

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

Catherine M. Little, Emma Rand, Megan MacIsaac, Lise Charbonneau, and N. Kirk Hillier

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.

RÉSUMÉ

Le suivi de la propagation des insectes envahissants dans de vastes territoires et en régions éloignées peut être coûteux et nécessiter beaucoup de temps. Grâce aux citoyens scientifiques bénévoles, les projets de recherche peuvent bénéficier de ressources humaines enthousiastes et de connaissances locales. Les citoyens scientifiques peuvent aussi faire économiser du temps et de l'argent aux chercheurs principaux. Avec l'aide de nos bénévoles et de nos partenaires de recherche, nous avons établi de nouvelles mentions de plantes hôtes facultatives pour le *Drosophila suzukii* au Canada atlantique et nous avons prélevé des échantillons de fruits en Nouvelle-Écosse et à Terre-Neuve-et-Labrador.

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 *Drosophila 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 *Drosophila suzukii* in Canada occurred in 2009 in the Okanagan Basin of British Columbia (Thistlewood et al. 2012). In 2010, populations of *Drosophila 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 *Drosophila 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 *Drosophila suzukii* has been detected every year since 2013 in Newfoundland, monitoring and mitigation programs through both federal and provincial agencies have as yet been

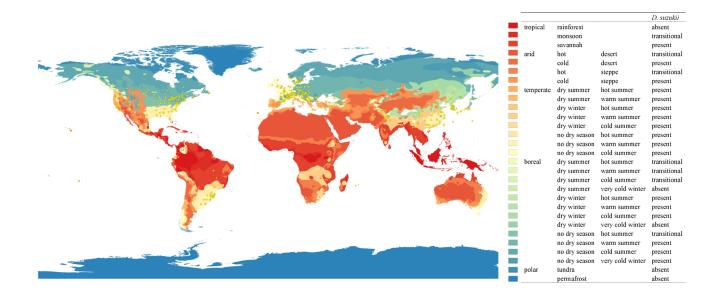
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Figure 1. Map of reported *Drosophila suzukii* collection records shown against Köppen climate classification scale (Peel et al. 2007a, b). **NOTE**: Presence or absence of *Drosophila suzukii* within each climate zone classification is shown within the legend. Zones labelled as transitional indicate that *Drosophila suzukii* has been reported at the margins between that zone and an adjacent climate zone generally thought to be more suitable to *Drosophila suzukii*.



unable to confirm if *Drosophila suzukii* populations have been overwintering in the region or have been reintroduced each year (AAFC Pest Management Centre 2013).

Comparing distribution records for *Drosophila suzukii* against global climate records (Peel et al. 2007a, b), it is evident that *Drosophila suzukii* can withstand a broad range of environments in terms of temperature and humidity (Figure 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 Drosophila 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 Drosophila 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 Drosophila 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 Drosophila suzukii in Canada have focused on protection of commercially grown tender fruits (cane berries - raspberries (Rubus idaeus L. (Rosaceae)) and blackberries (Rubus spp. (Rosaceae)), and blueberries (*Vaccinium* spp. (Ericaceae), grapes (Vitis vinifera L. (Vitaceae)), and cherries (Prunus avium L. (Rosaceae) and Prunus cerasus L. (Rosaceae))) in response to concerns of commercial fruit growers (AAFC Pest Management Centre 2013). Additional commercially grown soft fruits, such as strawberries (Fragaria×ananassa Duchesne (Rosaceae)) and currants (Ribes rubrum L. (Grossulariaceae) and Ribes nigrum L. (Grossulariaceae)), are also susceptible to damage (Lee et al. 2011; Lee and Sial 2016; Little et al. 2017). It is anticipated that climate change will result in the geographic ranges of invasive Drosophila suzukii and temperate zone plant species to converge with boreal plant species (Gauthier et al. 2015). Additionally, Drosophila 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, *Drosophila 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' *Drosophila 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 *Drosophila suzukii* and related *Drosophila* spp. Fallén (Diptera: Drosophila) 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. Nonscientist 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).

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 Drosophila 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, from geographic areas not otherwise easily accessible for study.

We beta-tested a citizen science initiative in Nova Scotia and Newfoundland and Labrador to identify wild and ornamental fruits used as hosts by *Drosophila suzukii*. 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 *Drosophila suzukii* at the presumed northern limit of its geographic range in North America.

METHODS

Citizen science FlySpotter participant kits, containing everything required to collect and submit four fruit samples, were distributed 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 Drosophila suzukii and

the citizen science initiative with members of the public.

Instructions included in each kit provided examples of how to use each of the four 50-ml FalconTM 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 <u>flyspotter@acadiau.ca</u>. Both the website and starter kits provided participants with suggestions of useful botanical field guides (Roland and Zinck 1998; Scott 2010; Boland 2011; Fernald and 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 <u>iTunes Store</u> or <u>Google Play</u>), <u>Pl@ntNet (http://</u> 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. The FalconTM tube lids were replaced with a bonded cellulose acetate plug (Genesee Scientific Corporation, El Cajon, CA). Tubes were stored at room temperature (approximately 20 °C and 50-60% RH) and examined twice weekly for emerging insects until fruit degraded and no further insects eclosed. 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 and O'Grady 2005; Thistlewood and 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. eclosions were refrigerated at 4 °C for one week, moved to a freezer for two weeks at -4 °C, 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 eclosed after two weeks, the samples were thoroughly examined and discarded. Fruits with excessive mould or that liquified were also discarded as such conditions inhibited the rearing of *Drosophila* spp.

A sub-sample of eclosing insects (10 insects) was processed

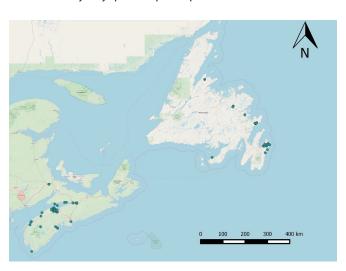
with Lifescanner® kits (http://lifescanner.net/) per package directions and sent to the Centre for Biodiversity Genomics (University of Guelph, Guelph ON) for DNA barcoding to obtain conclusive identification. Genetic data collected using multiple animal specific primers from DNA Genotek Inc. (https://www.dnagenotek.com/ROW/index.html) 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.

RESULTS

One hundred and twenty-five FlySpotter kits (4 sample collection vials per kit) were distributed 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 (Figure 2, Table 1). Fruits from 107 species representing 61 genera from 29 plant families were monitored daily for emerging insects (Table 1). Drosophila suzukii eclosed from 20 fruit samples (5.8% of fruit samples), representing 11 species (10.3% of species sampled) from 6 plant families (Table 1). Previous observations of host-plant use were confirmed through these samples. Adult Drosophila suzukii eclosed from fruits grown commercially in Nova Scotia, including Arctic kiwi fruit (Actinidia arguta Siebold and Zuccarini (Actinidiaceae)), wine grapes, apples, pears (*Pyrus communis* L. (Rosaceae)), highbush blueberries, (Vaccinium corymbosum L. (Ericaceae)), blackberries, and raspberries. Adult Drosophila suzukii also eclosed from introduced species Tatarian honeysuckle (Lonicera tatarica L. (Caprifoliaceae)) and crab-apple (Malus spp. (Rosaceae). Endemic plants were also suitable hosts for Drosophila suzukii in Nova Scotia. Adult flies eclosed from wild blackberry (Rubus allegheniensis Porter (Rosaceae)), wild raisin (Viburnum nudum cassinoides L. (Adoxaceae)), and common elderberry (Sambucus canadensis L. (Adoxaceae)). An expanded geographical range of Drosophila 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 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 *Drosophila suzukii* in Nova Scotia for Arctic kiwi, Lonicera spp., Malus spp., Pyrus spp., common

Figure 2. Map of collection sites for fruit samples submitted by FlySpotter participants in Atlantic Canada.



elderberry, wild raisin, and wine grapes. The sole previous record of Tatarian honeysuckle as a host was recorded in British Columbia (Thistlewood et al. 2018). Natural Drosophila suzukii infestations for wine grapes, Tatarian honeysuckle, 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 *Drosophila suzukii* eclosion occurred 1 September 2017 and the latest on 15 January 2018. All fruits from which *Drosophila suzukii* eclosed were collected between 21 August 2017 and 2 November 2017. Multiple species of *Drosophila* (Diptera: Drosophilaae), including *Drosophila simulans* Sturtevant, *Drosophila melanogaster* Meigen, *Drosophila affinis* Sturtevant, *Chymomyza fuscimana* Zetterstedt, and *Chymomyza amoena* Loew, eclosed from 18 fruit samples, representing 13 plant species from 5 families, beginning 5 September 2017 and ending 1 February 2018 (Table 1). Other insects eclosed from 69 fruit samples, representing 41 plant species from 12 families between 4 July 2017 and 19 December 2017 (Table 1). Other species of flies (Diptera), hymenopterans

(Hymenoptera), caterpillars (Lepidoptera), and weevils (Coleoptera) were also common eclosing 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 eclosion, across all groups, occurred 4 July 2017 and 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 eclosed post simulated winter treatment. The remainder of the fruit samples were discarded after fruit had degraded, insect eclosion had ceased, and no further signs of invertebrate life were observed. Results of DNA barcoding of a subset of 10 eclosing insects revealed that a variety of plant- and insect-feeding insects was collected, including two parasitoid wasps (Table 2).

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 confirm plant species identification. All fruit samples were identified to genus; however, species identification for 49 (14.2%) fruit samples could not be confirmed.

Low-cost participant kits were simple to prepare, costing less than \$6.00 per kit including postage. FalconTM 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™ tube or a larger

Table 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.

Introduced Actinidiaceae				Collection site		Insects eclosed			
Elacagnaceae	Source / Use	Plant family	Plant Species	NB	NL	NS			Other
Rosaceae			Zuccarini) Planchon ex Miquel)	,			Х	Х	
Rosaceae		=	** *						
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Rosaceae			· · · · · · · · · · · · · · · · · · ·	X					X
Rosaceae					Х		X	x	X
Rosaceae			` ′			X			
Rosaceae Rubus idaeus (L.)			* *		X				
Introduced							X	x	X
Nitaceae			* *		X	X	X	x	
Introduced		Solanaceae	Solanum lycopersicum (L.)			X			X
Aquifoliaceae		Vitaceae	Vitis vinifera (L.)			X	x	x	X
Aquininaceue lieta miserveue (weserve)		Apiaceae	Coriandrum sativum (L.)			х			
Berberidaceae	cultivated	Aquifoliaceae	Ilex x meserveae (Meserve)		x	X			
Grossulariaceae		Asparagaceae	Convallaria majalis (L.)			X			
Grossulariaceae Ribes rubrum (L.)		Berberidaceae	Berberis thunbergii (de Candolle)			X			
Grossulariaceae Ribes inva-crispa (L.)		Grossulariaceae	Ribes nigrum (L.)		x				
Oleaceae		Grossulariaceae	Ribes rubrum (L.)		x				
Rosaceae Chaenomeles japonica ((Thunberg) Lindley ex Spach) Rosaceae Cotoneaster horizontalis (Dacaisne) Rosaceae Malus sayentii (Rehder) x Rosaceae Malus sylvestris (I) Miller) x Rosaceae Malus sylvestris (I) Miller) x Rosaceae Rosa rubiginosa (I) x x Rosaceae Rosa rubiginosa (I) x x Sapindaceae Sorbus aucuparia (I) x x Sapindaceae Asexulus hippocastanum (I) x Sapindaceae Physalis pruinose (I) x Taxaceae Taxus baccata (I) x Taxaceae Taxus baccata (I) x Thymelaaceae Daphne mezereum (I) x Rosaceae Cartaegus mollis ((Torrey & x x x x x x x x x x x x x x x x x x		Grossulariaceae	Ribes uva-crispa (L.)			X			
Rosaceae		Oleaceae	Ligustrum vulgare (L.)			X			
Rosaceae		Rosaceae				X			
Rosaceae		Rosaceae	Cotoneaster horizontalis			x			
Rosaceae Malus sargentii (Rehder) X		Rosaceae				x			
Rosaceae Rosa rubiginosa (L.)		Rosaceae	, , ,		х				
Rosaceae Rosa rubiginosa (L.) x x x x		Rosaceae	= ' '			x			х
Rosaceae Sorbus aucuparia (L.) x x x					x				х
Sapindaceae Solanaceae Physalis pruinose (L) x Taxaceae Taxus baccata (L.) x Thymelaeaceae Daphne mezereum (L.) x Introduced / not cultivated not cultivated Remanaceae Frangula alnus (Miller) x Rosaceae Crataegus mollis (Torrey & x x Gray) Scheele) Endemic / agricultural Ericaceae Vaccinium angustifolium (Aiton) x x Ericaceae Vaccinium macrocarpon (Aiton) x x x Ericaceae Vaccinium myrilloides (Michaux) x x Ericaceae Vaccinium myrilloides (Michaux) x x Ericaceae Vaccinium myrilloides (Michaux) x x Endemic / Rosaceae Rubus allegheniensis (Porter) x x x x x Endemic / Caprifoliaceae Use verticillate ((L.innaeus) Gray) x Enditivated Caprifoliaceae Cornus alternifolia (L. filius) x x Cornaceae Cornus sericea (L.) x x x Cornaceae Cornus stolonifera (L.) x x x Ericaceae Arctostaphylos uva-ursi (L.) x x x Ericaceae Ilex verticillate ((L.) Gray) x x x Ranunculaceae Ilex verticillate ((L.) Gray) x x x Rosaceae Amelanchier alnifolia (Nuttall) x x x Rosaceae Arenone canadensis (L.) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Arenone Canadensis (L.) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Arenone (L.) x x x Rosaceae Arenone (Michaux) x x x Rosaceae Areno			= ' ' '						
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Endemic	not cultivated	Rhamnaceae	Frangula alnus (Miller)			x			x
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Cornaceae Cornus stolonifera (L.) x x Cupressaceae Juniperus communis (L.) x Ericaceae Arctostaphylos uva-ursi ((L.) x Sprengel) x x Grossulariaceae Ribes hirtellum (Michaux) x x Iridaceae Ilex verticillate ((L.) Gray) x x Ranunculaceae Anemone canadensis (L.) x x Rosaceae Amelanchier alnifolia (Nuttall) x x Rosaceae Aronia (Medikus) x Sorbus (L.) x x hybrid x x x			, , ,			X			X
Cupressaceae Juniperus communis (L.) x Ericaceae Arctostaphylos uva-ursi ((L.) x Sprengel) x x Grossulariaceae Ribes hirtellum (Michaux) x x Iridaceae Ilex verticillate ((L.) Gray) x x Ranunculaceae Anemone canadensis (L.) x x Rosaceae Amelanchier alnifolia (Nuttall) x x Rosaceae Aronia (Medikus) x Sorbus (L.) x x hybrid Rosaceae Prunus nigra (Aiton) x					X	X		X	X
Ericaceae Arctostaphylos uva-ursi ((L.) x Sprengel) Grossulariaceae Ribes hirtellum (Michaux) x x Iridaceae Ilex verticillate ((L.) Gray) x Ranunculaceae Anemone canadensis (L.) x x Rosaceae Amelanchier alnifolia (Nuttall) x Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x		Cornaceae	* ' '		X	X			X
Sprengel Ribes hirtellum (Michaux) x x Iridaceae Ilex verticillate ((L.) Gray) x Ranunculaceae Anemone canadensis (L.) x x Rosaceae Amelanchier alnifolia (Nuttall) x Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x		Cupressaceae	Juniperus communis (L.)		X				
Iridaceae Ilex verticillate ((L.) Gray) x Ranunculaceae Anemone canadensis (L.) x x Rosaceae Amelanchier alnifolia (Nuttall) x Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x			Sprengel)						
Ranunculaceae Anemone canadensis (L.) x x x Rosaceae Amelanchier alnifolia (Nuttall) x Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x			1 /		Х				X
Rosaceae Amelanchier alnifolia (Nuttall) x Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x			** **						
Rosaceae Aronia (Medikus) x Sorbus (L.) x hybrid Rosaceae Prunus nigra (Aiton) x			` '		Х				X
Rosaceae Prunus nigra (Aiton) x			Aronia (Medikus) x Sorbus (L.)		x	Х			
		Rosaceae				x			
Rosaceae Prunus pensylvanica (L. filius) x x			Prunus pensylvanica (L. filius)		v				x

Table 1 (cont'd). Fruits collected by citizen science participants and identified to genus and species.

			Collection site		Insects eclosed			
Source / Use	Plant family	Plant Species	NB	NL	NS	Drosophila suzukii	Other Drosophila	Other
	Rosaceae	Prunus serotine (Ehrhart)			Х		,	X
	Rosaceae	Sorbus americana (Marshall)		X	х			X
	Rosaceae	Sorbus decora (Schneider)		X				
	Rubiaceae	Mitchella repens (L.)			x			
	Tiliaceae	Tilia Americana (L.)			х			
	Violaceae	Viola labradorica (Schrank)			х			
	Vitaceae	Parthenocissus quinquefolia ((L.) Planchon)			x			
Endemic / not	Adoxaceae	Sambucus canadensis (L.)			х	x	х	
cultivated	Adoxaceae	Sambucus pubens (Michaux)		x				
	Adoxaceae	Viburnum cassinoides (L.)			х	x		х
	Adoxaceae	Viburnum trilobum (Marshall)			х			
	Adoxaceae	Viburnum lantanoides (Michaux)			x			
	Aquifoliaceae	Ilex mucronate ((L.) Powell,		x	X			
	Asparagaceae	Savolainen, & Andrews) Maianthemum canadensis		х	x			
	1 isparagaeeae	(Desfontaines)						
	Asparagaceae	Maianthemum trifolium ((L.) Sloboda)			X			
	Caprifoliaceae	Lonicera canadensis (Bartram)		X	X			
	Cornaceae	Cornus canadensis (L.)		X	X			X
	Cornaceae	Cornus rugosa (Lamarck)			Х			
	Ericaceae	Empetrum nigrum (L.)		X				
	Ericaceae	Gaultheria hispidula ((L.) Muhlenberg ex Bigelow)		x				
	Ericaceae	Gaultheria procumbens (L.)			X			
	Ericaceae	Gaylussacia baccata ((Wangenheim) Koch)		х				
	Ericaceae	Kalmia angustifolia (L.)			Х			
	Ericaceae	Pyrola elliptica (Nuttall)			X			X
	Ericaceae	Vaccinium boreale (Hall & Aalders)		Х				
	Ericaceae	Vaccinium boreale (Hall & Aalders) x V. myrtilloides (Michaux)			Х			
	Ericaceae	Vaccinium oxycoccus (L.)			Х			
	Fagaceae	Fagus grandifolia (Ehrhart)			X			
	Geraniaceae	Geranium robertianum (L.)			X			
	Iridaceae	Iris versicolor (L.)			х			
	Liliaceae	Clintonia borealis ((Aiton) Rafinesque-Schmaltz)		X	x			X
	Myricaceae	Comptonia peregrina ((L.) Coulter)			x			
	Myricaceae	Morella pensylvanica (Mirbel)			x			
	Myricaceae	Myrica pensylvanica (Mirbel)			x			
	Ranunculaceae	Actaea pachypoda (Elliott)		X				
	Ranunculaceae	Actaea rubra ((Aiton) Willdenow)		X				
	Rosaceae	Amelanchier bartramiana ((Tausch) Roemer)		X				X
	Rosaceae	Amelanchier canadensis ((L.) Medikus)		X	Х			Х
	Rosaceae	Amelanchier laevis (Wiegand)		X				
	Rosaceae Rosaceae	Aronia melanocarpa ((Michaux) Elliott) Aronia prunifolia ((Marshall)		X X				
	Rosaceae	Rehder) Crataegus douglasii ((Loudon)		x				
	Rosaceae	Eggleston ex Rehder) Crataegus flabellate ((Bosc ex			х			
	Rosaceae	Spach) Rydberg) Crataegus brainerdii (Sargent)			х			
					А			
	Rosaceae	Fragaria vesca (L.)			X			

Source / Use	Plant family		Collection site			Insects eclosed		
		Plant Species	NB	NL	NS	Drosophila suzukii	Other Drosophila	Other insects
	Rosaceae	Geum rivale (L.)			x			X
	Rosaceae	Prunus virginiana (L.)	X	X	x			x
	Rosaceae	Rosa canina (L.)			X			X
	Rosaceae	Rosa Carolina (L.)			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 (L.)			x			

Table 1 (cont'd). Fruits collected by citizen science participants and identified to genus and species.

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 FalconTM 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.

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 as 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 and 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 and 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. <u>ca/#/</u>) 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 *Drosophila 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 Drosophila

Table 2. Eclosing 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 (L.)	Pseudanthonomus crataegi (Walsh, 1867)	Curculionidae	hawthorn weevil
Malus sylvestris ((L.) Miller)	Anthonomus rufus (Gyllenhal, 1836)	Curculionidae	weevil
Amelanchier canadensis ((L) Medikus)	* * * *	Pteromalidae	parasitoid wasp
Vaccinium corymbosum (L.)	Pseudanthonomus crataegi	Curculionidae	hawthorn weevil
Malus pumila (Borkhausen)	•	Braconidae	parasitoid wasp
Rubus allegheniensis (Porter)	Anthonomus signatus (Say, 1831)	Curculionidae	weevil
Viburnum cassinoides(L.)	Megastigmus aculeatus (Swederus, 1795)	Torymidae	Rose-hip chalcid was

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, *Drosophila suzukii* has spread to geographic regions that experience seasonal extremes of cold, hot, humid, or dry conditions. As *Drosophila 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, Drosophila suzukii is anticipated to further its spread across North America, South 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 °C 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 *Drosophila suzukii* populations. Regional changes in temperature and precipitation trends due to climate change will result in further range expansion. Over time, localized populations of Drosophila 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 and Ørsted 2018).

This initiative identified natural infestations by *Drosophila 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 *Drosophila suzukii* and endemic

Drosophila species. Localised areas with larger populations of *Drosophila suzukii* have smaller populations of other *Drosophila* species (Bombin and 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 Drosophila suzukii populations and are known to play a role in promoting the spread of Drosophila 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 Drosophila suzukii among climate zones (Langille et al. 2017). Ongoing climate change will further alter fruit phenology patterns, which could result in changed host use patterns for Drosophila 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 and 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 *Drosophila suzukii* infestation in Newfoundland, and identify role of climate zones to range expansion in Canada (Figure 1). We have demonstrated that fruit collected and transported from remote areas can be successfully used to monitor for an array of eclosing 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.

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