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Relative abundance and phenology of *Drosophila* Fallén, 1815 (Diptera: Drosophilidae) species in south-central Washington State

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Abstract. A monitoring program for a recently introduced vinegar fly, *Drosophila suzukii* Matsumura, 1931, was conducted in south-central Washington State, U.S.A. from March 2011 to November 2013. Along with *D. suzukii*, a complex of nine additional *Drosophila* Fallén, 1815 species were captured in baited traps and identified to species. The *Drosophila* were captured in Nalgene® and Haviland traps baited with apple cider vinegar or a sugar yeast mixture that were distributed among seven different horticultural crops or unmanaged habitats. All flies captured were identified to species and quantified for each sampling period. The species identified and quantified included *D. busckii* Coquillett, 1901, *D. funebris* Fabricius, 1787, *D. hydei* Sturtevant, 1921, *D. immigrans* Sturtevant, 1921, *D. melanogaster* Meigen, 1830, *D. simulans* Sturtevant, 1919, *D. obscura* Fallén, 1823, *D. subobscura* Collin, 1936, *D. subquinaria* Spencer, 1942 and *D. suzukii*. The predominant species in 2011 were the *obscura* group and *D. hydei*. In 2012, the predominant species were the *melanogaster* and *obscura* groups. The predominant species in 2013 were the *melanogaster* group and *D. suzukii*. Throughout the study, each species exhibited unique patterns in activity that varied from year to year. The results of this study reveal a greater diversity of *Drosophila* in the inland Pacific Northwest, U.S.A. Holarctic shrub-steppe environment than previously documented, highlighting the need for more in-depth research on any competition between *D. suzukii* and local *Drosophila* species.

Key Words. Survey, diversity, Pacific Northwest, *Drosophila*.

INTRODUCTION

The genus *Drosophila* Fallén, 1815 is a large taxon with over 1500 species globally, divided into 10 subgenera, the largest being the subgenus *Drosophila* (Sturtevant 1942, Ashburner 2004). Despite the extensive use of *Drosophila* in biological and genetic research, there remains a considerable level of controversy surrounding the phylogenetic relationships and taxonomy of the group. Studies regarding *Drosophila* taxonomy are based on morphological inferences (Throckmorton 1968, 1975; Grimaldi 1990) or molecular data (DeSalle 1992a, b; Kwiatowski et al. 1994, 1997; Martinez et al.

1988; Pelándakis & Solignac 1993; Powell 1997; Remsen & DeSalle 1998; Russo et al. 1995; Tatarenkov et al. 1999), and there are incongruences between the two methods (Kwiatowsky & Ayala 1999, Thomas & Hunt 1993). In spite of the difficulties in classification, it is well known that the species of this taxon play an important role at various trophic levels, primarily as decomposers. However, several *Drosophila* species are also known to have a deleterious effect on certain crops (Capinera 2001, van der Linde et al. 2006), particularly fruit crops with a soft or thin exocarp.

The recent invasion of an economically important species, *Drosophila suzukii* Matsumura, 1931 (Hauser et al. 2009, Hauser 2011), provided the impetus and opportunity to study not only the distribution and abundance of this species, but other species within the genus *Drosophila* that are also associated with cropping systems. Pest management programs are designed to monitor for the invasive species in question and tend to ignore closely related, endemic or naturalized species. While studying local fauna of *Drosophila* may not have immediate or direct economic advantages, understanding endemic fauna is important from an ecological perspective that could demonstrate the need for conservation efforts. It is well documented that invasive species can out-compete and cause decline in local species populations (Pimentel et al. 2005). While it is not known if *D. suzukii* has the ability to outcompete local fauna, monitoring for these species over time is essential to evaluate the stability of populations of the local *Drosophila* fauna.

Increased knowledge of *Drosophila* in the environment is important from an ecological perspective as well as from a management perspective. *Drosophila* are intimately associated with both human activity and cropping systems and play an important role in decomposition and nutrient cycles in both natural and agricultural environments. Aside from irrigated agricultural land in this region, a large percentage of the land surface is unmanaged and includes sagebrush steppe, riparian areas and wetlands. The region of study includes the lower Yakima Valley of Yakima and Benton Counties in south-central Washington, U.S.A., which produces a wide variety of fruit crops that include pome and stone fruits, wine and juice grapes, caneberries and blueberries. Due to the high diversity of fruit cropping systems and large areas of native habitat, this region of Washington is ideal for studying the diversity of *Drosophila* species.

The objective of this study was to quantify the diversity and relative abundance of the species of *Drosophila* that were found during a monitoring program for *D. suzukii*.

MATERIALS AND METHODS

Sample Collection. A survey was conducted from March 2011 to November 2013 at a total of 23 sites throughout the lower Yakima Valley. Traps were deployed in commercial orchards and vineyards, private property of anonymous volunteers, and unmanaged habitat. In 2011, the trap used at all sites was a 500-mL Nalgene® bottle (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) with a mesh insert in the lid to allow fly entry. In 2012, traps were constructed from 750-mL plastic containers (TakeAlongs Rubbermaid, Atlanta, Georgia, U.S.A.) with a mesh insert in the lid. Traps were baited either with 150 mL apple cider vinegar (Wal-Mart Stores, Bentonville, Arkansas, U.S.A.) or a mixture of 40 g of active dry yeast (Red Star Lasaffree Yeast Corporation, Milwaukee, Wisconsin, U.S.A.), 130 g refined white sugar and 3.785 L of water. Traps were checked weekly during the growing season (April–November)

and at monthly intervals during the winter months. The contents of the traps were removed at each visit and replaced with fresh bait. Adult *Drosophila* spp. were sorted from other by-catch arthropods and stored in 85% ethanol until samples were ready to be identified. Climate data were downloaded from AgWeatherNet, and average monthly temperatures were calculated based on data from stations located in three of the collection regions.

Species Identification Based on Morphology. The species were identified by use of dichotomous keys and species descriptions (Markow & O'Grady 2005, Grimaldi 1990, Ashburner et al. 2005, Thomas & Hunt 1993). Voucher specimens were sent to the James Entomological Museum at Washington State University in Pullman, Washington and to the California Department of Food and Agriculture (CDFA) in Sacramento, California, U.S.A. Due to the difficulty in identifying some species based on morphology, molecular identification was used to verify difficult species.

DNA Extraction, PCR Conditions and Sequencing. From single specimens, gDNA was extracted using a modified non-destructive insect protocol for the DNEasy Blood and Tissue Kit (Qiagen, Valencia, California). Whole specimens were placed in individual 1.5-mL microcentrifuge tubes with 20 μ L Proteinase K solution and 180 μ L ATL buffer and incubated for 24 hours at 55°C. Subsequently, 200 μ L of manufacturer's AL buffer was added to each tube, mixed briefly and incubated at 70°C for 10 minutes. To each tube, 200 μ L of 100% EtOH was added and mixed briefly. *Drosophila* specimens were retrieved and placed in 100% EtOH and retained as vouchers. The remaining mixture was pipetted into DNEasy Spin Column and standard kit protocol was followed for ethanol washes and elution in 50 μ L AE buffer. Primers TY-J-1460 (5'-TAC AAT TTA TCG CCT AAA CTT CAG CC-3') and C1-N-2191 (5'-GGA TCA CCT GAT ATA GC A TTC CC-3') (Simon et al. 1994) were used for amplification of the COI region of the mitochondrial DNA. Polymerase Chain Reaction (PCR) was carried out in a PTC-200 Thermal Cycler (MJ-Research: Bio-Rad, Hercules, California) with the following conditions: 94°C for 3 minutes, 32 cycles of 20 seconds at 94°C, 20 seconds at 50°C, and 30 seconds at 72°C, followed by a final extension of 5 min at 72°C. PCR was performed with the following parameters for each reaction: 5 U Platinum Taq (Invitrogen, Carlsbad, California), 5 μ L of manufacturer's 10X buffer (20-mM Tris-HCl pH 8.4 and 500-mM KCl), 2.5-mM MgCl₂, 10-M dNTP's (Sigma-Aldrich, St. Louis, Missouri, U.S.A.), 0.1-M each primer, 3 μ L of DNA template and ddH₂O to 501. Amplicons were purified using QIAquick PCR Clean-up kit (Qiagen) and eluted in 30 μ L of manufacturer's EB buffer. Sequencing reactions utilizing the same forward and reverse primers were performed using the Applied Biosystems Big Dye Terminator V3.0 sequencing chemistry on an ABI 3730 DNA sequencer. Electropherograms for the COI gene were edited and aligned with Sequencher version 4.6 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

RESULTS

Drosophila Species Detected, Relative Densities, and Seasonal Phenology. During the 34 months of the study, a total of 391,200 individuals representing ten species were captured in the survey traps from all crops and bait types (Table 1). The *obscura* group includes *D. obscura* Fallén, 1823 and *D. subobscura* Collin, 1936, and the *melanogaster* group includes *D. melanogaster* Meigen, 1830 and *D. simulans* Sturtevant, 1919 because of the difficulty of separating the species in these groups

Table 1. *Drosophila* species captured during study period.

Species	2011		2012		2013		Total	
<i>melanogaster</i> group	8946	22.79%	195,242	82.65%	45,493	39.32%	249,681	63.99%
<i>obscura</i> group	12,960	32.99%	15,710	6.65%	15,775	13.63%	44,445	11.39%
<i>D. busckii</i>	186	0.47%	407	0.17%	535	0.46%	1128	0.29%
<i>D. funebris</i>	135	0.34%	750	0.32%	1,022	0.88%	1907	0.49%
<i>D. hydei</i>	12,197	31.05%	2780	1.18%	8429	7.29%	23,406	6.00%
<i>D. immigrans</i>	432	1.11%	793	0.34%	446	0.39%	1671	0.43%
<i>D. subquinaria</i>	18	0.06%	27	0.01%	41	0.04%	86	0.02%
<i>D. suzukii</i>	4396	11.19%	20,521	8.69%	43,959	37.99%	68,876	17.65%

using morphological characteristics, especially females. Some of the more difficult to identify species presented in Table 1 were confirmed by comparing sequence data derived from the 5' end of the cytochrome oxidase subunit I gene and confirmed morphological identifications for *D. funebris* Fabricius, 1787, *D. hydei* Sturtevant, 1921, and *D. subquinaria* Spencer, 1942 and to validate the presence of *D. melanogaster*, *D. simulans*, *D. obscura* and *D. subobscura* within their corresponding groups.

Overall the most abundant species encountered over the entire study period were the *melanogaster* group, *D. suzukii*, the *obscura* group and *D. hydei* (Table 1). *Drosophila busckii*, Coquillett, 1901, *D. funebris* and *D. immigrans* Sturtevant, 1921 were present in relatively lower abundance than the aforementioned species each year, and *D. subquinaria* was the rarest species encountered overall and during each year of the study (Table 1). All species, with the exception of *D. subquinaria*, varied in abundance among years (Table 1).

Of the species detected in this study, *D. immigrans*, the *melanogaster* group and *D. suzukii* had one peak collection period for adult activity in late summer and fall (Figure 1), while *D. busckii*, *D. hydei*, the *obscura* group and *D. subquinaria* had two observable collection peaks in adult activity (Figure 1). *Drosophila funebris*, however, exhibited one activity peak in 2011 and 2012 while exhibiting two distinct activity peaks in 2013 (Figure 1). *Drosophila subquinaria* and *D. busckii* exhibited similar patterns in adult activity in all three years of the study (Figure 1). All other species monitored exhibited different patterns of adult activity relative to other species throughout the study period. Also, all species exhibited consistent patterns in terms of peak activity each year of the study with the exception of *D. funebris* and *D. subquinaria*. The former species exhibited two periods of activity in 2013, one larger peak period in 2012 and a smaller and shorter peak in 2011, while the latter species exhibited two periods of activity in 2012 and 2013 while exhibiting a single, extended period of activity in 2011 (Figure 1). All species were initially captured at earlier dates throughout the study with 2011 having the latest first-capture date and 2013 having the earliest first-capture date (Figure 1). Temperature data obtained from AgWeatherNet showed that summer temperatures increased over the study period with 2012 having the coolest spring temperatures, on average (Figure 2). Also, in 2011, spring temperatures were much lower than 2012 and 2013. The winter of 2012 and 2013 was also warmer than the previous years (Figure 2).

DISCUSSION

All of the captured species have a cosmopolitan distribution (Markow & O'Grady 2005, Adrion et al. 2014). Of the species captured in this study, *D. busckii*, *D. immigrans*,

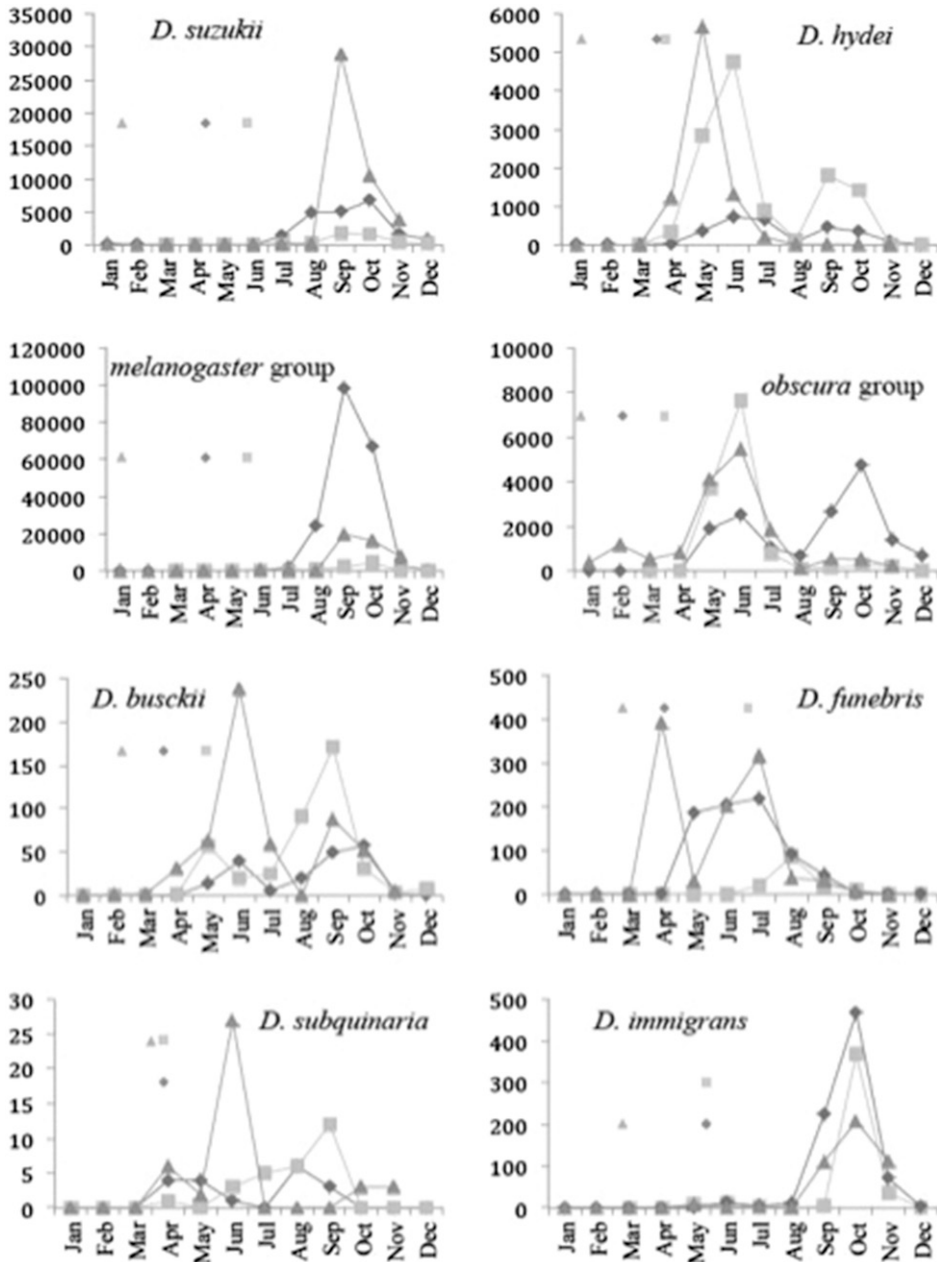


Figure 1. Season phenology of all species captured in 2011 (■), 2012 (◆) and 2013 (▲); Y-axis represents total number of flies captured, independent shapes on graphs correspond to year and represent first-capture date.

D. subquinaria, and *D. hydei* are native to North America, whereas the *melanogaster* group is native to Africa, the *obscura* group and *D. funebris* are native to Europe and *D. suzukii* is native to Asia (Markow & O’Grady 2005, Hauser 2011). Given that *D. melanogaster* is the most common and widespread species, the predominance of

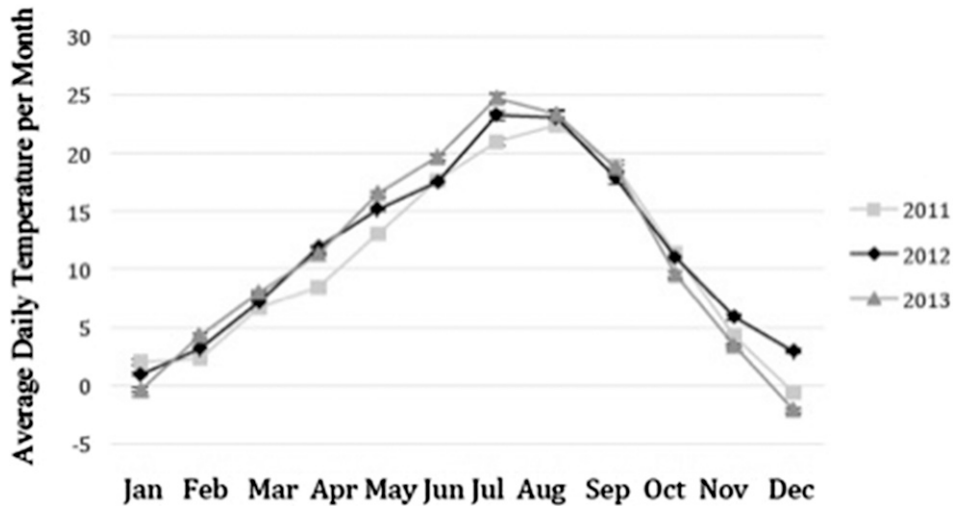


Figure 2. Average temperatures (C) per month from three AgWeatherNet Stations in the study area.

the *melanogaster* group is not unexpected. The high degree of variation in seasonal phenology for both *D. funebris* and *D. subquinaria* is interesting and suggests that these species may be sensitive to variation in local climate conditions, other abiotic factors, or biotic factors that were not addressed in this study. Other species did exhibit changes in the timing of peak activity, such as *D. busckii* that showed peak activity in June and September of 2013 while in 2011 exhibited peaks in May and September and in 2012 had peak activity in June and October. Similar variation was also seen in *D. suzukii*, *D. hydei* and the *melanogaster* group. Other species were more consistent from year to year in peak activity with the *obscura* group and *D. immigrans* exhibiting the same peaks in activity in the same month from year to year. While the overall cooler temperatures in 2011 likely had an impact on the development of all species, *D. suzukii* appears to have been the most impacted due to earlier capture dates of adults from 2011 to 2013. The more mild winters in 2012 and 2013 compared to 2011 likely allowed for better survival of the overwintering populations of all species, as indicated by earlier first-capture dates throughout the study period for each species observed in this study.

The order of magnitude increase in captures of *D. suzukii* from 2011 to 2013 is of particular interest and likely reflects establishment of this invasive species. According to climate models presented by Walsh et al. (2011), eastern Washington was not predicted as a region optimal for *D. suzukii*. However over a period of about three to four years, *D. suzukii* has become persistent and prevalent. *Drosophila suzukii*s pest status has varied among years. Cold winters, especially those with sudden hard freezes in spring or fall appear to delay population build up of *D. suzukii* (Walsh, personal observation). In these years, most spring bearing crops including cherries and blueberries can avoid infestation by *D. suzukii* if fruit is harvested prior to an increase in *D. suzukii* abundance (D. B. Walsh, personal observation). In years following mild winters *D. suzukii* populations recover and expand rapidly prior to commercial fruit harvest and *D. suzukii* becomes a more problematic economic pest (D. B. Walsh, personal observation). Based on population genetics of *D. suzukii* using microsatellite

variation, it does not appear that gene flow is occurring in eastern Washington. This population appears to be an established population capable of overwintering at low abundance rather than having local extinction events with seasonal reintroductions (Bahder et al. 2015). The recent global movement of *D. suzukii* has been documented by Adrion et al. (2014), and the establishment of *D. suzukii* in eastern Washington highlights the evolutionary potential that is characteristic of the genus.

Drosophila suzukii is of economic importance due to its ability to attack ripening and ripe fruit rather than overripe or rotting fruit, which is the case in many *Drosophila* spp. Because of this, it is unlikely to directly compete with other species of *Drosophila* (but see Mitusi et al. 2006, Poyet et al. 2014, and Stemberger 2015); however, it is unclear what impact, if any, the presence of this species will have on naturalized *Drosophila*. Hamby et al. (2013) found that there is a strong association between *D. suzukii* and the ascomycete *Hanseniaspora uvarum* (Niehaus) Shehata et al. (Saccharomycetaceae). If other species of *Drosophila* have a preference or association with a different strain of *H. uvarum* or other species of yeast, it is unclear what the impact that *H. uvarum* may have on the development of other *Drosophila* species that will colonize fruit after *D. suzukii*. Long-term monitoring of *Drosophila* species in eastern Washington would be desirable to study both the population of *D. suzukii* as well as the other species to understand the impact that *D. suzukii* is having on local species communities.

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