DROSOPHILA SUZUKII, OR SPOTTED WING DROSOPHILA, RECORDED IN SOUTHEASTERN PENNSYLVANIA, U.S.A.[1]

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ABSTRACT: Collections of *Drosophila* and their relatives were performed using bait traps on the campus of Saint Joseph's University, in Philadelphia and Lower Merion, Pennsylvania, between July and December 2011 and continuing in March of 2012. In the 2011 collection season, more than 200 specimens of *Drosophila suzukii* (Matsumura), or spotted wing Drosophila, were collected. In 2012, specimens started to appear in June and were collected until December. The appearance of *D. suzukii* in the Philadelphia and surrounding area has severe, negative implications for local agriculture. *D. suzukii,* unlike most other *Drosophila* species, is an agricultural pest that lays its eggs in soft-skinned, unspoiled fruits like cherries, grapes, and berries (Bolda et al., 2009).

KEY WORDS: Drosophila, *suzukii,* non-native, invasive, agriculture, agricultural, pest, Philadelphia, Pennsylvania, U.S.A.

INTRODUCTION

The goal of this study was to survey *Drosophila* biodiversity in the Philadelphia area over time. Previous work in this field discovered a number of species inhabiting the local area including *D. simulans*, *D. robusta*, *D. affinis*, *D. busckii*, *D. putrida, D. tripunctata*, and *D. melanogaster,* with *D. simulans* being the most prevalent (Roy 2009). The collection data from 2011 and 2012 is in concordance with the previous study but with the addition of a new, invasive species: *D. suzukii* (Matsumura), or spotted wing Drosophila. The purpose of this report is to inform local fruit growers, researchers, and other interested parties of the presence of *D. suzukii* in the Philadelphia area and Northeastern U.S.

Most species of the genus *Drosophila* lay eggs in rotten, spoiled fruits and vegetables and thus do not affect growing crops. *D. suzukii*, however, lays its eggs in unspoiled, soft-skinned fruits including berries, cherries, and grapes (Bolda et al., 2009). Due to the egg-laying method of *D. suzukii*, the potential for substantial crop loss may be great. Yield loss estimates vary widely, with negligible losses in some areas and close to 80% loss in others, depending on the crop and location (Caprile et al., 2011).

Female *D. suzukii* have a large, hardened, serrated ovipositor with dark teeth that is used to cut through the skin of fruit (Vlach 2010). Fruit with oviposition scars may be indicative of *D. suzukii* larvae present. Male *D. suzukii* are easily identified by the presence of wing spots centered on the first major wing vein (Vlach 2010). Additionally, male *D. suzukii* have a pair of sex combs on each

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front leg and unbroken bands at the end of each abdominal segment (Vlach 2010). Females lack wing spots but also have unbroken bands at the end of each abdominal segment. The main distinguishing feature of the female is the ovipositor. Both male and female adult *D. suzukii* are approximately 2-3 mm in length and have light yellow or brown bodies with red eyes (Vlach 2010).

Damage to fruit is caused by both the oviposition of the female and by the larvae feeding. The initial oviposition site begins to take on a sunken appearance and is subject to decay and secondary infection. After the larvae become larger, breathing holes are cut into the side of the fruit which further exacerbates decay (Beers et al., 2010).

The geographical range of *D. suzukii* has increased dramatically. The species was first described by Matsumura in 1931 and was widely observed in Japan, Korea, and China in the early 1930s (Kanzawa 1939). By the 1980s, *D. suzukii* was observed throughout Hawaii and first appeared in North America in California in 2008 (Walsh 2009). *D. suzukii* has been moving east, being observed as far east as Florida (Steck et al., 2009) and the Carolinas in 2010 (Burrack 2010).

METHODS

Bait traps were crafted from clear 20-oz or 2-L soda bottles (modified from Medeiros and Klaczko 1999). A curved slit was cut into the bottom portion of the trap as an opening through which bait could be added. The slit was covered with tape in spring and summer to inhibit wasps and bees from entering the trap. Small holes were punctured into the bottle at random locations for fly entry. The cap of the bottle was then removed and replaced with a standard shell vial that was attached onto the top with tape. The bait was comprised of mashed rotten banana, apple juice, and yeast. Traps were hung at two different locations on the campus of Saint Joseph's University in Philadelphia and Lower Merion, Pennsylvania.

Traps were maintained from July 2011 to December of 2011 and then again from March to December 2012. Collections were performed multiple times per week. Replacement traps were made at least once a week to ensure that emerging adults were not collected. Specimens were anesthetized with carbon dioxide, sorted, sexed, classified, and placed in 95% ethanol for later study.

D. suzukii was identified using morphological characters (Vlach 2010) and DNA sequencing. DNA was extracted (following a modified version of Gloor and Engels, 1992) from a male specimen collected on 18 October 2011. A Nano Drop spectrophotometer was used to estimate the DNA concentration (10.9 ng/μL). A voucher sequence (Sample ID and GenBank accession number GQ365213) of *cytochrome oxidase subunit I* (*COI*) was obtained from Barcode of Life Data Systems v. 2.5 (Ratnasingham and Hebert 2007). Two sets of primers were used for amplification with the polymerase chain reaction (PCR). The first were universal primers obtained from Folmer et al. (2004):

Forward: 5'-GGTCAACAAATCATAAAGATATTGG-3'

Reverse: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

A second set of primers was designed to match the voucher sequence of *D. suzukii* without mismatches:

Forward: 5'-TTTCTACAAATCATAAAGATATTGG-3'

Reverse: 5'-TAAACTTCAGGGTGTCCAAAAAATCA-3'

COI was amplified using the DNA isolated from the *D. suzukii* male as template (2 μL). The remaining components were: 1 μL (100 ng) of each primer (forward and reverse). 11.4 μL of sterile deionized H_2O, 4 μL of 5X buffer, 0.4 μL of nucleotide mix, and 0.2 μL of Phusion High Fidelity Polymerase Enzyme (New England Biolabs) for a total reaction volume of 20 μL. PCR protocol began with an initialization step at 98°C for five minutes. The following steps were repeated 35 times and included a denaturation step at 98°C for thirty seconds, an annealing step at 50°C for thirty seconds, and an extension step at 72°C for forty-five seconds. A final elongation step was then performed at 72°C for seven minutes. Amplifications were confirmed by gel electrophoresis using 1% agarose stained with SYBR Safe DNA gel stain (Invitrogen).

The PCR product was amplified using the universal primers and purified with the Qiagen PCR Clean-up kit. Sequencing was conducted using each of the universal primers by Genewiz, Inc. Sequences from both strands were aligned using Geneious Pro (Drummond et al. 2011) software to the voucher sequence provided by Barcode of Life Databases v. 2.5 to determine the identity of the specimen.

RESULTS AND DISCUSSION

After performing both morphological and genetic identification of the specimen, it was concluded that the flies captured are *D. suzukii*. Allen L. Norrbom, Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture, confirmed the identification of both male and female *Drosophila suzukii* specimens (Reference #1301082). Males are very distinctive when compared to other specimens of the genus because of the dark wing spots. Females, on the other hand, are not as easily detectable. The body coloration and structure resembles that of both *D. simulans* and *D. melanogaster*. The best course to take when attempting to identify *D. suzukii* females in the presence of other species in the *Sophophora* subgenus, is to use a dissecting microscope to locate the large, saw-like ovipositor. Voucher specimens will be deposited at the Academy of Natural Sciences in Philadelphia.

The genetic identification also confirms the taxonomy of the specimen. When compared to the voucher sequence provided, the 619 base pair *COI* fragment sequence obtained (GenBank JX272633) through extraction, amplification, and sequencing is identical (100% identity). This sequence is also nearly identical to those from Carvajal's (2010) five sequences from flies from the San Diego, CA, area, differing from their consensus at only one site. More specimens need to be sequenced to determine how much variability exists in the natural population for this portion of the *COI* gene.

In the 2011 collection season, 160 male and 44 female *D. suzukii* flies were identified. In the 2012 collection season, the first *D. suzukii* fly captured was a male in June. More *D. suzukii* flies were captured in 2012, perhaps because of the mild winter of 2011-2012. Capturing *D. suzukii* in both 2011 and now in 2012 in the Philadelphia area implies that a breeding population is now established in the area. This could have drastic implications for local fruit growers. Cini et al. (2012) hypothesized that introduction and re-infestation in Europe is due to international/national trade and undetected infested fruits. This is probably also true in the U.S.

It is unclear why the first *D. suzukii* flies were captured so late in 2011 compared to those first caught in 2012. Perhaps this is when *D. suzukii* was introduced to the area. The introduction could have been due to produce transportation or natural migration from the Carolinas to Pennsylvania. It would be valuable to focus future research on *D. suzukii* local adaptation and life-cycle as well as the impact *D. suzukii* will have on local biodiversity.

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