Research Article

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Trapping system comparisons for and factors affecting populations of *Drosophila suzukii* and *Zaprionus indianus* in winter-grown strawberry

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Abstract

BACKGROUND: *Drosophila suzukii* (Matsumura) is a major fruit pest in temperate regions worldwide, but in subtropical Florida, winter-grown strawberries have not been severely affected. *Zaprionus indianus* Gupta is another invasive drosophilid species and a pest of some tropical fruits. To improve monitoring, trapping systems for *D. suzukii* and *Z. indianus* were tested. Morphology, ovarian status and the suitability and availability of non-crop hosts as possible *D. suzukii* population-limiting factors were assessed.

RESULTS: Traps with commercial attractants captured more *D. suzukii* but fewer *Z. indianus* than those with a homemade mixture. In central and northern Florida, < 10% and 30-80% of *D. suzukii*, respectively, exhibited darker, winter morph coloration, and 55-75% of females from central Florida were carrying mature and/or immature eggs. Adult *D. suzukii* were reared from fruits of two of 28 potential hosts: elderberry (*Sambucus nigra*) and nightshade (*Solanum americanum*). Nightshade, but not elderberry, was common on field perimeters (21 and six of 36 fields, respectively). Traps placed in wooded or partially wooded field edges yielded the most *D. suzukii*.

CONCLUSION: Florida strawberry is at risk of *D. suzukii* infestation, as flies were captured throughout the growing season. However, fly captures remained relatively low, peaking at 1.5 flies per trap per day. In central Florida, the low availability and suitability of non-crop hosts likely limit population growth. The finding of few flies in northern Florida may additionally be attributable to a greater proportion of flies displaying winter morph coloration than in central Florida.

Keywords: spotted-wing drosophila; African fig fly; attractants; morphotype; alternative hosts; monitoring

1 INTRODUCTION

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), spotted-wing drosophila, is native to northern Asia but since it was first discovered in California, Italy and Spain in 2008, it has spread rapidly to many fruit-growing regions.[1–4] Female *D. suzukii* are unique among Drosophilidae because they possess large, serrated ovipositors, and damage to ripening and ripe fruit occurs as a consequence of oviposition punctures and larval feeding.[5] *Drosophila suzukii* has become a significant pest of berry and soft-fruit crops as a result of its high fecundity, short generation times and few known natural enemies in invaded regions.[6–9] In addition, suitable host plants that surround crop fields facilitate *D. suzukii* population build-up and fly dispersal into adjacent fields prior to and during harvest periods.[10–12] Frequent, regularly scheduled insecticide applications against flies are the backbone of current management strategies.[13–15] With improvements to monitoring techniques, management decisions based on accurate population estimates should enable strategies with fewer insecticide applications.

Larval infestation rates in fruit can be directly assessed.[16] However, it is desirable to detect adult flies so that management decisions are made prior to fruit infestation. Trap design and the relative attractiveness of commonly used baits affect capture rates of adult *D. suzukii*, and low captures may impede grower adoption of a monitoring program. Studies have shown that red or black traps and traps with an expanded entry area, a larger surface area for liquid attractants and increased headspace improved capture rates.[17–20] Apple cider vinegar is an easy-to-use attractant, but it is relatively inefficient at capturing *D. suzukii* compared with homemade yeast-sugar and yeast-flour mixtures or commercially available, synthetic lures.[7,21,22] Some synthetic pouch lures [e.g. Pherocon SWD dual-lure (Trécé Inc., Adair, OK, USA) and Scentry Lure (Scentry Biologicals, Billings, MO, USA)] are based on a four-compound blend,[23,24] but they vary in efficiency at capturing

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D. suzukii. Pherocon SWD dual-lure captured few *D. suzukii*, but also fewer non-targets, enabling rapid identification of *D. suzukii*, whereas Scentry Lure captured many *D. suzukii*, but also many non-targets, particularly other Drosophilidae.[22] Liquid attractants [Suzukii Trap (Bioibérica, S.A.U., Barcelona, Spain), DroskiDrink and Dros'Attract (Biobest®, Westerlo, Belgium)], typically based on mixtures of alcohols and fruit juices or extracts, also vary in their effectiveness at capturing *D. suzukii*.[21,22] In one study, the combination of Scentry Lure and Suzukii Trap caught more *D. suzukii* than the additive amount of either product alone, suggesting a synergistic effect.[22] As the number of available attractants increases and their compositions evolve to reflect current results,[25] there is a continued need to test trap type and attractant combinations ('trapping systems') in specific regions and crops so that growers can select optimal tools for monitoring programs.

Optimal trapping systems will consist of the best available traps and attractants, but trap placement will also influence *D. suzukii* captures, thus potentially effecting control decisions. Detecting fly activity in berry crops by placing traps in shaded areas of the canopy is recommended.[26] However, as *D. suzukii* use non-crop hosts in field margins[27,28] and tend to colonize crops in field areas adjacent to these hosts,[12] placing traps in margins where host plants are plentiful may provide early detection.[29] In addition, general landscape features may affect *D. suzukii* populations, as raspberry fields surrounded by a high amount of woodland had the earliest captures of *D. suzukii*.[30] Therefore, the most accurate determination of crop infestation risk may be made by placing traps in field margins where *D. suzukii* populations are most likely to occur.

In addition to trap features and placement, it will be important to know how morphological traits and the ovarian status of *D. suzukii* flies influence trap captures and the potential for causing crop damage. In temperate regions, *D. suzukii* displays a degree of phenotypic plasticity, with flies developing darker pigmentation during autumn in preparation for winter conditions.[31] Darker, winter morphs are more cold-hardy than lighter colored summer morphs, but adaptability to low temperature may be insufficient to explain overwinter survival in colder temperate regions.[32,33] Winter morphs also appear to undergo reproductive diapause, as preoviposition times were longer than for summer morphs.[34] In addition, female *D. suzukii* of varying reproductive status may be differentially attracted to baits in traps versus ripe fruit, as was observed during summer in North Carolina, or status may be related to host availability.[21,35] Therefore, the pest potential of *D. suzukii* depends on environmental cues affecting its morphology and egg-laying capacity, and effective management depends on detecting these differences to predict potential for crop losses.

Zaprionus indianus Gupta (Diptera: Drosophilidae) is an invasive vinegar fly also expanding its range in North and South America in recent years;[36–38] it was first found in Florida and North America in summer 2005.[39] Unlike *D. suzukii*, *Z. indianus* is not suspected to cause primary damage to soft fruits, although it has been reported as a pest of figs in Brazil.[40] However, it may be able to colonize fruit already compromised by *D. suzukii*, thus accelerating fruit decomposition and exacerbating damage. *Zaprionus indianus* is captured incidentally in traps for *D. suzukii*, as it is attracted to wine, vinegar and some of the same compounds as *D. suzukii*.[41,42] Determining which attractants are also most effective for *Z. indianus* will help combine trapping and monitoring efforts for both pests in regions in which they co-occur.

In Florida, strawberries are produced on >4000 ha primarily during the late fall and winter (November–March) and are an important agricultural resource for central areas of the state, with a production value of nearly $4.5 million.[43] Since its initial discovery in Florida in 2009, *D. suzukii* has not become a major pest in strawberry, but some growers routinely use traps to assess its presence. Little is known about suitable alternative host plants in subtropical Florida or the morphological and biological status of *D. suzukii* in the mild winter conditions of Florida. Understanding ecological factors unique to Florida that constrain or facilitate *D. suzukii* populations will be valuable for refining monitoring and management strategies. Therefore, our research objectives were to compare three trapping systems, assess the suitability and abundance of fruiting host plants, test the effect of field margin vegetation on trap captures, and determine the morphological and ovarian status of *D. suzukii* in Florida strawberry fields.

2 MATERIALS AND METHODS

2.1 Trapping system, fly morphotype, and female ovarian status

During winter 2015–2016, three *D. suzukii* trapping systems (attractant + trap type), namely (1) Dros'Attract (Biobest®) + DrosoTrap (Biobest®), (2) Scentry Lure (single pouch) (Scentry Biologicals) with water (250 ml) as a drowning agent + a homemade plastic jar trap (red; 1 L; eight 3.5-cm-diameter mesh-covered openings)[20] and (3) apple cider vinegar (ACV) (Publix Brand; Publix Super Market Inc., Lakeland, FL, USA) and beer (Steel® Reserve; 8.1% alcohol; Steel Brewing Company, Milwaukee, WI, USA) (1:1 by volume) + a clear plastic jar (1 L; 22 5-mm-diameter holes around the top), were compared in five central (Hillsborough County) and two northern Florida strawberry fields (Alachua and Bradford Counties). Apple cider vinegar and beer were evaluated because some Florida strawberry growers routinely use this mixture in homemade traps. Scentry Lures were hung with a plastic tie from the inside of trap lids 5 cm above the surface of the water. Unscented dish detergent (Free and Clear Dish Soap; Target Corp., Minneapolis, MN, USA) was added to all liquids (1.5 mL to 4 L) to reduce surface tension; each trap had 250 ml of liquid. The three systems are hereafter referred to as (1) 'commercial', (2) 'synthetic lure' and (3) 'ACV + beer'.

Traps were hung on the edges of fields with twist-ties from bamboo garden stakes that were inserted on an angle near the ends of raised strawberry beds, so that the bottom of the trap hung just below the top of the strawberry foliage. The three trapping systems were arranged in a randomized complete block design, with four replications per field. Traps within blocks were 5 m apart and blocks were at least 20–30 m apart. At the five fields in central Florida, traps were placed along one edge of each large field (10.1, 5.9, 14.0, 40.5, and 11.9 ha). As a consequence of the smaller size of strawberry fields in northern Florida (< 2 ha), traps were placed along different edges of a field, adjacent to unmanaged woodlands when possible.

In central Florida, traps were placed in four fields on 16 December and in the fifth field on 17 December. In northern Florida, traps were placed on 4 February. Traps were checked and serviced every 6 to 8 days. For traps with water or ACV and beer, contents were poured into a collection container and liquids were replaced with fresh solutions weekly. For Dros'Attract, the entire liquid was replaced every other week (per the manufacturer's recommendation). For the weeks between, trap contents were poured into a sieve above a graduated cylinder, trapped insects were washed into collection containers, and the amount of Dros'Attract was recorded and replenished (to 250 ml) before pouring back into traps. The trap order within blocks was re-randomized each week.

The Scentry Lures were replaced after 6 weeks in the field, per the manufacturer's recommendation. In central Florida, traps were removed from one field on 9 March (after 12 weeks) and from the other four fields on 16 March (after 13 weeks). In northern Florida, traps were removed on 18 and 17 March (after 7 weeks) from each field, respectively.

From all locations, numbers of *D. suzukii* males and females, *Z. indianus*, other Drosophilidae, and sap beetles (Coleoptera: Nitidulidae) were counted and the seasonal morph (summer or winter) of each *D. suzukii* was categorized based on abdomen coloration.[31] For males and females, the third and fourth abdominal segments were assessed, respectively. Winter morphs were those with segments completely dark and summer morphs were those with only a thin, dark line on the segment.[31] In addition, female *D. suzukii* from central Florida were dissected and categorized based on ovarian development status as no eggs present, only immature eggs present, only mature eggs present, or both immature and mature eggs present.[21] Mature eggs with a visible respiratory filament were counted.

2.2 Field perimeter host plants and vegetation type

Thirty-six strawberry fields in Hillsborough County, Florida were visited approximately weekly from 18 January to 20 March 2016. Field perimeters were walked, and fruiting plants that may serve as alternate hosts for *D. suzukii* were identified in hedgerows or up to about 5 m into forested edges using a field reference guide[44] and Internet searches on EDIS for specific plants.[45] Some leaf and fruit samples were brought to the laboratory and identified by Nathan Boyd and Andrew Koeser at the University of Florida. The geographic coordinates of each plant or small group (<5 m along the field perimeter) of plants of the same species were recorded (GNSS Surveyor, BE-GPS-3300; BadElf, Tariffville, CT, USA). A sample of ripe fruit was collected weekly from a potential host plant into a 500-mL or 1-L plastic container with a lid containing fine mesh for ventilation. Fruit availability was categorized as plants or small groups of plants with (1) < 25, (2) 25–100, (3) 101–1000 or (4) >1000 individual ripe fruits. Fruit sample size depended on fruit availability, and sampling occurred until no ripe fruits remained on a plant. Plastic containers were incubated at 25 °C for 2 weeks in a controlled environment growth room, after which emerged drosophilid flies were identified as male or female *D. suzukii*, *Z. indianus* or other Drosophilidae.

Field perimeter vegetation type was categorized as: woodland (large trees and few shrubs), partial-woodland (small trees, shrubs, vines and herbaceous plants, often in low-lying, wet areas), hedgerow (trees, bushes and other plants separating a strawberry field from other agricultural fields or pasture), open (immediately adjacent to agricultural fields, pasture, old fields, ponds or roads) and residential (front yards, backyards and fences). Field edge category was determined for partial sections of each field, and the 'stop' 'start' geographic coordinates of each category were used in Google Earth to measure distance.

In 2016, 15 traps were placed along perimeters of three contiguous strawberry fields that were in a low-lying area where fruiting elderberry (*Sambucus nigra* L.) was common. A third of the traps were placed in the largest elderberry patches, and other traps were placed where there were no potential fruiting hosts. Traps were at least 15 m apart. Each trap was assigned a vegetation-type category based on its location; there were no 'residential' perimeters along these three fields. Traps were homemade plastic jars baited with ACV that were hung 1.0–1.5 m above the ground from an elderberry bush from 16 February to 23 March (6 weeks). Traps were checked weekly, ACV was replaced, and captured *D. suzukii*, *Z. indianus* and by-catch were identified and counted.

In 2017, 20 traps were set along the same three strawberry fields as in 2016; 15 traps were in the same locations, with American black nightshade (*Solanum americanum* Mill.) occurring near one of the traps where it was not found in 2016, two of the new traps were near elderberry and the other three were near nightshade. The same procedures were used as in 2016, except that traps contained Scentry Lures and soapy water as a drowning agent and were in place from 1 February to 9 March (6 weeks; lures not replaced), and all captured *D. suzukii* were designated as a winter or summer morph based on abdominal coloration.

Ripe fruit samples from elderberry and American black nightshade were collected weekly from 3 February to 9 March 2017 from five large patches of each species along the edges of the three fields where traps were located. Fruits were counted and held as in 2016, and the number of emerged *D. suzukii* flies was counted.

2.3 Data analysis

Trapping system and morphotype data from central and northern Florida were analyzed separately. Effects of trapping systems on captures of male and female *D. suzukii*, the proportion of *D. suzukii*, and the numbers of sap beetles, *Z. indianus* and mature eggs per female were compared using a mixed model analysis of variance (ANOVA) with trapping system, date of capture (3 March 2016 was not included because there were no data from commercial traps and 2 February 2016 was not included for proportion of *D. suzukii* because no flies were caught in ACV + beer traps) and their interaction as fixed effects and field location and block nested within field as random effects. Because *D. suzukii* captures in northern Florida were low (many zeroes), males and females were analyzed together and numbers of *Z. indianus* were not analyzed (only means/trap shown). Egg data were combined across all females for trap types within fields and weeks to minimize the amount of missing data (when no females were captured), and only field location was used as a random effect. All data were square root transformed to normalize error variance, and back-transformed means (± 95% confidence interval) are shown. Tukey's honest significant difference (HSD) test was used to separate significantly different means ($\alpha = 0.05$).

Proportions of male and female summer and winter morphotypes and categories for female ovarian maturity were compared among trapping systems and dates using chi-square tests of independence. Subsequent tests were performed where differences occurred, using a Bonferroni correction (α/total df) to account for multiple pairwise comparisons. Dates in 2016 were categorized as early (16 December to 3 February), mid (4–25 February), and late (26 February to 17 March) winter according to the partitioning of the change in winter daylength into three equal periods. The dates 3 and 25 February were also days that trap contents were checked and serviced in most fields. We also evaluated whether the number of mature eggs and ovarian maturity status differed between female morphotypes from central Florida using a *t*-test and chi-square test, respectively. We only compared flies from 17 February 2016, as 50% of winter morphotypes were captured then.

Effects of trap location in 2016 and 2017 – whether near fruiting host plants and in four categorized vegetation types – on *D. suzukii* and *Z. indianus* were analyzed using separate mixed model ANOVAs. Date of capture and interaction terms between date and host plant proximity or vegetation type were included as fixed effects and the field in 2016 was used as a random effect.

Table 1. Least square mean (95% confidence interval) numbers of *D. suzukii*, *Z. indianus* and Nitidulidae captured per trap per day in three trapping systems from 16 December 2015 to 16 March 2016 at five strawberry fields in central Florida (Hillsborough County) and from 3 February to 18 March 2016 at two strawberry fields in northern Florida (Alachua and Bradford Counties)

		D. suzukii				
Field locations	Trapping systems	Males	Females	Proportion	*Z. indianus*[a]	Nitidulidae
Central FL	Commercial	0.14 (0.07-0.24) a	0.18 (0.11-0.27) a	0.71 (0.30-1.21) a	0.15 (0.02-0.39) b	0.02 (0.01-0.12) c
	Synthetic lure	0.11 (0.00-0.06) a	0.07 (0.02-0.13) b	0.61 (0.27-1.08) a	0.14 (0.02-0.38) b	0.20 (0.06-0.44) a
	ACV + beer	0.01 (0.05-0.21) b	0.02 (0.00-0.05) c	0.21 (0.03-0.50) b	0.40 (0.16-0.75) a	0.11 (0.01-0.30) b
Northern FL	Commercial	0.36 (0.19-0.57) a		0.03 (0.00-0.16)	0.046 ± 0.031	1.19 (0.72-1.65) ab
	Synthetic lure	0.13 (0.04-0.27) b		0.02 (0.01-0.13)	0.033 ± 0.016	0.98 (0.60-1.36) b
	ACV + beer	0.08 (0.01-0.19) b		0.02 (0.01-0.12)	0.009 ± 0.005	1.54 (0.97-2.11) a

Proportion is the number of *D. suzukii* out of all Drosophilidae.
[a] Mean (± standard error) for northern Florida captures.

As traps were not located in every host plant and vegetation type category, an interaction term was not included in the model. JMP® Pro 12.0.1 was used for all analyses.[46]

3 RESULTS

3.1 Trapping system, fly morphotype, and female ovarian status

In central Florida, male *D. suzukii* captures were affected by trapping system ($F_{2,636} = 45.2$; $P < 0.0001$), date ($F_{11,636} = 21.6$; $P < 0.0001$), and the trapping system by date interaction ($F_{22,636} = 3.4$; $P < 0.0001$). The commercial and synthetic lure systems captured more males than ACV + beer (Table 1). On two consecutive dates in late January, the commercial system captured more males than ACV + beer, and on two consecutive dates in February, commercial and synthetic lure systems captured more males than the ACV + beer system (Fig. 1A). Female *D. suzukii* captures were affected by trapping system ($F_{2,636} = 14.9$; $P < 0.0001$), date ($F_{11,636} = 31.2$; $P < 0.0001$) and the trapping system by date interaction ($F_{22,636} = 1.5$; $P < 0.0001$). The commercial system captured more females than the synthetic lure system, and the synthetic lure system captured more females than the ACV + beer system (Table 1). The commercial system captured more females than the ACV + beer system from 6 January to 17 February and more than the synthetic lure system on 20 January (Fig. 1B).

In northern Florida, *D. suzukii* captures were affected by trapping system ($F_{2,118} = 10.7$; $P < 0.0001$), date ($F_{5,118} = 2.3$; $P = 0.046$) and the trapping system by date interaction ($F_{10,118} = 2.8$; $P = 0.004$). More flies were captured in the commercial lure system than in the synthetic lure and ACV + beer systems (Table 1).

In central Florida, the proportion of *D. suzukii* out of all Drosophilidae was affected by trapping system ($F_{2,571} = 23.8$; $P < 0.0001$), date ($F_{10,571} = 5.9$; $P < 0.0001$), and the trapping system by date interaction ($F_{20,571} = 1.7$; $P = 0.023$). The proportion of *D. suzukii* was higher in the commercial and synthetic lure systems than in the ACV + beer system (Table 1). Tukey's HSD test did not separate by trapping system any of the within-week mean proportions (Fig. 1C). In northern Florida, the proportion of *D. suzukii* was affected by date ($F_{5,117} = 4.2$; $P = 0.002$) but not trapping system ($F_{2,117} = 2.0$; $P = 0.139$) or the trapping system by date interaction ($F_{10,117} = 1.8$; $P = 0.060$) (Table 1).

In central Florida, *Z. indianus* captures were affected by trapping system ($F_{2,636} = 27.2$; $P < 0.0001$), date ($F_{11,636} = 23.0$; $P < 0.0001$), and the trapping system by date interaction ($F_{22,636} = 5.7$; $P < 0.0001$). More *Z. indianus* were captured in ACV + beer than in the other two systems (Table 1), particularly for a few weeks in mid January and early February (Fig. 1D).

In central Florida, Nitidulidae captures were affected by trapping system ($F_{2,636} = 62.1$; $P < 0.0001$), date ($F_{11,636} = 8.6$; $P < 0.0001$), and the trapping system by date interaction ($F_{22,636} = 5.0$; $P < 0.0001$). The commercial system captured the fewest sap beetles and the synthetic lure system captured the most (Table 1). The largest differences in captures between the commercial and other systems occurred in late December and early February (Fig. 1E). In northern Florida, Nitidulidae captures were affected by trapping system ($F_{2,118} = 4.8$; $P = 0.010$), date ($F_{5,118} = 17.2$; $P < 0.0001$), and the trapping system by date interaction ($F_{10,118} = 3.5$; $P = 0.0004$). Fewer sap beetles were captured in the synthetic lure system than in the ACV + beer system (Table 1).

In central Florida, there were no differences in proportions of morphotypes among trapping systems (female: $\chi^2 = 2.91$; df = 2; $P = 0.233$; male: $\chi^2 = 4.04$; df = 2; $P = 0.133$). The proportion of female, but not male, morphotypes varied by season (female: $\chi^2 = 12.21$; df = 2; $P = 0.002$; male: $\chi^2 = 4.58$; df = 2; $P = 0.101$). The proportion of female winter morphs was greater in mid-season than in early season ($\chi^2 = 12.25$; df = 1; $P < 0.001$), but not greater than in late season ($\chi^2 = 0.19$; df = 1; $P = 0.664$) (Fig. 2A). Late and early season proportions were marginally insignificantly different ($\chi^2 = 3.36$; df = 1; $P = 0.067$). Overall, the proportion of winter morph *D. suzukii* caught in central Florida was never more than 7%, whereas in northern Florida, > 80% of females and 30–40% of males were winter morphs (Figs 2A, B). There were no differences in proportions of morphotypes attributable to season (female: $\chi^2 = 0.03$; df = 1; $P = 0.865$; male: $\chi^2 = 0.33$; df = 1; $P = 0.567$) or trapping system (female: $\chi^2 = 2.12$; df = 2; $P = 0.346$; male: $\chi^2 = 0.54$; df = 2; $P = 0.765$) in northern Florida.

Daylength was approximately 10 min shorter at the beginning of winter in northern Florida compared with central Florida, but by late winter, daylength was nearly the same in the two regions (Fig. 2C). In both regions, winter temperature varied from near or above 30 °C to near or below freezing (Figs 2D, E). In northern Florida, average daily minimum temperatures were 2–3 °C lower than in central Florida, and there were eight nights below 0 °C.

The trapping system affected the type of female *D. suzukii* captured ($\chi^2 = 28.07$; df = 6; $P < 0.001$) (Fig. 3A). Proportions of females with no eggs, only immature eggs, only mature eggs, or both immature and mature eggs differed between the synthetic lure system and the commercial ($\chi^2 = 40.69$; df = 3; $P < 0.008$) and ACV + beer ($\chi^2 = 18.44$; df = 3; $P < 0.008$) systems. There was also an effect of trapping date on the ovarian status of females

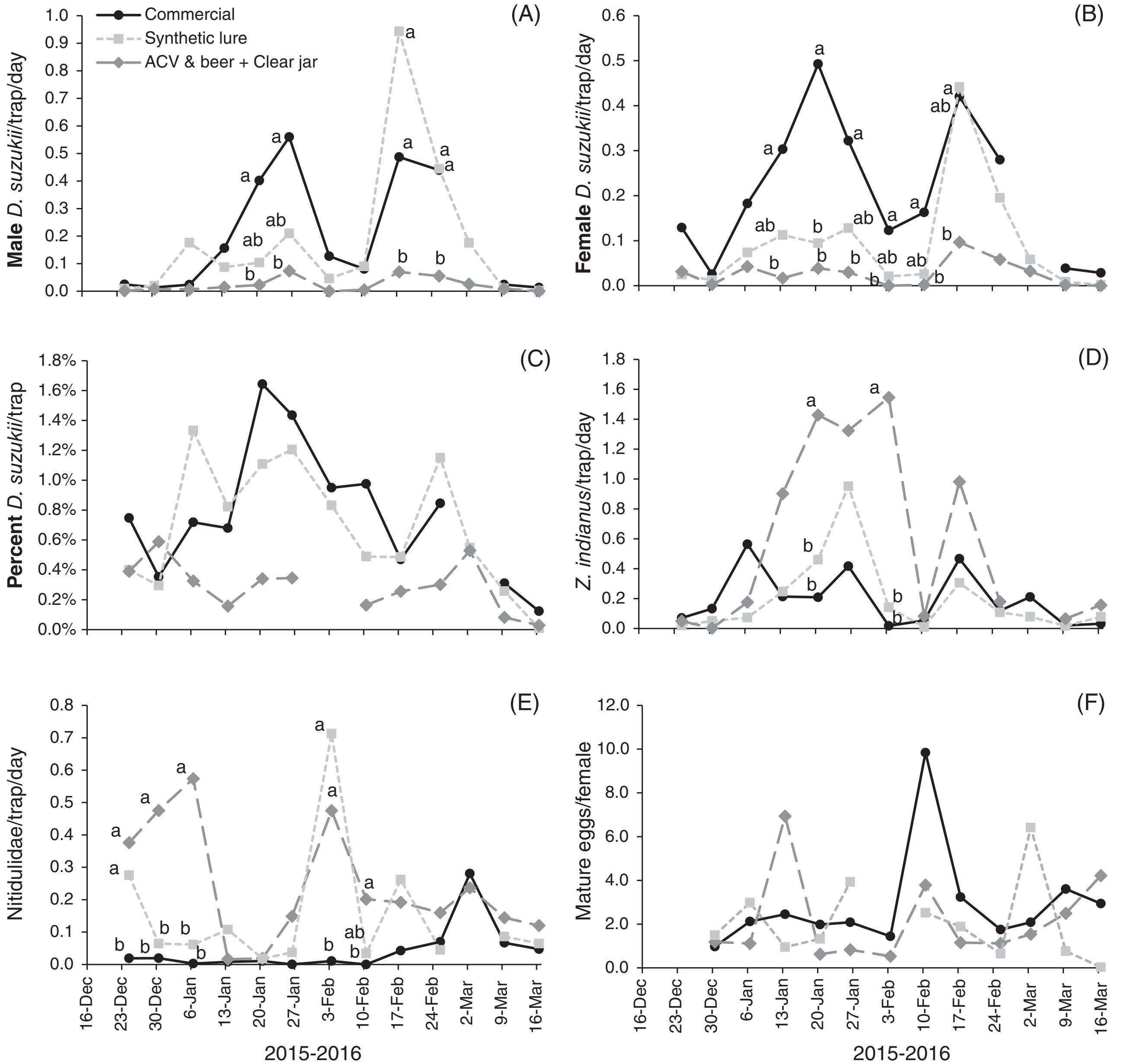

Figure 1. Mean (A) number of male and (B) number of female *D. suzukii*, (C) proportion of *D. suzukii* out of all Drosophilidae, (D) number of *Z. indianus*, (E) number of Nitidulidae and (F) number of mature eggs per female *D. suzukii* in three trapping systems from 16 December 2015 to 16 March 2016 at five strawberry fields in central Florida (Hillsborough County). For each week in each panel, means with different letters are significantly different (Tukey's HSD test; $\alpha = 0.05$).

($\chi^2 = 16.46$; df = 6; $P = 0.012$) (Fig. 3B), but no paired comparisons were significant ($P > 0.008$) when accounting for Bonferroni's correction. For *D. suzukii* captured in central Florida, there were no differences in egg loads for females captured in different trapping systems ($F_{2,92} = 2.2$; $P = 0.122$) or on different dates ($F_{9,91} = 1.2$; $P = 0.316$), and there was no trapping system by date interaction ($F_{18,90} = 1.1$; $P = 0.391$) (Fig. 1F).

Females with winter morphotype coloration captured on 17 February 2016 ($n = 20$) contained 3.05 ± 0.94 mature eggs per female [mean ± standard error of the mean (SEM)], which was marginally, but not significantly, less than the 3.39 ± 0.40 mature eggs per female in summer morphotypes ($n = 244$) ($t_{26} = 0.33$; $P = 0.746$). The distribution of ovarian maturity status categories did not differ by female morphotype ($\chi^2 = 1.23$; df = 3; $P = 0.747$).

3.2 Field perimeter host plants and vegetation type

In 2016, 404 fruit samples from 27 potential host plants (identified to genus or species) yielded just 17 total *D. suzukii* adults from two species: elderberry and American black nightshade (Table 2). American black nightshade was relatively common, with at least one fruit sample from 29 of the 36 fields. Elderberry was less common, with fruit samples from 12 of 36 fields. *Zaprionus indianus* was reared from mandarin oranges (*Citrus reticulata* Blanco), which occurred at two fields (Table 2).

Nine *D. suzukii* individuals were reared from elderberries from a single sample collected between 8 and 14 February (Fig. 4B). Six *D. suzukii* individuals were reared from American black nightshade fruits from a sample collected between 22 and 28 February, and the remaining two *D. suzukii* individuals were from two

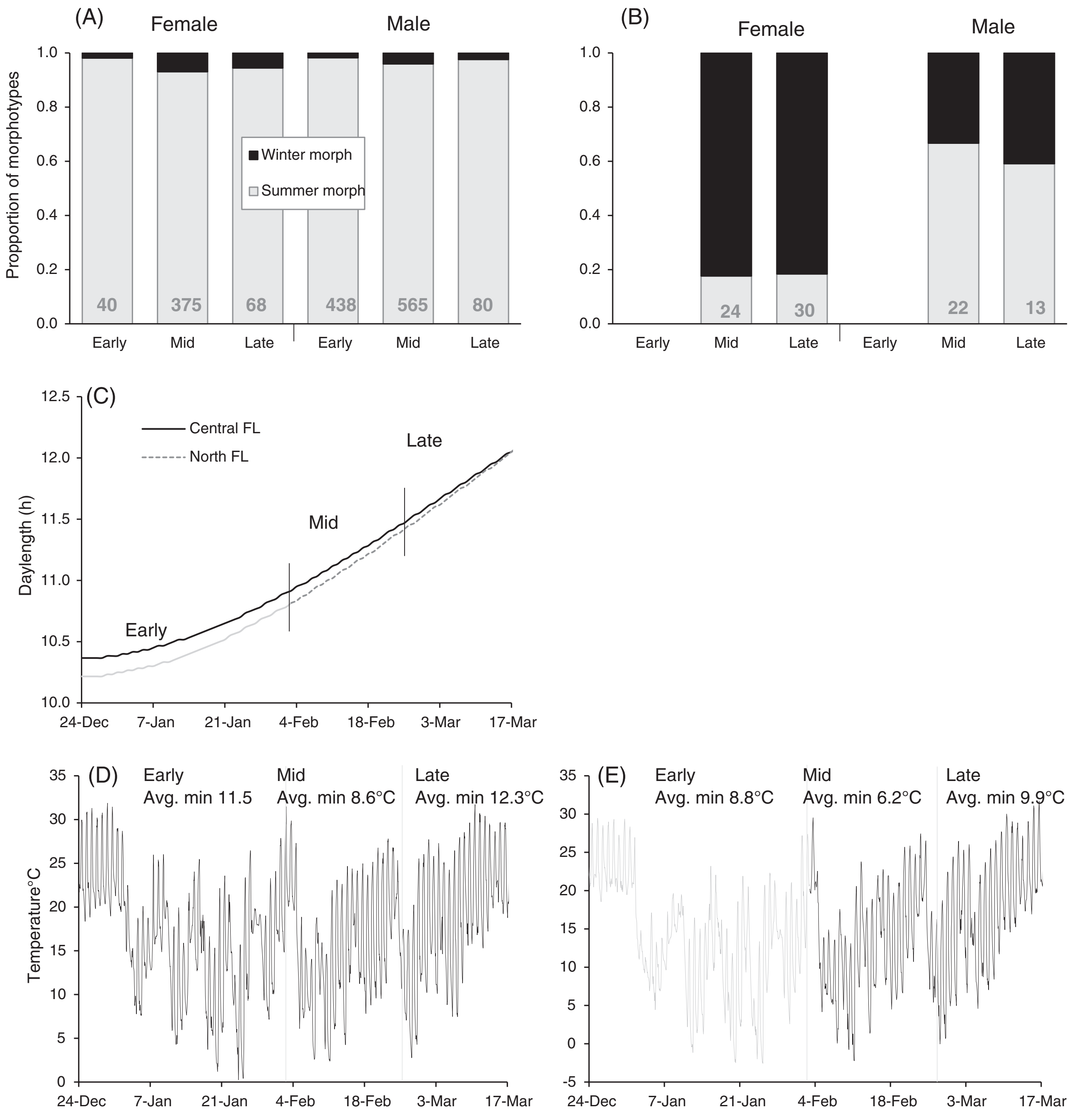

Figure 2. Morphotypes of *D. suzukii* from strawberry fields in (A) central Florida (Hillsborough County) and (B) northern FL (Alachua and Bradford Counties) based on abdomen coloration (summer morph has less melanization on the fourth segment for females and on the third segment for males). Environmental factors that may influence morphotype are (C) daylength in Plant City, central Florida and Lawtey, northern FL and temperature for (D) Dover, central Florida and (E) Putnam Hall, northern FL. Daily minimum temperatures were averaged for early, mid and late winter.

samples collected between 29 February and 6 March (Fig. 4A). American black nightshade fruits were collected from all vegetation categories, from 14% of samples in woodland to 27% in partial-woodland and open habitat, but elderberry was found almost exclusively in hedgerows or partial-woodland habitat (Fig. 4 insets). For all 36 surveyed strawberry fields combined, 42% of edge distance was categorized as open habitat, 20% as residential, 19% as woodland, 11% as hedgerow, and 8% as partial-woodland.

In 2016, trap location based on proximity to elderberry had no effect on captures of total ($F_{1,45} = 1.7$; $P = 0.201$), male ($F_{1,50} = 0.9$; $P = 0.343$), or female *D. suzukii* ($F_{3,45} = 2.2$; $P = 0.147$) or *Z. indianus* ($F_{1,42} = 0.02$; $P = 0.886$) (Table 3). Trap location based on vegetation category affected captures of female ($F_{3,45} = 5.0$; $P = 0.004$) and total *D. suzukii* ($F_{3,45} = 4.6$; $P = 0.007$), but not male *D. suzukii* ($F_{3,50} = 1.8$; $P = 0.161$) or Z. *indianus* ($F_{3,42} = 0.8$; $P = 0.517$). There were more female and total *D. suzukii* captured in traps in woodland than in partial-woodland or hedgerow habitat (Table 3).

In 2017, trap location based on proximity to fruiting hosts had no effect on captures of total ($F_{2,65} = 2.6$; $P = 0.085$), male ($F_{2,65} = 2.5$; $P = 0.089$), or female *D. suzukii* ($F_{2,65} = 2.0$; $P = 0.150$) but affected captures of *Z. indianus* ($F_{2,65} = 3.0$; $P = 0.056$) (Table 3).

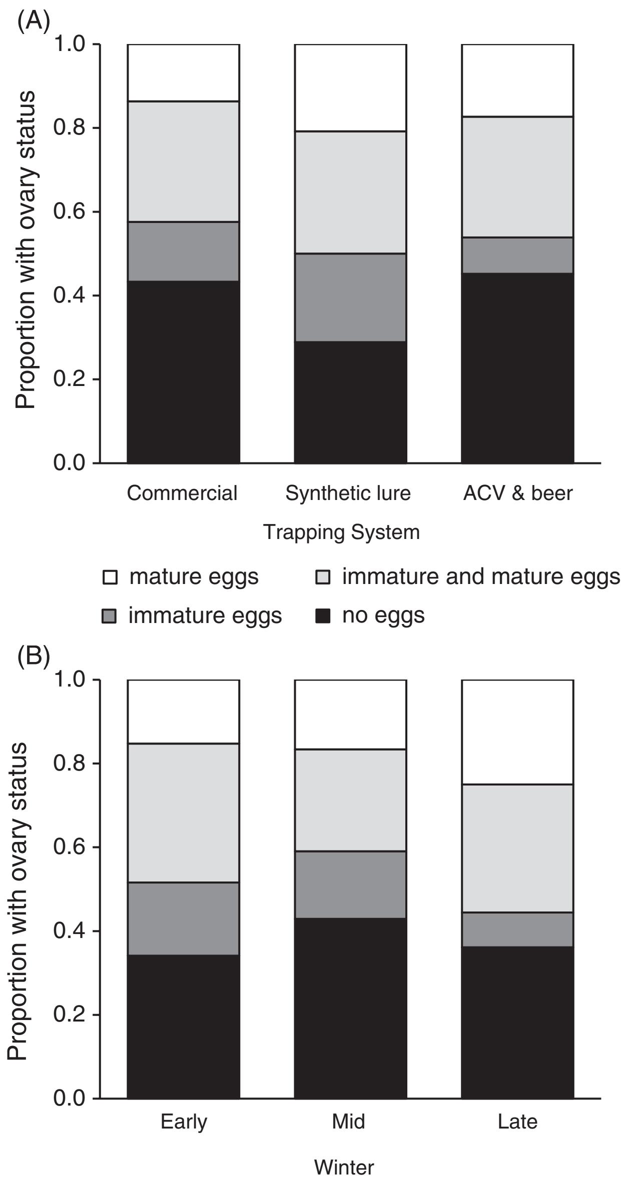

Figure 3. Proportions of female *D. suzukii* with different ovarian maturity status (A) in three trapping systems and (B) during early, mid and late winter periods from 16 December 2015 to 16 March 2016 at five strawberry fields in central Florida (Hillsborough County).

More *Z. indianus* were captured in traps near nightshade than those near neither host. Trap location based on vegetation category affected captures of total ($F_{3,65} = 11.8$; $P < 0.0001$), male ($F_{3,65} = 10.8$; $P < 0.0001$), and female *D. suzukii* ($F_{3,65} = 12.0$; $P < 0.0001$), and *Z. indianus* ($F_{3,65} = 9.8$; $P < 0.0001$). More total, male and female *D. suzukii* were captured in traps placed in woodland or partial-woodland vegetation than those in hedgerows or open field margins (Table 3). More *Z. indianus* were captured in traps placed in woodland than in partial-woodland and hedgerow field margin types (Table 3).

In 2017, American black nightshade consistently had ripe fruit, and *D. suzukii* were reared from nightshade fruits each sampling date (Table 4). Alternatively, elderberries did not consistently have ripe fruit, and *D. suzukii* were only reared from elderberries collected in March; however, numbers of *D. suzukii* per elderberry exceeded numbers per American black nightshade fruit (Table 4).

4 DISCUSSION

This study showed that Florida strawberries, produced primarily during late fall and winter, are continually at risk of infestation by *D. suzukii*. Flies were captured every week in central Florida fields, from the first peak harvest in mid/late December to the second peak harvest from late February to early March. In addition, temperatures are not low enough nor day length short enough to induce widespread winter morphotype coloration or reduced oviposition status in females. However, winter morphotypes were more common in *D. suzukii* populations in northern Florida where winter temperatures are cooler. Even though *D. suzukii* are continually present throughout the Florida winter, maximum captures were approximately 1.5 flies per trap per day, which are low compared with peak *D. suzukii* captures in other susceptible crops during late summer in temperate regions; for example, North Carolina, 93 flies; Ontario, 31 flies; Oregon, 55 flies per trap per day.[17,21,22] Captures varied by trapping system but also by week, corroborating previous results that widely variable winter temperatures over a few days (e.g. minimum 0.2 °C on 24 January 2016 to maximum 25.5 °C on 26 January 2016) may affect *D. suzukii* populations.[47] We found that adult *D. suzukii* emerged from just two fruiting plant species collected along strawberry field edges, suggesting that low availability of suitable non-crop hosts may also be an important limiting factor for *D. suzukii* populations during Florida winters.

In Florida strawberry, as in other crops and regions and/or states, commercially formulated *D. suzukii* attractants placed in visually attractive (red) traps outperformed apple cider vinegar (and beer) in simpler, clear traps.[21,22] Even though few data are available on relating trap counts to total *D. suzukii* populations in a field or infestation rates in fruit,[21] trapping systems and new formulations of attractants that consistently have greater *D. suzukii* captures and fewer non-targets should be used in future research focused on defining an action threshold using trap count data.[25] In previous comparative studies, the synthetic lure has generally been the most effective commercial attractant tested.[22,48] In this study, the synthetic lure and commercial systems were comparable, except that the commercial system caught more female *D. suzukii* and only 10% of the Nitidulidae, the primary non-target taxa, compared with the synthetic lure system. As trap physical features affect *D. suzukii* and non-target captures,[7,17,20] various commercial, synthetic lures will need to be further compared in the same trap types, and combinations of liquid and pouch-based attractants should also be evaluated, as there may be synergistic effects of combining volatiles/products on *D. suzukii* captures.[22]

Assessing not only numbers of captured *D. suzukii* but also their morphological and physiological characteristics is valuable for determining their pest potential. Morphologically, *D. suzukii* populations in central and northern Florida were distinct. Most females and about a third of males from northern Florida had winter morph coloration, whereas <10% of the population in central Florida were winter morphs. Darker pigmentation can be induced by rearing *D. suzukii* at 10 °C and enables greater survival of flies at 1 °C.[31] The average minimum temperature during the early winter period was <10 °C in northern Florida and > 10 °C in central Florida, and there were seven more days where the minimum temperature fell below 10 °C in northern compared with central Florida. While the exact accumulation of cool temperatures to induce winter morphotypes has not been determined (although longer wings, another measure of winter preparedness, could be induced at 20 °C and with a 12 h photoperiod[31]), slight differences in temperatures between northern and central Florida seem to be a tipping

Table 2. Numbers of fruit samples collected from plants on perimeters of 36 strawberry fields in central Florida (Hillsborough County) from 18 January to 20 March 2016, and the numbers of *D. suzukii*, *Z. indianus* and other Drosophilidae flies reared from fruit

Family Scientific name	Common name	No. samples with varying numbers of fruits[a] < 25	25–100	101–1000	> 1000	Total	No. fields	*D. suzukii*	*Z. indianus*	Other Dros. reared
Adoxaceae										
Sambucus nigra	Elderberry	4	6	2	0	12	6	9	0	0
Anacardiaceae										
Schinus terebinthifolius	Brazilian pepper	0	0	6	23	29	7	0	0	0
Apocynaceae										
Morrenia odorata	Milkweed vine	3	0	0	0	3	2	0	0	0
Aquifoliaceae										
Ilex cassine	Dahoon holly	3	1	0	0	4	3	0	0	0
Arecaceae										
Syagrus romanzoffiana	Queen palm	0	1	2	0	3	3	0	0	0
Asparagaceae										
Asparagus densiflorus	Asparagus fern	0	2	0	0	2	1	0	0	0
Caricaceae										
Carica papaya	Papaya	1	0	0	0	1	1	0	0	0
Cucurbitaceae										
Momordica charantia	Balsam apple	44	6	0	0	50	19	0	0	0
Lamiaceae										
Callicarpa americana	American beauty berry	3	8	5	0	16	6	0	0	0
Clerodendrum indicum	Tubeflower	1	1	0	0	2	1	0	0	0
Meliaceae										
Melia azedarach	China berry	0	0	1	0	1	1	0	0	0
Petiveriaceae										
Rivina humilis	Rouge plant	2	2	0	0	4	2	0	0	0
Primulaceae										
Ardisia crenata	Coral ardisia	5	13	0	1	19	5	0	0	0
Roseaceae										
Eriobotrya japonica	Loquat	0	6	1	0	7	2	0	0	0
Prunus caroliniana	Carolina laurelcherry	1	0	3	0	4	3	0	0	0
Rutaceae										
Citrus limon	Lemon	1	1	2	0	4	1	0	0	0
Citrus reticulata	Clementine	2	8	2	0	12	2	0	36	143
Citrus spp.	Wild orange	2	2	2	1	7	4	0	0	0
Citrus x sinensis, Citrus x aurantium	Cultivated orange	9	4	0	3	16	4	0	0	60
Citrus x paradisi	Grapefruit	2	0	0	0	2	2	0	0	0
Smilacaceae										
Smilax spp.	Greenbrier	1	3	1	0	5	3	0	0	0
Solanaceae										
Physalis angulata	Cutleaf groundcherry	2	5	0	0	7	2	0	0	0
Solanum americanum	American black nightshade	41	66	8	0	115	21	8	0	101
Solanum diphyllum	Twoleaf nightshade	1	0	0	0	1	1	0	0	0
Solanum viarum	Tropical soda apple	5	0	0	0	5	3	0	0	0
Unknown										
Unknown tree		1	0	0	0	1	1	0	0	0
Verbenaceae										
Lantana camara	Lantana	31	27	0	0	63	12	0	0	0
Vitaceae										
Vitis spp.	Wild grape	0	0	4	5	9	5	0	0	0
Totals		165	162	44	33	404		17	36	304

[a] Number of samples = number of plants with each fruit sample size category × number of sampling weeks.

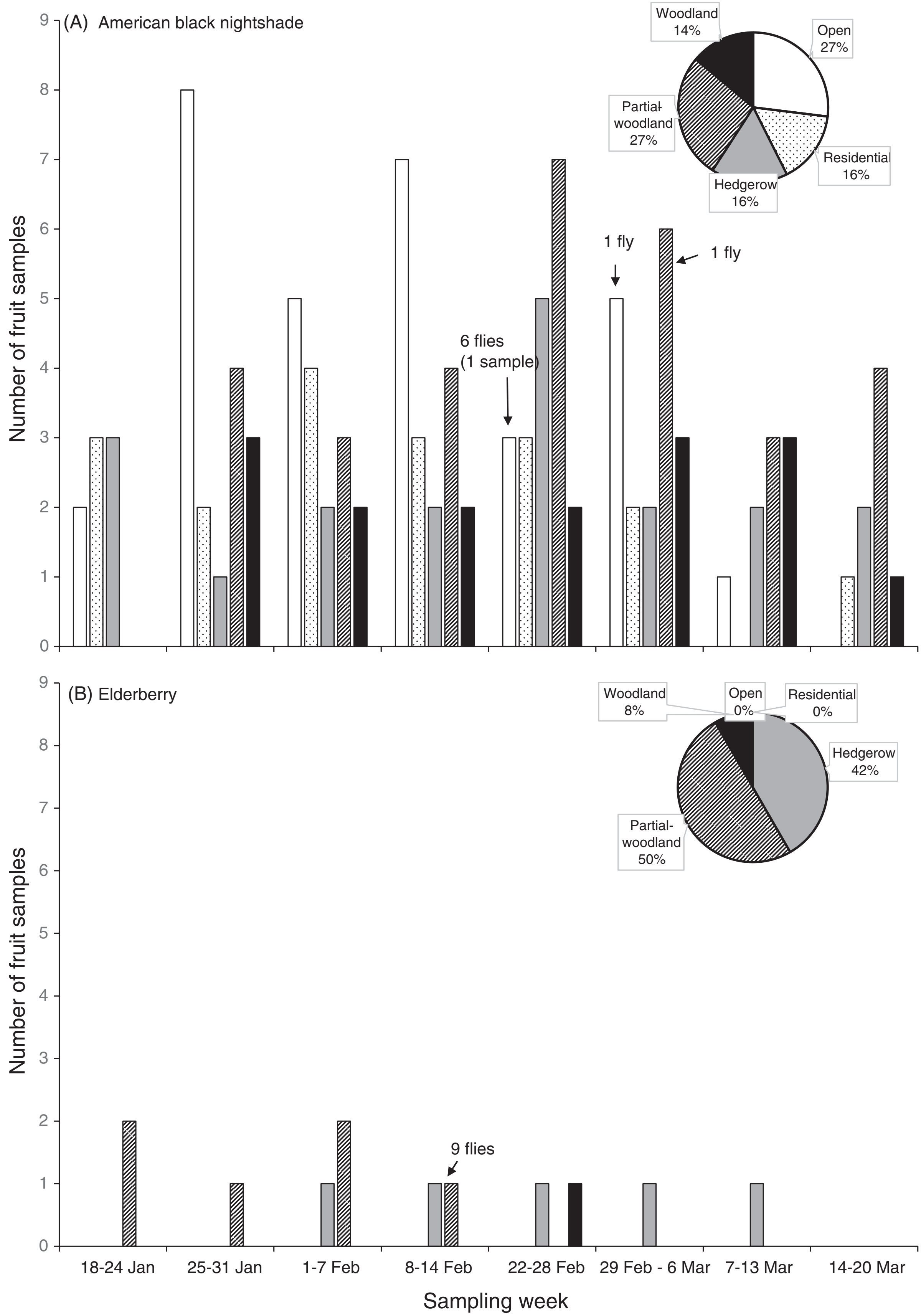

Figure 4. Weekly numbers of (A) American black nightshade and (B) elderberry fruit samples collected from 18 January to 20 March 2016 from perimeters of 36 strawberry fields in central Florida (Hillsborough County). Inset graphs show the proportion of fruit samples collected from five field perimeter vegetation-type categories. Arrows indicate from which samples *D. suzukii* flies were reared.

Table 3. Least square mean (95% confidence interval) numbers of *D. suzukii* and *Z. indianus* captured on margins of three strawberry fields in central Florida (Hillsborough County) from 1 February to 9 March 2016 and 2017. Traps baited with synthetic lures were placed near fruiting elderberry, nightshade or neither ('no hosts') in woodland, partial-woodland, hedgerow or open habitat

Year	Habitat	Trap location		*D. suzukii*			*Z. indianus*
				Male	Female	Total	
2016	Fruiting hosts	Near	Elderberry ($n = 5$)	5.9 (2.5-10.7)	4.7 (2.4-7.8)	12.2 (6.3-20.2)	0.3 (0.0-0.8)
			No hosts ($n = 10$)	3.9 (2.3-6.1)	2.9 (1.8-4.3)	8.0 (4.9-11.9)	0.3 (0.1-0.6)
	Vegetation category	In	Woodland ($n = 2$)	9.4 (3.7-17.8)	9.7 (5.1-15.8) a	24.5 (12.6-40.2) a	0.7 (0.1-1.9)
			Partial-woodland ($n = 8$)	2.8 (1.3-4.8)	1.8 (0.9-2.9) b	4.8 (2.6-7.9) b	0.3 (0.1-0.5)
			Hedgerow ($n = 3$)	3.2 (0.8-7.0)	2.1 (0.7-4.2) b	5.6 (1.9-11.2) b	0.2 (0.0-0.5)
			Open ($n = 2$)	5.2 (1.3-11.8)	3.4 (0.8-7.9) ab	9.8 (2.4-22.3) ab	0.2 (0.0-0.8)
2017	Fruiting hosts	Near	Elderberry ($n = 7$)	41.6 (26.0-61.0)	23.3 (15.5-32.7)	65.4 (42.3-93.6)	12.0 (7.3-17.7) ab
			Nightshade ($n = 5$)	80.2 (50.9-116.2)	39.4 (25.9-55.9)	122.1 (79.5-173-7)	19.7 (11.7-29.6) a
			No hosts ($n = 8$)	48.3 (34.9-63.9)	26.2 (19.6-33.6)	75.1 (55.4-97.7)	8.6 (5.6-12.4) b
	Vegetation category	In	Woodland ($n = 5$)	97.6 (71.9-127.1) a	48.4 (36.5-61.9) a	147.1 (110.1-189.4) a	26.0 (18.7-34.5) a
			Partial-woodland ($n = 8$)	87.0 (64.7-112.6) a	46.8 (36.0-59.0) a	135.4 (102.7-172.7) a	11.6 (7.3-17.0) b
			Hedgerow ($n = 5$)	24.2 (12.2-40.1) b	13.4 (7.5-21.1) b	37.8 (20.2-61.0) b	4.5 (1.8-8.4) b
			Open ($n = 2$)	32.4 (11.3-64.2) b	17.4 (7.1-32.5) b	51.1 (19.7-97.4) b	14.6 (6.5-26.1) ab

Proportion is the number of *D. suzukii* out of all Drosophilidae. Means in each column, with fruiting hosts and vegetation category analyzed separately, followed by the same letter are not significantly different (Tukey's HSD test; $\alpha = 0.05$).

Table 4. Fruit abundance and *D. suzukii* infestation rates in five patches of American black nightshade and elderberry sampled from three strawberry field margins in central Florida (Hillsborough County) in 2017

Host plant	Sample date		Plants with ripe fruit (%)	Mean (±SEM) fruits/plant	Total *D. suzukii*	Mean (±SEM) *D. suzukii*/fruit
American black nightshade	February	3	100	58 ± 17	4	0.013 ± 0.008
		8	80	65 ± 14	1	0.010 ± 0.010
		16	100	44 ± 13	6	0.024 ± 0.016
		23	80	57 ± 8	2	0.009 ± 0.005
	March	3	100	39 ± 17	3	0.009 ± 0.005
		9	60	42 ± 4	1	0.007 ± 0.007
Elderberry	February	3	20	100 ± 0	0	0 ± 0
		8	0	-	-	-
		16	20	100 ± 0	0	0 ± 0
		23	0	-	-	-
	March	3	80	90 ± 10	125	0.313 ± 0.229
		9	20	100 ± 0	84	0.710 ± 0.0

point for inducing darker pigmentation in *D. suzukii*. Lower proportions of winter morph male than female *D. suzukii* in northern Florida suggest that males are more susceptible to cold, survive winter at lower rates and, therefore, are a bottleneck in population development.[16,31] We also found that >50% of *D. suzukii* females in central Florida contained mature and immature eggs throughout the winter, whereas in cooler regions (including central California) only 0–20% of winter females contained mature eggs. However, when daily minimum temperatures began to increase in February, a greater proportion of females had mature eggs.[49,50] In addition, we found that winter morphotype females contained slightly fewer mature eggs on average than summer morphotype females. Therefore, reproductive diapause in *D. suzukii* does not appear to occur during a typical central Florida winter, and populations are continually able to infest fruit crops.

From all fruit collected along strawberry field margins, we found that *D. suzukii* emerged only from elderberry and American black nightshade. Fruits of elderberry, *Sambucus* spp.,[10,51,52] and nightshade, *Solanum* spp.,[10,52,53] have previously been reported to be infested with *D. suzukii*, and *D. suzukii* successfully developed in *Sambucus* and *Solanum* spp. fruits.[10] The list of suitable *D. suzukii* non-crop hosts from temperate regions contains dozens of species,[10,52] and, therefore, the risk of fruit crop invasion and infestation from field margins may be far greater than in Florida, where strawberry field margins contain relatively few suitable hosts during winter. However, *D. suzukii* has been found in Florida in relative abundance in orange jasmine fruits, *Murraya paniculata* (L.) Jack (Dean D, 2017, pers. comm.), and wild blackberry,[54] which are likely important, in addition to other fruiting plants,[47] for sustaining *D. suzukii* populations in agro-landscapes during spring, summer and autumn.

Nightshade plants consistently had ripe fruits in the latter half of the strawberry season (February to early March), whereas elderberry fruits were not prevalent until March. However, elderberry may be a better host, as more *D. suzukii* emerged from elderberries than nightshade berries. As nightshade was common (21 of 36 fields), grows only 1–1.5 m tall, and was often found as individual plants or in small groups, it is advisable to remove it from field margins. In contrast, as elderberry is taller (3–15 m), was often found among other dense vegetation, and only contributes to *D. suzukii* populations in the last week or two of the strawberry season, it is likely not practical or necessary to remove it. Nightshade was found in roughly equal proportions in all five field perimeter categories, whereas elderberry was almost exclusively found in partial woodland and hedgerow habitats. Therefore, efforts to identify and remove nightshade should focus on all edges of strawberry fields.

We categorized and quantified the type of vegetation surrounding Florida strawberry fields to recommend optimal trap placement for monitoring *D. suzukii*. Traps placed in wooded perimeters (2016) and wooded and partially wooded perimeters (2017) yielded the most *D. suzukii*, but traps near elderberry and nightshade did not capture more *D. suzukii* than traps near neither plant. In the upper Midwestern USA, greater amounts of woodland in the landscape were predictive of more and earlier *D. suzukii* in berry fields.[30] Wooded perimeters may serve as a refuge for flies during the overwintering period in temperate regions but also daily for flies seeking cooler temperatures and higher relative humidity.[55,56] Just over a quarter of the Florida strawberry field perimeter was woodland or partial-woodland, and we recommend focusing trap placement in these areas.

Zaprionus indianus was regularly captured in trapping systems designed for *D. suzukii* during the Florida winter strawberry season, although considerably fewer *Z. indianus* were captured at northern than at central Florida sites. *Zaprionus indianus* is native to tropical regions and sensitive to cold; consistent temperatures of ≤15 °C caused male sterility.[57–59] Therefore, slightly lower winter temperatures in northern than central Florida may have been sufficient to limit *Z. indianus* populations. Interestingly, *Z. indianus* captures in central Florida were more than twice as high in ACV + beer as in the synthetic lure or commercial trapping systems, particularly for a few weeks in January and early February. Previously, *Z. indianus* captures were improved when wine or ethanol was added to vinegar.[7,41] The combination of acetic acid, ethanol, acetoin and methionol was most attractive to *D. suzukii* (the components of the synthetic lure), and the same four compounds plus isoamyl alcohol and ethyl hexanoate were strongly attractive to *Z. indianus*.[24,25] Isoamyl acetate and hexanoic acids (ethyl hexanoate from the reaction of hexanoic acid and ethanol) are not only found in wine but are also present, usually in lesser amounts, in beer because of yeast fermentation.[60] Isoamyl acetate was antagonistic to *D. suzukii* using electroantennographic detection, and ethyl hexanoate did not elicit a response.[24] Therefore, even though it would be convenient to use a single, highly effective attractant for both drosophilids in places where or during periods when *Z. indianus* poses a significant secondary threat to crops (e.g. guava in Mexico),[61] differences in their behavioral ecology (oviposition in unripe/ripe versus overripe fruit) leading to differences in chemical ecology (orientation to different compound blends) may preclude development of a dual lure.

Drosophila suzukii and *Z. indianus* were captured in Florida strawberries throughout the growing season, but *D. suzukii* did not reach population levels that are commonly encountered in other crops and/or regions. The weather in northern Florida and agro-landscape features in central Florida during winter appear to be important limiting factors. However, other factors, such as soil conditions,[62] agronomic practices (especially short, 2–3 day harvest intervals), and frequent use of insecticides for control of other pests,[63] are also likely contributing to capturing few *D. suzukii*. We recommend that strawberry growers continue to use current knowledge and the best available tools to monitor *D. suzukii* and *Z. indianus*, and that future research address the relative importance of management versus environmental and/or landscape factors in regulating *D. suzukii* populations in Florida strawberries.

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