylvestris ((Linnaeus) Miller)	*Anthonomus rufus* (Gyllenhal, 1836)	Curculionidae	weevil
Amelanchier canadensis ((Linnaeus) Medikus)		Pteromalidae	parasitoid wasp
Vaccinium corymbosum (Linnaeus)	*Pseudanthonomus crataegi*	Curculionidae	hawthorn weevil
Malus pumila (Borkhausen)		Braconidae	parasitoid wasp
Rubus allegheniensis (Porter)	*Anthonomus signatus* (Say, 1831)	Curculionidae	weevil
Viburnum cassinoides (Linnaeus)	*Megastigmus aculeatus* (Swederus, 1795)	Torymidae	Rose-hip chalcid wasp

Low-cost participant kits were simple to prepare, costing less than $6.00 per kit including postage. Falcon^{TM} tubes used for sample collection were the highest cost item but could be washed and reused. Costs of participant kits and shipping were a fraction of the potential costs for researchers to visit remote collection sites personally. Business reply mail service was a cost-effective option for shipment of fruit samples. Participants were provided with pre-addressed, postage-paid envelopes to submit fruit samples. Fruit samples could be shipped a short distance without undue degradation. However, logistical delays were a significant issue. Samples received by mail from Newfoundland often arrived after a week or more in transit. These lengthy delays resulted in degraded fruit condition, in which dead larvae were sometimes observed but could not be definitively identified. Fruit samples faired best when returned in-person to the laboratory at Acadia University or to a partner site to be forwarded via bulk shipping. For future studies, small pinhole punctures in the lid of the Falcon^{TM} tube or a larger hole in the lid lined with 2-3 layers of cheesecloth would permit air exchange and improve fruit condition during transport. Improved air exchange could also be achieved during shipping by replacing Falcon^{TM} tube lids with acetate plugs (Genesee Scientific Corporation, El Cajon, CA). Fruit samples with little or no insect infestation degraded quickly in vials regardless of method of closure used. In a laboratory setting, the natural water content of individual fruits induced degradation issues including mould growth and desiccation. A cotton ball at the bottom of each vial alleviated this to some degree but was not sufficient to prevent natural decomposition processes.

2.3.5 Discussion

Citizen science initiatives can play an important role in disseminating information about invasive insects to the public and in collecting valuable data from a broad geographic area, including remote areas not normally accessible to researchers (Turrini et al. 2018). However, such projects can require considerable time investments by researchers since every sample submitted by participants must be validated and catalogued, emerging insects must be collected and identified, and results must be communicated with participants. Initial set-up of a citizen science network involves organizing participant kits, developing a website, recruiting partner organizations, and encouraging members of the public to participate.

We were fortunate to draw on the examples of previous citizen science initiatives. Citizen science is becoming the most common method of addressing large scale monitoring for biological systems, environmental conditions, and pollution (Savan et al. 2003, Conrad & Daoust 2008, Maisonneuve et al. 2009, Sullivan et al. 2009). However, some monitoring programs are not suitable to citizen science initiatives, including those with potential risk of exposure to toxic or harmful materials, those that require specialized skills, and those that require special care be taken to ensure data quality (Conrad & Hilchey 2011, Tregidgo et al. 2013). Programs can use volunteers for periodic annual or seasonal intervals or to monitor systems year-round. Volunteer contributions can be amassed over time and across geographical areas to map population movements of a target species or to monitor spread of pollution and debris from known events. The most well-known and possibly most successful citizen science entomology programs, such as eButterfly (http://www.e-butterfly.org/) and Monarch Watch (https://www.fs.fed.us/wildflowers/pollinators/Monarch_Butterfly/citizenscience/index.shtml), require participants to identify butterfly species and submit photos or identification records on-line. Biodiversity monitoring and Bio-blitz projects require participants to learn basic taxonomy and identification techniques. Other programs, including Budworm Tracker (https://budwormtracker.ca/#/) and our FlySpotter program, ask participants to submit samples for processing in-lab. As a general principle, simpler requirements for participants and a topical subject species can lead to greater public involvement.

Consumers are expressing greater interest in the buy-local movement and are becoming more aware of challenges facing agricultural growers. These interests sparked interest in local stakeholders and members of the public to join in the effort to monitor the invasive spread of *D. suzukii*. A common theme among many participants was a desire to know if fruits grown in their own gardens were at risk.

Recent studies have demonstrated that *D. suzukii* show extraordinary plasticity in response to temperature, humidity, and daylength (Jaramillo et al. 2015, Shearer et al. 2016; Wiman et al. 2016, Clemente et al. 2018; Fraimout et al. 2018; Guédot et al. 2018; Sánchez-Ramos et al. 2018). Since 2008, *D. suzukii* has spread to geographic regions that experience seasonal extremes of cold, hot, humid, or dry conditions. As *D. suzukii* in regions at the current limit of their range continue to adapt, populations could evolve increased tolerance for extreme temperature and humidity.

Based on current climate conditions, *D. suzukii* is anticipated to further its spread across N. America, S. America, and Europe, and to expand into regions of Africa and Oceania (dos Santos et al. 2017). *Drosophila suzukii* are most likely to occur in areas with mean annual temperatures between 5 and 20°C and annual rainfall between 500 and 2,500 mm (dos Santos et al. 2017). These ranges represent differences between upper and lower mean annual temperature of 15 °C and differences between upper and lower mean annual precipitation of 2000 mm. This suggests that environmental conditions are conducive to establishment of *D. suzukii* populations. Regional changes in temperature and precipitation trends due to climate change will result in further range expansion. Over time, localized populations of *D. suzukii* will further adapt to regional climate conditions, evolving greater tolerance to temperature and humidity at their previous tolerance limits (Gibert et al. 2016, Shearer et al. 2016, Wiman et al. 2016, Clemente et al. 2018, Fraimout et al. 2018, Guédot et al. 2018, Sánchez-Ramos et al. 2018). This invasive pest insect will continue to expand its range and infest novel fruits (Asplen et al. 2015, Poyet et al. 2015, Benito et al. 2016, Gutierrez et al. 2016, dos Santos et al. 2017, Langille et al. 2017, Ørsted & Ørsted 2018).

This initiative identified natural infestations by *D. suzukii* in introduced plant species, including commercially grown agricultural crops and ornamental species, and in endemic Atlantic Canadian plant species. In separate studies, we have observed an inverse relationship between populations of *D. suzukii* and endemic *Drosophila* species. Localised areas with larger populations of *D. suzukii* have smaller populations of other *Drosophila* species (Bombin & Reed 2016). Further research is needed to assess the effects of competitive pressures depressing endemic Drosophilid populations on biodiversity, and ecosystem health and sustainability.

Non-crop host fruits, both ornamental and endemic species, are widely considered a risk as refuges for *D. suzukii* populations and are known to play a role in promoting the spread of *D. suzukii* into fruit crops (Lee et al. 2011, 2015, Haviland et al. 2016, Kenis et al. 2016, Thistlewood et al. 2018). Fruit and flower phenology can differ across a plant species' distribution and phenology patterns differ among species (Hopp 1974, Legave et al. 2015). These asynchronous patterns could alter the role for host use of a given plant species by *D. suzukii* among climate zones (Langille et al. 2017). On-going climate change will further alter fruit phenology patterns, which could result in changed host use patterns for *D. suzukii* (Chmielewski et al. 2004, Chapman et al. 2005, Cleland et al. 2007, Legave et al. 2015, dos Santos et al. 2017, Langille et al. 2017, Ørsted & Ørsted 2018).

We are pleased with the overwhelming response of our partner sites and public participation. This initiative represents the first attempt to determine the northern limit of *D. suzukii* infestation in Newfoundland, and identify role of climate zones to range expansion in Canada (Fig. 2.3.1). We have demonstrated that fruit collected and transported from remote areas can be successfully used to monitor for an array of emergent insect species. However, time is of the essence for transportation of samples and prolonged shipping delays reduce the probability of success. Perhaps the greatest benefits of this and any citizen science project are the inherent educational value to participants as well as the public engagement fostered toward environmental issues.

2.3.6 Acknowledgements

First and foremost, we wish to thank our many citizen-science volunteers and partners. We are also thankful for the financial support of Atlantic Canada Opportunities Agency Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21718-956). We also thank the reviewers for their invaluable suggestions.

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Chapter 3

Role of colour and visual cues

3.1 Effect of colour and contrast of highbush blueberries to host-finding behaviour by *Drosophila suzukii* (Diptera: Drosophilidae)

A version of this chapter section has been published in Environmental Entomology:
Little, C.M., Chapman, T.W., and Hillier, N.K. 2018. Effect of color and contrast of highbush blueberries to host-finding behavior by *Drosophila suzukii* (Diptera: Drosophilidae). Environ Entomol **47**(5): 1242–1251. doi:10.1093/ee/nvy102.

Author Contribution Statement
CML and NKH designed research. TWC advised on methodology. CML conducted and analyzed research and wrote manuscript. All authors read, edited, and approved manuscript.

3.1.1. Abstract

Drosophila suzukii Matsumara (Diptera: Drosophilidae) has become a serious pest in soft-skin fruits and berries, infesting both ripe and ripening fruits. Crop damage in highbush blueberry have been particularly severe. During blueberry fruit development, fruits of various degrees of ripeness are present simultaneously. In addition, foliage colour changes as the season progresses. We investigated the influence of blueberry fruit and leaf colour on host-finding behavior in *D. suzukii*. Opposing shifts between reflectance spectra of ripening fruits and senescing leaves increased contrast between ripe fruit and senesced foliage. Developmental changes in contrast between fruit colour and leaf colour may act as a visual contextual cue in finding suitable host fruits. Opposing shifts in reflectance spectra of ripening fruits and senescing leaves increased the contrast between ripe fruit and senesced foliage. These opposing changes in colour may contribute to the attractiveness of blueberry fruit as a late season host for *D. suzukii*.

3.1.2. Introduction

Since 2008, *Drosophila suzukii* Matsumara (Diptera: Drosophilidae) (spotted wing drosophila) has spread across much of North and South America and Europe (Walsh et al. 2011). Unlike most vinegar flies, *D. suzukii* is a serious pest of soft fruits and berries. Female flies use a heavily serrated ovipositor to lay its eggs in ripening rather than over-ripe fruits, causing the fruit to spoil and become unmarketable (Bolda et al. 2010). A wide variety of soft fruits and berries of commercial and cultural significance are suitable hosts to *D. suzukii* infestation (Lee et al. 2011, Walsh et al. 2011, Burrack et al. 2013, Little et al. 2017). Although fruit phenology can have a significant role in fruit susceptibility, *D. suzukii* shows distinct preferences among fruits that ripen at the same time. For example, raspberries and blackberries are generally preferred to blueberries, but differences in attraction have been observed among blueberry varieties (Lee et al. 2011, Little et al. 2017).

Female *D. suzukii* can oviposit on various stages of ripening fruits (Lee et al. 2011). Susceptibility of many commercially grown soft fruits and berries begins with the earliest stages of fruit ripening, as fruits increase in sweetness and acidity, but decrease in firmness (Lee et al. 2011). Fruit colour changes during ripening and has been suggested as a reliable proxy for changes in firmness, sweetness, and acidity (Sinelli et al. 2008; Leiva-Valenzuela et al. 2013). Many frugivorous insects rely on visual cues as well as olfactory cues for detecting suitable fruits for feeding and oviposition (Owens and Prokopy 1986; Brévault and Quilici 2007; Fadzly and Burns 2010). Previous research suggests *D. suzukii* are most strongly attracted to the colour red (Basoalto et al. 2013; Renkema et al. 2014; Rice et al. 2016). As blueberries develop and ripen, fruit colour progresses from white to pink to red to blue, and that partially ripened (pink or red) blueberry fruits would, therefore, be most at risk from this invasive pest (See Suppl. Fig. S1).

Depending on the location and the type of crops grown, highbush blueberries (*Vaccinium corymbosum* L.) are among the soft fruits and berries least preferred by *D. suzukii* (Little et al. 2017). Despite preference for other fruits over blueberries, North American blueberry growers are experiencing increased economic losses due to increased fruit damage, reduced crop quality, increased pesticide use, and increased labor costs (Lee et al. 2011; Burrack 2014). Blueberries are among the most important agricultural crops in eastern Canada and northeastern United States. Infestation by *D. suzukii* can result in millions of dollars in lost revenue and food waste annually due (Lee et al. 2011).

In addition, blueberry leaves simultaneously change colour during the time fruits are ripening, progressing from green to red-green to bright red as chlorophyll degrades and anthocyanin synthesis increases (Routray and Orsat 2014). Leaf colour can be representative of plant health and be an indicator of environmental stress or nutritional deficiency (Routray and Orsat 2014). Conspicuousness due the contrast between fruit and foliage colours is more influential to food selection preference in fruit-eating bird species than fruit colour itself (Schmidt et al. 2004; Schaefer et al. 2006). Contrast cues are also important to host selection by *Rhagoletis* spp. (Diptera: Tephritidae) (Teixeira et al. 2010).

Highbush blueberries are an ideal subject for this study given that a wide variety of fruit and leaf colours can occur simultaneously within a single crop area and even within a single plant. Thus, we are able to assess and compare attraction to colour and contrast among various stages of fruit ripening and leaf senescence.

Previous studies pertaining to colour preference in *D. suzukii* have used a wide variety of methods to quantify colour as a means of identify specific colours; however, they do not use those measures as a factor in their analysis (Basoalto et al. 2013, Kirkpatrick et al. 2016, Rice et al. 2016). This is the first study to investigate relationships between quantitative measures of colour and *D. suzukii* host-finding behavior. Our objectives were to: a) quantify progressive colour changes in both fruit and foliage and the resulting changes in contrast; and b) evaluate if such shifts in visual cues may increase attractiveness of blueberry fruit to *D. suzukii*.

Insights into the role of opposing colour changes in ripening blueberry fruit and senescing leaves to attraction and host-finding behavior of *D. suzukii* will inform blueberry growers about the relative potential susceptibility of different varieties of blueberries. Growers can then select varieties which minimize visual attraction to *D. suzukii* based on fruit and foliage colour over the harvest season in their specific geographic region. Growers may also be able to use these insights to breed new varieties that minimize risk of attraction by *D. suzukii*. Understanding the role of visual colour and contrast cues in *D. suzukii* attraction to blueberries may also help to inform our understanding of host-finding behavior in this economically important fruit pest species.

3.1.3 Materials and Methods

Highbush blueberry plants continuously produce new flowers from July to September and produce fruits until mid-October in Atlantic Canada. Several dozen blueberry fruits and leaves of differing degrees of ripening were collected simultaneously from commercial growers in the Annapolis Valley, Nova Scotia in late afternoon on multiple days during August - October 2015 and 2017, refrigerated to maintain freshness, and used within 48 h (Supp. Fig. S3.1.1). Many available measures of colour were explored and discarded. For example, previous studies have used measures such as hue and saturation to describe colour (Lee et al. 2013, Renkema et al. 2014, Kirkpatrick et al. 2015). Both parameters are measures of colour as perceived by humans and are quantitative measures of subjective values. Hue is a measure of similarity to other colours (depicted as relative position in a three-dimensional colour space) or dominant spectral wavelength, and saturation is a relative measure of brightness. We have chosen to use a more objective measure of the colour associated with each object rather than our perception of that colour. We assessed fruit and foliage colour based on wavelength reflectance. In addition to measuring reflectance across a wide spectral range, spectral measures can be used to assess perceived values such as hue by using the values of reflectance at each wavelength relative to each other to determine dominant and secondary wavelengths which could be compared to predetermined colour standards. Percentage reflectance also intrinsically incorporates an objective measure for brightness similar to saturation measures. Comparing the percentage reflectance across the spectra relative to other spectra would reveal the colour associated with one object as brighter than the other (see Supp. Fig. S3.1.2a). using an Alta II reflectance spectrometer (Vernier Software & Technology, Beaverton OR USA). Percentage reflectance measures were obtained for seven visible colour wavelengths (470 – 700 nm) and four near-infrared wavelengths (735 – 940 nm; hereafter, infrared). Fresh fruits were categorized for ripening based on colour as pink (early stage ripening), red (mid-stage ripening), and blue (fully ripe).

Blueberry leaves also progress through a colour change in autumn, coinciding with the peak ripening period for blueberry fruits in Atlantic Canada. We categorized leaves as green, red-green (mid-change of colour), or red (Supp. Fig. S3.1.1). Leaves of blueberry plants which have experienced environmental stress or nutritional deficiencies may turn yellow. Leaves were measured for wavelength reflectance as above.

Fruit assays – odor not isolated

Fruits, leaves, and leaf~fruit pairs were assessed for preference by *D. suzukii* in 2-choice bioassays. A contrast score based on the Weber contrast (Shapley and Enroth-Cugell 1984) was calculated for percentage reflectance at each wavelength for each fruit ~ leaf pair.

$$Contrast = (I_F - I_L) / I_L$$

I_F is reflectance (%) of blueberry fruit.

I_L is reflectance (%) of blueberry leaf

Drosophila suzukii were obtained from colonies maintained at Acadia University since 2014, cultivated from stock originally reared by the Kentville Research and Development Centre (Agriculture and Agri-Food Canada, Kentville, NS, Canada). Colonies were housed in 250-ml *Drosophila* flasks (Genesee Scientific, San Diego, CA, USA) containing 50 ml Formula 4-24 Instant Drosophila medium (Merlan Scientific Ltd., Mississauga, ON, Canada) mixed with 50 ml dH_2O. Approximately 2 h prior to the start of each assay, reproductively-mature females aged up to 2 weeks were removed from colonies created 1 month earlier.

Two-choice assays were conducted within a sealed 600 ml airtight arena (18 x 12 x 6.5 cm) using a modified version of previously described trap assays (Larsson et al. 2004; Dekker et al. 2006; Little et al. 2017). Moistened filter paper was placed in the centre of each experimental arena to provide the flies with access to water and prevent desiccation. Each arena contained two transparent plastic traps, each consisting of a 30 ml portion cup with a truncated 200 μl micropipette tip inserted through the lid. Effects of the plastic cup on colour reflectance were tested by comparing percentage reflectance measurements of coloured paper (white, blue, green, yellow, and red) through a piece of cup plastic against unobstructed coloured paper.

Approximately 10 ml of fruit (2 berries) were placed in each trap for blueberry-ripening trials. Approximately 5 ml of fruit (1 berry) and one leaf were placed in each trap for berry-leaf colour trials. Fruits and leaves were clearly visible through the sides and top of each cup. Fifteen female *D. suzukii* were placed in each arena. Host choices were checked after 24, 48, and 72 h. Each blueberry fruit ripening category and was tested against each other and against a blank (control) trap. Fruits of each colour category were tested against each other four times and against blank controls twice. Each blueberry-leaf colour pairing was tested against each other. We completed five replicates each of ripe blueberries with red leaves against fruits with red-green leaves, five replicates of fruits with red leaves against fruits with green leaves, and nine replicates of fruits with green leaves against fruits with red-green leaves. Response Index scores ranging from -1 to +1 were calculated as a measure of host preference based on Dweck et al. (2013). Positive response index values represent preferred fruits or fruit-leaf pairs.

$$\mathrm{RI} = (A-B)/T$$

A and B are the number of flies in each trap
T is the total number of flies per trial

No odor assays

In all assays described below, moistened filter paper was placed in the centre of each experimental arena to provide the flies with access to water and prevent desiccation.

1. Card stock. Two-dimensional colour contrast targets were created using coloured card stock. Targets of green or red card stock disks 5 cm in diameter overlaid with blue card-stock disks of 2.5 cm diameter were placed at opposite ends of a sealed 600 ml airtight arena (18 x 12 x 6.5 cm) as above. Targets were sealed with clear packing tape and coated with TangleTrap Sticky Coating^{TM} (The TangleFoot Company, Grand Rapids, MI). Card stock colours were measured as above for fruit and leaf colours. Male and female *D. suzukii* were tested separately. Two trials each of 15 individuals and three trials each of 25 individuals were conducted. All results for these trials are expressed as percentage response.

2. Photographs. We created 2-dimensional fruits and leaves from printed photographs of blueberry fruits and leaves. Photographs were digitally manipulated to approximate mean reflectance measures of actual fruit and leaves used in fruit trials above (see Supp. Fig. S3.1.2). Proxy fruits were created to represent the pink and blue ripeness stages. Proxy leaves were created to represent the green, red-green, and red stages of senescence. Additionally, white leaf shapes were used to assess attraction based on fruit colour alone. Fruit and leaf shapes were cut using a template to ensure consistent size and shape of all proxy fruits and all proxy leaves (berry diameter = 1.2 cm, leaf width = 4 cm, and leaf length = 8 cm). A single paper berry was positioned centrally on a paper leaf and placed face-down on a piece of transparent packing tape, which was then used to secure the fruit/leaf pair to the sides of an assay arena as above. TangleTrap Sticky Coating^{TM} (The TangleFoot Company, Grand Rapids, MI) was applied to the surface of each paper leaf~fruit pair. Fifteen mature female *D. suzukii* were released into each arena. Host choices were checked after 48 h. A choice was determined to have been made when a fly was adhered to a fruit or leaf. Trials were shorter in duration since flies did not have to navigate the trap entrance as in trials using actual fruits and leaves.

3. Sealed fruit and leaves. Fresh blueberry fruits, leaves, and leaf~fruit pairs were enclosed within 60 x 15 mm plastic petri dishes and sealed with Parafilm® (Bemis Company, Inc., Oshkosh WI USA). In 2017, leaves on bushes experiencing some environmental stress (prolonged hot, dry weather) turned from green to yellow. Therefore, 2-choice assays were conducted using red and blue fruits and green, red-green, red, and yellow leaves. White labelling tape (Fisher Scientific Company, Ottawa ON Canada) was used to obscure the contents from view from the sides, leaving contents visible from the top only. A piece of transparent sticky-trap plastic (Alpha Scents, Inc., West Linn OR USA) was secured across the top of each petri dish. Two-choice assays were completed in arenas as per above and checked after 24 h. A choice was determined to have been made when a fly was adhered to the sticky-trap plastic. We completed eight replicates of each two-choice assay with 15 mature female *D. suzukii* per trial.

In all choice assays, a 4 x 4 cm piece of moistened paper towel was placed centrally in each arena to control for humidity. Percentage reflectance measures were obtained for all no odor assays using the same methods as above.

Statistical analysis

We used ANOVA and Tukey Post Hoc tests ($P < 0.05$) to analyze differences in spectra among stages of fruit ripening, leaf senescence, and contrast. Effects of plastic cups used in choice assays on colour reflectance were analyzed using Wilcoxon rank sum and Spearman's rank correlation tests. Because response index data for choice assays in which odors were not isolated, assays using card stock disks, and assays using paper photograph were not normally distributed, we used nonparametric measures to analyze response index data. We accounted for effect of treatment (fruit ripening stage or contrast between fruit and leaf senescence stage), time and trial on response indices for choice assays in which odors were not isolated using repeated measures Friedman analyses. Analysis of response index by fruit ripening stages and by leaf senescence stages of fruit-leaf pairs were conducted using Kruskal-Wallis χ^2 tests and Tukey and Kramer (Nemenyi) Post Hoc tests ($P < 0.05$). We investigated correlation between response indices and colour measurements at each wavelength (reflectance or contrast) using Spearman's rank correlation. Response index data for choice assays with sealed fruit and leaves were normally distributed. We therefore analyzed those data using Pearson's correlation. Paired t-tests were used to compare responses in two-choice trials. All statistical analyses were conducted and graphs generated using RStudio (Version 1.1.383 – © 2009-2017, RStudio, Inc., http://www.rstudio.org/, using R version 3.4.3 [2017-11-30, The R Foundation, https://www.r-project.org/]).

3.1.4 Results

Fruit assays – odor not isolated

Mean percentage reflectance values were 6.36% lower for coloured paper measured through the cup plastic versus unobstructed coloured paper. However, this difference was not significant (Wilcoxon rank sum W = 1798.5, p = 0.09) and differences were strongly correlated (Spearman's rank correlation $Rho_1 = 0.85$, $p < 0.0001$). Therefore, it was not meaningful to adjust fruit reflectance values for the minimal effect of light refraction through the plastic cup.

Highbush blueberry fruit colours progress from white (not tested) to pink to red to blue as they ripen. Reflectance spectra for fully ripened fruits were significantly different than for ripening fruits (ANOVA (Reflectance ~ [Wavelength:Stage]), $F_{32,297} = 69.59$, $P < 0.0001$). Percentage reflectance values at each wavelength were higher for early stages of ripening than for fully ripened blueberries (Supp. Fig. S3.1.2a). As blueberry fruits ripened, their colour deepened and reflected less light. Reductions in reflectance were most pronounced in the orange to infrared portion of the light range (≥ 600 nm). Significant differences in reflectance between fully ripened blue fruits and both unripe (pink fruits) and partially ripened (red fruits) were observed across the visible and infrared spectra (Table 3.1.1a).

Highbush blueberry leaves also changed colour over the course of the fall harvest season, progressing from green to red-green to red as they senesced. As leaves senesced, visible leaf colour differences among the three leaf senescence stages were evident as leaves brightened and reflected more light. Significant differences in reflectance values were observed across most of the spectrum measured (ANOVA (Reflectance ~ [Wavelength:Stage]), $F_{32,385} = 208.4$, $P < 0.0001$). Increases in reflectance were most prominent in the cyan to infrared range of the spectrum (525 mn – 735 nm) (Supp. Fig. S3.1.2b). Significant differences between green and senesced leaves were observed across the visible and infrared spectrum (Table 3.1.2a). Percentage reflectance values were highest in the infrared range among leaves of all stages of senescence. Green leaves had significantly higher reflectance values at shorter (cyan and green) wavelengths; whereas, red leaves had significantly higher reflectance values in the mid-range (yellow to red) wavelengths (Table 3.1.3).

Reflectance measures were consistent among fully ripe fruit used in leaf~fruit pairings where odor was not isolated with no significant differences in fruit colour observed among the three fruit~leaf pairings. When paired with fully ripe blueberry fruits, contrast between leaf colour and fruit colour were significantly different among stages of leaf senescence (ANOVA (Contrast ~ [Wavelength : Stage]), $F_{32,385} = 13.04$, $p < 0.0001$) (Supp. Fig. S3.1.3). Contrast scores at longer wavelengths were comparable among leaf~fruit pairings. Contrast between fully ripe (blue) blueberry fruit and leaves among all leaf~fruit pairs, regardless of leaf stage, contrast between ripe fruits and leaves was highest at blue - cyan wavelengths (470 - 525 nm) (Supp. Fig. S3.1.3). Differences among leaf~fruit pair stages were observed throughout most of the visible light spectrum; however, differences in contrast among categories of leaf ~ leaf pairs were most pronounced at the shorter wavelengths (535 – 645 nm) (Table 3.1.2b).

Table 3.1.1. a) We observed significant differences in fruit reflectance measures among blueberry fruit ripening stages at measured wavelengths in fruit used for choice tests a) with odors and b) without odors (ANOVA). Significant values in bold. Different letters represent differences among fruit ripening stages (Tukey Post Hoc, P < 0.05).

	a)	Choice assays with odor				b)	Choice assays without odor		
Wavelength	Fruit reflectance		Differences among stages			Fruit reflectance		Differences among stages	
	$F_{2,27}$	P	Pink fruit	Red fruit	Blue fruit	$F_{1,226}$	P	Red fruit	Blue fruit
Blue (470 nm)	**3.47**	**0.05**	b	ab	a	1.78	0.18	a	a
Cyan (525 nm)	**4.77**	**0.02**	b	ab	a	0.92	0.34	a	a
Green (560 nm)	**8.27**	**<0.005**	b	a	a	2.55	0.11	a	a
Yellow (585 nm)	**19.37**	**<0.0001**	b	a	a	**11.00**	**0.001**	b	a
Orange (600 nm)	**17.92**	**<0.0001**	b	a	a	**17.26**	**<0.0001**	b	a
Red (645 nm)	**25.31**	**<0.0001**	b	b	a	**33.60**	**<0.0001**	b	a
Deep Red (700 nm)	**38.08**	**<0.0001**	b	b	a	**99.80**	**<0.0001**	b	a
Infrared 1 (735 nm)	**78.30**	**<0.0001**	b	b	a	**178.70**	**<0.0001**	b	a
Infrared 2 (810 nm)	**15.39**	**<0.0001**	b	b	a	**20.02**	**<0.0001**	b	a
Infrared 3 (880 nm)	**4.51**	**0.02**	b	ab	a	1.33	0.79	a	a
Infrared 4 (940 nm)	**5.15**	**0.01**	b	ab	a	0.01	0.91	a	a

Table 3.1.2. We observed significant differences in a) blueberry leaf reflectance measures and b) contrast among blueberry fruit~leaf pair categories used in choice assays where odor was not isolated based on leaf senescence stages at each wavelength measured (ANOVA). We also observed significant differences in c) blueberry leaf reflectance and d) contrast among blueberry fruit~leaf pair categories used in no-odor choice assays conducted with sealed fruit. Significant values in bold. Different letters represent differences among blueberry fruit ripening stages (Tukey Post Hoc, $P < 0.05$).

a) Leaf reflectance			Differences among leaf stages			c) Leaf reflectance		Differences among leaf stages			
With odor						Without odor					
Wavelength (nm)	$F_{2,35}$	P	Green	Red-green	Red	$F_{3,224}$	P	Green	Red-green	Red	Yellow
Blue (470)	2.04	0.15	a	a	a	0.71	0.55	a	a	a	a
Cyan (525)	**38.49**	**<0.0001**	b	a	a	**23.28**	**<0.0001**	b	ab	a	c
Green (560)	**15.84**	**<0.0001**	b	a	a	**31.98**	**<0.0001**	b	ab	a	c
Yellow (585)	**10.89**	**<0.0005**	a	a	b	**41.12**	**<0.0001**	a	a	a	b
Orange (600)	**24.89**	**<0.0001**	a	a	b	**64.30**	**<0.0001**	a	b	b	c
Red (645)	**35.25**	**<0.0001**	a	a	b	**62.11**	**<0.0001**	a	a	b	c
Deep Red (700)	**20.30**	**<0.0001**	a	a	b	**69.94**	**<0.0001**	a	b	c	d
Infrared 1 (735)	**8.28**	**<0.005**	a	ab	b	**66.65**	**<0.0001**	a	b	c	c
Infrared 2 (810)	**5.82**	**<0.01**	b	b	a	**7.79**	**<0.0001**	b	a	b	a
Infrared 3 (880)	**5.92**	**<0.01**	b	b	a	0.81	0.49	a	a	a	a
Infrared 4 (940)	**4.88**	**<0.05**	b	b	a	**5.00**	**<0.01**	b	ab	b	a

b) Contrast			Differences among leaf stages			d) Contrast		Differences among leaf stages			
With odor						Without odor					
	$F_{2,35}$	P	Green	Red-green	Red	$F_{3,224}$	P	Green	Red-green	Red	Yellow
Blue (470)	0.90	0.41	a	a	a	3.02	0.03	a	ab	b	ab
Cyan (525)	**9.64**	**<0.0005**	a	b	b	2.44	0.07	a	a	a	a
Green (560)	**10.55**	**<0.0005**	a	b	a	**27.71**	**<0.0001**	b	b	c	a
Yellow (585)	**5.33**	**<0.01**	b	b	a	**21.90**	**<0.0001**	c	b	b	a
Orange (600)	**5.58**	**<0.01**	b	ab	a	**39.95**	**<0.0001**	c	b	b	a
Red (645)	**7.93**	**<0.005**	b	ab	a	**47.76**	**<0.0001**	c	b	b	a
Deep Red (700)	**4.46**	**0.02**	b	ab	a	**53.02**	**<0.0001**	c	b	b	a
Infrared 1 (735)	**3.30**	**0.05**	b	ab	a	**30.39**	**<0.0001**	c	b	b	a
Infrared 2 (810)	0.71	0.50	a	a	a	1.61	0.19	a	a	a	a
Infrared 3 (880)	0.02	0.98	a	a	a	0.93	0.43	a	a	a	a
Infrared 4 (940)	0.12	0.89	a	a	a	**9.91**	**<0.0001**	ab	bc	a	c

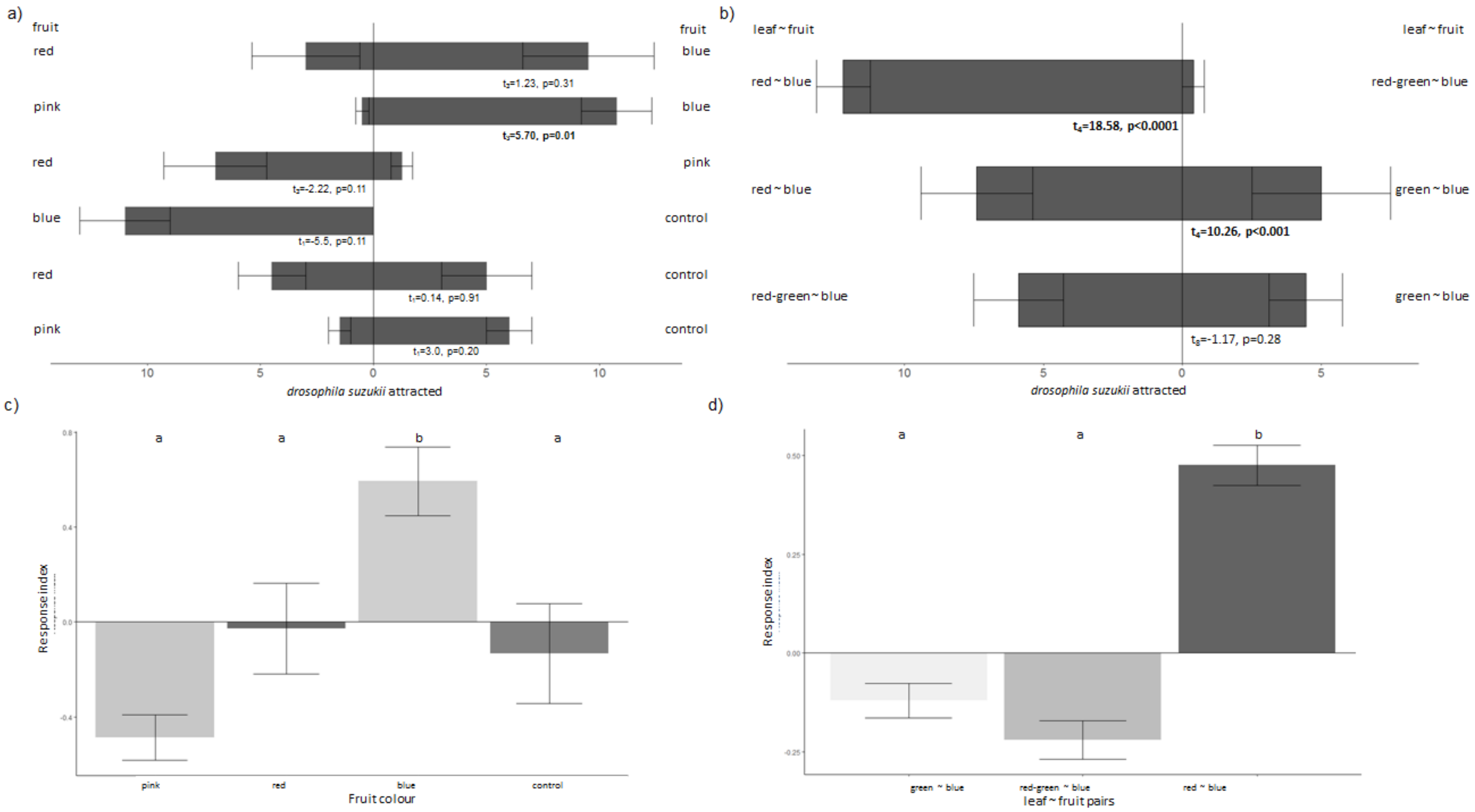

Fig. 3.1.1 a) Riper, more deeply coloured blueberry fruits were more preferred in host preference 2-choice assays where odor was not isolated. Results of Paired t-tests are shown within the figure (significant differences are in bold). b) Therefore, mean *Drosophila suzukii* response index scores were highest for fully-ripe blue fruits (Kruskal-Wallis χ^2 = 21.919, df = 3, p < 0.0001). c) After 72 h, ripe blueberry fruits paired with red leaves had attracted more female *D. suzukii* than ripe blueberries paired with red-green or green leaves during 2-choice assays with odor. Results of Paired t-tests are shown within the figure (significant differences are in bold). d) Resulting mean response index values were highest for Red~blue leaf~fruit pairs (Kruskal-Wallis χ^2 = 85.077, df = 2, p-value < 0.0001). Different letters above the figures (b & d) denote significant differences among fruits (Tukey and Kramer (Nemenyi) Post Hoc, p < 0.05).

In fruit-only 2-choice assays, female *D. suzukii* preferred the riper of the two fruits available (Fig. 3.1.1a). Differences in attraction among the fruit stages were observed at 24 h, but intensified after 48h and 72h. Repeated measures analysis confirms that differences in host selection between blueberry ripening stages are significant, accounting for within-trial variability in choice decisions between days (Friedman $\chi^2 = 42.554$, df = 2, p < 0001); therefore only 72 h responses were used for analyses. Female *D suzukii* were more strongly attracted to fully ripe blueberries (blue stage) than to blueberries at earlier stages of ripening in two-choice tests (Fig. 3.1.1c). Attraction to partially ripened blueberries (pink and red stages) was not different than from control traps. Response indices were negatively correlated with reflectance intensity of ripening fruit in the green to infrared range (560 nm – 940 nm) (Table 3.1.3a).

In leaf~fruit pair 2-choice assays where fruits were equally ripe, female *D. suzukii* preferred the more senesced of the two leaves available (Fig. 3.1.1b). Repeated measures analysis confirms that differences in host selection between leaf~fruit pairings are significant, accounting for within-trial variability in choice decisions between days (Friedman $\chi^2 = 36.383$, df = 2, p < 0.0001); therefore only 72 h responses were used for analyses. Female *D. suzukii* were most strongly attracted to ripe blueberries paired with fully senesced foliage (red leaves) in 2-choice tests (Fig. 3.1.1d). Percentage reflectance of leaves in leaf~fruit pairs contributed to attraction by *D. suzukii* in 2-choice trials; however, leaf colour was not predictive of attraction (Kruskal-Wallis $\chi^2 = 354.34$, df = 365, p = 0.65). Response indices were negatively correlated with reflectance intensity of senescing leaves in the yellow to orange range (585 nm – 600 nm) (Table 3.1.3a).

Contrast between blueberry fruit and leaf reflectance contributed to attraction by *D. suzukii*, but was not predictive of attraction (Kruskal-Wallis $\chi^2 = 411.53$, df = 415, p = 0.54). Response indices were negatively correlated with contrast in leaf~fruit pairs in the infrared range (735 nm & 880 nm) (Table 3.1.3a).

No odor assays

1. Paper disks. Differences in spectra between blue and green card stock had comparable spectra at wavelengths of 645 nm and above, with most noticeable differences occurring at blue (470 nm) and cyan (525 nm) (Supp. Fig. S3.1.2c). Reflectance measures for red card stock were distinctly lower at shorter wavelengths and distinctly higher at all higher wavelengths (600 nm and above) (Supp. Fig. S3.1.2c). Contrast scores for both green~blue and red~blue disks were highest at shorter wavelengths (470 nm – 525 nm), with greatest contrast occurring in red~blue disks (Supp. Fig. S3.1.2c & S3.1.3b).

There was no difference in attraction of *D. suzukii* between green~blue and red~blue card stock disks in 2-choice assays (females: $t_4 = 1.6$, $p = 0.19$; males: $t_4 = 0.7$, $p = 0.50$). Differences in responses between sexes were not statistically significant (Paired t-test, $t_9 = 0.75$, $p = 0.47$). Preferences were correlated with differences in contrast intensity across the full spectrum (Spearman's rank correlation; $Rho_{218} = 0.15$, $p = 0.03$). However, given lack of variability in reflectance within each colour card stock and only two-colour combinations, correlation analysis at each wavelength would not be meaningful.

2. Photographs. Paper photographs of blue fruits had lower reflectance across the full spectrum than those of pink fruits (Supp. Fig. S3.1.2d). Photographs of all stages of senescing leaves had higher reflectance across most of the spectrum than did fruit photographs, with later stages of leaves having progressively higher reflectance values in the yellow to lower infrared range (600 nm – 735 nm) (Supp. Fig. S3.1.2d). Contrast within photographed leaf~fruit pairs was most prominent in green~pink and red~blue pairs (Supp. Fig. S3.1.2d & S3.1.3c).

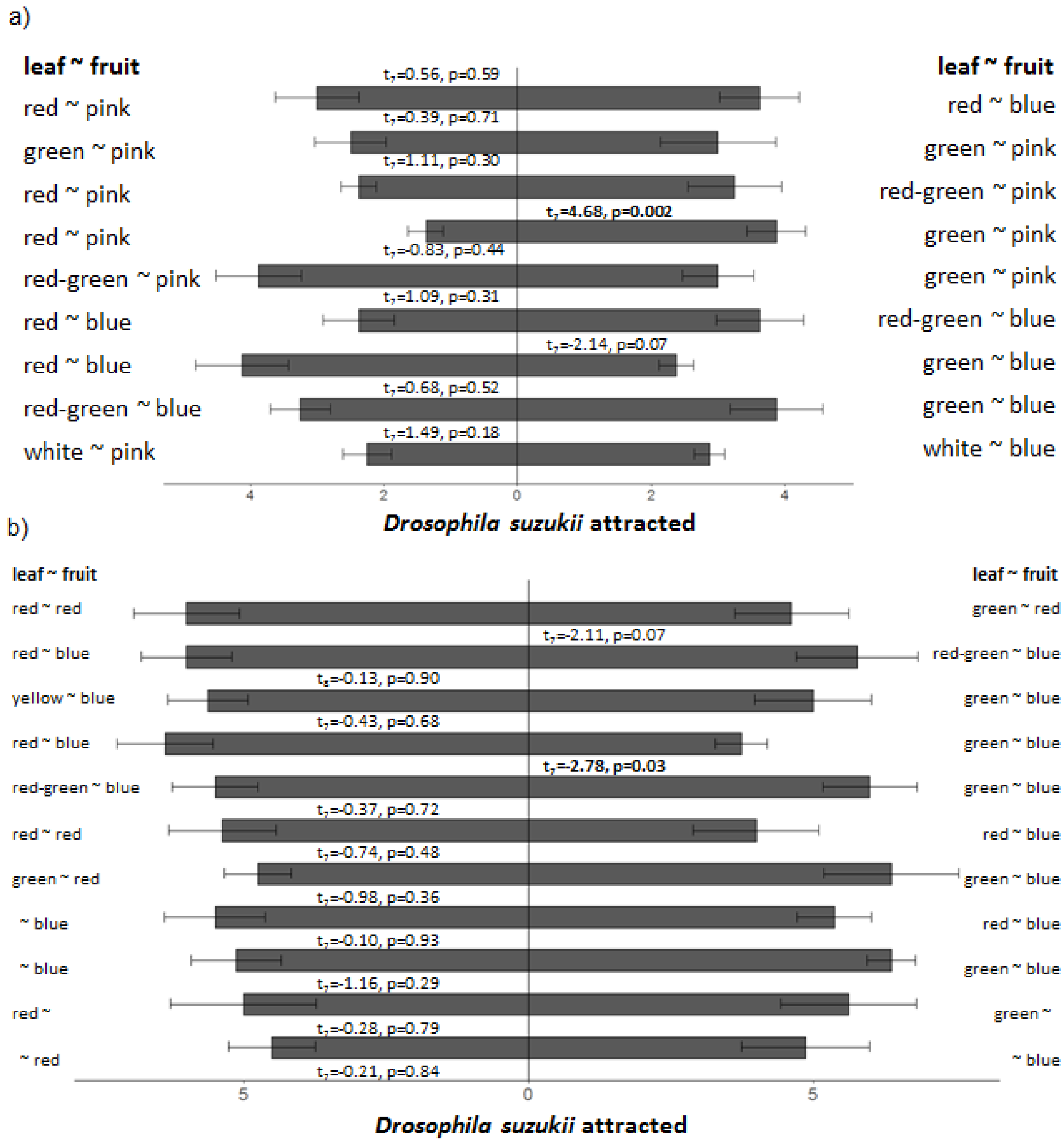

Fig. 3.1.2. a) Mean counts of female *D. suzukii* attracted (± SE) to each paper photograph leaf~fruit pair after 48h. b) Mean counts of female *D. suzukii* attracted (± SE) to each no-odor leaf~fruit pair after 48h. 15 mature female flies per trial. Results of paired t-tests are shown within the figures (significant differences are in bold).

Table 3.1.3. Correlation between *Drosophila suzukii* response index values to fruit and leaf reflectance and to contrast scores during 2-choice trials in fruit choice assays where odors were not isolated (Spearman's rank correlation). b) No significant correlations were observed between response index values and fruit reflectance during 2-choice assays with no odors (Pearson's correlation). However, correlations with leaf reflectance and contrast scores were significant at yellow and orange wavelengths (585 and 600 nm).

	a) choice assays with odor						**b) choice assays with no odor**					
Wavelength	Fruit reflectance		Leaf reflectance		Contrast		Fruit reflectance		Leaf reflectance		Contrast	
	Rho$_{28}$	P	Rho$_{28}$	P	Rho$_{28}$	P	R_{226}	P	R_{226}	P	R_{226}	P
Blue (470 nm)	-0.20	0.30	0.07	0.69	0.01	0.94	0.00	0.96	-0.01	0.93	0.04	0.56
Cyan (525 nm)	-0.19	0.31	-0.17	0.31	0.24	0.15	-0.01	0.91	0.03	0.64	0.11	0.11
Green (560 nm)	**-0.45**	**0.01**	0.04	0.81	0.05	0.76	0.01	0.82	0.12	0.08	-0.10	0.12
Yellow (585 nm)	**-0.58**	**<0.001**	**0.39**	**0.02**	-0.20	0.23	0.04	0.53	**0.17**	**0.01**	**-0.19**	**0.005**
Orange (600 nm)	**-0.58**	**<0.001**	**0.38**	**0.02**	-0.25	0.13	0.05	0.50	**0.16**	**0.02**	**-0.15**	**0.02**
Red (645 nm)	**-0.51**	**<0.005**	0.30	0.07	-0.26	0.12	0.04	0.54	0.10	0.13	-0.07	0.30
Deep Red (700 nm)	**-0.55**	**<0.005**	0.22	0.18	-0.06	0.73	0.06	0.38	0.09	0.16	-0.03	0.64
Infrared 1 (735 nm)	**-0.64**	**<0.0005**	-0.02	0.81	**-0.16**	**0.05**	0.06	0.34	0.09	0.19	-0.02	0.80
Infrared 2 (810 nm)	**-0.66**	**<0.0001**	-0.07	0.69	-0.22	0.18	0.02	0.80	-0.06	0.36	0.05	0.43
Infrared 3 (880 nm)	**-0.65**	**<0.0001**	-0.08	0.62	**-0.38**	**0.02**	0.05	0.44	0.06	0.36	0.07	0.30
Infrared 4 (940 nm)	**-0.57**	**<0.005**	-0.09	0.61	-0.30	0.07	0.02	0.81	0.02	0.73	0.01	0.91

Differences in attraction were not significant between paper targets with blue paper fruits and those with pink paper fruits (Kruskal-Wallis $\chi^2 = 2.12$, df = 1, p = 0.15). Differences in attraction among paper leaf colours were also not significant overall (Kruskal-Wallis $\chi^2 = 3.55$, df = 2, p = 0.17). However, printed photographic leaf~fruit pairs with greater visual contrast were more preferred by *D. suzukii* than pairs with fruit and leaf colours that were more similar (e.g. pink fruits with green leaves more attractive than pink fruits with red leaves) (Fig. 3.1.2a).

3. Sealed fruit and leaves. Reflectance values for fruits collected in 2017 and used in no-odor 2-choice assays (Supp. Fig. S3.1.2e) were comparable in colour to those collected in 2015 and used in 2-choice assays where odor was not isolated (Supp. Fig. S3.1.2a&b). However, differences in reflectance between fruit stages were significant over the yellow to infrared range (585 nm – 810 nm) (Table 3.1.1b). Reflectance values for leaves collected in 2017 (Supp. Fig. S3.1.2c) were considerably lower than those collected in 2015 (Supp. Fig. S3.1.2b); however, differences in reflectance among leaf stages were significant across most of the spectrum (525 nm – 810 nm) (Table 3.1.2c). Contrast scores for leaf~fruit pairs were most prominent in the shorter wavelengths (470 nm – 645 nm) (Supp. Fig. S3.1.2e & S3.1.3d) and differences between leaf~fruit pair categories were significant through the mid-range of the spectrum (560 nm – 735 nm) (Table 3.1.2d).

In choice tests of fruit only, no differences in preference were observed between red and blue fruit (Paired t-test, $t_7 = 0.21$, p = 0.84) and no correlations were observed between preference and reflectance at any wavelength (Pearson's correlation, p > 0.10). In choice assays where choices were fruit alone or fruit of the same stage paired with a leaf, higher numbers of female *D. suzukii* were attracted to leaf~fruit pairs; however, differences in preference were not significant (Paired t-test, $t_{15}=-0.68$, P=0.51) (Fig. 3.1.2b).

In choice tests with between differing leaf~fruit pairs, blue fruits paired with red or yellow leaves were more preferred than blue fruits paired with green or red-green leaves; however, differences were not significant (ANOVA, $F_{3,62} = 1.15$, $p = 0.34$) (Fig. 3.1.2b). Response index scores and fruit reflectance values were not correlated (Table 3.1.3b). Response index scores were correlated with leaf reflectance and contrast scores at yellow (585 nm) and orange (600 nm) wavelengths (Table 3.1.3b).

In 2-choice assays with sealed fruits and/or leaves, differences in response index scores were most strongly associated with differences in reflectance scores between the two choices for fruits at green (560 nm) and leaves at orange (600 nm), and differences in contrast scores at green, yellow (585 nm), and orange (Table 3.1.4). In a choice between two fruits or two leaf~fruit pairs, the choice with higher fruit reflectance at green (560 nm), higher leaf reflectance at orange (600 nm) or higher contrast in the green to orange range would be the more preferred.

As blueberry fruits ripened and blueberry leaves senesced, we observed a shift in relative intensity of reflectance from green (560 nm) to red (645 nm) (Fig. 3.1.3). However, relative reflectance in blueberry fruits shifted as they ripened from yellow (585 nm) to blue (470 nm); whereas, relative reflectance in blueberry leaves shifted from blue (470 nm) to yellow (585 nm) as they senesced (Fig. 3.1.3).

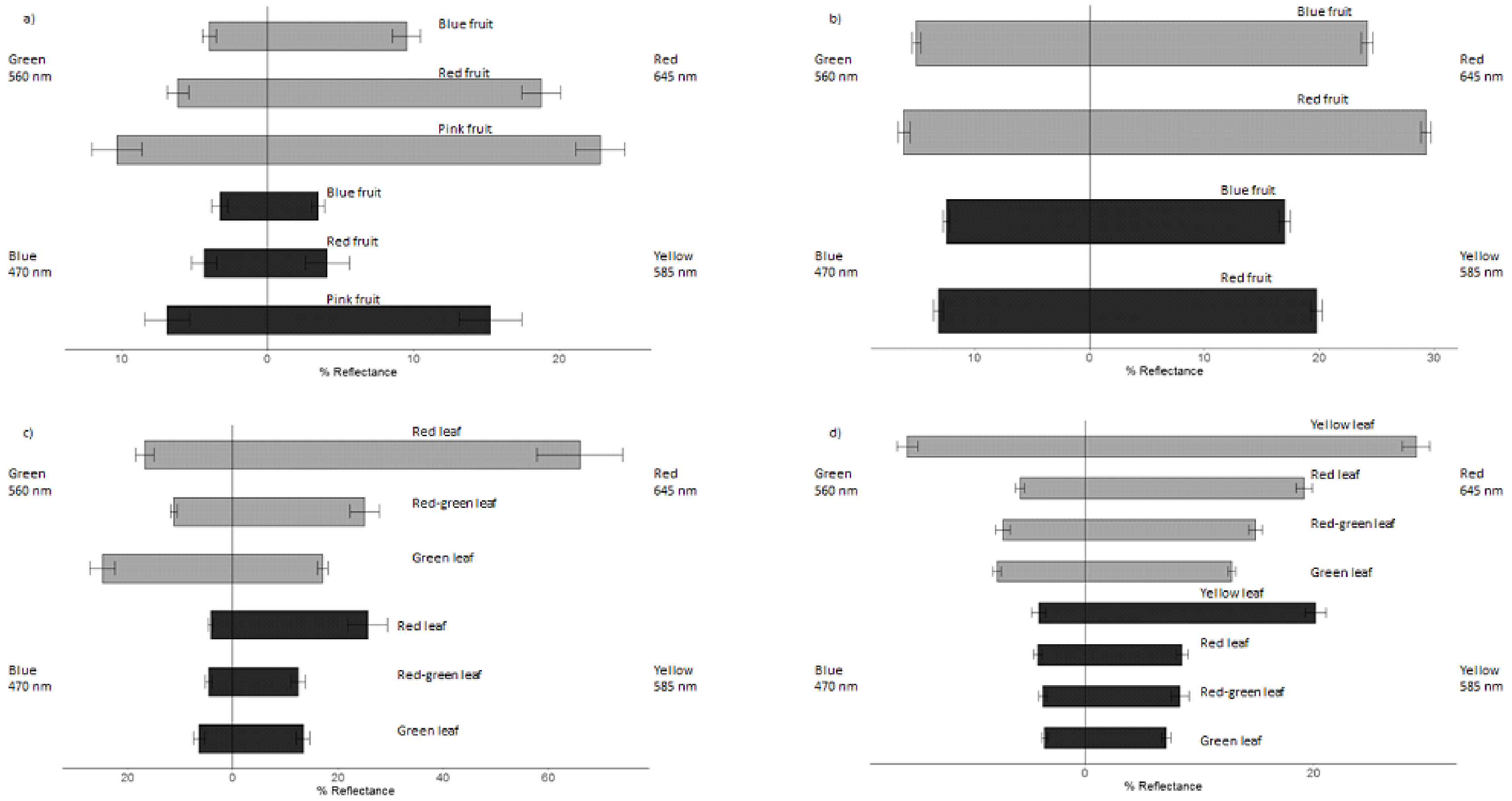

Fig. 3.1.3. Reflectance values of blueberry fruits diminished in intensity overall as fresh fruits ripened [a) choice assays with odor, b) choice assays with no odor], with greatest reductions in reflectance at yellow and red. However, as leaves senesced [c) choice assays with, d) choice assays without odor], reflectance intensity increased and shifted from blue to yellow and from green to red. (Blue and yellow reflectance shown in black, green and red reflectance shown in grey. Bars denote mean reflectance +/- SE.)

Table 3.1.4. Linear regression between *Drosophila suzukii* response index scores and blueberry fruit or leaf reflectance differences or contrast differences between choices during 2-choice assays with sealed fruit. Response indices and differences in overall fruit reflectance intensity overall were not correlated (Pearson's correlation, R = -0.01, P = 0.53); however, response index scores were correlated with differences in both leaf reflectance intensity (R = 0.08, P < 0.005) and contrast scores (R = -0.07, P = 0.02) across the full spectrum.

	Fruit reflectance		Leaf reflectance		Contrast		
Wavelength	$F_{1,60}$	P	$F_{1,128}$	P	F	df	P
Blue (470 nm)	0.95	0.34	0.11	0.74	<0.01	1,108	0.97
Cyan (525 nm)	2.28	0.13	0.03	0.87	2.00	1,110	0.16
Green (560 nm)	**5.83**	**0.02**	1.20	0.28	**4.08**	1,112	**0.05**
Yellow (585 nm)	3.18	0.08	3.36	0.07	**14.37**	1,112	**<0.0005**
Orange (600 nm)	1.01	0.32	**3.99**	**0.05**	**9.63**	1,112	**<0.005**
Red (645 nm)	0.03	0.86	1.46	0.23	1.97	1,112	0.16
Deep Red (700 nm)	0.91	0.34	0.53	0.47	0.26	1,114	0.61
Infrared 1 (735 nm)	0.74	0.39	0.20	0.65	0.11	1,112	0.74
Infrared 2 (810 nm)	0.09	0.76	3.34	0.07	2.36	1,112	0.13
Infrared 3 (880 nm)	0.54	0.46	0.70	0.40	2.46	1,110	0.12
Infrared 4 (940 nm)	<0.01	0.98	0.02	0.89	<0.01	1,112	0.95

3.1.5 Discussion

Percentage reflectance values were higher across most of the visible and infrared range for pink (early-stage ripening) blueberries than other fruit stages. Previous research on spectral analyses of blueberry fruit has focused on using colour images to map differences in harvest yield potential because several stages of fruit development can occur on a single highbush plant or even within a single cluster of fruit (Supp. Fig. S3.1.1) (Li et al. 2014). Differences in reflectance values among fruit stages were more pronounced at longer wavelength portion of the visible light spectrum into the shorter wavelength range of the near-infrared electromagnetic spectrum. Near-infrared reflectance is correlated with increased total soluble solids (a measure of sweetness), anthocyanins (pigments associated with blueberry ripening), and ultraviolet reflectance can be used to measure organic acid content in blueberry fruits (Kalt and McDonald 1996; Sinelli et al. 2008). Hyperspectral reflectance in the 500 – 1000 nm range is an effective predictor of blueberry fruit firmness and to a lesser extent is correlated with blueberry fruit sweetness (Leiva-Valenzuela et al. 2013). Analysis of photographs of blueberry fields have been demonstrated as an effective means of assessing relative proportions of different growth and ripening stages of blueberry fruits for estimating crop yields by relying on fruit colour differences (Li et al. 2014). Phenolic concentrations and antioxidant activity are highest in blueberry leaves with the highest levels of the red pigment, anthocyanin (Routray and Orsat 2014). The period when anthocyanin levels are increasing in blueberry leaves coincides with peak harvest periods for fully ripe fruit for most blueberry cultivars (Hampton et al. 2014). As blueberries ripened, differences in reflectance were most pronounced in the low infrared range (735 nm).

Our results were consistent with previous studies which found that while berry fruits were susceptible to *D. suzukii* at early stages of ripening, fully ripened berries were most preferred (Grassi et al. 2011; Arnó et al. 2016; Lee et al. 2016). Even in the absence of an olfactory cue from fruits or fruit-like substitutes, *D. suzukii* preferred darker, less reflective targets consistent with fully ripened fruits. Differences in percentage reflectance values of ripening blueberry fruits were correlated with host selection by *D. suzukii* in 2-choice assays.

We observed significant differences in the cyan to infrared range (525 – 810 nm) among senescence stages. We also observed significant differences in reflectance contrast of fruit and leaves among fruit ripeness and leaf senescence stages within the green to red range (560-645 nm). These differences in contrast were most pronounced between fully ripened (blue) fruit and fully senesced (red) or stressed (yellow) leaves. Contrast between ripe blueberry fruits and leaves of all senescence stages was highest at blue to cyan wavelengths (470 – 525 nm).

In 2-choice assays, more *D. suzukii* were attracted to the choice with the greater contrast between fruit or fruit-substitute and background leaf colour. Other Dipertan species, including apple maggot, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), and cherry fruit fly, *R. cingulata* Loew (Diptera: Tephritidae), were also found to be most strongly attracted to yellow or to strongly contrasting targets (Teixeira et al. 2010). Differences in contrast between target fruits or traps and background colour or foliage has been demonstrated to modify attraction behavior in the cabbage root fly, *Delia radicum* L. (Diptera: Tephritidae) (Finch 1995; Košťál and Finch 1996) and in the tomato fruit fly, *Neoceratitis cyanescens* Bezzi (Diptera: Tephritidae) (Brévault and Quilici 2007). We suggest that the contrast between fruit and foliage increases the conspicuousness of the fruit, allowing the insect to identify prospective host fruits from a distance. Other cues, including olfactory cues would reinforce or confirm the visual stimuli as the insect approaches. Although the spectral range of photoreceptors of some insect species, including some Lepidoptera, Hymenoptera, and Odonata are among the broadest described, most insects have photoreceptors which optimize over a narrower range of ultraviolet to green, but that may provide colour vision covering a range of wavelengths up to 700nm (Arikawa et al. 1987; Briscoe and Chittka 2001; Futahashi et al. 2015).

The simultaneous presence of short wavelength colours (blue or green) can increase the attractiveness of other colours including ultraviolet wavelengths in *D. melanogaster* (Fischbach 1979). Colour opponency hypothesis suggests that opponent neurons are specific for blue/yellow and green/red, thus these colours are seen separately. Specific colour opponency stimulus patterns of blue/yellow and green/red colour reception have been associated with host-finding behavior in aphids (Hemiptera: Aphididae) (Döring and Chittka 2007). Colour opponency may also explain reduced attraction in bumblebees (*Bombus impatiens*) to red flowers surrounded by green foliage compared to red flowers in a laboratory setting (Rivest et al. 2017). Colour discrimination in *D. melanogaster* relies on integration of signals from multiple visual receptors, with inner photoreceptors providing blue/green discrimination and outer photoreceptors providing a colour opponency dimension to improve discrimination at specific wavelengths (Schnaitmann et al. 2013). Colour opponency theory suggests that visual receptor neurons function in a binary system to identify colour. These opponent neurons pair short with mid-length wavelength colour stimuli (blue or yellow) or short with long wavelength colour stimuli (green or red) (Kien and Menzel 1977, Schnaitmann et al. 2013, Lunau 2014). Ergo, each neuron can signal in response to one but not both colour stimuli and excitatory stimulation from one wavelength may be inhibitory to signals for the opposing wavelength colour. Blueberry fruits reflectance decreased as they ripened, and leaf reflectance increased as they senesced across much of the spectrum. Both fruits and leaves reflected less green; however, whereas berries had small reductions in reflectance of red during ripening, leaves experienced marked increases in reflectance of red. The balance of blue and yellow reflectance demonstrated more appreciable differences between ripening fruit and senescing leaves. Light reflectance in ripening blueberries shifted from yellow to blue; whereas, the balance of reflectance in senescing leaves shifted from blue to yellow. Thus, separate spectral changes in blueberry fruits and in blueberry foliage would interact as part of a larger colour opponency mechanism to enhance host detection.

The contrast between ripening fruit and surrounding foliage plays a crucial role in locating and identifying suitable fruits for feeding among many fruit-eating and frugivorous insects (Burns and Dalen 2002, Schmidt et al. 2004, Schaefer et al. 2006, Teixeira et al. 2010). Thus, a similar mechanism of using contrast or relative conspicuousness of fruit against a contrast background of foliage may play a vital role in host-finding by *D. suzukii*. Both blueberry fruits and foliage change colour as plants synthesize increasing levels of anthocyanin in early autumn. We found significant differences in percentage reflectance values of senescing leaves and reflectance contrast between ripe fruit and senescing leaves that correlated with host selection by *D. suzukii*. We propose that increasing visual contrast between fruit and foliage, as they ripen or senesce respectively, results in stronger visual stimuli and greater attraction by *D. suzukii*. Visual stimuli due to strong contrast between fruit and foliage likely acts in concert with olfactory cues as part of a multi-modal sensory suite in host-finding behavior. This may account for increased late season preference for highbush blueberries and the significant crop losses experienced by fruit growers (Lee et al. 2011; Burrack et al. 2013). Specific fruit and foliage colours may be less important to *D. suzukii* than contrast of colour or intensity during host detection, as is the case with *Rhagoletis pomonella* (Owens and Prokopy 1986). Fruits of many blueberry species have a waxy coating (bloom) that alters both the visual appearance of the fruit and reduces reflectance, particularly in the ultraviolet and visual light ranges (<670 nm) (Willson and Whelan 1989). Thus, blueberry varieties bred to produce a bloom may have greater contrast with senescing leaves and be more susceptible to *D. suzukii* than non-bloom blueberry varieties. Fruit breeders and growers may also find it useful to select or breed blueberry varieties with foliage colours that minimize visual contrast against the fruits. Similar patterns of fruit ripening and leaf senescence may also occur in other at-risk fruits. Adopting fruit varieties that ripen before foliage colour changes may provide some protection to a range of soft fruits and berries.

This study also highlights the potential impact of environmental stress on insect-plant interactions. Blueberry plants that experience heat-or drought-stress will develop premature colour changes, particularly yellowing, have fruits that are visually more conspicuous to *D. suzukii* and, therefore, more attractive than fruits of unstressed plants. Climate change will likely exacerbate risks of environmental stresses. Growers will need to mitigate exposure to stress or switch to varieties more tolerant to changing environmental conditions to not only protect fruit abundance, but also to protect against increased *D. suzukii* attraction to more conspicuous fruits against stress-induced foliage colouration. In addition, use of stronger visual contrast signals may also improve the efficacy of monitoring traps catch-and-kill devices. Black and red traps currently in use in many areas offer little contrast within the visual range of *D. suzukii*. To our knowledge, this is the first study to investigate relationships between visual contrast cues and host selection in *D. suzukii*.

3.1.6 Acknowledgements

Funding for this work has been provided by Natural Sciences and Engineering Research Council #PGSD2-475743-2015 and #356109-2008 RGPIN, Atlantic Canada Opportunities Agency Atlantic Innovation Fund #197853, Growing Forward 2 in Newfoundland and Labrador Advancing Agriculture Innovation Program #GF21516-519 and #GF21718-956, Canada Foundation for Innovation #22087, and a Research Acceleration grant from the Nova Scotia Department of Agriculture. We are grateful for the generosity of Nova Agri Group and MacBerry Farms for their continuing support and access to their blueberry fields. Thank you to AR Rizatto and S Pawlowski for assistance with graphs and statistical analysis.

3.1.7 References

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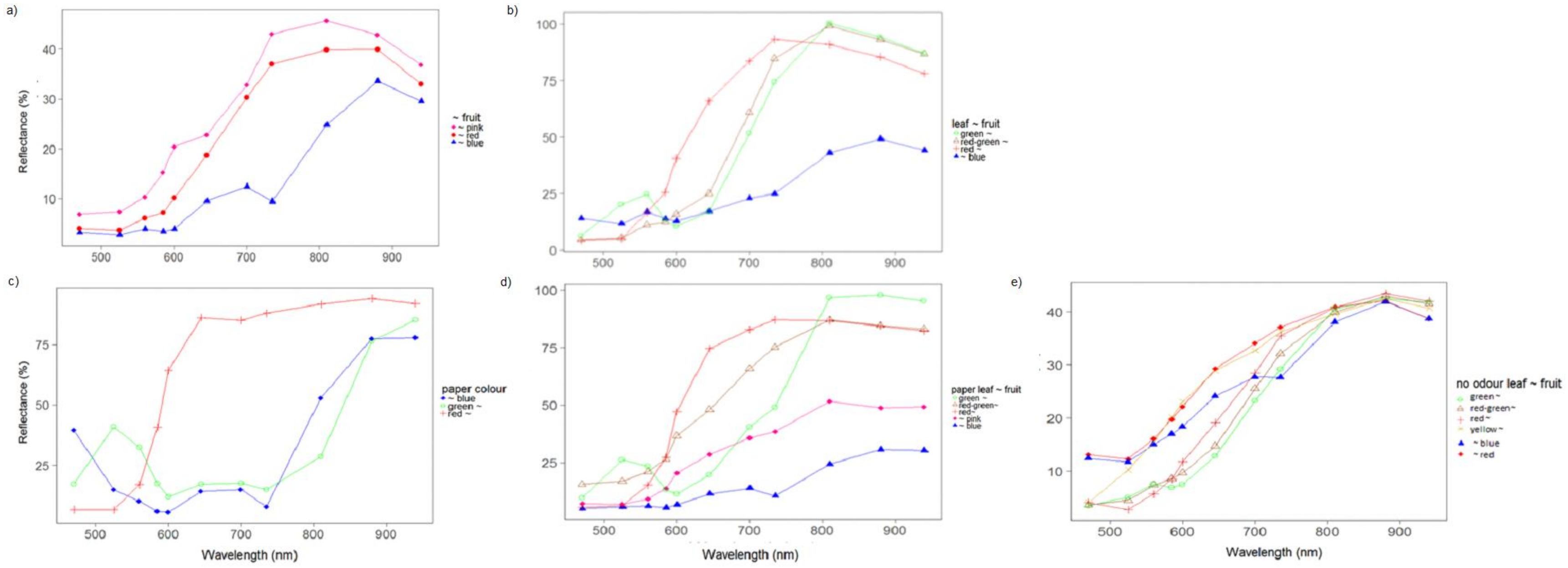
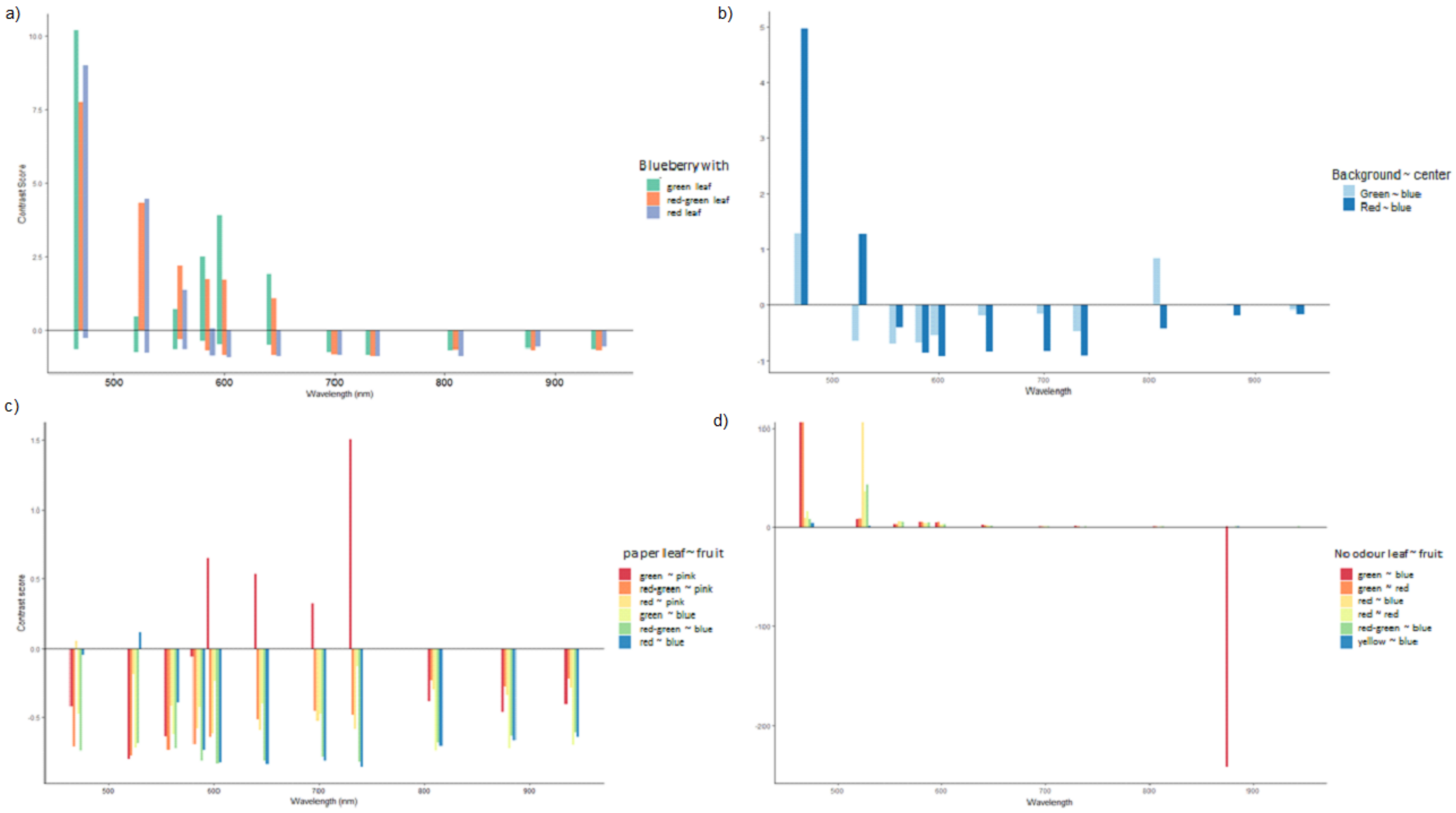
3.1.8 Supplementary information

Supplementary Figure S3.1.1. a) Blueberry fruits change colour as they ripen, progressing from white through pink and red to blue as colour pigments accumulate. b) Blueberry fruits and leaves of various stages occur on plants throughout the growing and harvest seasons. a) Blueberry leaves change from green to red-green to red as foliage senescence progresses.



Supplementary Figure S3.1.2. Spectra of blueberry a) fruit used in fruit-only choice trials b) leaves and fruits used in leaf~fruit choice trials where odor not isolated from cues available to *D. suzukii*. Comparison of percentage reflectance spectra among a) three categories of blueberry ripening used in fruit-only choice assays and b) leaves and fruits used in leaf~fruit choice assays where in each case odor not isolated from cues available to *D. suzukii*. Percentage reflectance across the measured visible and infrared spectra of c) paper disk colours, d) paper photos of blueberry leaves and fruits and e) sealed blueberry fruits and leaves used for no-odor choice trials. Points and vertical lines represent mean values ± SE.



Supplementary Figure S3.1.3. a) Differences in reflectance measures among leaf colours is reflected in higher contrast scores in the cyan-orange colour range for berries paired with red-green and red leaves, whereas contrast scores were higher for blueberries paired with green leaves in the orange-red colour range (Table 2b). Minimal contrast was observed between fruit and leaf colours in the infrared colour range. Contrast scores across the visible and infrared spectra for b) red~blue and green~blue contrast disk targets, c) printed photograph leaf~fruit pairs, and d) sealed leaf ~fruit pairs used in no-odor 2-choice trials.

3.2. Colour preference of the spotted wing Drosophila, *Drosophila suzukii*

A version of this chapter section has been published in Scientific Reports:
Little, C.M., Rizzato, A.R., Charbonneau, L., Chapman, T., and Hillier, N.K. 2019. Color preference of the spotted wing Drosophila, *Drosophila suzukii*. Sci Rep **9**(1): 16051. doi:10.1038/s41598-019-52425-w.

Author contribution statement
CL designed research. CL, AR, and LC conducted experiments. CL analyzed research and wrote manuscript. NH, AR, LC, and TC edited manuscript. All authors read and approved manuscript.

3.2.1. Abstract

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a significant invasive pest in soft-skin fruits and berries in Asia, Europe, and North and South America. Many herbivorous insects use multiple cues for host selection, particularly olfactory and visual stimuli. The visual system of closely-related *Drosophila melanogaster* is well-documented, expressing strong sensitivity to short-wavelength colours (ultraviolet to green) and only limited sensitivity to long-wavelength colours (red to infrared). We confirmed that visual sensitivity range was conserved within *Drosophila* species and that *D. suzukii* have limited sensitivity to clearly distinguish red, thus contrast rather than colour appearance may be of greater importance in orientation and attraction. This study suggests that differences in reflectance within opponent colour pairs are key to colour discrimination to provide contextual contrast between foreground and background, as occurs between fruit and foliage, during host-finding.

3.2.2. Introduction

Host-finding by insects often relies on the integration of a combination or sequential reception of olfactory, visual, tactile, and/or gustatory cues to identify suitable hosts (Bruce et al. 2005;

Gregg et al. 2018). Use of multi-modal cues for host-finding is widespread, and hierarchical sensory systems have been identified in numerous species from several insect orders, including Lepidoptera, Hymenoptera, Coleoptera, and Diptera (Aluja and Prokopy 1993; Rojas et al. 1999; Fischer et al. 2001; Couty et al. 2006; Stenberg and Ericson 2007; Burger et al. 2010; Goyret 2010). Even within a single insect species, separate host-races of *Rhagoletis pomonella* (apple versus hawthorn) can be distinguished by differences in attraction to both olfactory and visual cues (Forbes and Feder 2006).

The spotted wing Drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a highly polyphagous invasive pest insect in Asia, North America, South America, and Europe (Hauser 2011; Calabria et al. 2012; Cini et al. 2014; Asplen et al. 2015; Hamby et al. 2016; Funes et al. 2018; Schetelig et al. 2018). Female *D. suzukii* use their serrated ovipositor to lay eggs in soft-skinned fruits and berries, resulting in millions of dollars in damage to fruit crops (Farnsworth et al. 2017; Mazzi et al. 2017). Volatile organic compounds associated with ripening fruits and naturally occurring yeasts have been widely acknowledged as key factors in host-finding behaviour for *Drosophila* species, including *D. suzukii* (Yu et al. 2013; Abraham et al. 2015; Hamby et al. 2016). Visual cues are also important to host-finding behaviour (Little et al. 2018). To that end, monitoring traps in use for *D. suzukii* are red or employ a combination of black and red (Basoalto et al. 2013; Lee et al. 2013; Renkema et al. 2014; Kirkpatrick et al. 2018). Recent research supports the attractiveness of red and black against a white background (Kirkpatrick et al. 2016). However, monitoring traps used in fruit crops are normally deployed amongst foliage rather than a white background. This may explain why monitoring traps in a combination of clear plastic and yellow have been used with similar efficacy (Lee et al. 2013; Iglesias et al. 2014; Cha et al. 2017). Previous research has demonstrated that colour contrast between foreground and background can facilitate food search efforts by frugivorous birds and host search efforts by Tephritid flies (Burns and Dalen 2002; Teixeira et al. 2010). Similar mechanisms may play a role in host-finding by *D. suzukii*.

Colour vision can be defined as the ability to discriminate among colour stimuli based on wavelength composition (Kelber and Osorio 2010; Lunau 2014). Colour vision in a closely related species, *Drosophila melanogaster*, has been studied extensively (Yamaguchi et al. 2010; Paulk et al. 2013; Schnaitmann et al. 2013). Peak sensitivity in *D. melanogaster* occurs at 420 nm and 495 nm; however, visual sensitivity is relatively stable and consistent from 406 nm to 505 nm (Hernández de Salomon and Spatz 1983). Thus *D. melanogaster* are most sensitive to shorter wavelengths (ultraviolet, blue, and green), with only limited sensitivity to higher wavelengths (orange, red, and infrared). The colour vision system of *Drosophila* spp. is thought to be highly conserved (Kelber and Henze 2013).

Numerous methods of quantifying colour are currently in use, most based on human perception of colour appearance (Fairchild 2005). Concepts of colour brightness, hue, chroma, and saturation are comparative measures of colour perception based on human colour vision and can be influenced by the viewers assumptions about environmental conditions including assumed illumination of the object viewed (Fairchild 2005; Kelber and Osorio 2010; Lunau 2014; Cuthill et al. 2017). The XYZ colour space model used to quantify colour is also based on human colour perception. In addition, the XYZ model requires identical viewing conditions, including illumination and background, to compare differences among colours (Fairchild 2005). In contrast, measures of light wavelength and reflected wavelength are objective independent of the species perceiving the colour and of viewing conditions.

The goal of this study was to investigate the relative sensitivity and preference of *D. suzukii* to a range of colours to optimize monitoring and trapping efficacy. We tested sensitivity of *D. suzukii* to different colours of light, the relative attractiveness of coloured light, and of reflected colours alone and in combination. We hypothesize that visual spectral sensitivity is highly conserved within *Drosophila* species, and that visual sensitivity ranges are similar in *D. suzukii* and *D. melanogaster*. We previously demonstrated that *D. suzukii* are highly attuned to changes in foliage colours and are attracted to fruit colours which contrast against foliage colours (Little et al. 2018). This suggests that contrast between foreground and background colours, as is found between fruit and foliage, may be a key factor in host-finding behaviour.

3.2.3. Materials and methods

D. suzukii colony

Adult *D. suzukii* flies used for all laboratory experiments were sourced from colonies maintained at Acadia University, Wolfville, NS since 2013. Initially, *D. suzukii* used to found colonies were reared from cultivated blueberries by D. Moreau at the Kentville Research and Development Centre (Agriculture and Agri-Food Canada, Kentville, NS). Colonies were maintained in 250 ml flasks (Genesee Scientific, San Diego, CA) containing 50 mL of Formula 4-24 Instant Drosophila medium (Merlan Scientific Ltd, Mississauga, ON, Canada) mixed with 50 mL of dH2O. Sexually-mature mated *D. suzukii*, approximately two weeks of age, were removed from colony vials and starved for 2 h prior to start of each assay.

Sensitivity to colour (Electroretinography)

Colour sensitivity differs among insect orders and even among many species; however, the colour vision system in flies (Diptera) is believed to be relatively well conserved (Kelber and Henze 2013). We tested sensitivity of female and male *D. suzukii* to blue, green, and red light-emitting diode (LED) lights and a full spectrum white LED light using a Bluetooth-enabled Programmable BeeWi 9W SmartLite® LED Colour Bulb and SmartPad® app (VOXLAND, Marseilles, France). Analysis of light spectra for each LED light colour were conducted with advice and assistance of Dr. Michael Robertson (Department of Physics, Acadia University), who specializes in optics and optical properties. Spectra for each colour light were measured using an Ocean Optics USB4000 Spectrometer (corrected linearity >99%) and SpectraSuite® Spectrometer Operating Software (Ocean Optics, Inc., Dunedin FLA) (Fig. S3.2.1a). Nine replicates of blue wavelength spectra and ten replicates of green and red wavelength spectra were measured to ensure consistency of light colour (One-Way ANOVA; Blue: $F_{8,9387}$=0.40, P=0.92; Green: $F_{9,10430}$=0.21, P=0.99; Red: $F_{9,10430}$=0.77, P=0.64). Lights were set at maximum brightness of 756 lumens for all assays. Light intensity was comparable across white and coloured lights (Fig. S3.2.1a). Intermediate colours pink, turquoise, and yellow could also be emitted by BeeWi lights; however, these colours were achieved using a combination of blue, green, and red LED lights, not by emitting intermediate wavelengths, and so were not used for testing.

Changes in sensory receptor neuron activity were measured with electroretinograms[55,64] using an IDAC-2 signal connection controller and GC-EAD 2014 x1.2.5 software (Syntech Data Acquisition for Gas Chromatography with EAD, Syntech Equipment and Research, Kirschzarten Germany). Individual *D. suzukii* were mounted in 200 µl pipette tips, allowing only the head to emerge (Fig. S3.2.1b). All overhead laboratory lighting was extinguished once set-up was complete and not switched on until after the assay was complete. Each *D. suzukii* preparation was acclimatized for 10 minutes prior to start of electroretinogram assays.

The light source was enclosed within a cardboard box and light was directed at the fly's eye through a 12 mm X 12 mm hole covered by a flap of black foam-board. Light colours were changed with the box closed and flies were exposed to light colours in random order at one-minute intervals. Each fly was exposed to white light at the beginning, middle, and end of the trial as a positive control. Blue, green, and red lights were presented in random order twice during each trial. Eleven replicates were completed for each sex of fly, using a naïve fly for each replicate.

Preference among LED light colours

Drosophila suzukii preference among blue, green, and red light was assessed through two-choice assays using the same LED colour bulbs as in the electroretinograms. Light intensity was consistent among light colours (Fig. S3.2.1a). Mean intensity levels at spectral peaks are white: 58295 counts/ms at 449.46 nm, blue: 51471.3 counts/ms at 462.67 nm, green: 54904.9 counts/ms at 513.78 nm, and red: 51981.9 counts/ms at 629.47 nm. Arenas were constructed of 3-inch diameter (7.6 cm) black ABS (Acrylonitrile butadiene styrene) pipe fittings and cleanout T-fitting, using a modified set-up based on Diclaro et al. (2012) (Fig. S3.2.1c). Clear plastic sandwich bags coated with TangleTrap Sticky Coating^{TM} (The TangleFoot Company, Grand Rapids, MI) were placed over plastic drinking cups covered with black duct tape that were fitted into 3-4-inch (7.6-10.2 cm) diameter ABS adapters at either end of the arena. Coloured light was directed perpendicularly into the arena via a small 2 x 2 cm clear openings on the side of each cup to prevent flooding the arena with light and prevent blinding the insects. The position of each light colour was alternated relative to the other from one trial to the next to mediate positional effects. Male and female *D. suzukii* were tested separately. Twenty-five mature *D. suzukii* were inserted through the port located at the center of the arena (Fig. S3.2.1c, position A). Each paired colour choice was replicated 10 times for each sex. After 24 h, *D. suzukii* adhering to the TangleTrap at each end of the arena were counted.

Preference among solid colours

Two cylindrical arenas were constructed using vertical strips of coloured foam arranged around the circumference of an 11.8 L plastic container (circumference of 74 cm at top & 67 cm at bottom and height of 29.5 cm). Two strips each of black, blue, green, yellow, red, and white were repeated twice in each arena. Colour order was arranged to ensure that adjacent colours were different in each arena (Fig. S3.2.2a, arenas 1 and 2). The coloured foam surfaces were covered with clear cellophane tape and brushed with a 1.5 mm coating of TangleTrap Sticky Coating^{TM} per package directions. No change in colour reflectance was observed following application of sticky coating (Little et al. 2018). As *D. suzukii* alighted on a coated surface, they adhered to the coloured strip. Male and female *D. suzukii* were tested separately. Fifty mature *D. suzukii* were placed at the center of the arena. The top of the container was covered with cellophane. The container lid was cut open, leaving only the outer rim, which was used to secure the cellophane. Arenas were placed inside a box to reduce ambient light. A clear plexiglass panel placed over the arena supported a full spectrum light source (VX Series High CRI LED 14W Bulb, Yuji International, Beijing, China) that illuminated the entire arena area. After 24 h, flies adhered to each of the coloured strips were counted. Seven replicates of six-colour trials were conducted for both male and female *D. suzukii*, alternating arena used for each trial.

Multi-choice assays were also conducted in two arenas using eight colours of card stock in the same type of arenas (Fig. S3.2.2a, arenas 3 and 4). Two strips each of black, purple, blue, green, yellow, orange, red, and white were repeated twice in each arena. Ten replicates of eight-colour trials in each arena were conducted for both male and female *D. suzukii*.

Reflectance spectra for each colour were measured with Alta II reflectance spectrometer (Vernier Software & Technology, Beaverton, OR USA) to quantify colours used (Fig. S3.2.3). The spectrometer measures reflectance at 11 wavelengths covering a range of 470 nm to 940 nm.

Preference among contrast disks

Contrasting colour disks, based on those used by Kirkpatrick et al. (2016), were constructed of pairs of card stock disks 5 cm in diameter overlaid with card-stock disks of 2.5 cm diameter (same front and back of disk). Each disk was covered with clear cellophane tape and coated with TangleTrap Sticky Coating^{TM}. Disks were suspended from the top of a 30 x 30 x 30 cm plastic and mesh insect cage (BugDorm, MegaView Science Co. Ltd., Talchung, Taiwan). Disks were arranged in random order, equidistant from each other, and at a radius of 12.5 cm from the center of the cage. We recorded the order of the disks around the arena. Ambient light and external visual distractions were excluded from each arena with white cardboard trifold display boards. Natural field light conditions differ dependent upon time of day, time of year, geographic location, and other abiotic and biotic environmental conditions. It would not be feasible to replicate all possible natural light conditions. We have approximated field light conditions by illuminating arenas with a full spectrum light source ([5600K daylight spectrum, CRI typical 97, TLCI typical 99] VX Series High CRI LED 14W Bulb, Yuji International, Beijing, China). Female and male *D. suzukii* were tested separately. One hundred mature *D. suzukii* were released into the center of each arena. After 24 h, *D. suzukii* adhering to each disk were counted. Results of each set of assays were used to inform and refine the colour choices for the next iteration.

Contrast disk assay 1. Each of the eight colours used in solid colour multi-choice assays were used in contrast with black (Fig. S3.2.2b). Colour contrast pairs are hereafter denoted as "outer colour ~ inner colour" for clarity. Colours were paired with black as either foreground (inner portion) or background (outer portion of disk) to identify possible effects of contrast inversions. Solid white and solid black disks served as controls.

Contrast disk assay 2. Contrasting colour assays paired combinations of black, blue, green, purple, and yellow (Fig. S3.2.2c). Results of contrast disk assay 1 suggested that black and green backgrounds were attractive.

Contrast disk assay 3. Based on results of contrast disk assay 2, assays were conducted with four contrasting colour disks of green background with black, purple, red, and yellow and a fifth disk of black~ red representing the colours used most frequently for monitoring traps (Fig. S3.2.2d).

Contrast disk assay 4. To account for potential differences in attraction due to contrast inversions between black and red, disks of green~ purple were tested against black disks and disks that combined red and black (Fig. S3.2.2e).

Contrast disk assay 5. To ensure differences in counts were not attributable to differences in availability between colour options, green~ purple disks were paired against black~ red disks in two-choice assays (Fig. S3.2.2f). Because results of choice assays to this point revealed the importance of blue and yellow reflectance on behaviour, green~ purple disks were also paired against yellow~blue disks (Fig. S3.2.2g).

Ten replicates were completed for each sex and each multi-choice assay (assays 1-4) and five replicates for each sex for two-choice assays (assay 5).

Statistical analysis

A contrast score was calculated based on the Weber contrast[65] for percentage reflectance at each wavelength for each colour-contrast disk.

$$Contrast = (I_i - I_o) / I_o$$

I_i is reflectance (%) of inner ring.

I_o is reflectance (%) of outer ring

Statistical tests used for data analyses are described in the results for each assay. Responses of male and female flies were analyzed separately due to potential sex-specific physiology and behaviour. Analyses of choice assays were adapted from Kirkpatrick et al. (2016). All statistical analyses were performed in R version 3.4.3 (R Core Team 2017 [RStudio Version 1.1.419 - © 2009-2018 RStudio, Inc.]).

3.2.4. Results

Sensitivity to colour (Electroretinography)

Significant differences were observed among white and coloured lights (Fig. 3.2.1a & b). Light colour, sex of the *D. suzukii*, and the interaction of these factors all contributed significantly to differences in physiological responses (Fig. 3.2.1b) (1-way ANOVA; Females: $F_{3,96}$=33.79, P<0.0001; Males: $F_{3,95}$=15.96, P<0.0001; 2-way ANOVA; Colour: $F_{3,191}$=32.64, P<0.001; $Sex_{1,191}$=51.22, P<0.001; Colour:Sex: $F_{3,191}$=3.53, P=0.02). Responses from male *D. suzukii* were consistently stronger than from females (ANOVA, $F_{1,197}$=33.96, P<0.0001). Mean ERG response values to blue light were significantly different than to red light irrespective of *D. suzukii* sex (Tukey HSD, P=0.02).

Preference among LED light colours

Differences between responses by females and males in 2-choice assays were not statistically significant (Paired t-test; t_5=-1.35, P=0.18). (Fig.3.2.2a). Blue and green lights attracted more *D. suzukii* than red lights (ANOVA, $F_{2,117}$=64.61, P<0.0001; Tukey HSD, blue:green: P=0.36, blue:red: P<0.0001, green:red: P<0.0001) (Fig. 3.2.2b).

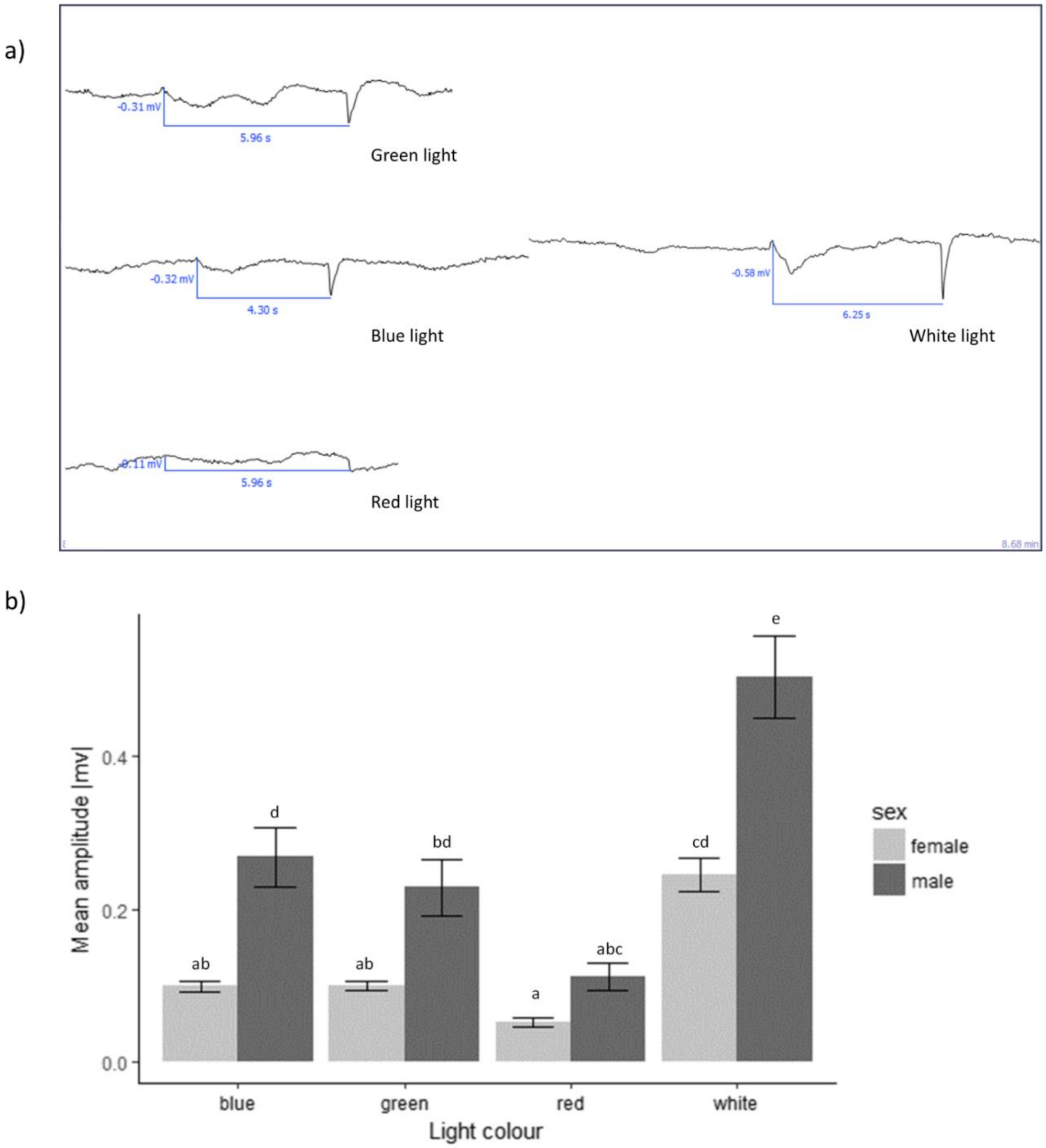


Figure 3.2.1. a) Representative electroretinogram responses to white and coloured lights. b) ERG results for each light colour by *D. suzukii* sex. Different letters denote significant differences light colours for each sex.

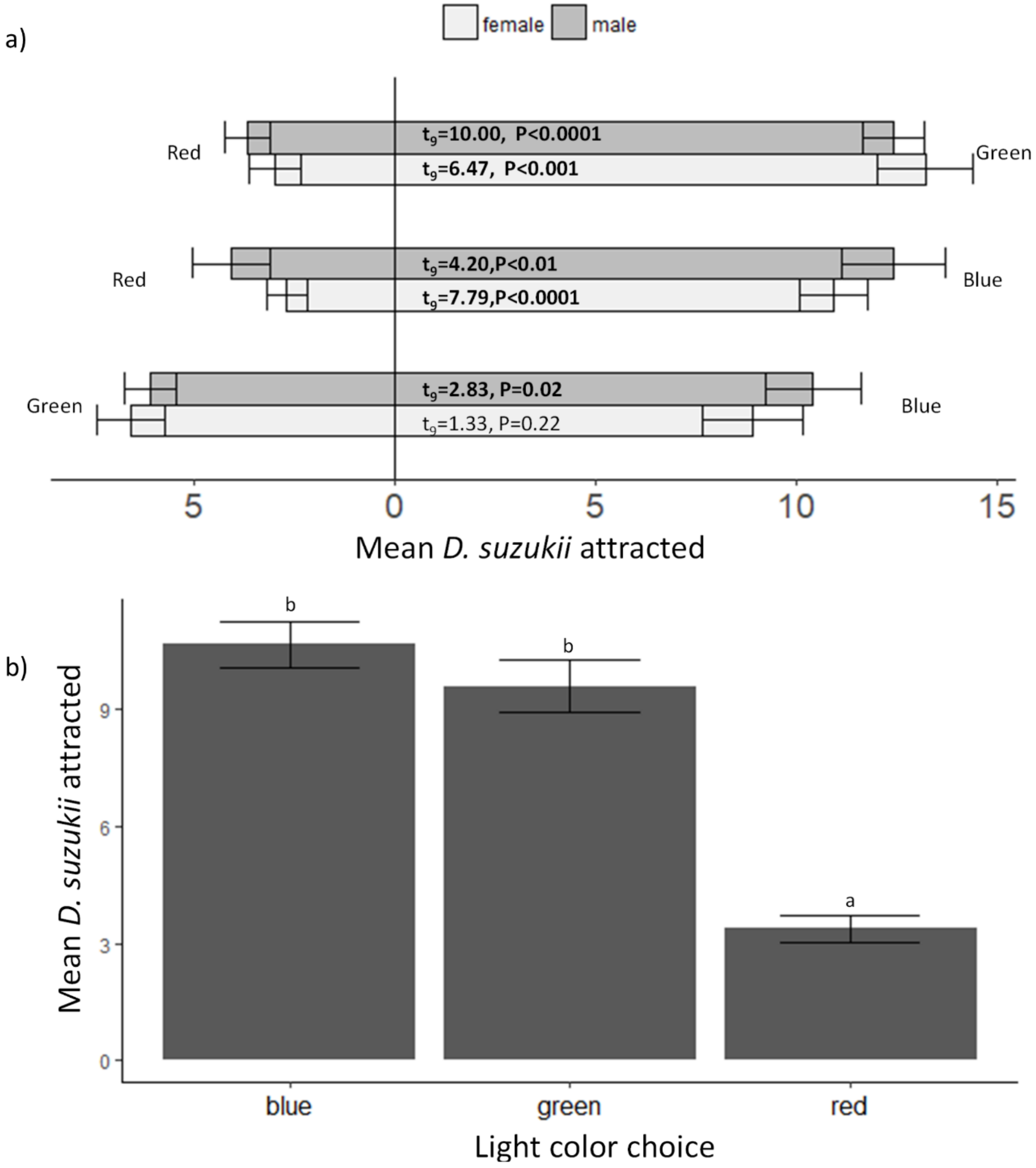


Figure 3.2.2. a) Mean count (± SE) of *D. suzukii* attracted to coloured lights in 2-choice trials. Results of Paired t-tests are shown within the figure (significant differences are in bold). b) Mean counts of male and female *D. suzukii* attracted to each colour in 2-choice assays.

Preference among solid colours

Although higher numbers of *D. suzukii* were attracted to green, red, and black bands, differences observed among foam colours during choice assays were not significant (1-way

ANOVA: Females: $F_{5,36}$=0.70, P=0.63; Males: $F_{5,36}$=2.31, P=0.06) (Fig. 3.2.3a). Mean responses to solid foam colours by female *D. suzukii* were higher than responses by males (paired t-test, t_5=3.01, P=0.03). Differences were for all colours; however, differences were significant only at blue (Welch's 2-sample t-test, $t_{11.8}$=2.48, P=0.03). No correlations were observed between mean number of flies choosing a colour (colour choice) and percentage reflectance at any wavelength for foam colours (Spearman's rank correlation, Females: P's>0.19; Males: P's>0.13).

However, more female *D. suzukii* were found adhered to black and red, and more male *D. suzukii* were adhered to red and yellow during choice assays among card stock colours (Fig. 3.2.3b). Preferences between red and black in female *D. suzukii* and between red and yellow in males did not differ significantly. Colour and the interaction of colour and *D. suzukii* sex contributed most strongly to differences in preferences. Differences in attraction between sexes were not significant; however, differences in colour preference were significant only within females, not within males (1-way ANOVA: Females: $F_{7,152}$=5.06, P<0.0001; Males: $F_{7,152}$=2.01, P=0.06; 2-way ANOVA; Colour: $F_{7,304}$=4.65, P<0.0001; Sex: $F_{1,304}$=1.44, P=0.23; Colour:Sex: $F_{7,304}$=2.24, P=0.03). Female colour choice was negatively correlated with percentage reflectance at blue (470 nm; Spearman's rank correlation; r_s=-0.39, P<0.0001), cyan (525 nm; r_s=-0.32, P<0.0001), and green (560 nm; r_s=-0.23, P<0.005), but not at other wavelengths. No correlations were observed between male colour choice and percentage reflectance (Spearman's rank correlation, P's>0.11). Mean responses to solid card colours were not significantly different between male and female *D. suzukii* (paired t-test, t_7=-0.80, P=0.45).

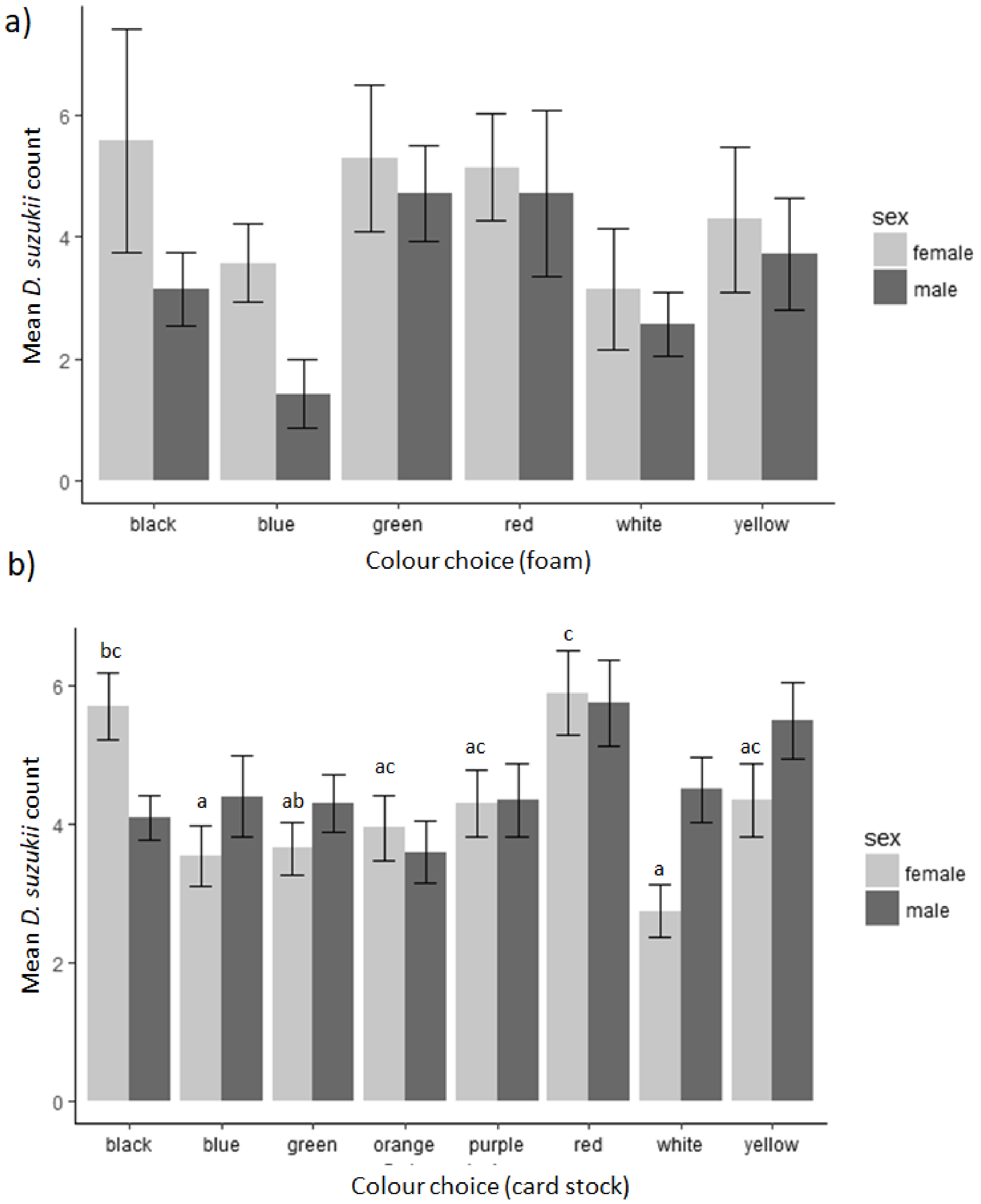


Figure 3.2.3. a) Mean count (± SE) of *D. suzukii* attracted to foam board colours during six-colour choice assays. b) Mean count (± SE) of *D. suzukii* attracted to card stock colours during eight-colour choice assays. Different letters represent statistically significant differences between contrast disks for each sex (Tukey Post-Hoc, P<0.05).

Preference among contrast disks

Contrast assay 1. During the contrasting-colour assays with eight colours contrasted against black, differences between contrast disks within each sex were not statistically significant (ANOVA, females: $F_{15,144}$=1.22, P=0.26; males: $F_{15,144}$=1.25, P=0.24). Responses to contrast disks were significantly different between male and female *D. suzukii* (paired t-test, t_{159}=-6.31, P<0.0001). Among disks with black centers (colour~black disks), female *D. suzukii* were most attracted to disks with green as the outer colour of the disk; however, differences in attraction were significant only in comparison to disks with blue as the outer colour (ANOVA, $F_{7,152}$=1.89, P=0.07; Tukey HSD (green:blue), P=0.05). Among disks with black as the outer colour (black~colour disks), male *D. suzukii* were most attracted to disks with yellow or blue as the inner colour of the disk (Fig. 3.2.4a); however, differences were not significant (ANOVA, $F_{7,152}$=1.77, P=0.10).

We conducted an ANOVA using the disk colours of the adjacent disks. There was no significant difference in attraction to any disk due to colours of adjacent disks for either sex (ANOVA, Females: $F_{15,304}$=1.48, P=0.11; Males: $F_{15,304}$=0.66, P=0.82). No correlation was observed between percentage reflectance of disk outer colours (Spearman's rank correlation, Females: P's>0.56; Males: P's>0.28), inner disk colours (Females: P's>0.08; Males: P's>0.44), or contrast scores (Females: P's>0.35; Males: P's>0.46). n-numbers necessary to conduct more detailed analyses of where flies did not alight were deemed excessive.

Contrast assay 2. Responses to contrast-colour disks were not significantly different between male and female *D. suzukii* (paired t-test, t_{79}=-1.57, P=0.12). Among contrast-colour disks comprised of the five most attractive colours in the previous assay (black, blue, green, purple, and yellow), disks of green~purple were most attractive to female *D. suzukii* (ANOVA, $F_{7,72}$=6.81, P<0.0001; Fig. 3.2.4b), although differences between green~purple and green~black and between green~purple and green~yellow were not significant. No significant differences in male *D. suzukii* preferences for contrasting-colour disks were observed (ANOVA, $F_{7,72}$=1.39, P=0.22).

Disks with green outer rings were significantly more attractive to female *D. suzukii* than other colours (ANOVA, $F_{2,77}$=14.06, P<0.001). Disks with green outer rings were significantly more attractive to male *D. suzukii*, but differences among colours were significant only between green and purple (ANOVA, $F_{2,77}$=3.81, P=0.03; Tukey HSD, P=0.02). Purple was the most attractive colour of inner ring on contrast disks for female *D. suzukii* (ANOVA, $F_{3,76}$=8.03, P=0.0001; Fig. 3.2.3b). No significant differences in preference for inner ring colour were found for male *D. suzukii* (ANOVA, $F_{3,76}$=0.42, P=0.74). Disk colours of adjacent disks were not associated with any significant differences in preferences for either sex *D. suzukii* (ANOVA, Females: $F_{7,152}$=0.30, P=0.95; Males: $F_{7,152}$=0.42, P=0.89). Attraction to disks in female *D. suzukii* was correlated to percentage reflectance values for blue (470 nm) to yellow (585 nm) for outer colours (Spearman's rank correlation, P's<0.005), for yellow (585 nm) to infrared 4 (940 nm) for inner colours (P's<0.02), and for orange (600 nm) to infrared 2 (810 nm) for contrast between outer and inner disks (P's<0.01). No correlations were observed for responses of male *D. suzukii* to percentage reflectance or contrast scores (outer colour: P's>0.21; inner colour: P's>0.38, contrast score: P's>0.19).

Contrast assay 3. Responses to contrast-disks were significantly different between male and female *D. suzukii* (paired t-test, t_{48}=-2.37, P=0.02). Green~purple disks were more attractive to female *D. suzukii* than disks with red inner rings (Fig. 4c; ANOVA, $F_{4,44}$=6.07, P<0.001; Tukey

HSD black~red, $P<0.01$ and green~red, $P<0.001$). No significant differences in preference were observed for male *D. suzukii* (ANOVA, $F_{4,44}=0.82$, $P=0.52$). Disks with green outer rings were more attractive than disks with black outer rings, although not significantly so for male *D. suzukii* (ANOVA, Females: $F_{1,47}=4.09$, $P=0.05$; Males: $F_{1,47}=3.31$, $P=0.08$). Disks with red inner rings were significantly less attractive to females than disks with purple or yellow inner rings (ANOVA, $F_{3,45}=8.06$, $P<0.001$: Tukey HSD, purple: $P<0.001$ and yellow: $P=0.03$); however, no significant differences were observed for male *D. suzukii* (ANOVA, $F_{3,45}=0.68$, $P=0.57$). Disk colours of adjacent disks were not associated with any significant differences in preferences for either sex (ANOVA, Females: $F_{4,93}=0.39$, $P=0.82$; Males: $F_{4,93}=0.90$, $P=0.47$).

Contrast assay 4. Responses to coloured disks were different between sexes (paired t-test, $t_{39}=-3.10$, $p<0.005$); therefore, results were calculated separately for each sex. Disks with a green outer ring and purple inner ring (green~purple) attracted higher numbers of both male and female *D. suzukii* over black disks or disks combining red and black (Fig. 3.2.4d).

Contrast assay 5. In two-choice assays between black~red disks and green~purple disks, responses were not different between sexes (paired t-test, $t_1=0.-68$, $p=0.62$); therefore, results for both sexes have been combined. Both male and female *D. suzukii* were attracted in higher numbers to green~purple disks than black~red disks (Fig. 3.2.4e [top bar]).

In two-choice assays between green~purple disks and yellow~blue disks, responses were not different between sexes (paired t-test, $t_1=0.-83$, $p=0.56$); therefore, results for both sexes have been combined. Both male and female *D. suzukii* were attracted in higher numbers to green~purple disks than yellow~blue disks (Fig. 3.2.4e [bottom bar]).

Analysis of the colour spectra for colour used in choice assays shows that black cardstock had characteristically low reflectance at all wavelengths (Fig. 3.2.5). Red cardstock showed comparatively more reflectance at 585 nm and 645 nm wavelengths (yellow and red) than at 470 nm and 560 nm wavelengths (blue and green). The center red portion of black~red disks reflects more light at all wavelengths than the outer black portion of the disk (Fig. 3.2.5).

Yellow cardstock had high but comparatively equal reflectance values at 560 nm and 645 nm wavelengths (green and red), but higher reflectance at 585 nm (yellow) than at 470 nm (blue) wavelengths (Fig. 3.2.5). Reflectance was proportionately equal between 560 nm (green) and 645 nm (red) for blue cardstock, but relatively higher at 470 nm (blue) than at 585 nm (yellow). The outer yellow portion of yellow~blue disks reflected more light at all wavelengths except blue compared to the inner blue portion of the disks (Fig. 3.2.5).

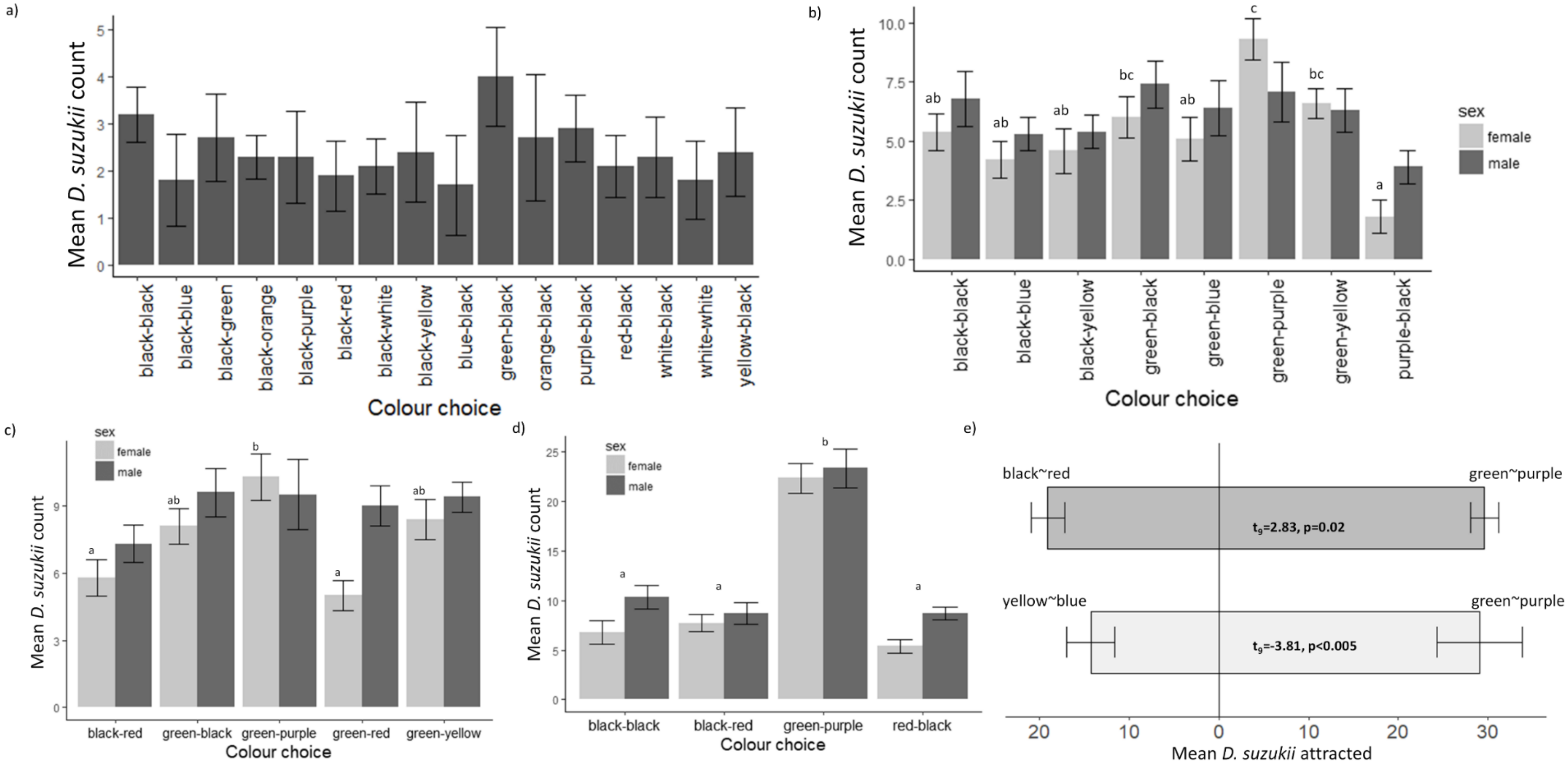


Figure 3.2.4. a) Mean count (± SE) of *D. suzukii* attracted to coloured disks in multi-choice trials (contrast assay 1). Results shown are pooled responses of male and female *D. suzukii*. b) Mean count (± SE) of *D. suzukii* attracted to coloured disks in multi-choice trials (contrast assay 2). The first colour in each pair represents the outer ring colour and the second represents the inner colour ring. c) Mean count (± SE) of *D. suzukii* attracted to coloured disks in multi-choice trials (contrast assay 3). d) Mean count (± SE) of *D. suzukii* attracted to coloured disks in multi-choice trials (contrast assay 4). Different letters represent statistically significant differences between contrast disks for each sex (Tukey Post-Hoc, P<0.05) in assays 2, 3, and 4. e) Mean count (± SE) of *D. suzukii* attracted to coloured disks in 2-choice trials. Results of black~red versus green~purple disks (contrast assay 5a) are shown in the top bar and results of yellow~blue versus green~purple disks (contrast assay 5b) are shown in the bottom bar. Results of paired t-tests are shown within the figure (significant differences are in bold).

Green cardstock reflected relatively more light at 560 nm (green) than at 645 nm (red) (Fig. 3.2.5). Reflectance values were higher at 585 nm (yellow) than at 470 nm (blue). Purple cardstock reflected more light at 645 nm (red) than at 560 nm (green) and reflected more light at 470 nm (blue) than at 585 (yellow). Higher reflectance at 560 nm (green) of the outer green portion of green~purple disks contrasted with the higher reflectance at 645 nm (red) of the purple portion of the disk. In addition, higher reflectance at 585 nm (yellow) in the green portion of the green~purple disks contrasted with higher reflectance at 470 nm (blue) in the purple portion (Fig. 3.2.5).

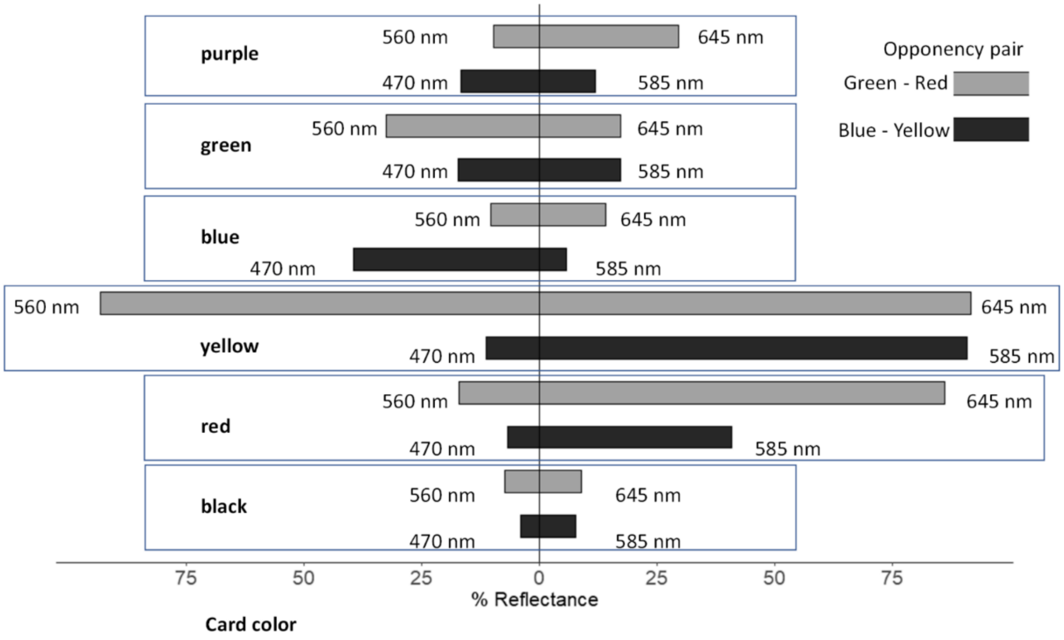


Figure 3.2.5. Comparison of the reflectance spectra for outer and inner card-stock colours of a) black-red, b) yellow-blue, and c) green-purple contrast disks used in 2-choice assays.

3.2.5. Discussion

The spectral sensitivity range of invertebrates differs from that of humans. This study reinforces the principle that research into the sensory discrimination and preferences of non-human species must use objective measures, and not measures that are biased by human perceptions or based on colour appearance based on human perception. Spectral sensitivity experiments demonstrate that colour discrimination by dipterans may be limited to just four broad colour categories: ultraviolet, purple, blue, and green (Troje 1993; Lunau 2014). In general terms, Dipteran spectral sensitivity would limit colour discrimination to wavelengths less than 600 nm (Hardie 1979). Colour recognition and preference have been noted in *Drosophila melanogaster* with strong colour discrimination and sensitivity occurring between 406 nm and 505 nm (Menne and Spatz 1977; Hardie 1979; Hernández de Salomon and Spatz 1983; Heisenberg and Wolf 1984; Salcedo et al. 1999; Washington 2010; Marcus et al. 2018).

Most *Drosophila* species are differentially sensitive to ultraviolet, blues, and greens (Bertholf 1932; Tang and Guo 2001; Yamaguchi et al. 2010; Kelber and Henze 2013; Paulk et al. 2013). Sensitivity drops rapidly at longer wavelengths, with up to 25 times less sensitivity at 606 nm than at 505 nm (Hernández de Salomon and Spatz 1983). The compound eye of *D. melanogaster* contains eight different photoreceptors expressing five spectrally distinct types of opsins (Schnaitmann et al. 2013). In *D. melanogaster*, inner photoreceptors R7 and R8 are sufficient to distinguish between blue and green and provide limited colour discrimination over a wider range (Schnaitmann et al. 2013). Four types of opsins are expressed on the inner photoreceptors R7 and R8. At the eye margin, both R7 and R8 express opsin Rh3, sensitive to ultraviolet. Elsewhere in the eye, R7 and R8 photoreceptors come in two forms, pale (p) and yellow (y). In pale forms, R7 expresses Rh3 (ultraviolet) and R8 expresses Rh5 (blue). In yellow forms, R7 expresses Rh4 (longer UV wavelengths) and R8 expresses Rh6 (green) (Yamaguchi et al. 2010). However, broader spectrum colour discrimination requires input from outer photoreceptors (photoreceptors

R1-R6), expanding visual sensitivity range to between 306 nm and 540 nm. Photoreceptors R1-R6 express the same type of opsin (Rh1), which is broadly tuned to blue and ultraviolet light. These outer photoreceptors are critical for motion detection and vision under low light conditions. Distinction of colour by *D. melanogaster* requires stimulation of two or more photoreceptors of different spectral sensitivities; however, all photoreceptors in the *D. melanogaster* eye are selectively tuned to the ultraviolet to green, effectively limiting colour vision to the shorter wavelengths. Thus, colour vision in *D. melanogaster* occurs via "interommatidial'' opponency photoreceptors (i.e., Rh3-Rh4 and Rh5-Rh6 in R7 and R8) and a possible additional opponency dimension from outer photoreceptors (Rh1 in R1-R6 interacting with Rh4 in R7) which serve to enhance colour discrimination (Schnaitmann et al. 2013). Although most long-wavelength light is reflected by the *D. melanogaster* eye, small amounts of red light can enter the eye at an oblique angle to re-sensitize photosensitive pigments enabling increased sensitivity to ultraviolet (Minke and Kirschfeld 1979; Lunau 2014). Even accounting for the shift in spectral sensitivity due to retinal pigments, *D. melanogaster* are not able to distinguish distinct colours above 600 nm (Vogt et al. 1982). Therefore, it is unsurprising that *D. melanogaster* phototactic behaviour reflects a preference for short wavelength (UV to green) light over long wavelength (red) light by almost 2 orders of magnitude (Gao et al. 2008).

Previous studies on *D. suzukii* attraction to colour have gauged behavioural responses to single colours in choice assays against a uniform white or black background (Lee et al. 2012, 2013; Kirkpatrick et al. 2016). However, we find that the physiological responses of *D. suzukii* to coloured light (strong responses to blue and green, and a weak response to red) are consistent with previous findings that *Drosophila* species perceive red poorly relative to other colours (Menne and Spatz 1977; Hernández de Salomon and Spatz 1983; Heisenberg and Wolf 1984; Dolph et al. 2011; Kelber and Henze 2013). We observed greater sensitivity in *D. suzukii* at the shorter wavelength range (blue-green) of the spectrum than at longer wavelengths (red). It has been suggested that *Drosophila* species perceive red as something akin to a dull green or yellow-green (Chittka and Raine 2006; Glover and Whitney 2010). Thus, attraction of *D. suzukii* to red may be in response to darkness, iridescence, or ultraviolet reflectance rather than colour and does not imply that the colour that we perceive as red is visible to the fly (Glover and Whitney 2010; Lunau 2014).

As in other studies, we found that *D. suzukii* were attracted to red, black, and yellow targets (Lee et al. 2012, 2013; Iglesias et al. 2014; Renkema et al. 2014). However, the attraction to single colour targets was correlated with reflectance at short wavelengths (blue [470 nm] and green [560 nm]), rather than the overall colour that we perceive. We also found strong attraction to green targets that was comparable to responses to red targets. Given the lack of visual sensitivity and visual discrimination at longer wavelengths (red [645 nm]) by *D. suzukii*, the common practice of pairing red and black results in decreased attractiveness. We found that colour combinations pairing green as a background colour against other colours within the optimal sensitivity range of *Drosophila* species resulted in higher attractiveness. This is consistent with naturally occurring conditions for host-finding, where potential host fruits of various colours would normally be near, typically, green foliage.

The colour opponency model suggests that opposing values between blue and yellow and between green and red are important to colour discrimination. Thus, visual contrast is emphasized by pairing a shorter and longer wavelength as a binary system within each type of visual receptor neuron, such that each neuron can signal in response to only one of the two opposing colour stimuli, not both, and that excitatory stimulation from one wavelength might be inhibitory to signals for the opposing wavelength colour (Kien and Menzel 1977; Fairchild 2005; Schnaitmann et al. 2013; Song and Lee 2018). The green~purple colour pairing preferred by *D. suzukii* in our experiments exploits this colour opposition. The outer green portion has higher reflectance values at green (560 nm) than at red (645 nm), while having lower reflectance at blue (470 nm) than at yellow (585 nm). In comparison, the inner purple portion has lower reflectance values at green than at red, while having higher reflectance at blue than at yellow (Fig. 3.2.5). Consequently, green~purple should appear as high contrast and a strong visual cue for *D. suzukii*. Colour discrimination could be further improved by refining colour choice so that peak reflectance at blue vs. yellow opposes reflectance at green vs. red in each colour. Contrast could be further refined by ensuring the colour opposition pattern of inner and outer portions of the contrast disks are the reverse of each other.

For both feeding and oviposition, *D. suzukii* must locate small ripening fruits and berries of various colours within a background of predominantly green foliage. We have previously demonstrated that *D. suzukii* use contrast in colour between ripening fruits and surrounding foliage to identify suitable host fruits (Little et al. 2018). While olfactory cues are the primary driver of host-finding behaviour in many Drosophila species and thought to be the primary driver in *D. suzukii*, we have presented evidence to suggest that colour can play a significant role in host-finding and potentially other behaviours (Becher et al. 2012; Yu et al. 2013; Sachse and Beshel 2016; Dweck et al. 2018; Keesey et al. 2019). Differences in reflectance within opponent colour pairs (green vs. red and blue vs. yellow) contributes to colour discrimination in *D. suzukii* and these differences promote host-finding through contrast between foreground (fruit) and background (foliage) colours.

3.2.6. Acknowledgements

We gratefully acknowledge the advice and suggestions of D. Moreau and members of the Acadian Entomological Society. Funding was provided by Atlantic Canada Opportunities Agency

Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21516-519, GF21617-773, and GF21718-956).

Data availability

Data has been stored as open access at: https://doi.org/10.5061/dryad.vhhmgqnpn.

3.2.7. References

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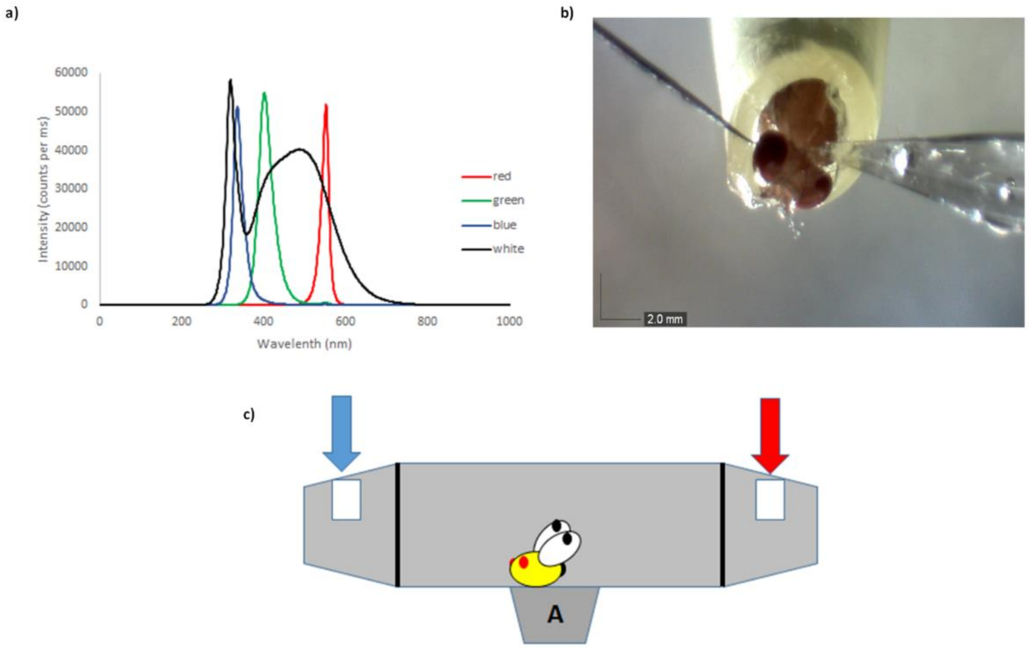
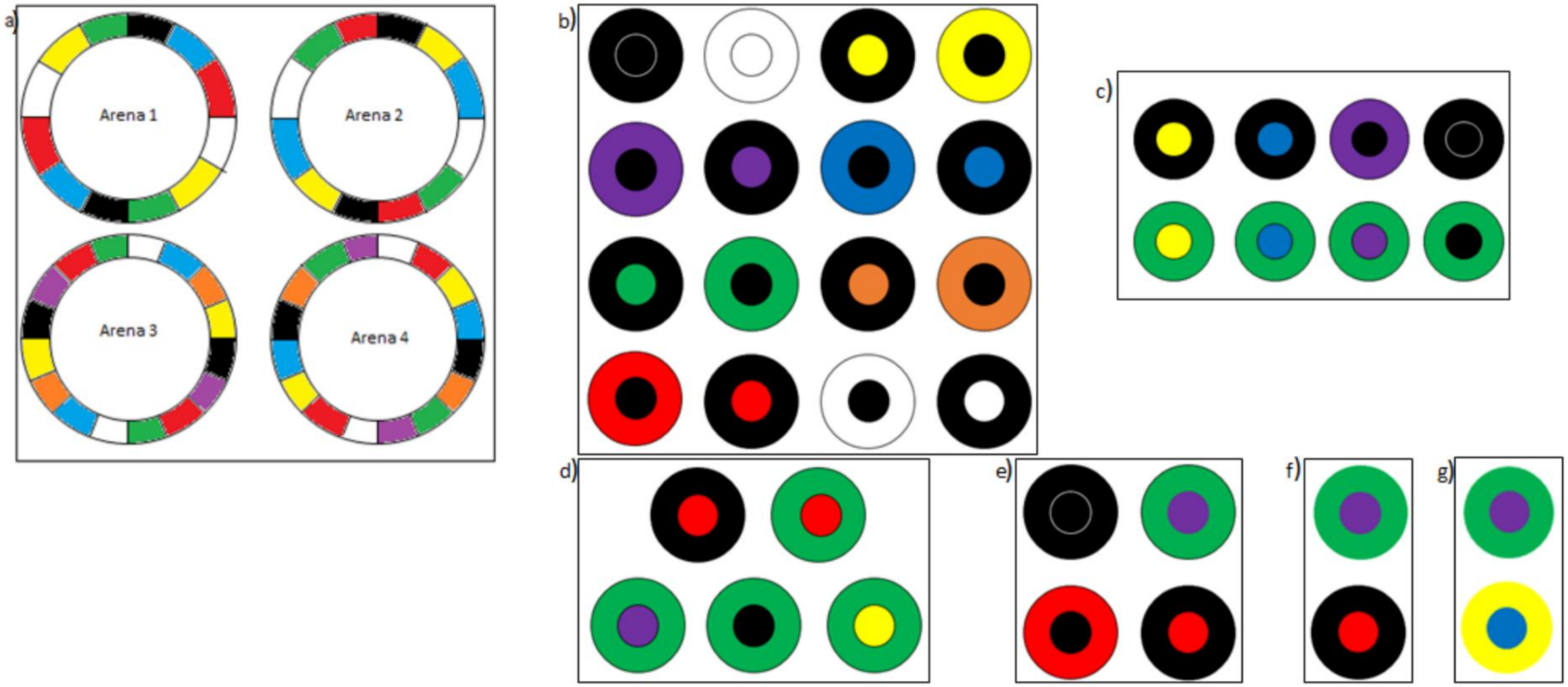
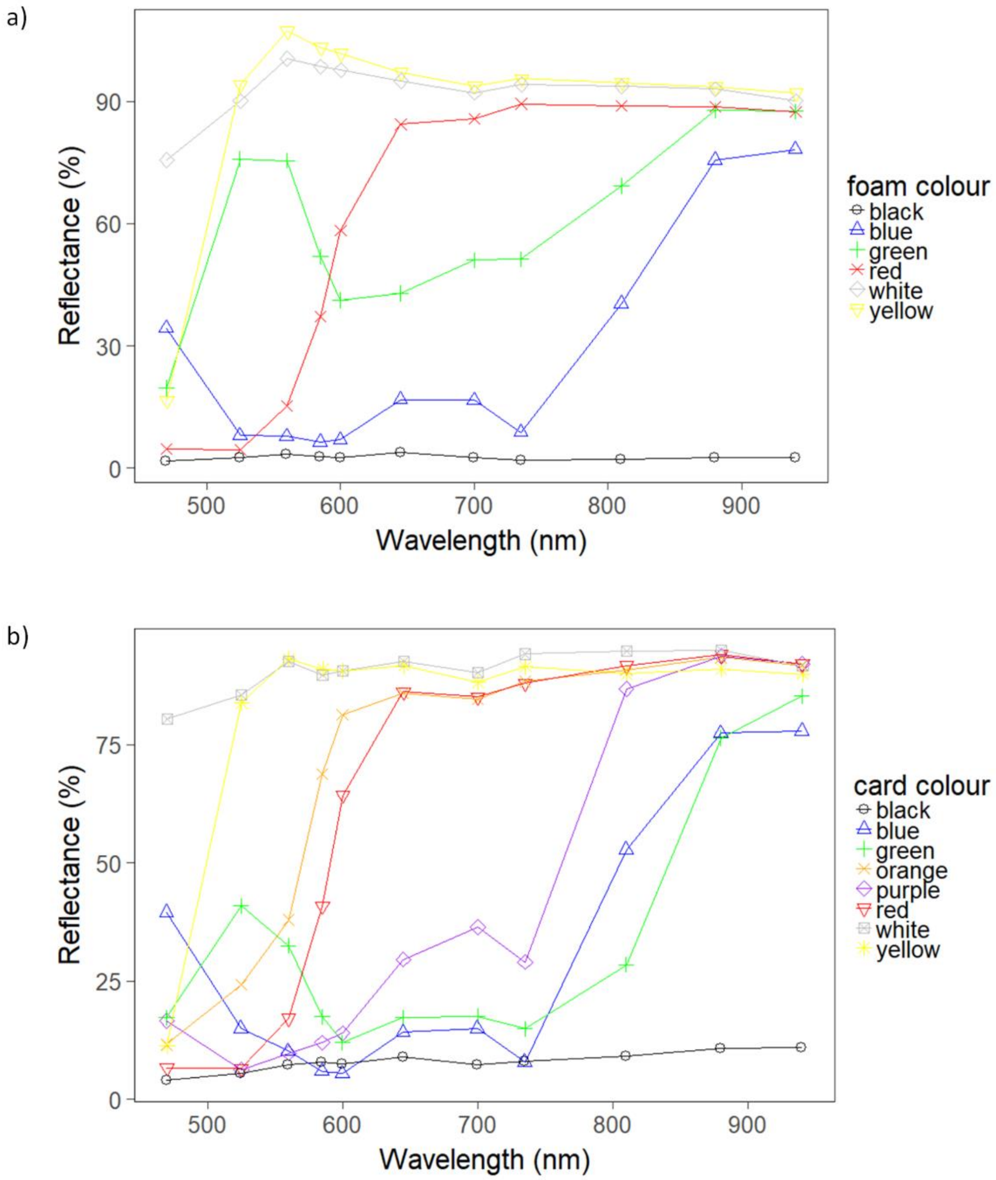
3.2.8 Supplementary information



Supplementary Figure S3.2.1. a) Mean spectra were calculated from measured wavelength emissions of blue, green, red, and white lights emitted from BeeWi SmartLite® bulb. b) A ground electrode comprised of a glass electrode containing a tungsten wire filament and insect saline was inserted at the base of the *Drosophila*'s head. A sharpened tungsten wire probe was inserted into the Drosophila's right eye at a 90° angle to act as the recording electrode. c) Arena set up for 2-choice light attraction assay (Diagram not to scale). *D. suzukii* were released into center of arena (position A). Light was directed perpendicularly into the arena through clear ports at either end of the arena. *Drosophila suzukii* attracted to the lights were trapped on Tangle-Trap coated clear plastic (black lines).



Supplementary Figure S3.2.2. a) Setup of choice assays used to determine colour preferences. *Drosophila suzukii* were released into arenas with six different colours of foam board (arenas 1 & 2) and with eight different colours of card stock (arena 3 & 4). Two-colour disks were hung in random order equidistant from the center of a 30 x 30 x 30 cm plastic and mesh cage. Disks in all choice assays were 5 cm in diameter with 2.5 cm diameter centers. b) Each of the eight colours of card-stock were used in combination with black for contrast. c) Two-colour disks comprised of black, green, blue, purple, and yellow were used for a second round of contrasting colour choice assays. d) A third round of multi-choice contrast assays consisted of four green disks with centers of black, purple, red, and yellow and one black disk with a red center. e) Green disks with purple centers were tested against disks of black and red, the most commonly used colours for *D. suzukii* traps, and against black disks. Two-choice assays paired f) green~purple disks against black~red disks and g) green~purple disks against yellow~blue disks.



Supplementary Figure S3.2.3. Reflectance spectra for each colour used in choice assays were measured. a) Six colours of foam board were used in multi-choice assays. b) Eight colours of card stock were used in multi-choice and 2-choice assays.

Chapter 4

Odorants and olfactory cues

4.1 Assessment of attractant lures and monitoring traps for *Drosophila suzukii* (Diptera: Drosophidae) using electrophysiology, laboratory choice assays, and field trials.

A version of this chapter has been submitted for publication:
Little, C.M., Dixon, P.L., Moreau, D.L., Chapman, T.W., and Hillier, N.K. 2021. Assessment of attractant lures and monitoring traps for *Drosophila suzukii* (Diptera: Drosophidae) using electrophysiology, laboratory choice assays, and field trials. J Econ Entomol: toab006. doi:10.1093/jee/toab006.

Author Contribution Statement

CL, and NH designed research. PD, DM, and TC advised on methodology. CL, PD, and DM conducted research. CL analyzed data and prepared manuscript. NH, PD, DM, and TC edited manuscript. All authors read and approved manuscript.

4.1.1 Abstract

Monitoring is critical to control efforts for *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive polyphagous fly that has the potential to cause significant losses in commercial soft fruit and berry production worldwide. We used an iterative process to identify trap colours, trap designs, and volatile mixtures to improve monitoring efforts in commercial blueberry, raspberry, and blackberry crops. Our results suggest that the selection of trap colour and design and attractant lures should be customized to the crop in which they are deployed. In raspberries grown in high tunnel systems, DrosaLure® paired with Drosal® traps painted green and purple were highly specific to *D. suzukii* although actual capture counts were low. However, in field grown raspberries, BioLure® and Multilure traps were most effective, but with significant non-target bycatch. In blueberries, we had greatest success with a 5µg:50ng mixture of ethyl acetate–acetoin in a green/purple coloured jar-style trap with large (5 cm) mesh covered openings.

4.1.2. Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), commonly known as spotted wing Drosophila, was first described in Japan (Matsumura 1931) but is believed to have originated in mainland Asia (Hauser 2011, Calabria et al. 2012, Cini et al. 2014, Asplen et al. 2015). Shortly thereafter, it was identified as the source of damage in fruit crops in Japan (Kanzawa 1935, 1939). Established populations of *D. suzukii* have been reported in Hawaii since the early 1980's (Kaneshiro 1983). Since 2008, invasive populations of *D. suzukii* have been confirmed throughout most of North America and Europe, and, more recently, in parts of South America (Hauser 2011, Calabria et al. 2012, Thistlewood et al. 2012, Saguez et al. 2013, Cini et al. 2014, Deprá et al. 2014, Asplen et al. 2015, Lasa and Tadeo 2015, Andreazza et al. 2017, dos Santos et al. 2017). The heavily serrated ovipositor of female *D. suzukii* allows it to lay its eggs in ripening soft fruits and berries. Fruit producers are facing increased costs estimated in the millions of United States dollars annually, due to unmarketable *D. suzukii* damaged fruit and increasing mitigation costs, such as integrated pest management and post-harvest treatments (Bolda et al. 2010, Walsh et al. 2011, Follett et al. 2014, Farnsworth et al. 2017, Mazzi et al. 2017). Effective monitoring traps can improve fruit growers' efforts to target *D. suzukii* populations and minimize impact of associated insecticidal treatments on non-target species. However, monitoring traps currently in use are estimated to capture as few as 10-30% of *D. suzukii* which encounter the traps (Hampton et al. 2014). In addition, current monitoring systems capture significant numbers of non-target insects, particularly other *Drosophila* species, as bycatch (Basoalto et al. 2013, Cha et al. 2013, Burrack et al. 2015, Jaffe et al. 2018, Cloonan et al. 2019).

Drosophila suzukii can use a wide range of host plant species for feeding and laying its eggs and flies are thought to use multiple sensory cues to identify host fruits, including fruit and foliage odors, visual cues, and physical characteristics of fruits (Lee et al. 2011, 2015, Keesey et al. 2015, Little et al. 2017, 2018, Cloonan et al. 2018). The majority of *D. suzukii* monitoring traps currently in use are some combination of red, black, and clear. We had previously found that a combination of green and purple was more attractive in a laboratory setting. This study investigated using visual cues, in particular trap colours, and olfactory cues, specifically odorant compounds and mixtures, to improve monitoring trap efficacy. We used an iterative process over a span of five years to develop an attractant lure mixture comprised of odorant compounds for use in apple cider vinegar-baited traps. We tested odorants and odorant mixtures associated with fruits, foliage, and odorants known to be behaviourally active in *D. melanogaster* Meigen (Diptera: Drosophilidae), using a combination of laboratory assays and field trials to narrow the scope of attractant cues. Building on previous studies, we also used an iterative process to test novel trap colours (Little et al. 2019) and alternative trap designs (Leblanc et al. 2009, Renkema et al. 2014) to improve trapping efficacy. Results of physiological and behavioural assays, and field trials from each year informed changes to attractant lures and trap designs for subsequent years. Our goal was to develop a trap design and attractant lure that improved *D. suzukii* capture numbers, reduced bycatch, and was reliable across a variety of fruit crop systems.

4.1.3. Materials and methods

Source of insects

Drosophila suzukii flies used for all laboratory studies were obtained from managed colonies maintained in a quarantine facility at Acadia University, Wolfville, Nova Scotia (Little et al. 2020a). Colonies were maintained on a diet of 50-ml Formula 4‑24 Instant Drosophila medium (Merlan Scientific Ltd., Mississauga, ON, Canada) and 50-ml dH2O. in 250-ml *Drosophila* flasks (Genesee Scientific, San Diego, CA). Fly colonies were maintained, and laboratory choice assays were conducted at 25+/-4ºC with 45-50% relative humidity and photoperiod of 14:10 light : dark cycle. Each fly was used in a single trial of a single experiment and discarded.

2014

Electroantennography

Preliminary electroantennogram screening of fruit and plant associated odors was conducted in 2012-2013 (results not shown). Follow-up behavioural assays reported here were conducted using a modified FlyWalk setup based on Steck et al. (2012) (results not shown). Promising candidate odorants from these screening trials were included for continued assessment in 2014. We selected primary component compounds of BioLure® (putrescine and trimethylamine hydrochloride) and additional compounds for which other *Drosophila* species have odorant receptors or exhibit physiological or behavioural responses, and for relevance to fruit ripening and fermentation (Table S4.1.1). Individual compounds were tested for physiological activity in *D. suzukii* using electroantennography (Table S4.1.1).

For each run, stimulus cartridges were made at one of three discrete doses for each of 56 compounds and two or more stimulus cartridges of each solvent (ethanol, hexane, and methanol) or controls (air puff and blank stimulus cartridge) were tested. Stimulus cartridges were prepared for doses of 10 ng (0.01 µg), 1 µg, and 100 µg. For each stimulus cartridge, 10 µl of compound diluted to one decade-step below the required dose was applied via micropipette to a 1 cm x 5 cm strip of filter paper and inserted into a glass Pasteur pipet. Stimulus cartridges were wrapped in aluminum foil and stored at -20ºC until use. Stimulus cartridges were replaced every five runs. Thirteen to fifteen runs were completed with a single naïve fly from each treatment group (male or female, virgin or mated fly) at each dosage. Runs in which electroantennogram signals were unstable were excluded.

The best 12 complete runs were analyzed for each of virgin and mated female flies. Male flies were more prone to desiccation and were more likely to die during electroantennogram runs. This was consistent with other studies which found that male *D. suzukii* are less tolerant of desiccation than female flies (Tochen et al. 2016, Terhzaz et al. 2018). We analyzed 11 complete runs for virgin male flies and 6 complete runs for mated male flies.

Laboratory two-choice trials

Electroantennogram results were assessed to identify potential attractant compounds. Given the number of compounds tested using electroantennography, we selected compounds for further study based on strongest physiological responses for each treatment group (virgin or mated, male or female flies) at each dosage (Table 4.1.1). We selected 22 compounds for female *D. suzukii* and 16 compounds for male *D. suzukii* to confirm relative attractiveness versus their respective solvents (Table 4.1.1, Table S4.1.1). Male and female flies were tested separately. Arenas were constructed from 600ml plastic food containers (16 cm x 11 cm x 6 cm) (Plastico®, China). Compounds (1 µg = 10 µl x 100 ηg/µl) and solvents (10 µl) were applied to individual 1 cm x 3 cm filter paper strips, which were placed in 30 ml lidded portion cups. A truncated 1-200 µl pipet tip was inserted through the portion cup lid to make a one-way entrance (Little et al. 2017). Compound and control 'traps' were placed in opposite corners of arenas. A moistened 2 cm x 2 cm square of paper towel was placed in the centre of each arena to control humidity. Fifteen flies aged approximately 2 weeks were placed in the centre area of each arena. Flies in each 'trap' and in the remaining area of each arena were counted after 48 h. Each trial was replicated seven times.

Table 4.1.1. Compounds selected for 2014 choice trials based on 2014 electroantennography results.

	virgin flies			**mated flies**		
Compound	**10ng**	**1ug**	**100ug**	**10ng**	**1ug**	**100ug**
A) Female *D. suzukii*						
2 methyl butanoic acid	top 10				top 20	
2,3 butanedione		top 10			top 5	
2-heptanol				top 5	top 20	
2-phenyl ethanol	top 5			top 5		top 20
acetoin	top 5	top 20		top 5		top 20
acetyl furan (2-furyl methyl ketone)			top 5	top 20	top 5	
benzaldehyde	top 20				top 5	
butyric acid (butanoic acid)				top 5	top 10	top 20
ethyl 3-hydroxyhexanoate				top 10	top 20	top 10
ethyl hexanoate			top 20			top 5
furfural (2-furaldehyde)	top 20		top 20		top 10	top 5
geranyl acetone	top 20	top 20	top 20		top 5	top 10
hexanal		top 20	top 20		top 20	top 5
hexanoic acid			top 20		top 5	
isoamyl acetate	top 20			top 20		
methyl salicylate						top 5
nonanone			top 20			top 10
phenylethylamine	top 5		top 10	top 5		top 5
propanol		top 10	top 10	top 10		
putrescine (1,4 diaminobutane)	top 5	top 5	top 5	top 10		top 10
trimethylamine hydrochloride	top 20	top 10	top 20	top 20		top 20
ursolic acid	top 20	top 5	top 5	top 10	top 10	top 20
B) Male *D. suzukii*						
1-hexanol	top 5			top 20		
1-octanol			top 5	top 20		
2-phenyl ethanol	top 10			top 20		
acetyl furan (2-furyl methyl ketone)	top 10					top 5
benzaldehyde	top 5	top 5	top 10			top 20
benzyl acetate	top 10			top 5	top 10	top 5

geranyl acetone	top 10	top 20		top 10	top 10	top 5
heptacosane		top 5				
isoamyl alcohol	top 5		top 20		top 5	top 10
methyl salicylate		top 10	top 5			top 10
nonanol	top 5	top 10			top 5	top 10
phenylethylamine	top 10	top 20	top 20	top 10		top 20
propionic acid		top 10				
putrescine (1,4 diaminobutane)	top 20	top 20	top 5	top 5	top 20	top 20
trimethylamine hydrochloride	top 5	top 20	top 20	top 20	top 10	
ursolic acid	top 20	top 20	top 20	top 10	top 5	top 5

Field trials

Unless stated otherwise, contents of traps, including lures, sticky cards (if any), and drowning solutions, were checked and replaced weekly. Captured insects were collected weekly for counting. Field trials in Nova Scotia were conducted independently; however, field trials in Newfoundland were conducted in conjunction and cooperation with on-going monitoring by Agriculture and Agri-Food Canada (AAFC, St. John's Research and Development Centre). Drowning solutions used in traps in Nova Scotia consisted of 100 ml apple cider vinegar with unscented dish soap as a surfactant (5 ml soap : 4 l vinegar), or 100 ml water with unscented dish soap (5 ml soap : 4 l vinegar), or 100 ml Drosolure. Traps deployed in Newfoundland used a drowning solution of 100 ml apple cider vinegar with ethanol (5 ml ethanol : 4 l vinegar). Yeast lures deployed in Newfoundland control traps consisted of 5 ml *Saccharomyces cerevisiae*, 5 ml sugar, and 10 ml water in a Falcon tube with a mesh lid held upright within the solo cup trap. Attractant compounds and mixtures used as lures were loaded into rubber septa which were then suspended within the traps.

Traps deployed in 100 m long raspberry tunnels at a commercial farm in Nova Scotia trials were hung within the plant canopy using support lines at a height of 1.5 m. All other traps deployed in Nova Scotia were hung at height of 85 cm from a pigtail fencepost (Gallagher Animal Management Systems, Owen Sound, Canada) that were placed within the plant structures. Traps were deployed in a similar fashion in Newfoundland using wooden stakes at a height of 90 cm. Traps deployed in Nova Scotia field trials were rotated weekly within each block. Traps deployed in Newfoundland were stationary through each trial.

Nova Scotia attractant trials

Based on results of preliminary laboratory assays and field trials conducted in 2013 (results not shown), we selected benzyl acetate for further field trials as a potential attractant. We selected five additional potential attractant compounds (acetoin, benzaldehyde, furfural, methyl salicylate, and ursolic acid) for field testing based on results of 2014 electroantennography and 2-choice assays (Table 4.1.2). Methyl salicylate elicited strong physiological responses in *D. suzukii* and other Drosophila species and has been suggested as a potentially important attractant for *D. suzukii* (Figure S4.1.1) (de Bruyne et al. 2001, Hallem et al. 2004, Walsh et al. 2011, Revadi 2015).We also tested a commercially available lure mixture (BioLure®, Suterra LLC, Inc., Bend, OR) that had been reported as an effective lure for *D. suzukii* in Hawaii (Leblanc et al. 2009, 2010). All compounds were field tested in 16-ounce (473.18 ml) red/black Solo® cup traps (Solo Cup Company, Urbana, IL) with cover plates providing shade (Figure S4.1.1a, Table S4.1.2) (Leblanc et al. 2009). A 7.5 cm x 12.5 cm yellow sticky card was placed inside each trap. A yellow visual stimulus within each trap had been proposed to improve attraction of *D. suzukii* (Burrack et al. 2012, Iglesias et al. 2014). Each trap contained 100 ml of apple cider vinegar as drowning solution. Three replicates of each compound and control traps were deployed at each test site, including a 34 hectare commercial highbush blueberry (*Vaccinium corymbosum* L.

(Ericales: Ericaceae)) field, a 4 hectare commercial high tunnel raspberry (*Rubus idaeus* L. (Rosales: Rosaceae)) farm, and a 1 hectare research field of mixed highbush and lowbush blueberry species (*Vaccinium* spp. (Ericales: Ericaceae)).

Table 4.1.2. Response index values and results of paired t-tests for 2-choice assays of prospective attractant odorants and their respective solvents conducted in 2014. Positive RI value denotes compound is more attractive than its respective solvent. Significant differences ($P \leq 0.05$) shown in bold.

	female *D. suzukii*				**male *D. suzukii***				**field**
Compounds	**mean RI**	**t**	**df**	**p**	**mean RI**	**t**	**df**	**p**	**test 2014**
1 octanol					0.05	0.59	10	0.57	
1 hexanol					-0.07	-1.06	10	0.31	
2 heptanol	-0.15	-1.13	7	0.30					
2 methyl butanoic acid	-0.06	-0.41	7	0.69					
2 phenyl ethanol	0.00	0.00	8	1.00	0.10	1.10	10	0.30	
2,3 butanedione	0.02	0.33	8	0.75					
acetoin	0.04	0.20	8	0.85					x
acetyl furan	-0.03	-0.20	8	0.85	0.00	0.00	10	1.00	
benzaldehyde	0.17	1.35	9	0.21	0.02	0.27	10	0.79	x
benzyl acetate					0.00	0.00	10	1.00	x
butyric acid	-0.07	-0.09	9	0.93					
ethyl hexanoate	0.19	1.28	9	0.23					
ethyl-3-hydroxyhexanoate	0.06	0.38	8	0.71					
furfural	0.11	0.88	8	0.40					x
geranyl acetone	-0.04	0.00	8	1.00	0.05	0.67	10	0.52	
heptacosane					-0.07	-0.73	10	0.48	
hexanal	0.23	1.30	8	0.23					
hexanoic acid	-0.06	-0.26	8	0.80					
isoamyl acetate	-0.15	-0.82	7	0.44					
isoamyl alcohol					**-0.20**	**-2.27**	**10**	**0.05**	
methyl salicylate	-0.06	-0.64	8	0.54	-0.12	-1.36	10	0.22	x
nonanol					0.01	0.17	10	0.87	
nonanone	-0.24	-2.01	9	0.08					
phenylethylamine	**0.44**	**5.84**	**8**	**0.0004**	**0.29**	**2.52**	**10**	**0.03**	
propanoic acid					-0.05	-0.55	10	0.59	
propanol	-0.21	-1.08	8	0.31					

putrescine	0.12	1.02	8	0.34	0.10	1.80	10	0.10	
trimethylamine hydrochloride	-0.14	-1.72	7	0.13	0.07	1.07	10	0.31	
ursolic acid	0.09	0.56	7	0.59	0.05	1.34	10	0.21	x

Figure 4.1.1. a-d) Trap designs field tested in 2014. b & e-f) Trap designs field tested in 2015. g-i) Trap designs field tested in 2016. g & j-l) Trap designs tested in laboratory in 2017. l-m) Trap designs field tested in 2017. l-o) Trap designs field tested in 2018.

Nova Scotia trap design trials

A variety of trap designs were in use for monitoring *D. suzukii* in Europe, the United States, and Canada; however, they could be categorized in into three broad groups: cup traps, jar traps, and dome traps (Landolt et al. 2012, Lee et al. 2012, 2013, Basoalto et al. 2013, Renkema et al. 2014). Four trap designs were field tested using two potential attractant compounds (furfural and ursolic acid), a commercially available lure mixture (BioLure®), and a control with no lure (Table S4.1.2). Red/black Solo cup traps (unshaded) based on Moreau et al. (2013, red/black Solo® cup traps with a cover to deflect rain and provide shade (shaded) based on Lee et al. (2013) and Renkema et al. (2014), and yellow/clear Multilure (modified McPhail) traps (Better World Manufacturing, Fresno, CA) based on Leblanc et al. (2010), hereafter referred to as MultiTraps, were deployed in heritage raspberries and two highbush blueberry sites at a 18 hectare commercial fruit farm on 11 September 2014 (Figure 4.1.1a-c). Unshaded Solo® cup traps were replaced with 473 ml jar style traps based on Renkema et al. (2014) on 18 September 2014 (Figure 4.1.1d). Therefore, trap captures in week 1 were analysed separately from weeks 2-6. Two replicates of each trap/lure combination were deployed at each site. A 7.5 cm x 12.5 cm yellow sticky card and 100 ml of apple cider vinegar were placed inside each trap based on Iglesias et al. (2014). All traps were checked, and sticky card, lures, and vinegar were replaced, weekly until 21 October 2014.

2015

Laboratory two-choice trials

We conducted 2-choice assays in four steps to assess relative attractiveness of prospective attractant lure compounds and mixtures (Table S4.1.2). In each trial, 15 flies were transferred to an arena (as above) for each assay. Male and female flies were tested separately, and results were combined for analysis. Assays of male flies were checked after 48h and female flies after 72h, except in step 4 where all assays were checked after 48h.

Step 1.

Leblanc et al. (2009, 2010) found significant attraction of *D. suzukii* to BioLure® traps in forested areas of Hawaii. We used two-choice assays to compare relative attraction of *D. suzukii* to BioLure® primary components (putrescine and trimethylamine hydrochloride) (1 µg load [10µl x 100ηg/µl]) or 2-component mixtures (1 µg load [5µl x 100ηg/µl of each component compound]). We conducted 6-7 replicates of each 2-choice trial per sex.

***Step 2*.**

To identify potential attractants that might could minimize bycatch during field trials, two-choice assays were performed to compare relative attraction of *D. suzukii* to 9 potential attractant compounds (1 µg load [10µl x 100ηg/µl]) versus 2-component mixtures containing that compound (1 µg load [5µl x 100ηg/µl of each component compound]) (see Table 4.1.3). Attractant compounds were chosen based on previous results. Furfural, methyl salicylate, and ursolic acid were selected based on prior year's field trial results. Putrescine was selected based on Step 1 of 2015 choice assay results. Phenylethylamine was selected based on 2014 choice assay results. We also selected compounds attractive to related Drosophila species (ethyl acetate, hexyl acetate, and phenylacetaldehyde) based on 2014 electroantennography. We conducted 3 replicates of each of the 72 permutations of 2-choice trials per sex.

Table 4.1.3. In behavioural 2-choice trials conducted in 2015 (step 2), flies were made to choose between 2-compound mixtures and each of the component compounds. Mean *D. suzukii* counts and statistics on the left side of the table shows results of 2-choice trials where component compounds were more attractive than the mixture, whereas on the right side of the table, mixtures were more attractive than the component compound. Paired t-test, df = 5. P values ≤ 0.10 are shown in bold.

	Compound more attractive than mixture					Mixture more attractive than compound				
Mixture	Compound	Mean (mixture)	Mean (compound)	T	P	Compound	Mean (mixture)	Mean (compound)	T	P
ethyl acetate + methyl salicylate	methyl salicylate	1.83	4.50	**-2.46**	**0.06**	ethyl acetate	3.17	2.83	0.15	0.89
ethyl acetate + putrescine	putrescine	3.67	3.83	-0.12	0.91	ethyl acetate	3.33	3.00	0.25	0.81
furfural + ethyl acetate	furfural	2.83	4.17	-0.64	0.55	ethyl acetate	4.00	3.33	0.28	0.79
hexyl acetate + ethyl acetate	hexyl acetate	3.50	4.00	-0.34	0.75	ethyl acetate	4.50	3.50	0.46	0.67
ethyl acetate + phenylethylamine	phenylethylamine	2.33	6.17	-1.53	0.19	ethyl acetate	4.50	2.83	0.92	0.40
ursolic acid + ethyl acetate						ethyl acetate	4.00	3.33	0.93	0.39
						ursolic acid	3.67	3.50	0.08	0.94
phenylacetaldehyde + putrescine	putrescine	2.33	5.00	**-2.39**	**0.06**	phenylacetaldehyde	6.17	2.67	1.71	0.15
cis-3-hexen-1-ol + ethyl acetate	cis-3-hexen-1-ol	3.50	6.50	**-2.02**	**0.10**	ethyl acetate	5.67	4.00	1.04	0.35
phenylacetaldehyde + ethyl acetate						ethyl acetate	6.00	3.67	1.18	0.29
						phenylacetaldehyde	5.83	4.33	0.65	0.55
ursolic acid + furfural	furfural	3.00	4.50	-1.42	0.22					
	ursolic acid	2.83	4.33	-0.87	0.42					
furfural + putrescine	furfural	3.50	6.17	-1.28	0.26					
	putrescine	3.83	4.83	-0.50	0.64					

furfural + hexyl acetate	furfural	2.00	2.50	-1.17	0.30					
	hexyl acetate	2.17	2.67	-0.41	0.70					
furfural + methyl salicylate	furfural	1.83	2.67	-0.96	0.38	methyl salicylate	2.83	2.00	1.05	0.34
phenylacetaldehyde + furfural	furfural	4.17	4.67	-0.37	0.73					
	phenylacetaldehyde	3.83	5.50	-1.07	0.34					
furfural + phenylethylamine	phenylethylamine	3.83	4.00	-0.10	0.93	furfural	4.33	3.83	0.21	0.85
cis-3-hexen-1-ol + furfural						furfural	4.17	3.67	0.36	0.73
						cis-3-hexen-1-ol	4.33	3.33	0.63	0.56
hexyl acetate + phenylethylamine	hexyl acetate	3.00	5.67	-0.84	0.44	phenylethylamine	5.83	3.33	**2.95**	**0.03**
ursolic acid + hexyl acetate	hexyl acetate	3.50	5.83	-0.84	0.44					
	ursolic acid	2.83	4.50	**-2.08**	**0.09**					
hexyl acetate + putrescine						hexyl acetate	4.17	4.17	0.00	1.00
						putrescine	3.67	3.33	0.14	0.89
cis-3-hexen-1-ol + hexyl acetate						hexyl acetate	4.17	4.00	0.12	0.91
						cis-3-hexen-1-ol	4.17	3.33	0.70	0.52
hexyl acetate + methyl salicylate						hexyl acetate	5.67	2.83	0.75	0.49
						methyl salicylate	6.00	3.50	1.56	0.18
phenylacetaldehyde + hexyl acetate	phenylacetaldehyde	3.17	4.50	-0.59	0.58	hexyl acetate	4.33	3.00	1.23	0.27
cis-3-hexen-1-ol + methyl salicylate	methyl salicylate	1.17	2.50	-0.73	0.50					
	cis-3-hexen-1-ol	4.33	5.50	-0.48	0.65					
methyl salicylate + putrescine	methyl salicylate	2.50	3.00	-0.30	0.78					
	putrescine	1.83	2.67	-0.63	0.56					
methyl salicylate + phenylethylamine	phenylethylamine	2.50	4.17	-1.89	0.12	methyl salicylate	2.83	2.67	0.06	0.95
phenylacetaldehyde + methyl salicylate	phenylacetaldehyde	1.83	3.83	-1.31	0.25	methyl salicylate	3.83	3.00	0.38	0.72
ursolic acid + methyl salicylate	ursolic acid	2.50	3.67	-1.23	0.27	methyl salicylate	3.17	2.67	0.45	0.67
phenylacetaldehyde + ursolic acid	phenylacetaldehyde	3.17	5.17	-1.13	0.31	ursolic acid	3.67	3.17	0.30	0.78

phenylacetaldehyde + cis-3-hexen-1-ol	phenylacetaldehyde	4.33	6.17	-0.60	0.57	cis-3-hexen-1-ol	3.50	3.33	0.16	0.88
cis-3-hexen-1-ol + ursolic acid	cis-3-hexen-1-ol	3.17	5.17	-1.17	0.30	ursolic acid	4.83	3.83	0.47	0.66
phenylacetaldehyde + phenylethylamine	phenylacetaldehyde	3.67	3.83	-0.10	0.93	phenylethylamine	3.83	3.00	0.39	0.71
ursolic acid + phenylethylamine						phenylethylamine	2.83	2.33	0.22	0.83
						ursolic acid	2.67	2.00	0.73	0.50
phenylethylamine + putrescine						phenylethylamine	6.83	4.00	1.46	0.20
						putrescine	5.67	3.83	0.79	0.47
cis-3-hexen-1-ol + phenylethylamine						phenylethylamine	4.67	2.83	1.81	0.13
						cis-3-hexen-1-ol	6.67	2.00	**3.16**	**0.03**
cis-3-hexen-1-ol + putrescine	putrescine	3.33	6.33	**-2.09**	**0.09**					
	cis-3-hexen-1-ol	3.83	5.33	-0.83	0.45					
ursolic acid + putrescine						putrescine	4.00	3.50	0.52	0.62
						ursolic acid	4.50	3.00	1.28	0.26

Step 3.

To identify potential attractant lures that are improve attraction of *D. suzukii* in apple cider baited monitoring traps, fourteen of the compounds (phenylacetaldehyde and methyl salicylate) (1 µg load [10µl x 100ηg/µl]) and 2-component mixtures (ethyl acetate + putrescine, furfural + putrescine, hexyl acetate + methyl salicylate, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, ursolic acid + ethyl acetate, ursolic acid + hexyl acetate, cis-3-hexen-1-ol + ethyl acetate, cis-3-hexen-1-ol + phenylethylamine, cis-3-hexen-1-ol + putrescine, and cis-3-hexen-1-ol + ursolic acid) (1 µg load [5µl x 100ηg/µl of each component compound]) which captured the most *D. suzukii* flies from step 2 were tested in combination with apple cider vinegar (10 µl on a separate 1 cm x 3 cm filter paper strip within the same portion cup), commonly used in monitoring traps as a lure and drowning solution, against apple cider vinegar alone. We conducted 3 replicates of each permutation of 2-choice trials per sex.

Step 4.

We had previously observed a strong preference for sea buckthorn (*Hippophae rhamnoides* L. (Rosales: Rosaceae)) fruit during choice trials among soft fruits and berries (Little et al. 2017). Choice assays were conducted using 1 l jar traps (Figure 4.1.1f) in 60 cm X 30 cm X 30 cm mesh enclosure (BugDorm). We tested the effect of using 100 ml apple cider vinegar or water as a drowning solution in 2-choice assays using five 2-component mixtures (furfural + putrescine, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, phenylethylamine + putrescine, and cis-3-hexen-1-ol + ursolic acid) (1 µg load [5µl x 100ηg/µl of each component compound]) which in combination with apple cider vinegar were more attractive than apple cider vinegar alone during step 3 trials and *H. rhamnoides* fruits. Attractant mixtures were loaded into rubber septa as in field testing. Three whole *H. rhamnoides* fruit were placed within a 30 ml portion cup with a mesh lid and suspended inside a trap. We conducted 2 replicates of each permutation of 2-choice trials per sex.

Field trials

Nova Scotia attractant trials

The five 2-part compound mixtures (furfural + putrescine, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, phenylethylamine + putrescine, and cis-3-hexen-1-ol + ursolic acid) (1 µg load [5µl x 100ηg/µl of each component compound]) from step 4 of choice assays were field tested as attractant lures in 500 ml jar style traps in black/red based on Basoalto et al.

(2013) and Renkema et al. (2014) with apple cider vinegar as a drowning solution (Figure 4.1.1f, Table S4.1.2). Three crushed *H. rhamnoides* fruits in a portion cup with a mesh lid suspended within the trap and with water as a drowning solution were also tested as a potential attractant. Jar traps containing only apple cider vinegar, but no chemical attractant lures were used as controls at each site. Three blocks of 7 traps were deployed in random order at each of 3 sites (unmanaged 1-hectare low bush blueberry (*Vaccinium angustifolium*), commercial 34-hectare high bush blueberry, and commercial 4-hectare raspberry tunnels) on 17 August 2015. We checked traps, replenished lures and drowning solutions, and rotated trap positions within each block weekly until 28 October 2015 (7 traps x 9 blocks for 10 weeks).

Nova Scotia trap design trials

Three trap designs were field tested using a randomized block design in open field raspberry (3 blocks) and highbush blueberry (4 blocks) crops at an 18-hectare commercial fruit farm. We tested a novel 500 ml jar style trap with eight equally spaced 2 cm entrance holes based on Basoalto et al. (2013) and Renkema et al. (2014) versus a traditional yellow Multitrap and a Multitrap painted red based on Leblanc et al. (2010) (Figure 4.1.1b & e-f). Individual traps of each design were tested using apple cider vinegar as drowning solution and attractant lures of 10 ug total load of phenylacetaldehyde - ethyl acetate or phenylethylamine - putrescine, or without attractant lures (control) (Table S4.1.2). Traps were deployed 18 August 2015 and checked weekly until 29 September 2015 (3 treatments x 5 blocks for 6 weeks). Deployment of one block in blueberry was delayed 2 weeks and one block in raspberry was delayed 1 week (3 treatments x 2 blocks for 5 weeks). Traps were rotated one position within each block weekly to prevent positional bias.

Newfoundland attractant trials

Additional field sites were added in 2015 to test the efficacy of the optimized trap (Figure 4.1.1f) as used in the Nova Scotia attractant trials and attractant mixture lure in a region actively engaged in surveillance for *D. suzukii* invasion, but did not yet have confirmed well established populations. Traps were deployed 13 August 2015 in a variety of fruit crops, including highbush blueberry, lowbush blueberry (*Vaccinium angustifolium* Aiton (Ericales: Ericaceae)), currants (*Ribes* spp. L. (Saxifragales: Grossulariaceae)), strawberries (*Fragaria* × *ananassa* Duchesne (Rosales: Rosaceae)), raspberries, haskap (*Lonicera caerulea* L. (Dipsacales: Caprifoliaceae)), sea buckthorn, grapes (*Vitis vinifera* L. (Vitales: Vitaceae)), and blackberry (*Rubus* spp. (Rosales: Rosaceae)) at commercial and research sites across Newfoundland. At each site, 1-2 Solo-cup traps with yeast lures and 1 jar-style trap with 2-part attractant lure mixture (10 ug total load of phenylethylamine + ethyl acetate) were deployed (Figure 4.1.1f-g, Table S4.1.2). Apple cider vinegar with ethanol was used as drowning solution in all traps deployed in Newfoundland. Yeast lures were replaced weekly. Two-part mixture lures were replaced 6 weeks into the study. Traps were checked weekly until 3 November 2015 (3 treatments x 14 sites for 14 weeks).

2016

Laboratory choice assays

Based on 2015 results, we created 3-component and 4 component mixtures containing phenylethylamine, phenylacetaldehyde, ethyl acetate, and putrescine. We conducted multiple choice assays in a 30 cm X 30 cm X 30 cm mesh cage (BugDorm). Each assay consisted of six attractant choices: four 3-component mixtures (phenylethylamine + phenylacetaldehyde + ethyl acetate, phenylethylamine + phenylacetaldehyde + putrescine, phenylacetaldehyde + putrescine + ethyl acetate, and phenylethylamine + putrescine + ethyl acetate), one 4-component mixture (phenylethylamine + phenylacetaldehyde + putrescine + ethyl acetate), and one blank control (Table S4.1.2). Each choice was presented on filter paper within a 30 ml portion container with mesh lid and coated with 0.5 mm layer of TangleFoot. Portion cups were suspended at a radius of 10 cm from the centre of the arena and equidistant from each other. Attractant mixture order was randomized for each trial. Each trial was conducted with 100 female and 100 male *D. suzukii* tested together. These refinements in choice assay methodology allowed us to compare relative attractiveness of multiple lure mixtures simultaneously and reduce assay duration to 24 h. We conducted 21 replicates of this assay.

Field trials

Nova Scotia attractant mixture and trap design trials

Potential attractant lure mixtures were field tested in commercial raspberry tunnels and blueberry fields using 1 l jar style traps with 2 cm entrance holes based on results of 2015 field trials. Lures were comprised of 3.3 µg (3.3 µl x 1 µg/µl) of each of phenylethylamine, phenylacetaldehyde, and ethyl acetate impregnated into a rubber septum and hung from the inside of the trap lid (Table S4.1.2). The majority of traps used for *D. suzukii* are some combination of red, black, yellow, and clear plastic (Landolt et al. 2012, Lee et al. 2012, 2013, Basoalto et al. 2013, Renkema et al. 2014). We had previously identified green and purple as a more attractive colour combination in laboratory trials (Little et al. 2019). We compared jar style traps in traditional red and black against novel green and purple jar style traps (Figure 4.1.1h-i). Traps of both colour combinations were tested with and without 3-component mixture attractant lures using apple cider vinegar as a drowning solution. Traps were deployed in a randomised block design on 22 August 2016 and checked twice weekly until 27 October 2016 (8 treatments x 8 blocks for 8 weeks). Traps were rotated one position within each block weekly to minimize positional bias.

Newfoundland attractant mixture trials

We deployed traps 17-18 August 2016 at commercial fruit growing sites across southern Newfoundland. We used red/black Solo cup traps with a baker's yeast lure (*Saccharomyces cerevisiae*) as controls (Figure 4.1.1g). Novel green/purple 1 l jar-style traps (Figure 4.1.1h) were deployed with the same phenylethylamine + phenylacetaldehyde + ethyl acetate attractant lure. An equal number of green/purple jar-style traps were deployed without an attractant lure (Table S4.1.2). Traps were checked weekly until 1 December 2016 (3 treatments x 10 sites for 7-8 weeks).

2017

Laboratory choice assays

Trap choice trials

Renkema et al. (2014) found that larger entrance holes increased capture numbers and that fiberglass drywall mesh with 2.5 by 2.5 mm2 openings could reduce bycatch. Two-choice assays were conducted to further refine jar-style trap designs. The green/purple trap from 2016 was modified by adding purple-painted mesh screening in the entrance holes (Figure 4.1.1h & j, Table S4.1.2). Traps were also constructed with green tape on the exterior and purple mesh in larger 5 cm diameter holes (Figure 4.1.1k). Traps with larger 5 cm holes and mesh were further modified by adding purple tape to both the trap exterior as on previous traps and the interior of the traps opposite the entrance holes (Figure 4.1.1l). Four versions of the jar traps were tested with apple cider vinegar and using water in control traps as a drowning solution. Unscented dish soap (5 ml / 4 l vinegar) was used as a surfactant. Trials were conducted with and without attractant mixture lures (phenylethylamine + phenylacetaldehyde + ethyl acetate at total dose of 9.9 µg as was used in 2016 field trials). For each trial, 25 male and 25 female *D. suzukii* were placed into a 60 cm X 30 cm X 30 cm mesh enclosure (BugDorm). Four-five replicates were completed for each 2-choice assay trial. Flies were removed and counted after 24 h.

Lure choice trials

Two-choice assays were conducted to further refine attractant lure mixtures. Two- and three-component mixtures were tested in novel jar traps (Figure 4.1.1l) with apple cider vinegar and using water as a drowning solution to approximate field trapping conditions. We tested 8 mixtures including ethyl acetate + acetoin, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + ethyl acetate + acetoin, phenylacetaldehyde + ethyl acetate + ursolic acid, phenylethylamine + phenylacetaldehyde + ethyl acetate, cis-3-hexen-1-ol + ursolic acid, and cis-3-hexen-1-ol + ursolic acid + acetoin, and a blank control (Table S4.1.2). Lure mixtures were applied to rubber septa for each trial. Two-component mixtures comprised of 5 µg (5 µl x 1 µg/µl) of each component compound to yield a total load of 10 µg and three-component mixtures comprised of 3.3 µg (3.3 µl x 1 µg/µl) of each compound to yield a total load of 9.9 µg. As above, 25 male and 25 female *D. suzukii* were placed into a 60 cm X 30 cm X 30 cm mesh enclosure (BugDorm) for each replicate trial. Four-six replicates were completed for each 2-choice assay trial. Flies were removed and counted after 72 h.

Field trials

Nova Scotia adjuvant mixture and trap design trials

Jar-style green/purple traps with large (5 cm) mesh holes, with and without an attractant lure (ethyl acetate + acetoin @ 10 µg total load), were field tested versus similar control traps in black/red at four sites in Nova Scotia from 12 September to 24 October 2017 (Figure 4.1.1l-m, Table S4.1.2). Green/purple traps were deployed in highbush blueberries, field-grown raspberries, and tunnel-grown raspberries. Traps were deployed with apple cider vinegar and unscented dish soap as a drowning solution. An additional green/purple trap with an attractant lure and with water as a drowning solution was deployed at each site. Traps were checked weekly (4 treatments x 4 sites for 6 weeks).

Newfoundland trap design trials

Jar-style green/purple traps with large mesh holes were field tested versus Solo cup traps in Newfoundland (Figure 4.1.1g & l, Table S4.1.2). Jar style traps were tested with and without attractant lures (ethyl acetate + acetoin @ 10 µg total load). Solo cup traps were tested with a yeast adjuvant. All traps were deployed with apple cider vinegar and ethanol as drowning solution. Traps were deployed in a mix of raspberry, blueberry, and currants. Seven traps were deployed 28 September 2017 in eastern Newfoundland (for 5 weeks) and six traps were deployed 4 October 2017 in western Newfoundland (for 4 weeks). Traps were checked weekly until 1 November 2017.

2018

Field trials

In 2018, we modified the 2-component attractant lure mixture used in 2017 field trials by adjusting the relative concentrations of each component based on initial electroantennogram results . The revised mixture was comprised of ethyl acetate (5µl x 1µg/µl = 5µg) and acetoin (5µl x 10ηg/µl = 50ηg) based on dose-dependent responses of *D. suzukii* during earlier electroantennography trials. We compared our revised attractant mixture to a commercially available lure, Drosalure® (Sylvar Technologies Inc., Fredericton, Canada, member of Andermatt Biocontrol group, Grossdietwil, Switzerland) and our novel trap design to a commercially available *D. suzukii* trap, Drosal trap (Sylvar Technologies Inc., Fredericton, Canada, member of Andermatt Biocontrol group, Grossdietwil, Switzerland). Six trap treatments were field tested in Nova Scotia in 2018 (Table S4.1.2 & S4.1.3, Figure 4.1.1l-o). Field testing was conducted in highbush blueberry (4 blocks), field-grown raspberry (1 block), field-grown blackberry (1 block), and tunnel-grown raspberry (2 blocks). Traps were deployed 28 August 2018. Drowning solutions and attractant lures were replaced weekly. The attractant lure mixture was made with incorrect proportions of 2 component compounds in week 3, therefore that week's trap capture results were excluded from analysis. Trap captures were checked weekly until 1 October 2018 (6 treatments x 8 blocks for 4 weeks).

Data analysis

To correct for depreciation in signal quality over time during a single electroantennogram run, we calculated linear interpolated values of responses towards solvent controls. Variability in electrical responses among electroantennogram preparations can result from electrode placement and insect desiccation. To account for this variation, an absolute value was calculated for each compound, solvent, and control by normalizing amplitude values against the mean for ethanol in each run using the formula:

$$\textit{Normalized absolute amplitude} = \frac{mV_{compound} * 10}{mV_{(mean\ ethanol)}}$$

This calculation gives ethanol a mean normalized amplitude value of 10 for each run, allowing easier comparison among runs. Mean normalized electroantennogram valences for virgin and mated, male and female *D. suzukii* were assessed for relative sensitivity at each dose. Response data were modelled with a generalized linear model (Guassian distribution with an identity link function) and Type II Wald chi square tests on the model. We excluded responses to control stimuli to reduce the complexity of the model. We used interaction of “sex-mating status group : load” with additive compound, sex, and mating status effects.

Differences in attraction between treatments in 2-choice behavioural assays were assessed using paired t-tests in 2014. Results of 2-choice assays in 2015, 2016, and 2017 were assessed using ANOVA and paired-t tests.

Field trials in Nova Scotia and Newfoundland were assessed separately due to expected differences in *D. suzukii* population sizes and differences based on previous observations by Agriculture and Agri-Food Canada (AAFC). Previous monitoring efforts by AAFC had revealed large overwintering populations of *D. suzukii* in Nova Scotia but, in Newfoundland, *D. suzukii* captures were infrequent and potential for populations to overwinter was unknown (Moreau et al. 2013). Previous observations in Newfoundland suggested that populations on the eastern and western sides of the province could be distinct and potentially transitory, therefore, we analyzed the two regions separately. Newfoundland field trials were conducted in conjunction with AAFC monitoring trials using apple cider-baited traps with yeast attractant lures; whereas, Nova Scotia field trials were conducted independently using apple cider-baited traps without lures.

We used a generalized linear mixed model fit by maximum likelihood (Laplace Approximation) and Type II Wald chi square tests on the model to analyze capture data for 2014 Nova Scotia attractant trap field trials, 2015 Nova Scotia attractant field trials, 2015 Nova Scotia trap designs field trials, 2016 Nova Scotia field trials, 2017 Nova Scotia field trials, and 2017 Newfoundland field trials . Further assessment of differences among treatments in Nova Scotia trials were analyzed using ANOVA tests and in Newfoundland trials using paired-t tests.

Differences in mean *D. suzukii* captures among trap designs during 2014 field trials were assessed using 2-way ANOVA and paired t-tests. *Drosophila suzukii* captures Newfoundland field trials were assessed using 2-way ANOVA tests in 2015 and using Kruskal-Wallis rank sum test in 2016. Captures from 2017 Nova Scotia trap design field trials were assessed using 1-way and 2-way ANOVA tests. Captures from 2018 Nova Scotia field trials were assessed using 1-way ANOVA tests.

All statistical analyses were conducted and graphs generated using R version 3.4.3 [2017-11-30, The R Foundation, (https://www.r-project.org/]), using RStudio (Version 1.1.383 – © 2009-2017, RStudio, Inc., (http://www.rstudio.org/).

4.1.4 Results

Throughout this five-year study, we used an iterative process to investigate the efficacy of single compounds and compound mixtures as prospective attractant lures, and efficacy of trap designs and colours for use as monitoring traps. The results of trials described above in the methods are presented based on the focus of study rather than a strict chronological basis (Table S4.1.2).

Single compound trials

Electroantennography

In 2014, individual adult *D. suzukii* were tested for physiological responses to prospective attractant compounds at one of three discrete loads using electroantennography. All individual flies demonstrated neuronal responses to all compounds tested. We observed differences in physiological responses among compounds, between female and male flies, between virgin and mated flies, and among loads within sex-mating status groups of flies (GLM: Type II ANOVA, compounds: $\chi^2_{51} = 92.8$, $P < 0.0005$, sex: $\chi^2_1 = 25.2$, $P < 0.0001$, mating status: $\chi^2_1 = 43.1$, $P < 0.0001$, interaction of sex-mating status group and compound load: $\chi^2_4 = 23.1$, $P = 0.0001$) (Table 4.1.4, see Figure S4.1.1). Responses from male flies were significantly stronger than from female flies when averaged over compounds and loads (T-test, t = +5.2, df = 2976.4, $P < 0.0001$). Electroantennography illustrates relative physiological sensitivities to odorant stimuli; however, results do not reveal behavioural valence (attraction or repellence). Therefore, we ranked mean neuronal responses within each sex-mating status group to identify compounds for further study (Table 4.1.1, Table S4.1.1). Dose-dependent differences in electroantennogram responses were observed within sex-mating status group of flies (Table 4.1.1).

Table 4.14. Generalised linear model (family = *Guassian*) output quantifying effects of compounds, fly sex, fly mating status, and interaction of fly sex-mating status group and compound load (0.01, 1, or 100 ug) on the neuronal responses of individual *D. suzukii* flies during electroantennography. Only factors with P < 0.10 shown for clarity.

Factor	Estimate	SEM	t	P
intercept	7.56	0.68	11.11	**<0.0001**
furfural	1.82	0.94	1.94	**0.05**
phenylethylamine	2.45	0.93	2.63	**<0.01**
putrescine	3.76	0.93	4.05	**<0.0001**
trimethylamine-hydrochloride	1.74	0.93	1.87	0.06
ursolic acid	2.46	0.93	2.63	**<0.01**
male	1.22	0.24	5.02	**<0.0001**
virgin	1.53	0.23	6.57	**<0.0001**
female virgin : load	0.01	0.003	2.91	**<0.005**
male virgin : load	-0.01	0.004	-3.29	**0.001**

Laboratory two-choice trials

In 2014, differences in attraction between compounds tested and their respective solvents were informative but not statistically significant except for phenylethylamine which was significantly more attractive than ethanol (paired t-test, Females: T=5.84, df=8, P<0.0005; Males: T=2.52, df=10, P=0.03) and isoamyl alcohol which was significantly less attractive than ethanol to males (paired t-test, T=-2.07, df=10, P=0.05) (Table 4.1.2). Based on these results, we selected four compounds with positive responses indices (acetoin, benzaldehyde, furfural, and ursolic acid) for field testing. We also chose to field-test benzyl acetate based on positive results during electroantennography and preliminary studies in 2013 (data not shown) and we field-tested methyl salicylate based on recommendations in published literature (Lee 2010, Walsh et al. 2011, Abraham et al. 2015). Additional compounds with positive RI values but that are ubiquitous organic compounds, such as phenylethamine, were tested as components in attractant mixtures in subsequent years.

Field trials

In 2014, we used a generalized linear mixed model fit by maximum likelihood (Laplace Approximation) to analyze field trial data for traps deployed in Nova Scotia (formula = count ~ compound + crop (1| week)). Type II Wald chi square tests on the model show significant differences in *D. suzukii* trap captures by compound (χ^2= 51.01, df=7, p<0.0001) and crop (χ^2=211.53, df=2, P<0.0001). More *D. suzukii* were captured in shaded red-black cup traps (Figure 4.1.1a) deployed in field grown blueberries than in tunnel grown raspberries. Male and female *D. suzukii* responded differently to lure compounds (paired t-test, t=-2.94, df=7, P=0.02) (Figure 4.1.2). Total male *D. suzukii* captures outnumbered female captures in all crop systems. Responses were significantly different among crop systems and lure compounds for each sex (Type II Wald chi square tests on generalized linear mixed model: compound χ^2=22.70, df=7, p 0.005, crop F=117.74, df=2,384, P<0.0001; females: compound χ^2=38.14, df=7, p<0.0001, crop F=94.22, df=2,384, P<0.0001). However, differences among compounds within each crop system for each sex of fly were not significant (ANOVA, P's >0.05) (Figure 4.1.2). Attractant lures containing ursolic acid and furfural showed promise for attracting male *D. suzukii*, and to a lesser extent for female flies; however, differences in attraction were not statistically significant (ANOVA, P's >0.05). Traps deployed with methyl salicylate lures captured numerically higher counts of female *D. suzukii*.

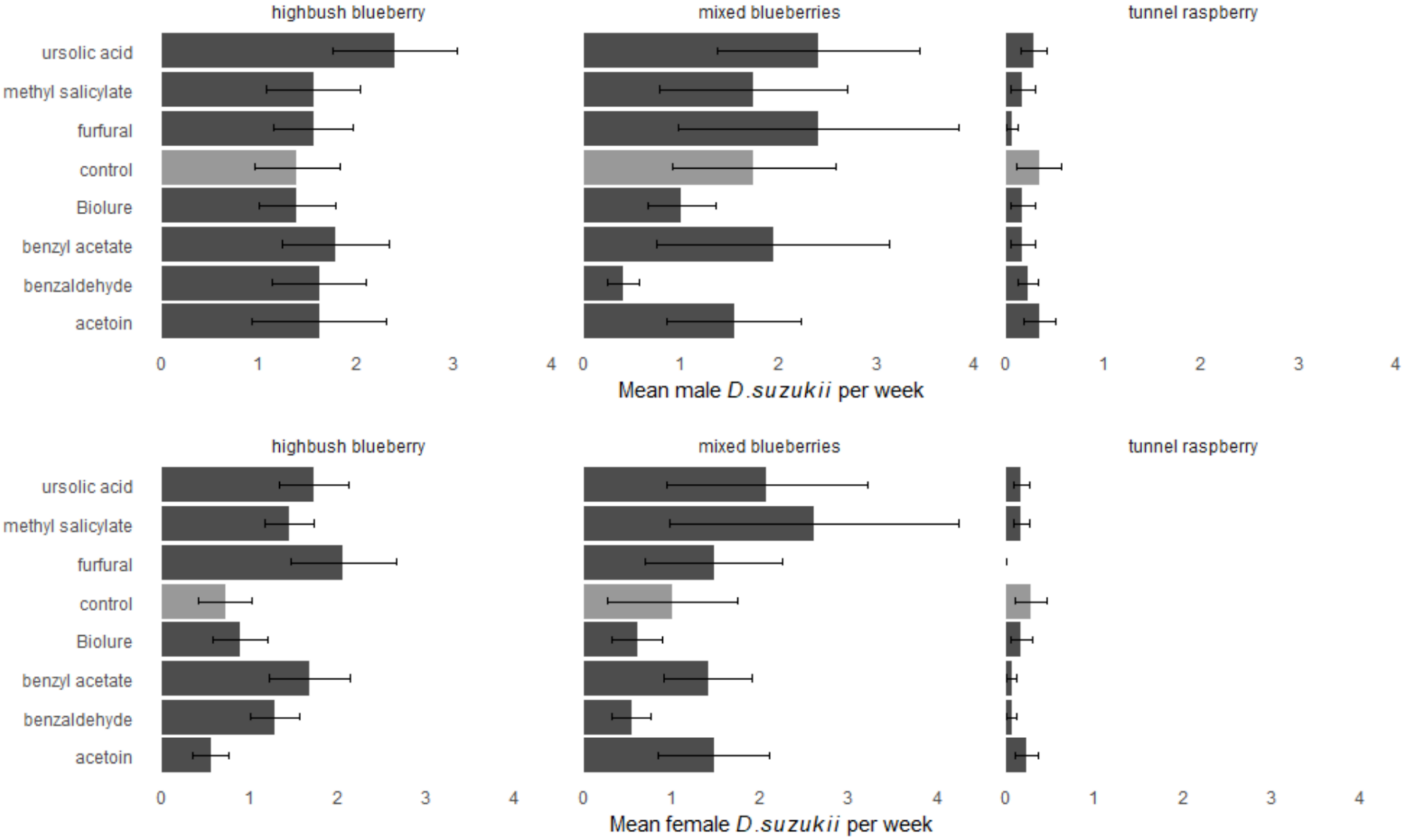


Figure 4.1.2. Male and female *D. suzukii* captures in 2014 Nova Scotia attractant field trials are shown separately for each attractant compound within each crop system where they were deployed.

Trap design trials

Field trials

In 2014, jar-style traps (Figure 4.1.1d) and MultiTraps (Figure 4.1.1b) attracted more *D. suzukii* than either version of Solo cup traps (Figure 4.1.1a&c and Figure 4.1.3a). Significant differences were observed among trap designs and between crop systems in 2014 (2-way ANOVA, Week 1: design F=10.83, df=2,66,P<0.0001, crop F=32.09, df=1,66, P<0.0001, interaction F=6.87, df=2,66, P<0.005; Weeks 2-6: design F=48.57, df=2,353, P<0.0001, crop F=84.63, df=1,353, P<0.0001, interaction F=19.71, df=2,353, P<0.0001) (Figure 4.1.3a). Traps deployed in field grown raspberries captured more *D. suzukii* than traps deployed in blueberries, irrespective of trap type. Capture counts also differed between male and female *D. suzukii* (paired t-test, t=2.92, df=431, P<0.005), so we assessed efficacy of trap + attractant lure combinations for each sex separately (Figure 4.1.3b). Efficacy of attractant compounds varied among trap designs but differences in attraction were significant only in Solo cup traps (2-way ANOVA, males: compound F=0.34, df=3,347, P=0.80, trap F=25.67, df=2,347, P<0.0001, interaction F=0.71, df=6,347, P=0.65; females: compound F=4.21, df=3,347, P<0.01, trap F=34.48, df=2,347, P<0.0001, interaction F=1.32, df= 6,347, P=0.25) (Figure 4.1.3b). Traps deployed with Biolure® attracted more *D. suzukii* than other attractants tested, except in shaded cup traps which had highest captures with furfural lures.

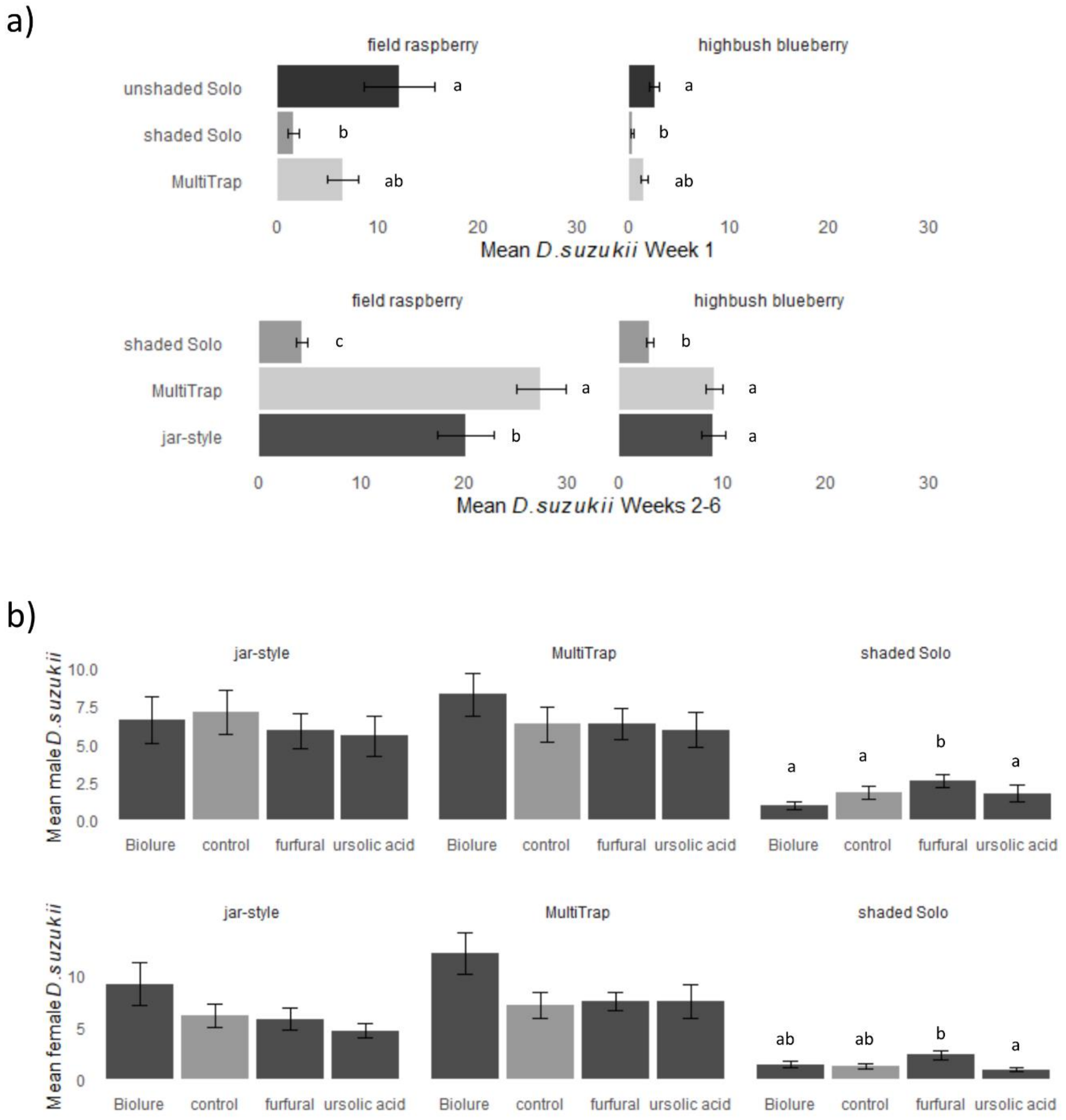


Figure 4.1.3. a) Mean *D. suzukii* trap captures for each trap design in field-grown highbush blueberry and tunnel-grown raspberry during 2014 trap design field trials. Solo traps (unshaded in week 1, shaded in weeks 2-6) were used as controls. b) Mean trap captures of male and female *D. suzukii* for each experimental treatment of trap design and attractant lure during 2014 trap design field trials. Control traps contained no attractant lures.

In 2015, more female than male *D. suzukii* were captured in all trap designs tested in Nova Scotia (Figure 4.1.1b & e-f) (paired t-test, t=-4.83, df=338, P<0.0001). We used a generalized linear mixed model fit by maximum likelihood (Laplace Approximation) to assess attraction to trap designs with different attractant lures with crop type as a random effect. Both trap design and attractant lure mixture were significant (Type II Wald chi square tests, trap design: χ^2=333.69, df=2, P<0.0001, attractant mixture: χ^2=73.70, df=2, P<0.0001) (Figure 4.1.4). Jar style traps (Figure 4.1.1f) attracted more *D. suzukii* than either yellow or red McPhail traps (Figure 4.1.1b&e), regardless of attractant lure used in 2015 (2-way ANOVA, trap design: F=6.09, df=2,330, P<0.005, adjuvant: F=1.31, df=2,330, P=0.27, interaction: F=0.98, df=4,330, P=0.42) (Figure 4.1.4). Overall differences in attraction were not significant between traps deployed in blueberries and raspberries (1-way ANOVA, F=1.78, df=1,337, P=0.67). Jar style traps outperformed McPhail traps in both raspberry and blueberry fields; however, differences were only significant between jar style traps and yellow McPhail traps in blueberry fields (2-way ANOVA, trap design: F=6.06, df=2,333, P<0.005, crop: F=0.22, df=1,333, P=0.64, interaction: F=0.94, df=2,333, P=0.39). Attractiveness of attractant lures differed among the trap designs. Phenylacetaldehyde + ethyl acetate was most attractive in jar style traps and yellow McPhail traps, but red McPhail traps were most attractive without an attractant lure (Figure 4.1.4).

In 2017, more *D. suzukii* were captured in traps with large holes and purple mesh inserts regardless of the treatment (drowning solution or presence of an attractant lure) (2-way ANOVA, design: F=6.80, df=3,82, P<0.0005, treatment: F=0.88, df=3,82, P=0.46, interaction: F=1.13, df=9,82, P=0.35). Among traps which were deployed with apple cider vinegar but not with attractant lures (phenylethylamine + phenylacetaldehyde + ethyl acetate), traps with small mesh covered holes attracted the least flies (ANOVA, F= 5.74, df=3,20, P= 0.005).

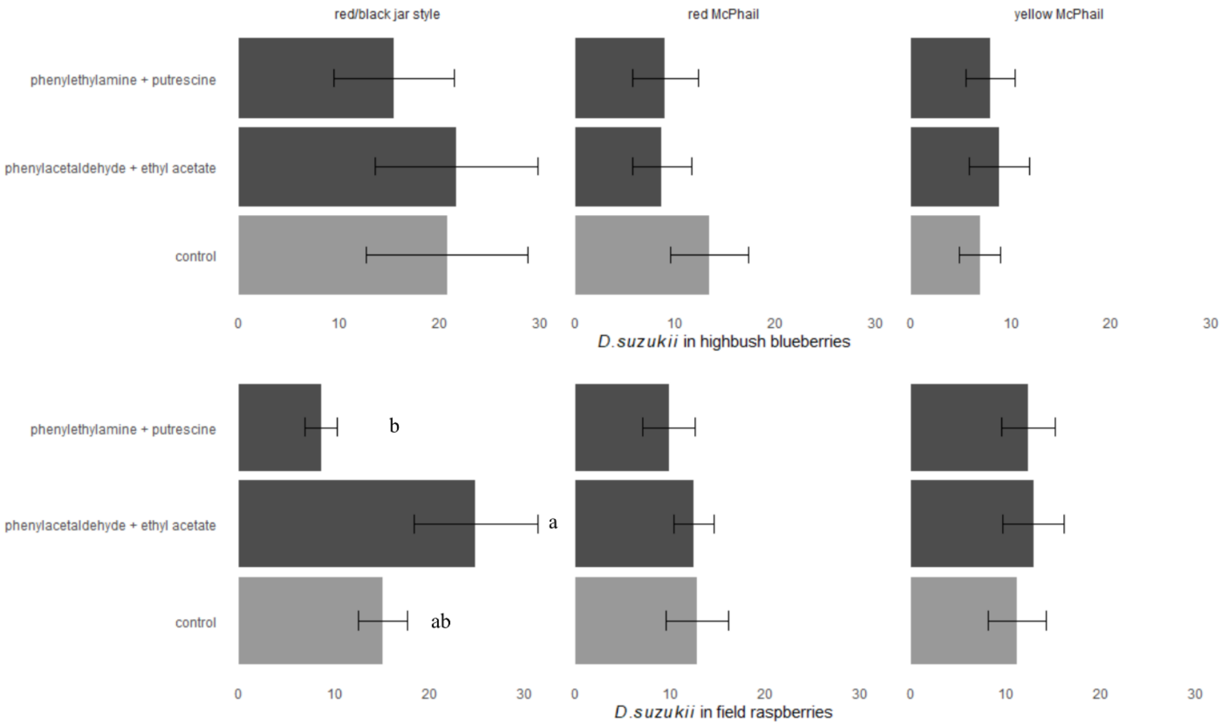


Figure 4.1.4. Mean weekly *D. suzukii* captures for each attractant mixture lure and trap design treatment during 2015 Nova Scotia trap design field trials in field-grown highbush blueberry and tunnel-grown raspberry. Differences in attraction among attractant mixture lures deployed in field raspberries are denoted by lower case letters within figure (1-way ANOVA, F=3.57, df=2,46, P=0.04, Tukey Post-Hoc [P<0.05]). Control traps contained no attractant lures.

Single compounds vs. compound mixture trials

Laboratory two-choice trials

In 2015, we employed a five-step iterative process to compare relative responsiveness of *D. suzukii* to individual compounds versus 2-component mixtures.

Step 1.

Putrescine was more attractive to *D. suzukii* than either trimethylamine hydrochloride or a mixture of the two compounds as would be found in BioLure® (ANOVA, F=6.12, df=2,113, P<0.005). However, differences were significant only in choice trials between putrescine and trimethylamine hydrochloride (paired t-test, t=2.94, df=17, P=0.01).

Step 2.

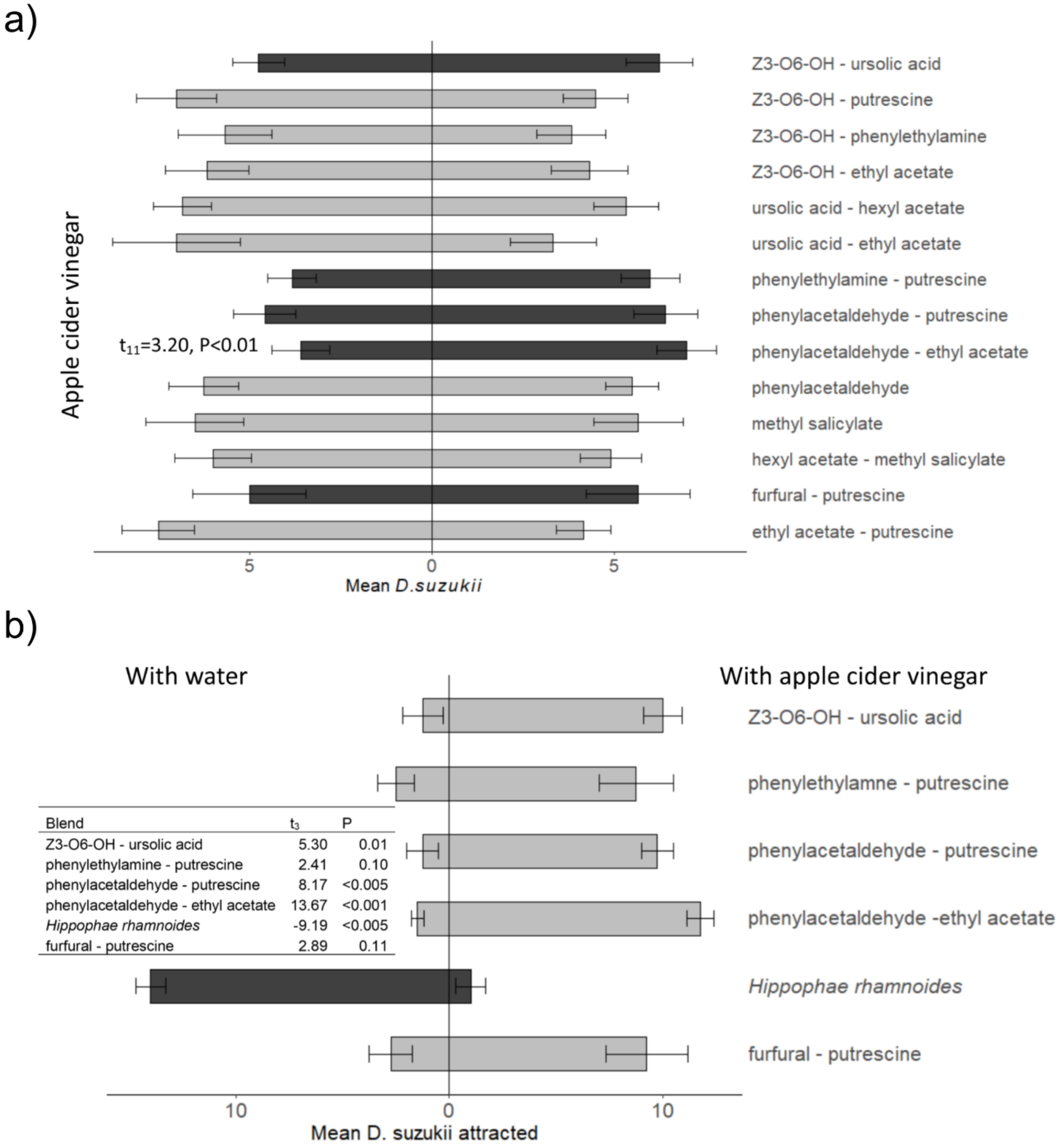
Attraction to 2-compound mixtures were tested against each of the component compounds in the mixture (e.g. mixture AB was compared against compound A and against compound B). Significant differences were observed among responses to compounds and mixtures, and more female flies than male flies responded to both compounds and mixtures, but the interaction of these factors was not significant (2-way ANOVA, compound: F=1.85, df=44,774, P<0.001, sex: F=167.64, df=1,774, P<0.0001, compound:sex: F=0.77, df=44,774, P=0.86) (Table 4.1.3).

Step 3.

Five attractant lure mixture + drowning solution combinations (cis-3-hexan-1-ol + ursolic acid, phenylethylamine + putrescine, phenylacetaldehyde + putrescine, phenylacetaldehyde + ethyl acetate , and furfural + putrescine) were more attractive than drowning solution alone, but differences were only significant for phenylacetaldehyde + ethyl acetate (Figure 4.1.5a).

Step 4.

More *D. suzukii* flies were attracted to 2-component attractant lure mixtures (cis-3-hexan-1-ol + ursolic acid, phenylethylamine + putrescine, phenylacetaldehyde + putrescine, phenylacetaldehyde + ethyl acetate , and furfural + putrescine) when combined with apple cider vinegar than when combined with water. *Hippophae rhamnoides* fruit plus water attracted more flies than fruit plus vinegar (Figure 4.1.5b).



Blend	t_3	P
Z3-O6-OH - ursolic acid	5.30	0.01
phenylethylamine - putrescine	2.41	0.10
phenylacetaldehyde - putrescine	8.17	<0.005
phenylacetaldehyde - ethyl acetate	13.67	<0.001
Hippophae rhamnoides	-9.19	<0.005
furfural - putrescine	2.89	0.11

Figure 4.1.5. a) Mean counts of *D. suzukii* attracted to apple cider vinegar alone or to potential attractant compounds or mixtures in combination with apple cider vinegar in 2015 choice trials. Dark bars denote attractants that attracted more flies than vinegar alone. b) Mean counts of *D. suzukii* attracted to attractant mixtures or *H. rhamnoides* fruit with either apple cider vinegar or with water as drowning solutions. Results of paired t-test experiment are shown within the figure.

Compound mixture trials

Field trials

In green/purple jar traps deployed in Nova Scotia in 2015 (Figure 4.1.1f), more male than female *D. suzukii* were captured in traps (paired t-test, t=-4.59, df=628, P<0.0001). Fewer *D. suzukii* were captured than other *Drosophila* bycatch regardless of attractant lure mixture (paired t-test, t=-5.72, df=628, P<0.0001), but more *D. suzukii* were captured than non-*Drosophila* insect bycatch (paired t-test, t=6.66, df=628, P<0.0001). *Hippophae rhamnoides* fruit plus water were not effective as *D. suzukii* attractants in field testing. We used a generalized linear mixed model to assess attraction to 2-part attractant lure mixtures in different crop types with early versus late season as a random factor. Both attractant mixture lure and fruit crop were significant (Type II Wald chi square tests; adjuvant: χ^2=2552.5, df=6, P<0.0001, crop: χ^2=23259.4, df=2, P<0.0001); however, no single attractant mixture lure was most effective in all crop systems (Table 4.1.5).

Traps deployed in Newfoundland in 2015 (Figure 4.1.1f-g) captured fewer *D. suzukii* than traps deployed in Nova Scotia (mean *D. suzukii*/trap ± SE, NL trials mean=0.47±0.06, n=444; NS attractant mixtures trials mean=64.8±7.61, n=629; NS trap designs trials mean=12.9±1.06, n=339). *Drosophila suzukii* captured in traps in Newfoundland were almost exclusively female (205 female / 208 *D. suzukii*). More *D. suzukii* were captured in traps with 2-part mixture attractant lures (phenylethylamine + ethyl acetate) than in traps with yeast, but differences were not significant (2-way ANOVA, adjuvant: F=2.33, df=1,436, P=0.13; region: F=15.68, df=3,436, P<0.0001; interaction: F=3.48, df=3,436, P=0.02). More *D. suzukii* were captured in the central and western regions than in the Avalon Peninsula and eastern regions of Newfoundland.

Table 4.1.5. Mean trap capture counts for each attractant lure treatment (mixture) by crop in which it was deployed during 2015 field trials in Nova Scotia. Different letters denote significant differences in mean *D. suzukii* captures among lures within each crop in early season (weeks 1-5) and late season (weeks 6-10) (N=968 samples, Tukey post-hoc P<0.05).

	Early season							**Late season**							
	Tunnel raspberry		**Highbush blueberry**		**Lowbush blueberry**			**Tunnel raspberry**			**Highbush blueberry**		**Lowbush blueberry**		
Mixture	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Tukey**	**Mean**	**SEM**	**Tukey**	**Mean**	**SEM**	**Mean**	**SEM**	**Tukey**
Control (no lure)	27.00	10.11	4.87	1.86	9.87	2.48	ab	12.93	2.97	ab	419.60	128.26	69.67	21.05	ab
Furfural + putrescine	35.13	14.25	5.67	2.29	8.60	2.18	ab	12.87	3.77	ab	273.13	41.78	63.40	14.53	ab
H. rhamnoides	0.67	0.37	0.20	0.14	0.33	0.16	a	0.13	0.09	a	3.07	1.09	0.33	0.16	a
Phenylacetaldehyde + ethyl acetate	29.33	12.93	8.93	3.56	8.93	2.21	ab	10.80	2.51	ab	368.33	149.84	82.93	24.84	b
Phenylacetaldehyde + putrescine	39.13	15.61	10.13	4.59	11.40	3.01	b	15.53	3.70	b	370.67	151.29	51.47	12.06	ab
Phenylethylamine + putrescine	32.67	13.59	10.87	4.81	11.13	2.34	b	11.33	2.76	ab	220.80	42.88	39.53	10.10	ab
Cis-3-hexen-1-ol + ursolic acid	29.20	11.74	8.07	4.49	9.07	2.43	ab	14.87	4.26	b	297.00	57.29	78.60	21.26	b

In 2017, traps (Figure 4.1.1g & l) deployed in Newfoundland captured fewer *D. suzukii* than traps deployed in Nova Scotia (mean ± SE; n=96; NL trials mean=5.15±1.58, n=59, NS trials mean=381±65.5). More *D. suzukii* were captured on the west coast of Newfoundland than on the east coast (t-test, t=-3.50, df=23.09, p-value=0.002) (Figure 4.1.6a). Eight of the 10 *D. suzukii* captured over 5 weeks on the east coast were caught in jar-traps with an attractant lure (ethyl acetate + acetoin). Traps on the west coast of Newfoundland captured 294 *D. suzukii* over 4 weeks, of which 72.4% were female flies. Green/purple jar-style traps (Figure 4.1.1l) captured more *D. suzukii* than did Solo cup traps (Figure 4.1.1g) in both locations. We used a generalized linear mixed model to assess attraction to trap design treatments and geographic region with trapping week as a random factor. Both treatment and region were significant factors to number of *D. suzukii* captured (Type II Wald chi square tests; trap-attractant lure combination: χ^2=104.07, df=2, P<0.0001, region: χ^2=119.49, df=1, P<0.0001).

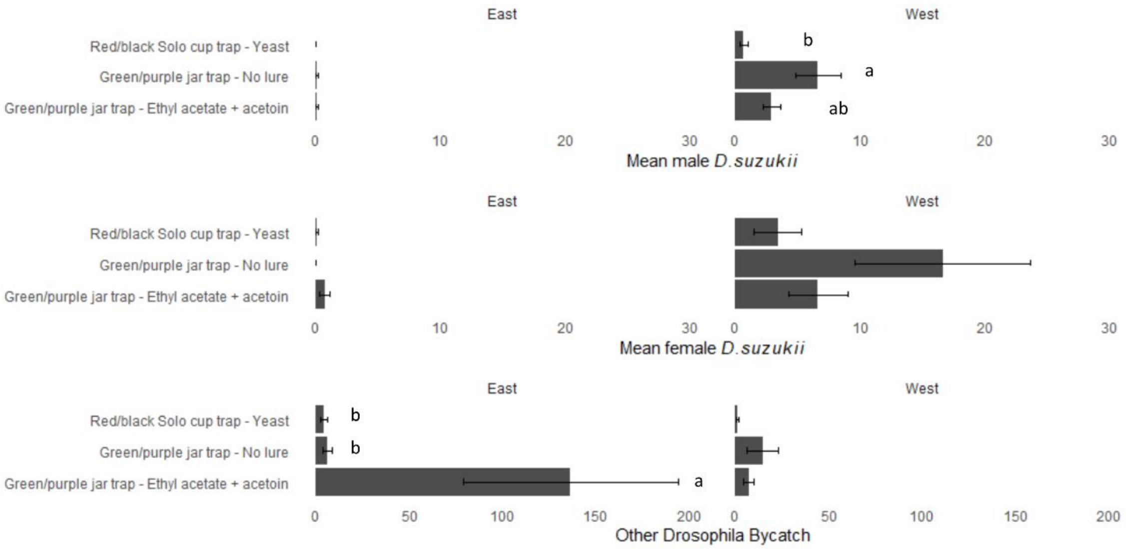


Figure 4.1.6. Mean trap captures of male and female *D. suzukii* and other *Drosophila* bycatch for trap design-attractant lure treatments during 2017 field trials between east and west regions of Newfoundland. Significant differences among treatments within each crop system are shown within figures (Tukey post hoc on 1-way ANOVA, P<0.05).

Laboratory choice assays

In 2016, more male *D. suzukii* were captured than females in choice assays (paired t-test, t=6.78, df=125, P<0.05). Overall, phenylethylamine + phenylacetaldehyde + ethyl acetate mixtures attracted the most *D. suzukii*; however, differences in attraction among mixtures and control were not significant (1-way ANOVA, F=1.59, df=5,120, P=0.17).

In 2017, an ethyl acetate + acetoin mixture was most attractive to *D. suzukii* compared to the 2- and 3- component mixtures tested in 2-choice assays (Table 4.1.6). However, differences in attraction among mixtures were not significant with either drowning solution (1-way ANOVA, vinegar solution: F=0.23, df=7,116, P=0.98, water: F=2.82, df=7,116, P=0.01, Tukey post-hoc, P’s>0.05).

Table 4.1.6. Mean counts of *D. suzukii* from 2-choice assays conducted in 2017 using 2- and 3-component mixtures tested with a) apple cider vinegar and b) water respectively as drowning solution. Paired t-test values in bold denote significant differences in attraction between mixtures. Mean counts denoting highest attraction for mixtures specified and significant statistical analyses are shown in bold.

a) With apple cider vinegar

		Mixture A		**Mixture B**				
Mixture A	**Mixture B**	**Mean**	**SEM**	**Mean**	**SEM**	**t**	**df**	**P**
phenylacetaldehyde + ethyl acetate	phenylethylamine + phenylacetaldehyde + ethyl acetate	**27.00**	**3.42**	22.33	3.44	0.68	5	0.53
phenylacetaldehyde + ethyl acetate	phenylacetaldehyde + ethyl acetate + acetoin	19.67	1.91	**24.00**	**2.88**	-1.03	5	0.35
phenylacetaldehyde + ethyl acetate	phenylacetaldehyde + ethyl acetate + ursolic acid	22.33	2.36	**25.33**	**1.74**	-0.84	5	0.44
phenylacetaldehyde + ethyl acetate + ursolic acid	cis-3-hexen-1-ol + ursolic acid	**25.67**	**4.19**	23.33	3.99	0.29	5	0.79
phenylacetaldehyde + ethyl acetate + ursolic acid	cis-3-hexen-1-ol + ursolic acid + acetoin	21.17	1.89	**25.33**	**1.99**	-1.12	5	0.32
control (no lure)	cis-3-hexen-1-ol + ursolic acid + acetoin	24.50	2.40	24.25	1.65	0.06	3	0.95
control (no lure)	cis-3-hexen-1-ol + ursolic acid	24.00	2.65	**24.75**	**3.38**	-0.12	3	0.91
cis-3-hexen-1-ol + ursolic acid + acetoin	cis-3-hexen-1-ol + ursolic acid	21.50	3.01	**27.00**	**2.55**	-1.01	3	0.39
cis-3-hexen-1-ol + ursolic acid	phenylacetaldehyde + ethyl acetate + acetoin	22.00	1.78	**26.25**	**1.49**	-1.44	3	0.25
phenylacetaldehyde + ethyl acetate + ursolic acid	phenylacetaldehyde + ethyl acetate + acetoin	19.00	2.89	**29.00**	**3.76**	-1.5	3	0.23
control (no lure)	phenylacetaldehyde + ethyl acetate + acetoin	22.50	2.90	**24.00**	**1.15**	-0.38	3	0.73
ethyl acetate + acetoin	phenylacetaldehyde + ethyl acetate + acetoin	**24.75**	**3.77**	20.25	3.25	0.7	3	0.53

ethyl acetate + acetoin	control (no lure)	25.00	2.27	24.75	2.25	0.06	3	0.96

b) With water

		Mixture A		Mixture B				
Mixture A	**Mixture B**	**Mean**	**SEM**	**Mean**	**SEM**	**t**	**df**	**P**
phenylacetaldehyde + ethyl acetate	phenylethylamine + phenylacetaldehyde + ethyl acetate	**24.83**	**1.78**	18.83	2.14	**2.90**	**5**	**0.03**
phenylacetaldehyde + ethyl acetate	phenylacetaldehyde + ethyl acetate + acetoin	**20.83**	**2.01**	20.00	2.29	1.00	5	0.36
phenylacetaldehyde + ethyl acetate	phenylacetaldehyde + ethyl acetate + ursolic acid	22.83	1.85	23.00	1.59	-0.08	5	0.94
phenylacetaldehyde + ethyl acetate + ursolic acid	cis-3-hexen-1-ol + ursolic acid	18.33	1.78	**27.17**	**1.38**	**4.26**	**5**	< **0.01**
phenylacetaldehyde + ethyl acetate + ursolic acid	cis-3-hexen-1-ol + ursolic acid + acetoin	18.33	1.26	**25.33**	**2.50**	**-2.74**	**5**	**0.04**
control (no lure)	cis-3-hexen-1-ol + ursolic acid + acetoin	**23.75**	**2.06**	21.50	2.47	0.67	3	0.55
control (no lure)	cis-3-hexen-1-ol + ursolic acid	18.00	1.68	**25.00**	**0.71**	-3.74	3	0.03
cis-3-hexen-1-ol + ursolic acid + acetoin	cis-3-hexen-1-ol + ursolic acid	**23.25**	**1.93**	21.75	1.11	-0.96	3	0.41
cis-3-hexen-1-ol + ursolic acid	phenylacetaldehyde + ethyl acetate + acetoin	20.50	1.55	**23.50**	**3.01**	-0.67	3	0.55
phenylacetaldehyde + ethyl acetate + ursolic acid	phenylacetaldehyde + ethyl acetate + acetoin	**25.50**	**1.32**	19.00	1.96	**7.51**	**3**	< **0.005**
control (no lure)	phenylacetaldehyde + ethyl acetate + acetoin	21.25	1.38	21.50	2.72	-0.17	3	0.88
ethyl acetate + acetoin	phenylacetaldehyde + ethyl acetate + acetoin	**28.50**	**1.71**	16.00	0.71	**6.76**	**3**	< **0.01**
ethyl acetate + acetoin	control (no lure)	**22.25**	**1.38**	21.50	2.10	0.79	3	0.49

Trials combining compound mixture lures and trap designs

Field trials

In 2016, traps deployed in Nova Scotia (Figure 4.1.1h-i) captured numerically more *D. suzukii* than traps deployed in Newfoundland (see Figure 4.1.1g-h**)** (mean ± SE; NS trials mean=66.5±5.41, n=818; NL trials mean=3.29±0.42, n=160).

Some traps (Figure 4.1.1h-i) deployed in Nova Scotia in 2016 contained 500 g or more of insect captures. All traps from these blocks for those collection dates were excluded from analysis due to time limitations for counting. We considered counting a sub-sample from these traps; however, we were concerned that the odorant profile of the traps in question was compromised by the odor of large numbers of bycatch flies. We used a generalized linear mixed model to assess *D. suzukii* responses to trap colours and attractant lures in different crop settings using trap collection date as a random effect. Efficacy of trap colour and attractant lures differed among fruit crops (Type II Wald chi square tests; trap colour: χ^2=70.25, df=1, P<0.0001, adjuvant: χ^2=229.83, df=1, P<0.0001, crop: χ^2=2724.74, df=2, P<0.0001). For example, green/purple traps with no lure attracted more *D. suzukii* in raspberries and lowbush blueberries, but black/red traps with no lure were more attractive in highbush blueberries (Table 4.1.7a). Mean counts of *D. suzukii* captured in traps in 2016 Nova Scotia field trials were highest in highbush blueberry (N=818 samples, 1-way ANOVA, F=6.90, df=2,815, P=0.001). Green/purple traps contained fewer other *Drosophila* bycatch; however, differences among trap treatments were not significant (1-way ANOVA, F=0.49, df=3,814, P=0.69).

Table 4.1.7. Mean weekly counts of *D. suzukii* captured during 2016 field trials are shown for each trap treatment a) in Nova Scotia by crop system in which traps were deployed and b) mean counts per trap treatment in Newfoundland field trials.

Treatment	Tunnel raspberry		Highbush blueberry		Lowbush blueberry	
a) Nova Scotia	Mean	SEM	Mean	SEM	Mean	SEM
Black/red jar trap & phenylethylamine + phenylacetaldehyde + ethyl acetate	27.08	9.41	75.49	13.77	32.52	15.35
Black/red jar trap + no lure (control)	15.00	4.85	88.30	15.09	38.17	16.28
Green/purple jar trap & phenylethylamine + phenylacetaldehyde + ethyl acetate	17.58	5.70	71.16	12.49	31.96	13.49
Green/purple jar trap + no lure	48.08	18.21	75.75	13.76	41.39	21.58

b) Newfoundland	Mean	SEM
Green/purple jar trap + no lure	17.90	6.78
Green/purple jar trap & phenylethylamine + phenylacetaldehyde + ethyl acetate	20.70	10.44
Red/black Solo cup trap & yeast (control)	6.40	1.80

Due to low population densities of *D. suzukii* in Newfoundland, trap captures in 2016 were small. We calculated mean values for each trap design (see Figure 4.1.1g-h**)** using total seasonal captures per trap rather than captures/week. Green/purple traps with a 3-component (phenylethylamine + phenylacetaldehyde + ethyl acetate) attractant lure attracted more *D. suzukii* than other trap treatments and green/purple jar traps captured more *D. suzukii* than red/black Solo® cups with a yeast lure; however, differences in mean *D. suzukii* captures among trap treatments in 2016 Newfoundland field trials were not significant (N=60 samples, Kruskal-Wallis rank sum test, χ^2=1.31, df=2, P=0.52). (Table 4.1.7). All trap treatments captured more female than male *D. suzukii* (406/450 flies).

In 2017, among traps with apple cider vinegar as drowning solution, mean weekly *D. suzukii* counts in green/purple jar traps (see Figure 4.1.1l-m**)** were higher than black/red cup traps in Nova Scotia (n=24 for each treatment, Green/purple-Vinegar-Ethyl acetate+acetoin: mean=549.5, SEM=770.1, Green/purple-Vinegar- No lure: mean=548.9, SEM=789.0, Red/black-Vinegar-No lure: mean=422.5, SEM=524.8). Green/purple jar traps containing ethyl acetate + acetoin lures but using water as a drowning solution captured the least *D. suzukii* in all crop settings (N=24,mean=1.5, SEM=2.3) (Figure 4.1.7). In field-grown raspberries, green/purple traps with ethyl acetate + acetoin attractant lures and vinegar attracted more *D. suzukii* than other traps (Figure 4.1.7). In blueberries, green/purple traps with no attractant lure were more efficacious at capturing *D. suzukii*. In tunnel-grown raspberries, *D. suzukii* counts were lower than at other sites and *D. suzukii* numbers were a smaller proportion of the total *Drosophila* community. Green/purple traps with the attractant lure attracted more female *D. suzukii* overall than other traps, while green/purple traps without a lure attracted more male *D. suzukii*. The attractant lure improved overall trap specificity to *D. suzukii* (Table 4.1.8). We used a generalized linear mixed model to assess attraction to trap design treatments and site locations with trapping week as a random factor. Both trap treatment and location were significant (Type II Wald chi square tests; treatment: χ^2=1726.3, df=3, P<0.0001, location: χ^2=10302.8, df=3, P<0.0001).

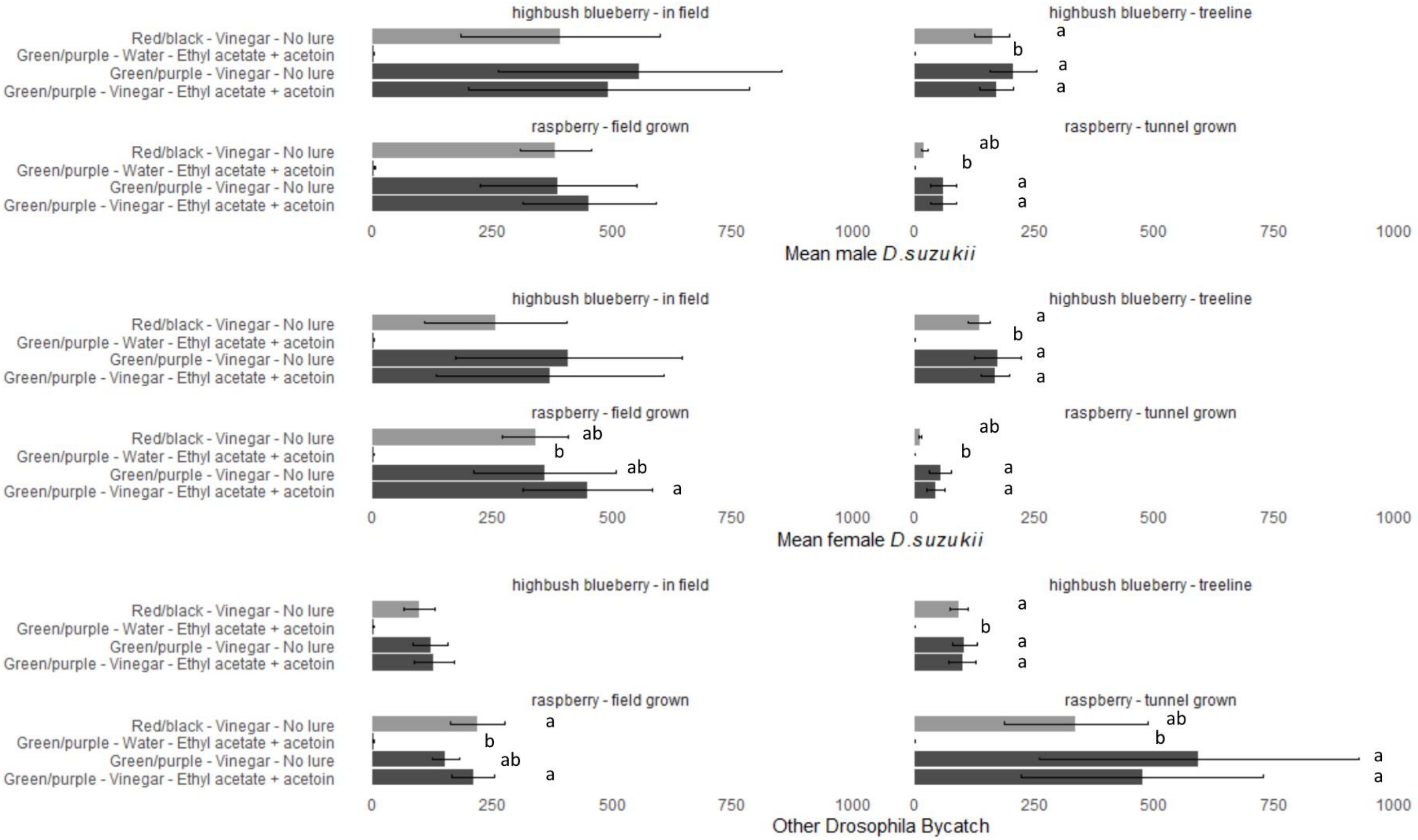


Figure 4.1.7. Mean trap captures of male and female *D. suzukii* and other *Drosophila* bycatch for trap design-attractant lure treatments during 2017 field trials among the field sites in Nova Scotia. Significant differences among treatments within each crop system are shown within figures (Tukey post hoc on 1-way ANOVA, P<0.05).

Table 4.1.8. Mean weekly trap captures of *D. suzukii* and other *Drosophila* (± SEM) and specificity of each trap treatment (percentage of total *Drosophila* captures were *D. suzukii*) during 2017 field trials in a) Newfoundland by geographic region and b) Nova Scotia. Mean values in bold denote treatment with highest *D. suzukii* attraction.

a) Newfoundland - Treatment					**Males**		**Females**		**total *D. suzukii***		***Drosophila* bycatch**		
Trap	**Attractant lure**	**Drowning solution**	**Region**	**n**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**Specificity**
Black-red Solo cup (control)	yeast	apple cider vinegar	East	15	0.00	0.00	0.07	0.07	0.07	0.07	4.20	1.86	1.56
			West	8	0.63	0.32	3.38	1.93	4.00	2.19	1.00	0.57	80.00
Green-purple jar (control)	n/a	apple cider vinegar	East	10	0.10	0.10	0.00	0.00	0.10	0.10	6.40	2.51	1.54
			West	8	**6.63**	**1.84**	**16.63**	**7.01**	**23.25**	**8.66**	14.50	8.35	61.59
Green-purple jar	ethyl acetate + acetoin	apple cider vinegar	East	10	0.10	0.10	0.70	0.40	0.80	0.49	136.30	57.42	0.58
			West	8	2.88	0.69	6.63	2.36	9.50	2.61	7.00	2.81	57.58

b) Nova Scotia - Treatment				**Males**		**Females**		**Total *D. suzukii***		***Drosophila* bycatch**		**Specificity**
Trap	**Attractant lure**	**Drowning solution**	**n**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	
Black-red jar (control)	n/a	apple cider vinegar	24	237.75	61.54	184.75	46.41	422.5	107.13	185	43.56	69.55%
Green-purple jar (control)	n/a	apple cider vinegar	24	**301.33**	**88.94**	247.58	72.76	548.92	161.06	240.83	89.42	69.51%
Green-purple jar	ethyl acetate + acetoin	apple cider vinegar	24	292.54	85.35	**256.92**	**72.46**	**549.46**	**157.2**	227	68.32	70.76%
Green-purple jar	ethyl acetate + acetoin	water	24	0.92	0.39	0.58	0.15	1.5	0.46	0.13	0.07	92.02%

Trap captures (see Table S4.1.3, Figure 4.1.1l-o) in Nova Scotia in 2018 suggest that *D. suzukii* populations in Nova Scotia remain relatively high (N=192, mean=76.6, SEM=7.55). Drosal® traps captured fewest numbers of *D. suzukii* of the six trap treatments (1-way ANOVA, F=4.37, df=5,186, P<0.001). Jar style traps with apple cider vinegar drowning solutions attracted more *D. suzukii* than Drosal® traps or jar style traps with DrosaLure® solution overall (1-way ANOVA, F=10.89, df=2,189, P<0.0001). However, efficacy of trap treatment combinations differed among crop systems (Table 4.1.9). Specificity for *D. suzukii* differed among treatments and among crop systems; however, specificity rates often exceeded 70% (Table 4.1.10).

Table 4.1.9. Mean weekly *D. suzukii* counts for each of six trap treatments among different crop systems during the five-week 2018 Nova Scotia field trials. Highest mean values shown in bold for each block and crop system. (N=240 samples)

a)

Trap treatment			**Blueberry**									
			block 1		block 2		block 3		block 4		all blueberry	
Trap	Attractant lure	Drowning solution	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
green/purple jar	5µg ethyl acetate + 50ηg acetoin	apple cider vinegar	**192.75**	**344.88**	92.75	175.02	**81.50**	**81.31**	**130.00**	**77.68**	**124.25**	**34.62**
green/purple jar (control)	n/a	apple cider vinegar	107.00	66.42	143.00	83.42	72.50	33.67	60.75	18.86	95.81	26.68
black/red jar (control)	n/a	apple cider vinegar	131.50	76.02	**187.25**	**122.63**	55.00	25.70	62.50	35.29	109.06	36.49
green/purple jar	Drosalure	Drosalure	72.50	27.67	55.00	17.70	52.25	9.13	49.50	13.18	57.32	8.50
green/purple Drosal	Drosalure	Drosalure	43.25	21.31	34.00	11.87	35.75	13.75	31.50	10.91	36.13	6.81
black/white Drosal	Drosalure	Drosalure	60.25	24.45	56.00	17.89	38.50	4.56	46.25	13.02	50.25	7.76

b)

Trap treatment			**Tunnel raspberry**						**Field raspberry**		**Field blackberry**	
			block 5		block 6		all tunnel raspberry		block 7		block 8	
Trap	Attractant lure	Drowning solution	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
green/purple jar	5µg ethyl acetate + 50ηg acetoin	apple cider vinegar	11.75	1.65	14.50	3.43	13.13	1.84	123.25	13.40	187.25	55.54
green/purple jar (control)	n/a	apple cider vinegar	40.25	26.19	10.50	2.63	25.38	13.42	114.25	49.26	**309.75**	**116.85**
black/red jar (control)	n/a	apple cider vinegar	13.75	5.96	15.25	9.62	14.50	5.25	**208.25**	**112.79**	257.25	91.92
green/purple jar	Drosalure	Drosalure	**42.00**	**12.83**	**22.75**	**5.86**	**32.38**	**21.14**	70.25	15.88	108.25	33.60
green/purple Drosal	Drosalure	Drosalure	12.50	2.50	5.25	1.25	8.88	1.88	42.00	7.38	49.00	13.00
black/white Drosal	Drosalure	Drosalure	23.25	11.32	13.25	6.34	18.25	6.30	35.75	8.50	54.75	9.00

Table 4.1.10. Efficacy (mean weekly trap captures) and specificity (*D. suzukii* as percentage of total *Drosophila* captures) for trap treatments among crops and growing conditions for each trap treatment within each crop system sampled during the five-week 2018 Nova Scotia field trial.

Trap treatment			**Blackberry**					**Blueberry**				
Trap	Attractant lure	Drowning solution	*D. suzukii*		Other *Drosophila*		Specificity	*D. suzukii*		Other *Drosophila*		Specificity
			Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
green/purple jar	5µg ethyl acetate + 50ηg acetoin	apple cider vinegar	228.33	52.85	43.67	16.17	83.95%	124.25	34.62	151	22.25	45.14%
green/purple jar (control)	n/a	apple cider vinegar	309.75	116.85	54.25	8.98	85.10%	95.81	26.68	161.31	22.25	37.26%
black/red jar (control)	n/a	apple cider vinegar	257.25	81.92	47	19.63	84.55%	109.06	36.49	152.38	20.11	41.72%
green/purple jar	Drosalure	Drosalure	108.25	33.6	5.25	3.07	95.37%	57.31	8.5	8.75	1.63	86.75%
green/purple Drosal	Drosalure	Drosalure	49	13	2	0.41	96.08%	36.13	6.81	5.5	1.15	86.79%
black/white Drosal	Drosalure	Drosalure	54.75	9	5.5	3.52	90.87%	50.25	7.76	11.31	1.99	81.62%

Trap treatment			Field-raspberry					Tunnel-raspberry				
Trap	Attractant lure	Drowning solution	*D. suzukii*		Other *Drosophila*		specificity	*D. suzukii*		Other *Drosophila*		specificity
			mean	se	mean	se		mean	se	mean	se	
green/purple jar	5μg ethyl acetate + 50ηg acetoin	apple cider vinegar	111.4	15.75	48.2	11.5	69.80%	13.13	1.84	76	14.43	14.73%
green/purple jar (control)	n/a	apple cider vinegar	114.25	49.26	41.75	7.35	73.24%	25.38	13.42	62	9.9	29.04%
black/red jar (control)	n/a	apple cider vinegar	208.25	112.79	45	6.1	82.23%	14.5	5.25	56.75	13.85	20.35%
green/purple jar	Drosalure	Drosalure	70.25	15.88	6	1.83	92.13%	32.38	7.47	27	8.75	54.53%
green/purple Drosal	Drosalure	Drosalure	42	7.38	3.5	0.96	92.31%	8.88	1.88	10.38	4.12	46.10%
black/white Drosal	Drosalure	Drosalure	35.75	8.5	14.25	7.8	71.50%	18.25	6.3	7.75	2	70.19%

4.1.5 Discussion

Through an iterative process, we tested a variety of trap design attributes, including trap size, trap shape, trap colour, and trap entry hole size. We also tested the effects of providing shade over the entry holes and of introducing mesh screening across entry holes. Our field trials confirm the findings of Renkema et al. (2014) that jar style traps with larger entry holes are more effective for capturing *D. suzukii*. We further found that mesh screening across the trap entry holes limited the capture of non-target bycatch without compromising *D. suzukii* captures.

Efficacy of trap colours differed dependent upon the fruit crop in which traps were deployed. Generally, green/purple traps were more effective than traditional red/black trap colours, particularly in blueberries and blackberries; however, results were mixed in raspberries (see Little et al. 2019). This suggests that crop systems must be considered in the selection of trap colours or designs.

Owing to differences in trapping regimes and crop systems between Nova Scotia and Newfoundland, statistical analysis of field trial captures between provinces could not be analysed statistically. However, throughout this study, *D. suzukii* captures were consistently lower in Newfoundland than in Nova Scotia, suggesting that current environmental conditions in Newfoundland limit *D. suzukii* population growth (Langille et al. 2017). However, the presence of *D. suzukii* in traps deployed in Newfoundland and the apparent increase in captures year over year suggests that conditions are suitable for overwintering and that further climate change could elicit *D. suzukii* population increases.

Across all years of this study, traps deployed among raspberries grown in high tunnel systems consistently captured low numbers of *D. suzukii* regardless of trap design or adjuvant used. This was consistent with lower infestation rates observed in tunnel systems versus field systems in previous studies (Rogers et al. 2016), perhaps due in part to temperatures within tunnel systems exceeding conditions suitable for *D. suzukii* mating, oviposition, and larval development (Rogers et al. 2016; Drummond et al. 2019). However, where high tunnels are managed to keep temperatures more moderate, tunnel structures can become protected environments which permit populations of *D. suzukii* and other pest insects to thrive (Ingwell et al. 2017).

We also employed an iterative process to identify compounds and mixtures for use as an attractant lure. Recognizing that physiological responses of adult flies could differ dependent upon the sex and mating status, we used electroantennography to identify compounds and relative dosages which elicited strongest physiological responses from adult virgin and mated male and female *D. suzukii*. Consistent with other studies, some fruit ripening associated compounds elicited a positive dose-dependent response; however, we observed other relationships between dose and responses among the compounds we tested (Bolton et al. 2019). The results of these tests informed the selection of compounds and relative doses of compounds in mixtures for further testing in laboratory and field trials.

We had mixed results using commercially available lures, drowning solutions, and traps. BioLure® was effective at capturing *D. suzukii* when used in MultiTraps® with apple cider vinegar as a drowning solution, but this efficacy was limited to traps deployed within field-grown raspberries. MultiTraps® were also prone to large numbers of non-target bycatch, although this issue may be resolved by covering the trap opening with mesh. DrosaLure® was effective in tunnel-grown raspberries but results were mixed in other fruit systems and growing conditions. Drosal® traps, particularly those recoloured in green and purple, captured limited numbers of *D. suzukii*, but also had almost no bycatch.

Efficacy of attractant compounds and mixtures varied dependent upon crops in which traps were deployed and fruit growing conditions (e.g. open field versus high tunnels). By using an iterative process, we were able to reassess and refine odorant mixtures through incremental steps toward an improved attractant lure. Differences among attractant compounds and mixtures, however slight, were informative to this process. In some cases, such as 2016 field trials in Newfoundland, small capture numbers resulted in differences that were informative but not statistically significant. In 2016 laboratory choice trials, five different blends of the same four compounds were tested against each other and a blank control in the same arena. Differences in attraction among the choices were slight, in large part because differences in the odorant profiles of the choices were slight and odorant fields could have spread and overlapped over the course of the assay. Our final 2-component mixture of ethyl acetate + acetoin (5µg:50ng mixture) was most effective when deployed in blueberries, but less effective in cane fruits (e.g. raspberries and blackberries). Jar style traps with mesh covering large diameter entrance holes afforded the best balance of high *D. suzukii* captures and reduced bycatch.

Drosophila suzukii is a highly adaptable frugivore, able to use multiple physical, chemical, and visual cues to identify suitable fruits for feeding and oviposition (Lee et al. 2012, Bellamy et al. 2013, Poyet et al. 2015, Lee and Sial 2016, Karageorgi et al. 2017, Lasa et al. 2017, Little et al. 2017, 2018, Zerulla et al. 2017). This invasive insect has become an important agricultural pest in a large number of commercially grown soft fruit and berry crops across at least four continents, representing a diverse set of environmental conditions with a broad spectrum of sensory cues (Hauser 2011, Walsh et al. 2011, Calabria et al. 2012, Thistlewood et al. 2012, Cini et al. 2014, Deprá et al. 2014, Asplen et al. 2015, Kenis et al. 2016, Lee and Sial 2016, Andreazza et al. 2017, dos Santos et al. 2017). It is therefore not surprising that efficacy of any single adjuvant, trap design, or drowning solution would differ among crop systems. Current trapping systems are of limited efficacy in controlling *D. suzukii* populations, sometimes resulting in increases in infestation rates rather than mitigating the problem (Cloonan et al. 2018). Standard monitoring and trapping efforts for *D. suzukii* use traps and drowning solutions and sometimes lures which lack specificity and capture high proportions of non-target bycatch (Cloonan et al. 2018). Careful consideration of trap design, trap colour, drowning solution, and attractant lures in relation to the fruit crop system can achieve high *D. suzukii* capture rates and/or high specificity. The relative importance of either trapping metric would depend on the purpose of the trapping effort, be it mass-trapping, population monitoring, or early detection.

Overall, we found that jar-style traps were more effective than other trap designs that we tested; however, efficacy of trap colour differed dependent upon the crop system in which the trap was used. Green/purple traps were more effective in blueberry crop systems, while red-black were more effective in *Rubus* spp. systems. Consistent with Renkema et al. (2014), we found that larger entrance openings in traps improved capture rates and mesh barriers over these opening reduced bycatch numbers without limiting the efficacy of traps. Although promising in laboratory trials, *H. rhamnoides* fruit were ineffective in laboratory behavioural assays (Little et al. 2017) and field trapping trials, possibly because the odour cues from a small volume of fruit were overwhelmed by ambient environmental odours. Attractant lures paired with apple cider vinegar drowning solutions were effective in increasing *D. suzukii* capture numbers. By using relative proportions of attractant lure compounds based on odorant sensitivities pertinent to fly sex and mating status, it is possible to target sub-populations of *D. suzukii* and reduce captures of non-target insects, including native *Drosophila* spp. However, efficacy of any lure was also dependent upon trap design used and crop system in which the traps were deployed. *Drosophila suzukii* has demonstrated that it is highly adaptable capable of using a wide range of fruits for feeding and reproduction, able to adapt to diverse environmental conditions, and expand into new geographic regions across much of the globe (Hauser 2011, Walsh et al. 2011, Cini et al. 2014, Deprá et al. 2014, Asplen et al 2015, Poyet et al. 2015, dos Santos et al. 2017, Langille et al. 2017, Lee and Sial 2016, Little et al. 2020b). This plasticity has contributed to the species' success as an invasive and continues to present challenges to efforts to monitor populations and mitigate its effects on crops systems or natural ecosystems.

We suggest that future research should consider means to address infestations within each crop system separately, using existing research and commercially available solutions as starting points upon which to build. Potential solutions should consider olfactory and visual cues and environmental conditions present within the crop system when considering prospective traps, attractant odorant lures, and drowning solutions.

4.6.1 Acknowledgements

We are grateful for the many fruit growers in Nova Scotia and Newfoundland who generously allowed us access to their crops. We also wish to thank the many people who assisted us in this endeavour or offered invaluable advice, including H. Caravan, A. Chaulk, L. Charbonneau, H. Crozier, L. Gray, R. Hallett, R. Isett, D. Kristie, C. MacKay, L. Madore, C. Parsons, S. Pawlowski, T. Power, M. Prieztniz, J. Renkema, E. Rand, A.R. Rizzato, B. Robichaud, and M. Snyder. We gratefully acknowledge the financial support of Atlantic Canada Opportunities Agency Atlantic Innovation Fund (197853), Canada Foundation for Innovation (22087), Natural Sciences and Engineering Research Council of Canada (356109-2008 RGPIN and PGSD2-475743-2015), and Growing Forward 2 in Newfoundland and Labrador (GF21415-309, GF21516-519, GF21617-773, and GF21718-956).

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4.8 Supplementary information

Table S4.1.1. List of compounds tested through electroantennography in 2014 and resulting selection of compounds for further testing in 2014 behavioural 2-choice trials.

Compound	CAS #	Solvent	Relevance	Reference	2014 choice trials	
					females	males
acetate esters						
ammonium acetate	631-61-8	methanol	Biolure component	Leblanc et al. 2010		
benzyl acetate	140-11-4	hexane	plant odor / attractive to *D. melanogaster*	Root et al. 2007 / Bruce & Pickett 2011		x
ethyl acetate	141-78-6	hexane	*Drosophila* have receptors/ assoc. with ripening fruits	de Bruyne et al. 2001 / Hallem et al. 2004 / Larsson et al. 2004		
geranyl acetate	105-87-3	ethanol	toxic to some flies	de Bruyne et al. 2001 / Hallem et al. 2004 / Date et al. 2013		
hexyl acetate	142-92-7	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Stökl et al. 2010 / Schubert et al. 2014		
isoamyl acetate	123-92-2	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Hallem et al. 2004 / Larsson et al. 2004 / Root et al. 2007 / Schubert et al. 2014	x	
isopentyl acetate (pentyl acetate)	628-63-7	hexane	vinegar odor	de Bruyne et al. 2001 / Hallem et al. 2004 / Silbering and Galizia 2007		

aldehydes

benzaldehyde	100-52-7	hexane	aversion odor for *D. melanogaster*	Rodrigues 1980 / Hallem et al. 2004 / Stensmyr et al. 2012	x	x
furfural (2-furaldehyde)	1998-01-01	hexane	*Drosophila* have receptors / vinegar	Stensmyr et al. 2003 / Qian et al. 2013	x	
heptanal	111-71-7	hexane	fruit odor			
hexanal	66-25-1	hexane	fruit odor	Larsson et al. 2004 / Date et al. 2013	x	
nonanal (nonyl aldehyde)	124-19-6	hexane	floral odor	Georgilopoulos & Gallois 1987		
phenylacetaldehyde	122-78-1	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Grosjean et al. 2011		
amines						
dimethyl amine	14802-36-9	hexane	plant odor / decomposition odor / attractive to *D. melanogaster*	Min et al. 2013		
phenylethylamine	64-04-0	hexane	floral odor	Grosjean et al. 2011	x	x
putrescine (1,4 diaminobutane)	110-60-1	ethanol	fruit odor / decomposition odor / attractive to *D. melanogaster*	Leblanc et al. 2010 / Min et al. 2013	x	x
trimethylamine hydrochloride	593-81-7	ethanol	fruit odor / fruit cuticle	Leblanc et al. 2010	x	x
carboxylic acids						
2 methyl butanoic acid	116-53-0	hexane	fruit odor	Pyysalo 1977 Thesis	x	
acetic acid	64-19-7	hexane	fruit odor / vinegars / attractive to *D. melanogaster*	Pyysalo 1977 Thesis / Hoffmann & Parsons 1984 / Qian et al. 2013		

butyric acid (butanoic acid)	107-92-6	hexane	vinegars	Qian et al. 2013	x	
hexanoic acid	142-62-1	hexane	fruit odor / *D. melanogaster* receptor	Pyysalo 1977 Thesis / Qian et al. 2013	x	
isovaleric acid	503-74-2	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Qian et al. 2013		
propionic acid	1979-09-04	hexane	attractive to *D. melanogaster* / Biolure component	Hoffmann & Parsons 1984 / Silbering and Galizia 2007		x
ursolic acid	77-52-1	ethanol	fruit odor / fruit cuticle	Peschel et al. 2007	x	x
carboxylic esters						
benzyl benzoate	120-51-4	hexane	floral / *Drosophila* have receptors	DeCoursey 1925		
butyl butyrate	109-21-7	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Schubert et al. 2014		
ethyl 3-hydroxybutyrate	5405-41-4	ethanol	*Drosophila* have receptors	Stensmyr et al. 2003 / Date et al. 2013		
ethyl 3-hydroxyhexanoate	2305-25-1	ethanol	*Drosophila* have receptors	Stensmyr et al. 2003	x	
ethyl butyrate	105-54-4	hexane	*Drosophila* have receptors / fruit odor	de Bruyne et al. 2001 / Stensmyr et al. 2003 / Hallem et al. 2004 / Grosjean et al. 2011 / Schubert et al. 2014		
ethyl hexanoate	123-66-0	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Stökl et al. 2010	x	

methyl hexanoate	106-70-7	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Larsson et al. 2004		
methyl salicylate	119-36-8	hexane	fruit odor / fruit cuticle	de Bruyne et al. 2001 / Hallem et al. 2004	x	x
hydrocarbons						
hentriacontane	630-04-6	hexane	fruit odor / fruit cuticle	Peschel et al. 2007		
heptacosane	593-49-7	hexane	fruit fly aggregation (Tephridids)	Peschel et al. 2007		x
hexane	110-54-3	n/a	fruit odor / vinegars			
nonacosane	630-03-5	hexane	fruit odor	Peschel et al. 2007		
paraffin oil	8012-95-1	hexane	promotes male *Drosophila* courtship / fruit odor	de Bruyne et al. 2001 / Hallem et al. 2004		
triacontane	638-68-6	hexane	Biolure component	Peschel et al. 2007		
ketones						
2,3 butanedione	431-03-8	hexane	*Drosophila* have receptors	de Bruyne et al. 2001 / Hallem et al. 2004	x	
acetoin	513-86-0	ethanol	in wine/vinegar	Stensmyr et al. 2003 / Becher et al. 2010/ Stökl et al. 2010 / Landolt et al. 2012 / Cha et al. 2013 / Date et al. 2013	x	
acetyl furan (2-furyl methyl ketone)	1192-62-7	ethanol	*Drosophila* have receptors	Stensmyr et al. 2003	x	x
furaneol (strawberry furanone)	3658-77-3	hexane	fruit odor / wine & fermenting grapes	Barata et al. 2011		
geranyl acetone	3796-70-1	ethanol	fruit odor / fruit cuticle	Mann et al. 2010 / Kaufman et al. 2011	x	x

nonanone	821-55-6	hexane	promotes male *Drosophila* courtship / fruit odor	Pelz et al. 2006	x	
primary alcohols						
1-hexanol	111-27-3	hexane	fruit odor / physiologically active in *D. melanogaster*	de Bruyne et al. 2001 / Stensmyr et al. 2003 / Hallem et al. 2004 / Root et al. 2007 / Silbering and Galizia 2007 / Stökl et al. 2010		x
1-octanol	111-87-5	hexane	blackberry odor	Georgilopoulos & Gallois 1987 / Date et al. 2013		x
2-phenyl ethanol	1960-12-08	hexane	fruit odor / physiologically active in *D. melanogaster*	Pyysalo 1977 Thesis / Stensmyr et al. 2003 / Larsson et al. 2004 / Becher et al. 2010/ Stökl et al. 2010 / Date et al. 2013 / Qian et al. 2013	x	x
cis-3-hexen-1-ol (z3-O6-OH)	928-96-1	hexane	leaf volatile	Reddy & Guerrero 2004 / Barata et al. 2011 / Date et al. 2013		
ethanol	64-17-5	water	fruit fermentation odor / solvent / control / attractive to *D. melanogaster*	Parsons 1981 / Becher et al. 2012		
isoamyl alcohol	123-51-3	hexane	fruit odor / physiologically active in *D. melanogaster*	Stensmyr et al. 2003 / Date et al. 2013		x
methanol	67-56-1	n/a	physiologically active in *D. melanogaster*			
nonanol	143-08-8	hexane	fruit odor	Neckameyer et al. 2007		x

propanol	67-63-0	hexane	*Drosophila* have receptors		x
secondary alcohols					
1-octen-3-ol	3391-86-4	hexane	fungal odor / neurotoxin / *Drosophila* have receptors	de Bruyne et al. 2001 / Hallem et al. 2004 / Larsson et al. 2004 / Inamdar et al. 2010 /	
2-heptanol	543-49-7	hexane	fruit odor	Georgilopoulos & Gallois 1987	x
uvaol	545-46-0	methanol	fruit odor / fruit cuticle	Peschel et al. 2007	
other compounds					
geosmin (+/-)	16423-19-1	hexane	aversion odor for *D. melanogaster* / mold & toxic bacteria odor	Gerber & Lecevalier 1965 / Mattheis & Roberts 1992 / Stensmyr et al. 2012	

Table S4.1.2. Individual trials conducted over the five years of this study investigated a combination of single compounds and compound mixtures for use as attractant lures, and trap designs for use as monitoring traps. Experiments included controls without lures. Field trials conducted in Newfoundland used controls with a yeast (*Saccharomyces cerevisiae*) lure.

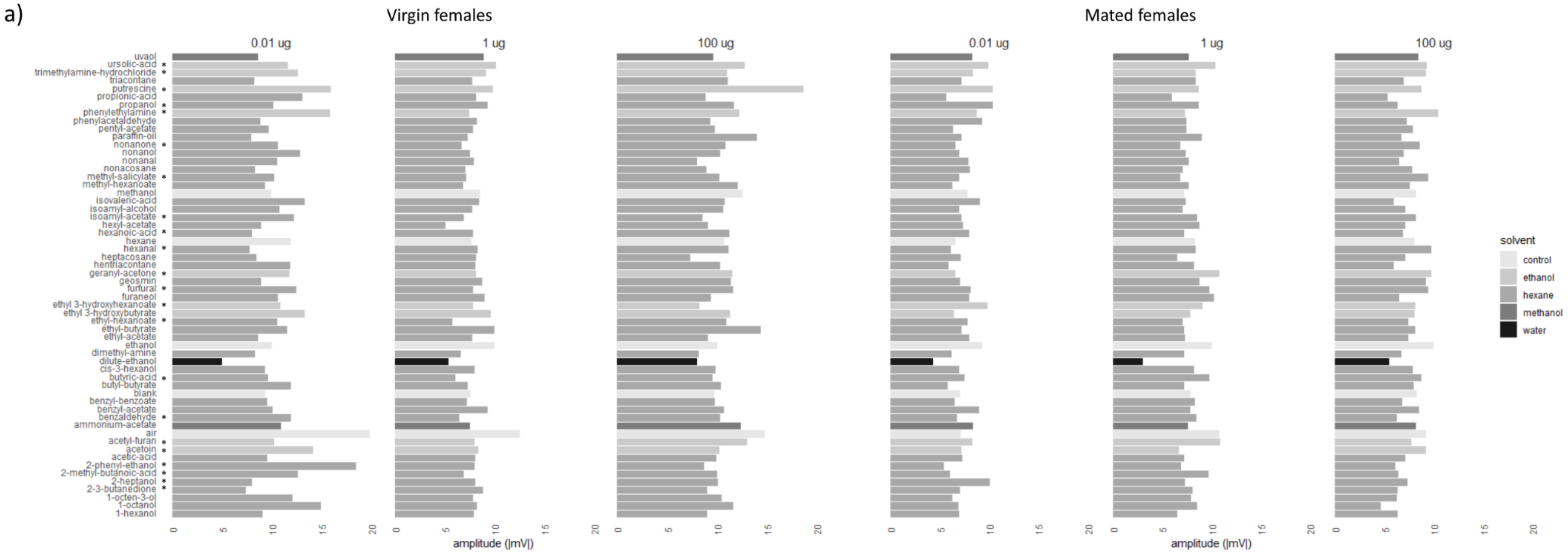
Year	Experiments	Focus	Lure compounds/mixtures	Trap designs
2014	Electroantennography	single compounds	see Table S1	n/a
	Laboratory two-choice trials	single compounds	see Table 1	n/a
	Field trials			
	NS trials	single compounds	acetoin, benzaldehyde, benzyl acetate, furfural, methyl salicylate, ursolic acid, BioLure	see Figure 4.1.1a
		trap designs	furfural, ursolic acid, BioLure	see Figure 4.1.1a-d
2015	Laboratory two-choice trials	single compounds vs. compound mixtures	*Step 1.* putrescine, trimethylamine hydrochloride, putrescine + trimethylamine hydrochloride mixture	n/a
			Step 2. see Table 3	n/a
			Step 3. compounds: phenylacetaldehyde, methyl salicylate	n/a
			mixtures: ethyl acetate + putrescine, furfural + putrescine, hexyl acetate + methyl salicylate, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, ursolic acid + ethyl acetate, ursolic acid + hexyl acetate, (cis-3-hexen-1-ol + ethyl acetate, cis-3-hexen-1-ol + phenylethylamine, cis-3-hexen-1-ol + putrescine, and cis-3-hexen-1-ol + ursolic acid	
			Step 4. H. rhamnoides fruits, furfural + putrescine, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, phenylethylamine + putrescine, cis-3-hexen-1-ol + ursolic acid	n/a

	Field trials				
		NS trials	compound mixtures	*H. rhamnoides* fruits, furfural + putrescine, phenylacetaldehyde + ethyl acetate, phenylacetaldehyde + putrescine, phenylethylamine + putrescine, cis-3-hexen-1-ol + ursolic acid	see Figure 4.1.1f
			trap designs	phenylacetaldehyde + putrescine, phenylethylamine + ethyl acetate	see Figure 4.1.1b & e-f
		NL trials	compound mixtures	phenylethylamine + ethyl acetate, yeast	see Figure 4.1.1f-g
2016	Laboratory multiple-choice trials		compound mixtures	phenylethylamine + phenylacetaldehyde + ethyl acetate, phenylethylamine + phenylacetaldehyde + putrescine, phenylacetaldehyde + putrescine + ethyl acetate, phenylethylamine + putrescine + ethyl acetate, phenylethylamine +-phenylacetaldehyde + putrescine + ethyl acetate	n/a
	Field trials				
		NS trials	compound mixtures & trap designs	phenylethylamine + phenylacetaldehyde + ethyl acetate	see Figure 4.1.1h-i
		NL trials	compound mixtures	phenylethylamine + phenylacetaldehyde + ethyl acetate, yeast	see Figure 4.1.1g-h
2017	Laboratory choice trials				
		Trap choice trials	trap designs	phenylethylamine + phenylacetaldehyde + ethyl acetate	see Figure 4.1.1h & j-l

	Field trials				
		NS trials	compound mixtures & trap designs	ethyl acetate + acetoin	see Figure 4.1.1l-m
		NL trials	compound mixtures	ethyl acetate + acetoin, yeast	see Figure 4.1.1g and l
2018	Field trials				
		NS trials	compound mixtures & trap designs	5µg ethyl acetate + 50ηg acetoin	see Figure 4.1.1l-o

Table S4.1.3. Trap treatments field tested in Nova Scotia in 2018. Trap designs are shown in Figure S4.1.1l-o.

Trap ID	Trap style	Colour	Attractant lure	Drowning solution
A	Drosal	Black/white	None	DrosaLure
B	Drosal	Green/purple	None	DrosaLure
C	Jar	Green/purple	ethyl acetate (5µg) + acetoin (50ηg)	apple cider vinegar
D	Jar	Green/purple	None	DrosaLure
E	Jar	Green/purple	None	apple cider vinegar
F	Jar	Black/red	None	apple cider vinegar


a)
Virgin females
Mated females
0.01 ug
1 ug
100 ug
0.01 ug
1 ug
100 ug
uvaol
ursolic-acid *
trimethylamine-hydrochloride *
triacontane
putrescine *
propionic-acid
propanol *
phenylethylamine *
phenylacetaldehyde
pentyl-acetate
paraffin-oil
nonanone *
nonanol
nonanal
nonacosane
methyl-salicylate *
methyl-hexanoate
methanol
isovaleric-acid
isoamyl-alcohol
isoamyl-acetate *
hexyl-acetate
hexanoic-acid *
hexane
hexanal *
heptacosane
hentriacontane
geranyl-acetone *
geosmin
furfural *
furaneol
ethyl 3-hydroxyhexanoate *
ethyl 3-hydroxybutyrate
ethyl-hexanoate *
ethyl-butyrate
ethyl-acetate
ethanol
dimethyl-amine
dilute-ethanol
cis-3-hexanol
butyric-acid *
butyl-butyrate
blank
benzyl-benzoate
benzyl-acetate
benzaldehyde *
ammonium-acetate
air
acetyl-furan *
acetoin *
acetic-acid
2-phenyl-ethanol *
2-methyl-butanoic-acid *
2-heptanol *
2-3-butanedione *
1-octen-3-ol
1-octanol
1-hexanol
0
5
10
15
20
amplitude (|mV|)
amplitude (|mV|)
solvent
control
ethanol
hexane
methanol
water

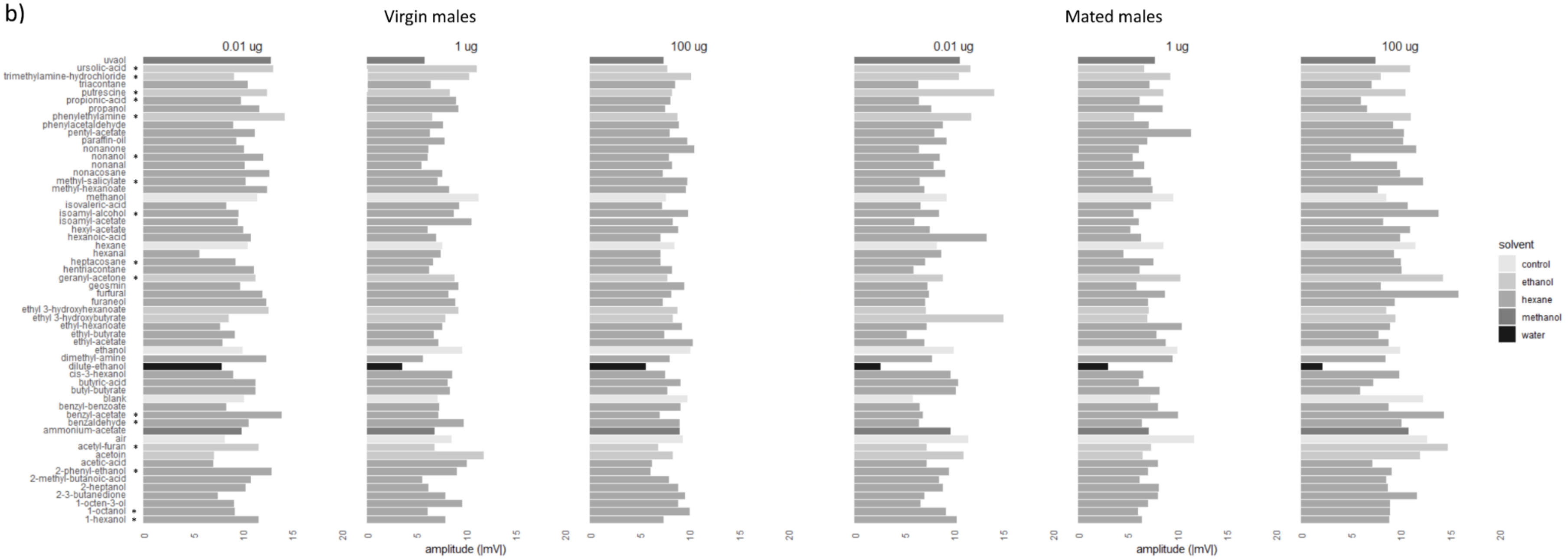


Figure S4.1.1. Mean standardized amplitudes (absolute mV value) from electroantennograms completed in 2014 for virgin and mated a) female and b) male flies at three discrete doses. SEM not shown to improve clarity of figure. Asterisks denote compounds tested in choice assays in 2014.

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Chapter 5

General conclusion

5.1 General conclusion

Drosophila suzukii has become an invasive fruit pest of economic importance throughout Asia, North America, Europe, and most recently South America. Efforts to develop an effective integrated pest management system are ongoing worldwide. Effective monitoring protocols are instrumental to optimizing timing for mediation efforts.

Chapter 1 explored aspects of *D. suzukii* physiology and behaviour that contributed to the species' introduction and invasiveness across diverse climates and geography. In this review, we discussed physiological plasticity that permits *D. suzukii* to adapt to diverse climatic conditions, including a broad range of temperature and humidity values. Distinct summer and winter morph variants allow *D. suzukii* to overwinter successfully in boreal regions of Canada and northern Europe. Individual *D. suzukii* flies can adapt their behaviour to take advantage of microclimate conditions and to seek out alternative feeding and reproductive sites when preferred hosts are unavailable.

Chapter 2 investigated host preference and host selection among introduced and endemic fruits in Atlantic Canada. We observed a sort of pragmatism, in that the level of acceptance of any fruit by *D. suzukii* was dependent upon the alternative available. Fruit characters that are indicative of fruit ripening, including fruit firmness, sweetness, and acidity, were assessed as factors in larval success and host selection by gravid female flies. Female *D. suzukii* can use these characters to assess fruit ripeness and generally preferred more acidic fruits. We observed mixed responses between fruit sweetness (brix) and attraction of *D. suzukii*. These results were consistent with results in other studies (Burrack et al. 2013, Lee et al. 2016). A characteristically robust ovipositor allows female *D. suzukii* to use a broad range of fruits and berries at earlier stages of ripeness than competitor Drosophila species; however, fruit firmness is still a limiting factor in successful oviposition. Where suitable alternative fruit species are not available, female *D. suzukii* will oviposit in damaged fruits or oviposit on the surface of firm, undamaged fruits which could then soften sufficiently before eggs hatch for larvae to infest the fruit.

Among individual fruit species, changes in fruit colour are indicative of ripeness. *Drosophila suzukii* uses a wide range of fruit species that ripen to colours that human observers would perceive as incorporating all colours of the colour wheel. We investigated objective measures of fruit colour (wavelength reflectance) within *D. suzukii* sensitivity range to determine what aspects of fruit colour use as cues to identify suitable host fruits.

Drosophila suzukii has been established in Nova Scotia since 2011. Records from Newfoundland and Labrador have shown that *D. suzukii* were present in low numbers since 2013; however, it was not yet known if populations were overwintering successfully or being reintroduced annually. We beta-tested a citizen science initiative to determine the extent of the distribution of *D. suzukii* across Nova Scotia and Newfoundland and to explore the range of host fruit species used by *D. suzukii* across this geographic range.

Chapter 3. Fruit colour and contrast with surrounding foliage are commonly used by frugivores, including birds, mammals, and insects, to identify ripe or ripening fruits (Schmidt et al. 2004, Schaefer et al. 2006). Throughout the late summer and autumn in Atlantic Canada, blueberry (*Vaccinium* spp.) fruits and foliage change in colour, indicative of each fruit's ripeness and the plant's condition. We explored the relationship between these colour changes, the contrast between fruit and foliage, and attraction behaviour in *D. suzukii*. We found that *D. suzukii* preferred fruits with colour indicative of being fully ripe. Blue ripe fruits which contrasted strongly with autumnal red senescing leaves or stressed yellow leaves were also preferred. Differences between fruit and leave colours at these stages were due primarily to contrasts in reflected light wavelengths in the blue to cyan range (470‑525 nm).

Drawing on this understanding of the relationship between colour and contrast in host selection among blueberry fruits, we explored *D. suzukii* preferences for colours using a wider array of colour choices. Again, we used an objective measure of wavelength reflectance to quantify the colour choices and determine what aspect of the visual signals act as attraction cues to *D. suzukii*. As in other *Drosophila* visual systems, colour opponency plays a strong role in *D. suzukii* discerning among colours. We found *D. suzukii* attracted most strongly to use of 2-colour targets in which the colour opponency patterns of reflected wavelengths contrasted strongly.

In **chapter 4**, we investigated odorants associated with fruits and foliage, and odorants known to be attractive to *D. melanogaster* as potential attractant odorants for *D. suzukii*. We used electroantennography to assess the relative sensitivity of 56 odorant compounds at three loads (10 ng (0.01 µg), 1 µg, and 100 µg). Responses of individual *D. suzukii* flies, categorized as virgin or mated and male or female, were assessed to narrow the field for further testing in 2-choice assays. We then field tested the six most promising odorant compounds as adjuvants and a commercially available lure against a control trap with no lure. Simultaneously, we began testing variants of cup traps commonly in use in North America against commercially available modified McPhail traps.

Through an iterative process over the next four years, we used choice assays and field trials to test blends of odorants and refinements of trap design, including lessons learned from our colour research and design developments based on Renkema et al. (2014). We field tested in a variety of conditions including field and tunnel grown raspberries (*Rubus idaeus*), high bush blueberries

(*Vaccinium corymbosum*), and mixed blueberries. (*Vaccinium* spp.), and field grown blackberries (*Rubus* spp.). Jar-style traps based on Renkema et al (2014) were most effective over a variety of fruit crops. Using larger (5 cm) entrance holes covered with 0.1 cm mesh screening increases captures of *D. suzukii* and reduces bycatch. Monitoring traps were most effective when trap colours were consistent with surrounding fruits, green/purple in blueberries and black/red in *Rubus* species. Similarly, adjuvant and lure efficacy differed among fruit crops.

We found a pattern in the composition of recently developed attractant lures and our final 2-component attractant mixture of ethyl acetate + acetoin (5µg:50ng mixture), which we used with an apple cider vinegar drowning solution. In each case, the mixture was comprised of a ketone, specifically acetoin, and a combination of an ester and/or an acid and/or an alcohol (Cha et al. 2013, Feng et al. 2018, Urbaneja-Bernat et al. 2021). This may provide an insight into the chemical ecology of *D. suzukii* host-finding behaviour.

Our research into the visual ecology of *D. suzukii* reveals that careful consideration of trap colour and design, in relation to the crop system in which traps are deployed, are essential to trap efficacy. Our investigation into colour cues was limited to wavelengths within the visual spectrum. Given the known visual spectral range of related Drosophila species, we recommend that future research investigate the role of ultraviolet (UV) light and reflectance.

We anticipate that the lessons learned through this research can contribute to a fully integrated pest management (IPM) approach to mitigating *D. suzukii*. Pairing insights from our work on attractant odorant mixtures with recent advances in identifying deterrent or repellent odorants could lead to an effective push-pull system (Wallingford et al 2016, 2017, Cha et al. 2020, Eben et al. 2020, Urbaneja-Bernat et al. 2020, Wang et al. 2021). Advances from our research can also contribute to IPM efforts in conjunction with trap crops, cultural controls, biological controls, selective crop breeding (Lee et al. 2019, Ulmer et al. 2020, Schöneberg et al. 2021). In more temperate regions with a pronounced diapause and reproductive periods, our advances could contribute to IPM efforts using sterile insect technique (Nikolouli et al. 2018).

The plasticity which enabled *D. suzukii* to successfully invade new regions and to adapt to novel fruit hosts may yet hold a key to effective trapping and monitoring. However, this plasticity also means that *D. suzukii* is able to use a range of signals, including olfactory and visual signals, as cues to mediate attraction. Our results suggest that *D. suzukii* could filter these cues in response to the context in which they are perceived. Thus, efficacy of trap design, trap colour, and adjuvant lure depend upon the crop in which they are deployed.

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