THE SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE): A NEW PEST OF CONCERN FOR BLACK CHERRY, *PRUNUS SEROTINA*, ON THE HIGH ALLEGHENY PLATEAU IN PENNSYLVANIA[1]

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ABSTRACT: *Drosophila suzukii* (Matsumura) is an invasive insect pest native to Southeastern Asia that is now reported to have established populations in North America. We used traps baited with red wine vinegar to detect and monitor *D. suzukii* in four black cherry (*Prunus serotina* Ehrh.) and maple (*Acer* spp.) stands in the Allegheny National Forest in northwestern Pennsylvania. Stands ranged in age from 70 to 110 years old, with average basal area from 40 to 45 m^2/ha. All stands are characterized as the intermediate Allegheny hardwood forest type with black cherry being the dominant species making up > 70% of the total stand basal area at each site (Eyre, 1980). Traps were placed in the crowns of mature black cherry from May to October 2012. In total, 11,000 *D. suzukii* were collected. The appearance of *D. suzukii* on the High Allegheny Plateau in northwestern Pennsylvania could have negative implications for fruit production, dispersal, and seed viability of black cherry and other forest species. With *D. suzukii's* predilection toward *Prunus* spp. and its unusual method of oviposition, this could be a contributing factor that impacts black cherry regeneration on the Plateau.

KEYWORDS: Spotted wing drosophila, *Drosophila suzukii*, black cherry, *Prunus serotina*, introduced species

INTRODUCTION

The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an invasive insect pest native to Southeast Asia that feeds on a wide range of cultivated plants including berries (*Rubus* spp., *Vaccinium* spp., and *Fragaria* spp.), grapes (*Vitis* spp.), peaches, plums, and cherries (*Prunus* spp.) (Lee et al., 2011a; Walsh et al., 2011; Bellamy et al., 2013).

The first reported introduction in North America was in Hawaii in 1980, then California in 2008; the pest is increasingly reported throughout the Eastern United States (Steck et al., 2009; Hauser, 2011; Lee et al., 2011b; Fig. 1). Unlike other vinegar or pomace flies that are considered a nuisance because they mostly infest damaged and decaying fruit, *D. suzukii* damages intact, ripening fruit, causing significant economic damage to cultivated crops (Beers et al., 2010; Hauser, 2011; Lee et al., 2011b; Ioriatti et al., 2015). Little, however, is known

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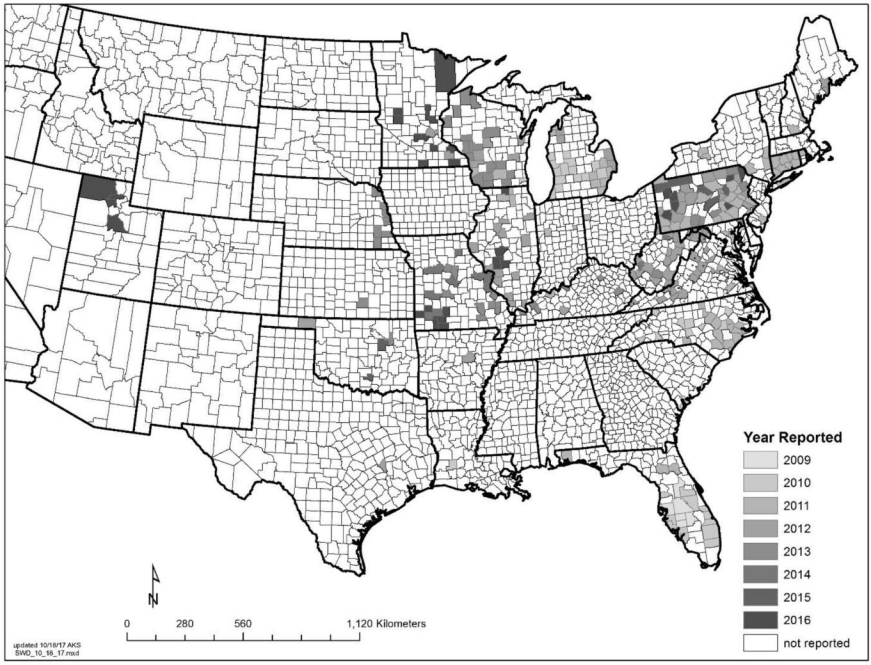

Fig. 1. The current range of *Drosophila suzukii* in the eastern United States.

about the economic and ecological impact this invasive species may have on forest ecosystems.

D. suzukii adults are small flies (2-3 mm long) with red eyes, yellow to brownish thorax, and black stripes on the abdomen (Cini et al., 2012). They have 7 to 15 generations per year and are sexually dimorphic; males display a dark spot on the leading edge of the wings and females possess a large serrated ovipositor (Walsh et al., 2011; Cini et al., 2012). Adult females typically lay 1-3 eggs per fruit and oviposit in 7-16 fruits per day (Kanzawa, 1939; Cini et al., 2012). Damage to fruit is caused by both oviposition and larval feeding (Freda and Braverman, 2013). Eggs are laid in fruit throughout the summer and hatch within 2-72 hours after being laid. Larvae feed and mature inside the fruit; the pupal stage usually occurs inside the fruit and lasts 3-13 days (Kanzawa, 1939; Cini et al., 2012). Kanzawa (1936, 1939) reported that adults overwinter in sheltered places in late November when temperatures drop.

Infested fruits take on a sunken appearance and are subject to pathogens (fungi, yeast, and bacteria), which may cause the fruit to rot and drop with immature seeds (Beers et al., 2010; Walsh et al., 2011; Poyet et al., 2014). These impacts may reduce the viability of black cherry fruits and may change the attac-

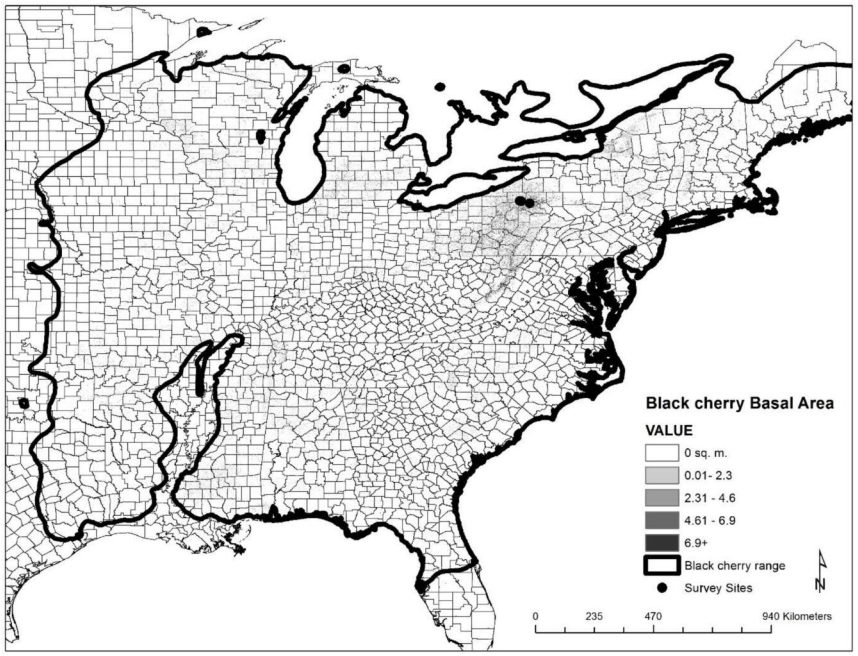

Fig. 2. Survey sites and range and basal area of *Prunus serotina* (Ehrh.).

tiveness and dispersal efficiency of the fruit by birds and other animals (Poyet et al., 2014). Observations of the decline in frugivorous birds at two sites with known infestations of *D. suzukii* have been noted using mist net surveys. According to the surveys, the average number of primary frugivores captured per 100 net hours decreased approximately 70% from previous years (personal communication Scott Stoleson).

METHODS

We conducted this project in four Allegheny hardwood (mixed black cherry) and maple stands located in the Allegheny National Forest in Warren and Elk Counties, Pennsylvania, USA, in 2012 (Fig. 2). Two stands were near Cherry Grove (CG) (41°41'46.24"N, 79°08'40.15"W); the other two stands were in the Kane Experimental Forest (KEF) (41°35'52.69"N, 78°45'56.04"W). Stands ranged in age from 70 to 110 years old, with average basal area from 40 to 45 m^2/ha. All stands were characterized as the intermediate Allegheny hardwood forest type (Eyre 1980) with black cherry being the dominant species, along with white ash and/or yellow poplar making up >50% of the total stand basal area at each site. Other overstory species included American beech (*Fagus grandifolia* Ehrh.), red maple (*Acer rubrum* L.), sugar maple (*A. saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britton), and eastern hemlock (*Tsuga canadensis* (L.) Carr.).

To sample for *D. suzukii* we randomly selected three single-stem overstory black cherry trees in each stand and hung one 500-ml transparent plastic soda bottle with four 1.4-mm holes cut in the upper 2/3 of the trap (modified from Freda and Braverman, 2013) in each tree. Each trap was baited with ~150 ml red wine vinegar. Traps were deployed from May to October in 2014. Traps were raised and lowered into the crown on strings placed using a Big Shot Rope Launcher (Sherrill Tree Company, Greensboro, NC.). Traps were changed every 14 days, and samples were stored in 70% ethanol until sorted and identified.

Black cherry fruit was also sampled in these study locations to quantify seed fall per acre and identify any insects utilizing the fruit. This was accomplished by using a modified method outlined in the Bjorkbom (1979) paper. Falling black cherry fruit was collected in flower pots, placed in paper bags and shipped to the Morgantown field office. Upon arrival the samples were placed in plastic soufflé containers. These containers were kept at room temperature and inspected weekly for emerging arthropods and obvious signs of insect infestation or feeding on the epidermis of the fruit (e.g. entry/exit holes). After the fruit had dried completely and fly emergence had ceased, the fruit was dissected. In order to make dissection possible, the fruits were placed in nearly boiling water for rehydration. A scalpel was used to cut the fruit in half to examine for seed and flesh damage and presence of arthropods. Any adult or larval specimens collected were preserved in 70% ethyl alcohol and stored for later identification.

D. suzukii were identified using morphological characters and total genomic DNA. The DNA was extracted from individual, whole specimens using the QIAGEN DNA mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. A region of the ribosomal DNA (rDNA) was PCR amplified (following a modified version of Kim et al. 2014) using the barcoding primers LCO-1490 (GGTCAACAAATCATAAAGATATTGG) and HCO-2198 (TAAACTT CAGGGTGACCAAAAAATCA). Amplified DNA was purified and sent to the DNA Sequencing Facility at University of California Davis for direct sequencing in both directions. BLASTn searches of the sequences resulted in 100% homology with KJ463777, described by Kim et al. (2014), and with six other *Drosophila* accessions. In addition to *D. suzukii* we also collected *D. quinaria* Loew, *D. putrida* Sturtevant, and *D. melanogaster* Meigen. Voucher specimens of *Drosophila* were deposited with the Pennsylvania Department of Agriculture in Harrisburg, PA.

RESULTS AND DISCUSSION

Female and male *D. suzukii* were first detected in June and July, respectively, at both sites (Fig. 3). In total, 11,683 *D. suzukii* flies were identified. The biweekly trap catches were low in June and increased throughout the trapping period (Fig. 3). To our knowledge, this detection is the first evidence of activity in black

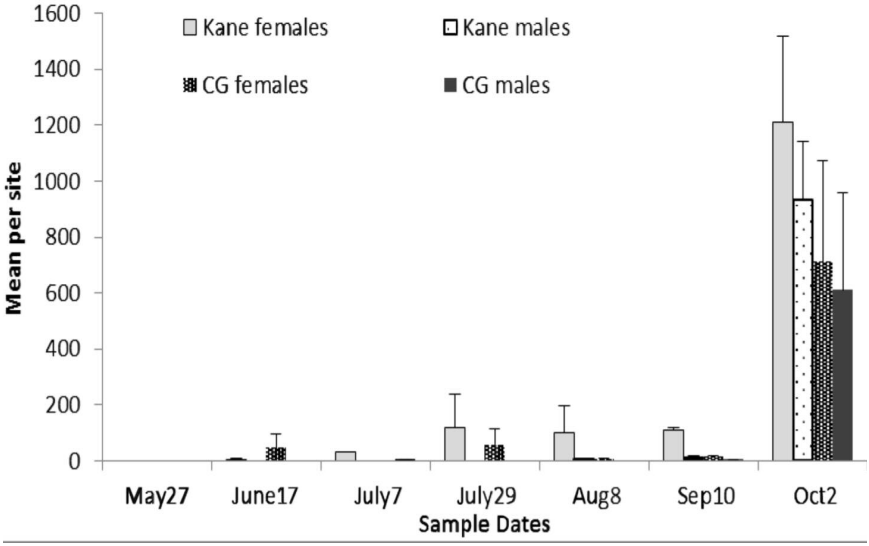

Fig. 3. Biweekly captures of female and male (mean ± SEM) *Drosophila suzukii* in *Prunus serotina* stands in Kane and Cherry Grove sections of the Allegheny National Forest in Pennsylvania during 2012.

cherry forest stands, although trapping and fruit surveys in Pennsylvania, Maryland, and West Virginia have detected *D. suzukii* in cultivated agricultural sites throughout the primary black cherry timber growing areas (pest tracker link; Fig. 2).

Out of the total number of fruits sampled (n=2158), an average of 37 ± 43 cherries per sampling period showed obvious signs of insect activity. However, preliminary results of dissections show that this type of damage only represents a fraction of the actual infestation rates of the fruit.

Dissections revealed dipteran maggots and puparia within the flesh of the fruit. Although *D. suzukii* larvae are nearly indistinguishable from other drosophilids, we were able to successfully rear and identify *D.suzukii* adults. It should also be noted that epidermal damage is not caused exclusively by *D. suzukii* and other arthropods have been found using the seed and fruit. Sawfly larvae were found feeding on the seed and were confirmed, using molecular and taxonomic characteristics, to be in the genus *Hoplocampa.* Other adult parasitic wasps and a weevil were also found in the flesh of the fruit and species determinations are ongoing.

Black cherry is an ecologically and economically valuable eastern hardwood timber species; some of the highest quality trees grow in northwestern Pennsylvania (Wiedenbeck et al., 2004). It is an important autumn soft mass species (October-November); it is high in kilocalories, protein, and nonfiber carbohydrates (Rose et al., 2014); it is used by a variety of animals (e.g., wild turkey and black bear; Ryan et al., 2004). Ripe seeds are typically dispersed in this area beginning August 1 and ending November 30 (Bjorkbom, 1979).

Open-grown black cherry begins producing seeds at 10 years of age and produces large fruit crops every 1-5 years (Grisez, 1975; Bjorkbom, 1979; Marquis, 1990). Good black cherry seed crops historically, on the Allegheny Plateau in northwestern Pennsylvania, have occurred about every other year (Marquis, 1990), but recent seed production has been less frequent and highly variable (Robert Long, per. comm. USDA Forest Service; Morden-Moore and Willson, 1982).

The High Allegheny Plateau in northwestern Pennsylvania has been experiencing a reduction in regeneration of black cherry, which could possibly be traced back to the invasion of *D. suzukii* into the area. This reduction, however, could also be attributed to a number of other different causes including climate change, deer browsing, decreased nitrogen and sulfate deposition, and interactions with other pathogens such as cherry leaf spot (*Blumeriella jaapii* [Rehm] Arx.) (Todd Ristau and Susan Stout, per. comm., USDA Forest Service). We considered *D. suzukii* to be a new pest of concern for black cherry. With *D. suzukii*'s predilection toward *Prunus* spp. and its unusual method of oviposition, this species could be a contributing factor impacting black cherry regeneration on the Plateau. However, further studies are necessary to understand the impact of this invasive insect pest on black cherry stand regeneration.

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Drosophila suzukii. Female (top), Male (bottom). Photos provided by Craig Larcenaire.

Drosophila suzukii. Serrated ovipositor. Photo provided by Craig Larcenaire.

As the Spotted Wing Drosophila is newly-introduced to eastern North America, these photos are included here as an aid to identification. For more information see paper by Turcotte et al. in this issue and the literature cited therein.