

Effect of Temperature and Humidity on the Seasonal Phenology of *Drosophila suzukii* (Diptera: Drosophilidae) in Wisconsin

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Abstract

Drosophila suzukii (Matsumura) is an invasive pest of soft-skinned fruits that has caused significant economic damage worldwide. In this study, we focused on the seasonal abundance of *D. suzukii* during the early years of establishment in Wisconsin. We explored the seasonal patterns of summer and winter morphs, their reproductive output, and the effect temperature and humidity may have on their seasonal phenology. The seasonal abundance of *D. suzukii* during 2 yr (2014–2015) revealed that flies were detected in Wisconsin from early July to late December, with winter morphs being trapped from August through December. The adult populations trapped spanned 1 mo longer in 2015 than in 2014. The peak proportional abundance of *D. suzukii* in 2015 was recorded in August which was about 2 mo earlier than that in 2014. The combined factor [maximum temperature and maximum humidity] explained the most amount of variation in *D. suzukii* abundance consistently across the 2 yr in Wisconsin. We did not find significant differences in the fat content, number of mature eggs, proportion of females with immature eggs, or proportion of mated females between summer morph females at the beginning, summer and winter morph females during the middle, or winter morph females at the end of the collecting season in 2015. Our results build on the body of work providing a better understanding of the *D. suzukii*–overwintering abilities and strengthen the importance of early crop risk assessment and targeted control strategies.

Key words: spotted wing drosophila, overwintering, reproductive output, seasonal morph, fat content

Invasive species demonstrate a diverse set of adaptations to novel environments (Lee 2002). The successful establishment of an invasive species in a new introduced range can be explained by multiple factors, such as their escape from native predators (Keane and Crawley 2002), their high competitive ability (Blossey and Notzold 1995), their phylogenetic relatedness to native species (Darwin 1859), the availability of resources in the introduced range (Davis et al. 2000), or the novelty of exotic species' traits (Callaway and Ridenour 2004). In addition, a combination of different factors, such as available resources combined with release from native natural enemies, or several combined biotic factors, might also affect the success of species invasions (Lau and Schultheis 2015). Chill-coma tolerance was reported as the main climatic adaptation in *Drosophila* species (Gibert et al. 2001). Cold tolerance may play an important role in successful colonization of the temperate and northern regions by many invasive insects, including *Drosophila suzukii* (Matsumura) which is the focus of this study.

Drosophila suzukii is an emerging invasive pest of soft-skinned fruit such as cherry, blueberry, strawberry, and raspberry (Lee et al.

2011; Burrack et al. 2012, 2013; Asplen et al. 2015). Native to Asia it was first reported in 1916 in mainland Japan (Kanzawa 1936). Due to its high dispersal capacity (Hauser 2011), *D. suzukii* is apparently able to invade new geographical regions very fast: several decades after the first reports in its native range, *D. suzukii* was detected in Europe, North America, and South America (Asplen et al. 2015, Fraimout et al. 2017). To date, seasonal monitoring of *D. suzukii* has been conducted in several states including California (Hamby et al. 2014, Harris et al. 2014); North Carolina, Georgia (Diepenbrock et al. 2017); Maryland, Pennsylvania (Joshi et al. 2016); Minnesota, Michigan, Wisconsin (Pelton et al. 2016), Washington (Bahder et al. 2016), and Tennessee (Addesso et al. 2015). Previous population monitoring in Wisconsin assessed the effect of surrounding landscape on infestation levels and population dynamics in raspberry (Pelton et al. 2016) and did not address the impact of abiotic factors, such as temperature and humidity, on seasonal population dynamics.

The successful invasion of *D. suzukii* in temperate regions may be associated with its preadaptations to temperate climate prior to

its actual introduction (Ometto et al. 2013). In temperate regions, *D. suzukii* shows remarkable phenotypic plasticity, producing seasonal morphs, with a more cold-tolerant winter morph which is darker and has longer wings than summer morphs (Stephens et al. 2015; Zerulla et al. 2015; Shearer et al. 2016; Jakobs et al. 2015, 2017). *Drosophila suzukii* individuals presumably overwinter as adult winter morphs that undergo a reproductive diapause (Dalton et al. 2011, Jakobs et al. 2015, Stephens et al. 2015, Zerulla et al. 2015, Wallingford et al. 2016). It has been proposed that such diapause can occur due to decreased expression of genes which regulate reproductive activities (Shearer et al. 2016). The ability to overwinter in reproductive diapause may be the main factor affecting invasion success of this species (Rossi-Stacconi et al. 2016). However, it is currently unclear whether *D. suzukii* undergoes a 'true' diapause (Toxopeus et al. 2016, Wallingford et al. 2016, Zhai et al. 2016). Toxopeus et al. (2016) found that *D. suzukii* winter morphs demonstrated delayed reproductive maturity that was mostly regulated by temperature and not photoperiod, whereas Wallingford et al. (2016) showed that photoperiod affected when females began producing offspring. Temperature and photoperiod combined seem to regulate ovary development (Zhai et al. 2016).

Our knowledge about overwintering behavior of *D. suzukii*, and specifically about microhabitats it might use for overwintering in the adult stage is limited (Wiman et al. 2014, Hamby et al. 2016, Pelton et al. 2016). *Drosophila suzukii* adults prefer overwintering habitats comprising leaves and dropped fruit compare with bare soil, leaves alone, or barky sticks (Wallingford et al. 2018) and can reproduce in dropped fruit in the fall, including less preferred hosts, such as apple, peach, and grape, which can provide suitable sources of food for late-season *D. suzukii* (Bal et al. 2017). However, the question of where *D. suzukii* overwinter, whether in man-made or natural shelters, remains to be explored further.

Abiotic factors, and, in particular, temperature and humidity, are among the key factors (along with food availability) which seem to affect *D. suzukii* survival and reproduction (Kacar et al. 2015, Rossi-Stacconi et al. 2016, Tonina et al. 2016, Wiman et al. 2016, Eben et al. 2018). It has been shown that *D. suzukii* adults are chill susceptible, and temperature affects *D. suzukii* survival, abundance, flight, fecundity, development, and reproductive activity (Harris et al. 2014, Kinjo et al. 2014, Tochen et al. 2014, Jakobs et al. 2015, Kacar et al. 2015, Hamby et al. 2016, Haviland et al. 2016, Tonina et al. 2016).

The effect of temperature on *D. suzukii* physiology and behavior has been extensively studied under both laboratory conditions (e.g., Tochen et al. (2014), Jakobs et al. (2015), Wallingford et al. (2016), Zhai et al. (2016), and Eben et al. (2018)) and field conditions (e.g., Jakobs et al. (2015) and Tonina et al. (2016)). Only few studies focused on the relationships between temperature changes and *D. suzukii* seasonal abundance (e.g., Tochen et al. (2014), Haviland et al. (2016), and Ryan et al. (2016)) or on temperature as it relates to the presence of seasonal morphs in *D. suzukii*. Consequently, our ability to predict changes in *D. suzukii* abundance during the season based on temperature data is currently limited and may significantly vary with geographical regions.

The effect of humidity, another potentially important factor affecting the activity of *D. suzukii*, as well as other *Drosophila* species, is also underrepresented in experimental studies (Aggarwal et al. 2013). Recently, under both laboratory and field conditions, Tochen et al. (2015, 2016) showed a strong positive relationship between mean daily humidity and *D. suzukii* population growth. Another study also found that increased ambient levels of relative humidity were correlated with fruit infestation and concluded that higher relative humidity may create ideal microhabitats promoting feeding and oviposition of *D. suzukii* (Diepenbrock and Burrack 2017). However,

studies considering whether temperature and humidity act alone or have a combined effect on *D. suzukii* seasonal abundance are largely lacking, though a recent study showed increased age-dependent mortality of adult *D. suzukii* when exposed to extreme heat and low humidity under laboratory conditions (Eben et al. 2018). Abiotic factors, such as temperature and humidity, are constantly interacting in the environment and determining which characteristics of these factors, i.e., maximum, mean, and minimum, are most influencing *D. suzukii* activities is important. This information would be invaluable for improving management of *D. suzukii* and could help better predict population abundance in a particular season and region.

Another aspect of the *D. suzukii* seasonal activity which might help us to understand how this species overwinter is the females' reproductive status before and after their exposure to winter conditions. Previous studies have suggested a decrease in the number of mature eggs in a female's reproductive tract throughout the season (e.g., Zerulla et al. (2015), Wallingford et al. (2016), and Grassi et al. (2018)) and that ovary size and development are influenced by cooler temperatures (Everman et al. 2018). However, little is known about a female's mating status during the winter season and how it might affect abundance of the subsequent generation. Ryan et al. (2016) found that 38% of *D. suzukii* females mated before a simulated cold exposure were still able to produce viable offspring when the temperature conditions returned to 22°C. This suggests that the sperm stored in the spermathecae during the winter season may remain viable until flies need it to fertilize their eggs once the environmental conditions become favorable for oviposition in the growing season. It is currently unclear, however, whether the proportion of mated females changes during the season, especially with regard to summer versus winter morphs. If females can use the stored sperm from prewinter matings (Ryan et al. 2016), then the information about the proportion of mated winter morphs could be especially helpful to better predict females spring fecundity and risk to crops.

Here we report the results of a 2-yr monitoring study of *D. suzukii* population dynamics in Wisconsin. Our first objective was to evaluate the seasonal phenology of *D. suzukii* in Wisconsin, with three subobjectives: 1) determine whether winter morphs are present in the Midwest; 2) describe when in the growing season the winter and summer morphs are present; and 3) determine changes in the reproductive status of winter and summer morph females throughout the season. Our second objective was to estimate the effect of 1) temperature, 2) humidity, and 3) a combined effect of temperature and humidity on *D. suzukii* seasonal abundance. Here, in addition to the effect of temperature and humidity on *D. suzukii* males and females, we also investigated the difference between winter and summer morphs.

Materials and Methods

Study Sites

Drosophila suzukii adults were monitored in raspberry crops in south-central Wisconsin on a weekly basis over a 24-wk period, from late spring into the winter of 2014 and 2015. Adults were monitored at three farms in Dane and Iowa Counties, WI in 2014 and at six farms in Dane, Iowa, and Rock Counties, WI in 2015. Each farm had three yeast-sugar traps in 2014 and two yeast-sugar traps set in 2015 placed in the fruiting zone of plants. Four of the farms did not apply any insecticide during the growing season. The other two farms applied either three or four applications of insecticides, including Malathion, Brigade, Assail, Delegate, or Mustang Maxx during the entire season. The trapping containers were 950-ml clear plastic cups and lids (Webstaurant Store, Lancaster, PA) with ten

5-mm entrance holes drilled around the top of the cup. Each trap was hung in the shade on the trellis or raspberry cane in the fruiting zone, about 1 m from the ground. The bait for each trap was made of 3.5 g of dry active baker's yeast (Red Star, Milwaukee, WI), 14 g of white cane sugar, and approximately 150 ml of water, and a drop of unscented dish soap (Seventh Generation, Burlington, VT). The minimum distance between any two traps was 30 m. Each week, samples from each trap were collected and the bait was replaced. Insects were stored in 70% ethanol until further identification in the laboratory. Trapping began mid-May before fruit set in both years and was terminated in late fall, following two consecutive weeks of no *D. suzukii* adults caught in traps.

Data Collection

Each week, trap contents in ethanol were returned to the laboratory and *D. suzukii* were sorted by sex. Following Pelton et al. (2016), samples with more than 200 *D. suzukii* adults (in 2014) and more than 100 *D. suzukii* adults (in 2015) were subsampled, using a 4 × 6 gridded tray and counting 20% of the tray cells (5 of 24 cells), and then calculating the sample total. Males and females were counted and further separated into seasonal morphs, as described below.

Seasonal Morphology

Adult flies caught in monitoring traps were sorted by pigmentation to quantify seasonal morphs. All adults were sorted by male winter morph, female winter morph, male summer morph, and female summer morph using pigmentation characteristics described in Shearer et al. (2016) for each week of the sampling period for each year. Samples containing more than 250 flies were subsampled to 250 *D. suzukii* flies to determine the seasonal morphs and the ratios of winter and summer morphs for both males and females were determined for all weekly samples in both years.

Based on Shearer et al. (2016), *D. suzukii* seasonal morphs were defined in this study as follows: 1) summer morph male characterized by a continuous dark band at the lower edge of the third abdominal segment; 2) summer morph female characterized by a continuous dark band at the lower edge of the fourth abdominal segment; 3) winter morph male characterized by the third abdominal segment completely melanized (filled in with dark pigmentation); 4) winter morph female characterized by the fourth abdominal segment completely melanized; and 5) unknown characterized by any specimen that did not clearly fall into one morph category for various reasons (e.g., damaged, bleached out, bloated, or unclear). This last category (5) was not included in the analysis.

To estimate changes in the proportional abundance of *D. suzukii* adults during the season, for each year, we calculated the mean weekly proportional abundance as [the total weekly proportional abundance for all farms/the number of farms]. Following Inamine et al. (2016), the total weekly proportional abundance for each farm was calculated as [the population index at each week/the total index for that year across all weeks]; and the total index for a year was calculated as [the total number of collected *D. suzukii* flies/the number of traps * the number of farms for that year]. The population index at each week was determined as [the number of collected *D. suzukii* flies per week/the number of traps * the number of farms] following Pelton et al. (2016).

Temperature and Humidity Effect

Both temperature and humidity data were retrieved from Weather Underground (www.wunderground.com; Dane County Regional-Truax, WI) for 2014 and 2015. The weather station was located

18–55 km from the test sites. These data were retrieved for each collection date during the trapping season (5/20/14–11/18/14 and 5/20/15–1/13/16). For both factors, the following data were used: 1) daily average; 2) daily minimum; and 3) daily maximum. For these analyses, the retrieved temperature and humidity data, as well as the calculated mean weekly proportional abundance of *D. suzukii* adults, were averaged for each month.

Female Reproductive Output

To further explore the seasonal phenology of *D. suzukii*, in 2015, the female reproductive output potential was additionally estimated. Ten *D. suzukii* females of both summer and winter morphs were dissected at each of three time points during the season: 1) early in the season (weeks 1 and 2; summer morphs only); 2) population peak (week 13, both summer and winter morphs); and 3) at the end of the season (week 20; winter morphs only).

For each dissected female, we recorded the fat content index (Jaffe et al. 2018), the presence or absence of immature eggs, the number of mature eggs, and their mating status (Avanesyan et al. 2017). The fat content of each female was rated as low, medium, or high. To compare the female reproductive potential between weeks, we used the following measurements: 1) the fat content mean index as [the sum of individual indexes/the number of flies for each time point]; 2) the mean number of mature eggs (\pm SE); 3) the proportion of females with immature eggs; and 4) the proportion of mated females.

Statistical Analysis

All analyses were conducted in R (R v.3.2.2). To compare the proportional abundance of males and females in both 2014 and 2015, as well as between years, a Kruskal–Wallis test was used, as the data did not meet the assumptions of normality and homogeneity of variances.

To estimate the effect of temperature and humidity, alone and as combined factors, on *D. suzukii* proportional abundance, we compared the following predictor variables: 1) minimum temperature (Min Temp); 2) mean temperature (Mean Temp); 3) maximum temperature (Max Temp); 4) minimum humidity (Min Humid); 5) mean humidity (Mean Humid); 6) maximum humidity (Max Humid); 7) a combined [Min Temp and Min Humid]; 8) a combined [Min Temp and Mean Humid]; 9) a combined [Min Temp and Max Humid]; 10) a combined [Mean Temp and Min Humid]; 11) a combined [Mean Temp and Mean Humid]; 12) a combined [Mean Temp and Max Humid]; 13) a combined [Max Temp and Min Humid]; 14) a combined [Max Temp and Mean Humid]; and 15) a combined [Max Temp and Max Humid].

The predictor variables were compared using regression in R (R v.3.2.2, package *lme4*). To achieve linearity, all data for *D. suzukii* proportional abundance (except the proportional abundance of male summer morphs in 2014) were square root transformed. For each *D. suzukii* morph (of both males and females) in each year, the regression model with the lowest Akaike Information Criterion (AIC) was considered to be the best model; consequently, it was concluded that the predictor variable used in such model was the best predictor variable for a particular *D. suzukii* morph.

Correlation between each single factor (Min Temp, Mean Temp, Max Temp, Min Humid, Mean Humid, and Max Humid) and the proportional abundance of *D. suzukii* males and females for both summer and winter morphs was also estimated.

To compare the reproductive output of females at different time points, two-way ANOVA with post hoc Tukey's HSD test (to

compare fat content and number of mature eggs), as well as chi-squared test (to compare proportions of females with immature eggs and proportion of mated females) were used.

Results

Population Monitoring

In both 2014 and 2015, *D. sukuzii* adults were present in all traps at all farms, from July to at least November (Fig. 1A and B). *Drosophila sukuzii* summer morphs were present almost every week until the last week of the collecting season and winter morphs were captured at all farms in both years (Fig. 2).

In 2014, both males and females *D. sukuzii* were first detected on July 14 (Figs. 2 and 3) and were all summer morphs. The first winter morph females ($n = 4$) were detected on July 21, whereas the first winter morph males were recorded in mid-August ($n = 2$ and $n = 5$ in 2014 and 2015, respectively). We did not find a significant difference between the proportional abundance of males and females, with seasonal morphs combined ($\chi^2(1, N = 54) = 0.005, P = 0.94$). The average proportional abundance of *D. sukuzii* males (\pm SE) during the collecting season was 0.012 ± 0.002 , whereas the average proportional abundance of *D. sukuzii* females was 0.014 ± 0.003 . The population peak was recorded on September 8 for summer morph males and females. The maximum proportional abundance of *D. sukuzii* winter morphs (for both males and females) was recorded on October 6. The last detection of *D. sukuzii* occurred on November 11, for males and females of both seasonal morphs.

In 2015, the first *D. sukuzii* males and females, all summer morphs, were captured on July 8, 1 wk earlier than in 2014 (Figs. 2 and 3). The first winter morphs were detected on August 12 for males and August 19 for females. We did not find a significant difference between the proportional abundance of males and females, with seasonal morphs combined ($\chi^2(1, N = 69) = 0.213, P = 0.64$). The average proportional abundance of *D. sukuzii* during the collecting season was 0.016 ± 0.004 and 0.022 ± 0.005 for males and females, respectively, which was up to 39% higher than in 2014 (although this difference was not significant; $\chi^2(1, N = 61) = 0.403, P = 0.53$). The population peak for summer morph males was recorded on August 12 and on August 5 for females, which was 1 mo earlier than in 2014; the maximum proportional abundance of summer morphs was approximately four times higher than the average proportional abundance

during the whole season. The maximum proportional abundance of *D. sukuzii* winter morphs, for both males and females, was recorded on October 7, similar to 2014. The last *D. sukuzii* were captured on December 16, for males and females of both seasonal morphs, except a single male summer morph captured on December 30.

Effect of Temperature on SWD Proportional Abundance

Overall, temperature did not have a significant effect on the proportional abundance of *D. sukuzii* males and females in 2014 and 2015 (Table 1; Fig. 3). In 2014, the weekly minimum, mean, and maximum temperature explained none of the variation in the proportional abundance of summer morphs and 21–24% of the variation in the proportional abundance of winter morphs (for both males and females). Minimum temperature explained the most amount of variation in the proportional abundance of female (24%) and male (22%) winter morphs.

In 2015, daily minimum, mean, and maximum temperature explained about 30–41% of the variation in proportional abundance of males, and none of the variation in the proportional abundance of females of either summer or winter morphs (Table 1). Maximum temperature explained the most amount of variation (41%) in the proportional abundance of males of both winter and summer morphs.

Effect of Relative Humidity on SWD Proportional Abundance

In 2014, maximum humidity had a significant effect on the proportional abundance for both male and female summer morphs, explaining 60 and 72% of the variation in the proportional abundance of males and females, respectively (Table 1; Fig. 4). Maximum humidity was also the best predictor for the proportional abundance of *D. sukuzii* winter morphs, though the effect was not significant and explained 18 and 19% of variation in the proportional abundance of winter morph males and females, respectively, in 2014 (Table 1; Fig. 4).

In 2015, humidity did not have a significant effect on the proportional abundance of *D. sukuzii* males and females. Minimum, mean, and maximum humidity explained from 0 to 16% of variation in the proportional abundance of *D. sukuzii* (Table 1; Fig. 4).

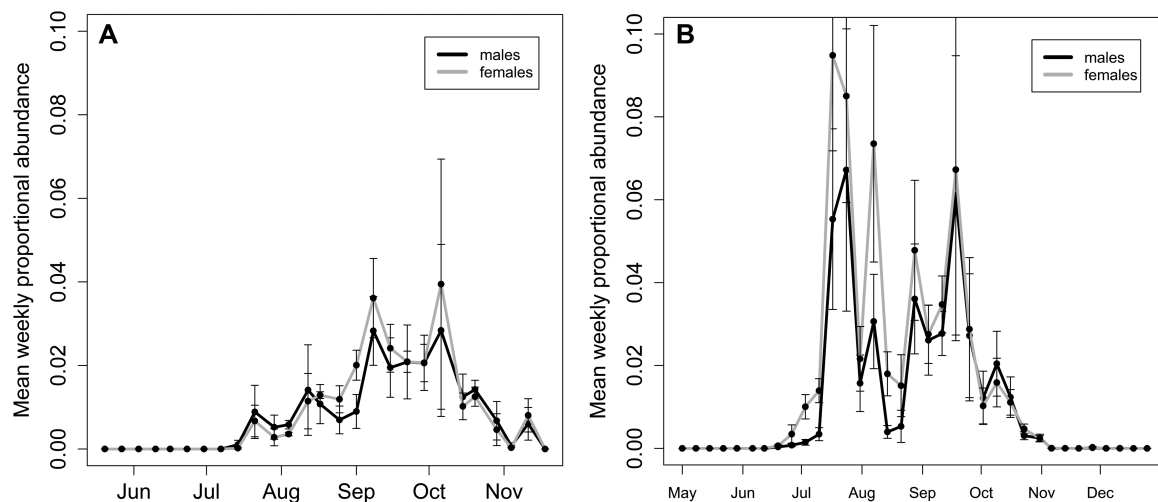


Fig. 1. Mean (\pm SEM) weekly proportional abundance of *D. sukuzii* adults in 2014 (A) and 2015 (B).

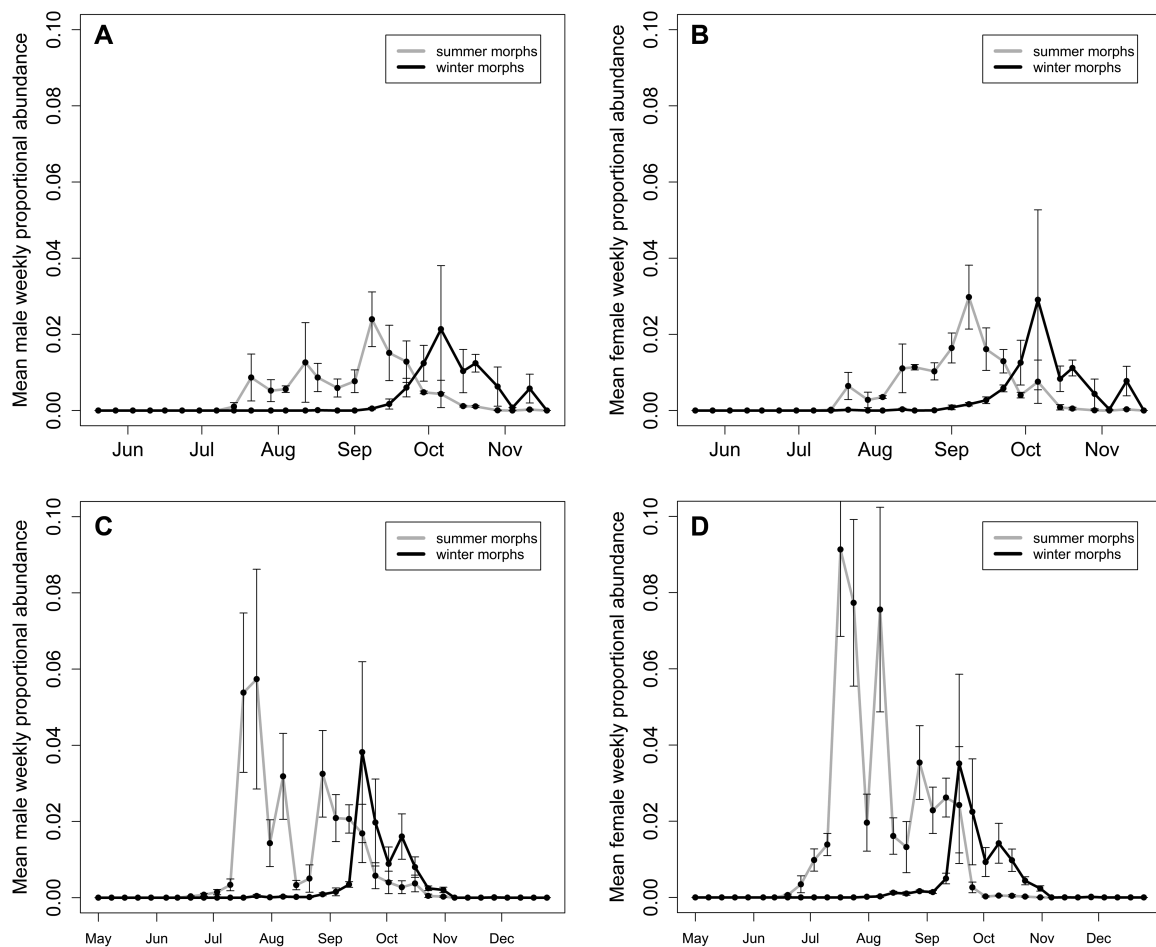


Fig. 2. Mean (\pm SEM) weekly proportional abundance of *D. suzukii* (A) males, (B) females in 2014, and (C) males and (D) females in 2015, for each seasonal morph.

Combined Effects of Temperature and Relative Humidity on SWD Proportional Abundance

The combination of maximum temperature and maximum humidity explained the most amount of variation (up to 66%) in *D. suzukii* proportional abundance in both 2014 and 2015 (Tables 2 and 3). This 'maximum temperature and maximum humidity' combination was the best predictor variable of the proportional abundance of *D. suzukii* winter morphs (both males and females) and *D. suzukii* males (both summer and winter morphs) in both years, when compared with temperature and humidity alone (Table 3).

Overall, when comparing humidity, temperature, and a combined [Temperature and Humidity] factor, maximum humidity was the best predictor variable for the proportional abundance of *D. suzukii* summer morphs and of females (Table 3).

Female Reproductive Status

The reproductive status of females collected the first week of trapping, during peak fly activity, and during the last week of trapping was not significantly different in the fat content index, the number of mature eggs, the proportion of female with immature eggs, and the proportion of mated females, among weeks or between morphs (Table 4).

Discussion

The 2 yr of monitoring *D. suzukii* populations in Southern Wisconsin showed consistent fly activity from July through

November–December across all sites monitored. Overall, our results indicate that the seasonal pattern of *D. suzukii* populations in Wisconsin varies from year to year and differs from seasonal patterns previously reported in other locations, such as California and Northern Spain, where populations tend to develop early in the spring, decrease significantly during the hot summer months, and increase again in the fall (Harris et al. 2014, Wiman et al. 2014, Arno et al. 2016, Wang et al. 2016). In more temperate climates, the first *D. suzukii* flies could be detected as early as May, with the population peak around July, and the decrease of *D. suzukii* seasonal abundance during October–December (Wiman et al. 2014, Hamby et al. 2016, Rossi-Stacconi et al. 2016). Similar to other studies in continental climates (Bahder et al. 2016, Joshi et al. 2016, Leach et al. 2016, Pelton et al. 2017), the *D. suzukii* abundance in Wisconsin showed that in both years, the first flies were detected in early July. This later fly emergence was followed with the population peak in September–October in 2014 and in July–September in 2015, and then a decrease in *D. suzukii* abundance in late October to December.

Drosophila suzukii winter morphs, both males and females, were detected in Wisconsin in both 2014 and 2015. To the best of our knowledge, this is the first report of the presence of winter morph *D. suzukii* in Wisconsin and the Midwest. The presence of winter morphs in Wisconsin may help explain their successful colonization in the Midwest (Shearer et al. 2016). In colder regions, such as the Midwest, evidence of whether *D. suzukii* overwinter or migrate seasonally from warmer regions has been lacking (Asplen et al. 2015, Hamby et al. 2016). However, in a recent study, Thistlewood et al.

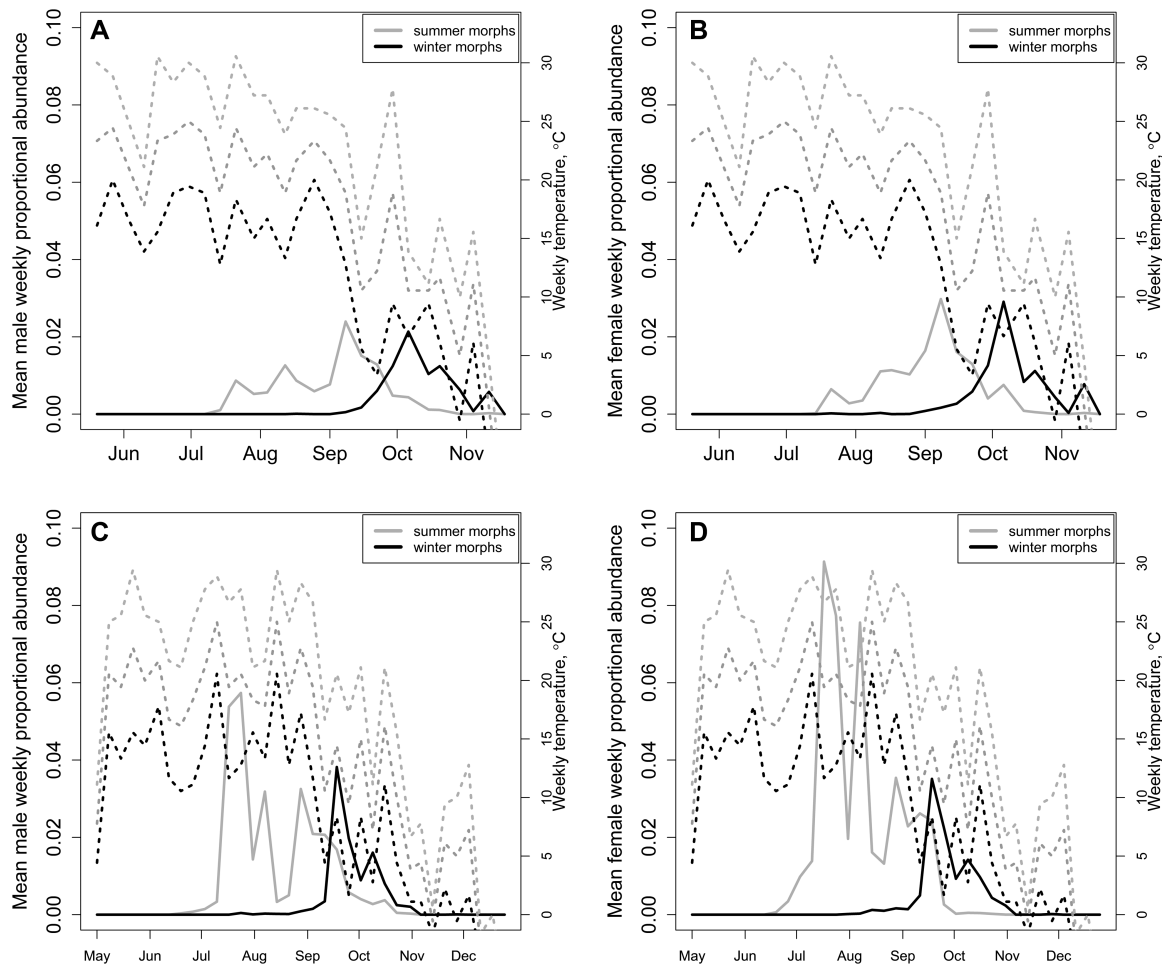


Fig. 3. Weekly temperature and mean weekly proportional abundance of *D. suzukii* (A) males in 2014, (B) females in 2014, (C) males in 2015, and (D) females in 2015 for each seasonal morph. Solid gray lines represent summer morphs, and solid black lines represent winter morphs. Dotted lines are maximum temperature (light gray), mean temperature (dark gray), and minimum temperature (black).

(2018) provided the first evidence of adult overwintering in cold climates and suggest that the patterns they observed do not support the hypothesis of reinfestation via passive dispersal or movement of fruit imports. Although our results support previous work showing that *D. suzukii* winter morphs are able to overwinter in northern climates (Thistlewood et al. 2018), we are yet to catch adults from January to June in Wisconsin (Christelle Guedot, unpublished data). Understanding the phenology of the two seasonal morphs is important as attractants vary in their attractiveness based on crop, region, timing in the season, and seasonal morphs (Jaffe et al. 2018, Kirkpatrick et al. 2018), suggesting that management strategies will likely need to be tailored to the specific morph, time of year, hosts, and regions to be most effective.

Temperature has been proposed as one of the primary drivers of *D. suzukii* population abundance (e.g., Wiman et al. (2014)) and was linked to fecundity and survival in previous studies (Dalton et al. 2011, Kinjo et al. 2014, Tochen et al. 2014, Wiman et al. 2014, Ryan et al. 2016). Average daily high temperature was negatively correlated with numbers of *D. suzukii* adults in weekly trap captures, with close to no trap captures above 35°C (Haviland et al. 2016). In contrast to some of the previous studies conducted in other regions (reviewed in Hamby et al. (2016)), in this study, none of the temperature levels (minimum, mean, or maximum) experienced in Wisconsin during our study had a significant effect on the number

of males or females captured, for either seasonal morphs. This result is not surprising for summer morphs, considering that summer temperatures in our study did not go above 30°C in either year of our study and 30°C has been suggested as a temperature threshold above which *D. suzukii* populations begin to decline (reviewed in Hamby et al. (2016)). Overall, the minimum and maximum temperature levels explained more variation in the proportional abundance of winter morphs than that of summer morphs and of males than females, suggesting that winter morphs and males may be more sensitive to temperatures than summer morphs.

Relative humidity has also been proposed to affect *D. suzukii* populations (Hamby et al. (2016) and references therein). In this study, maximum humidity had a significant effect on *D. suzukii* summer morph population abundance, but not on winter morph abundance. Overall our results were consistent with previous findings showing higher trap catches associated with higher relative humidity in the field (Tochen et al. 2016). Relative humidity was found to be higher inside the canopy of blackberry bushes where the highest fruit infestation was reported, suggesting within-crop microclimates that may enhance the reproductive potential of *D. suzukii* (Diepenbrock and Burrack 2017). In lab assays, fecundity and longevity were shown to increase with increasing humidity, with the highest reproductive rate and intrinsic rate of population increase recorded at 94% humidity (Tochen et al. 2016). It has been shown with *D. melanogaster*

Table 1. The relationship between two single factors (weekly temperature and weekly humidity alone) and mean weekly proportional abundance of *D. sukuzii* adults

Factor	Year	Sex [†]	Morph	Min [‡]			Mean			Max		
				Pearson's r	R ² -adjusted	AIC	Pearson's r	R ² -adjusted	AIC	Pearson's r	R ² -adjusted	AIC
Temperature	2014	M	Summer	0.15	-0.17	-49.58	0.15	-0.15	-49.71	0.24	-0.13	-49.83
			Winter	-0.59	0.22	-22.14	-0.59	0.21	-22.04	-0.58	0.21	-21.99
		F	Summer	0.08	-0.19	-17.47	0.08	-0.18	-17.53	0.15	-0.17	-17.59
			Winter	-0.60	0.24	-22.18	-0.60	0.23	-22.06	-0.59	0.22	-21.99
	2015	M	Summer	0.52	0.33	-41.96	0.54	0.38	-42.30	0.55	0.41	-42.56
			Winter	0.52	0.30	-48.87	0.55	0.36	-49.28	0.56	0.41	-49.62
		F	Summer	0.02	-0.16	-53.86	0.07	-0.15	-53.87	0.12	-0.13	-53.91
			Winter	-0.03	-0.17	-58.07	0.02	-0.16	-58.03	0.06	-0.16	-58.03
Humidity	2014	M	Summer	0.27	-0.11	-49.94	0.62	0.27	-52.87	0.81	0.59*[§]	-56.97
			Winter	0.26	-0.07	-19.92	0.48	0.17	-21.66	0.52	0.18	-21.72
		F	Summer	0.33	-0.06	-18.31	0.68	0.41	-22.40	0.84	0.72*[§]	-27.50
			Winter	0.27	-0.07	-19.81	0.49	0.17	-21.59	0.53	0.19	-21.73
	2015	M	Summer	-0.26	0.05	-41.12	0.62	-0.11	-39.76	0.21	-0.04	-40.34
			Winter	-0.28	0.05	-48.31	0.48	-0.12	-46.86	0.24	0.01	-47.95
		F	Summer	-0.09	-0.12	-54.02	0.19	-0.12	-54.02	0.54	0.12	-56.17
			Winter	-0.04	-0.14	-58.05	0.23	-0.09	-58.42	0.56	0.16	-60.84

Comparisons of linear models based on monthly data.

[†]Males are denoted as 'M' and females are denoted as 'F'.

[‡]Temperature and humidity factors at their minimum (Min), average (Mean), and maximum (Max).

^{||}Results for the best predictor variable within each sex/morph are in bold.

[§]Significant results at $\alpha = 0.05$ are denoted as '*[§]'.

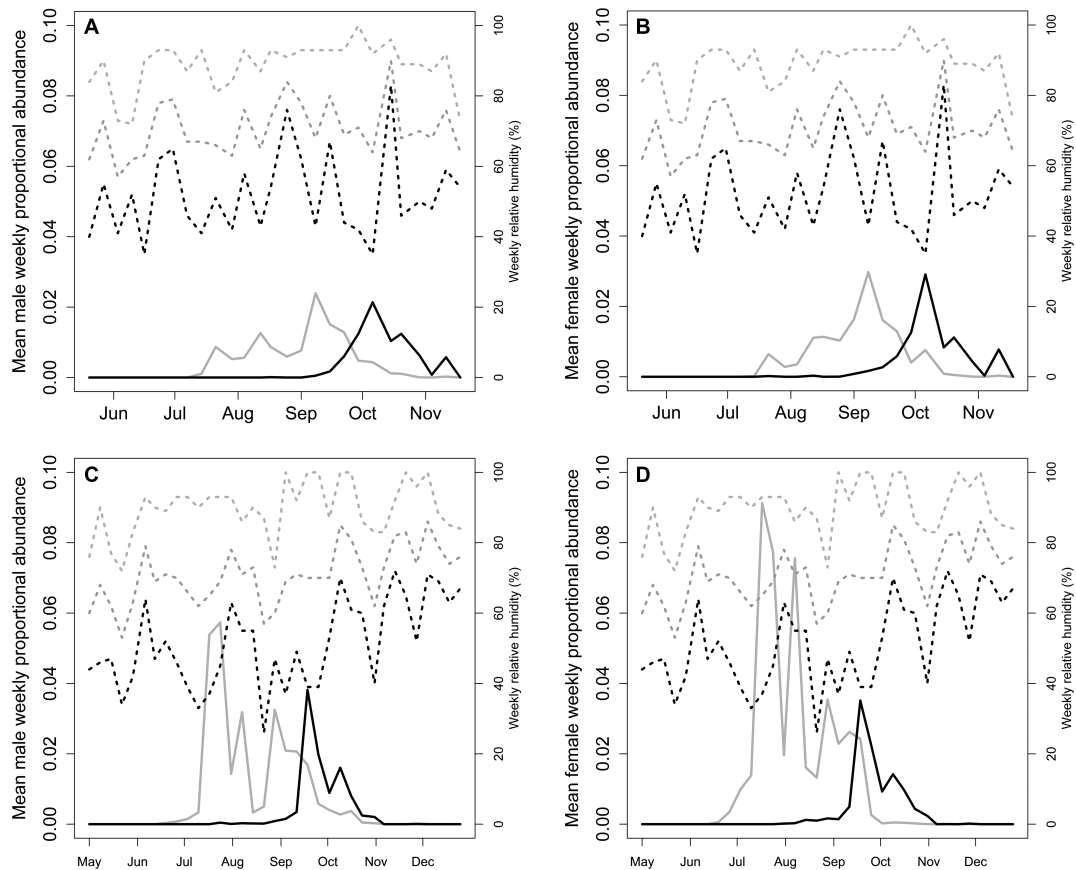


Fig. 4. Weekly humidity and mean weekly proportional abundance of *D. sukuzii* (A) males, (B) females in 2014, and (C) males and (D) females in 2015, for each seasonal morph. Solid gray lines represent summer morphs, and solid black lines represent winter morphs. Dotted lines are maximum humidity (light gray), mean humidity (dark gray), and minimum humidity (black).

Table 2. The relationship between combined factors (weekly temperature and weekly humidity) and mean weekly proportional abundance of *D. suzukii* adults

Year	Sex	Morph	Factors (Temperature / Humidity)	Min Temp [†]		Mean Temp		Max Temp	
				R ² -adjusted	AIC	R ² -adjusted	AIC	R ² -adjusted	AIC
2014	M [‡]	Summer	Min Humid	-0.29	-48.46	-0.23	-48.79	-0.17	-49.17
			Average Humid	0.20	-51.79	0.25	-52.24	0.30	-52.75
			Max Humid	0.50	-55.09	0.51	-55.19	0.51	-55.31
		Winter	Min Humid	0.06	-20.33	0.04	-20.19	0.02	-20.09
			Average Humid	0.33	-22.72	0.31	-22.57	0.30	-22.41
			Max Humid	0.59	-26.24	0.02	26.64	0.63	-26.93
	F	Summer	Min Humid	-0.25	-16.71	-0.21	-16.95	-0.16	-17.22
			Average Humid	0.34	-21.18	0.37	-21.53	0.41	-21.93
			Max Humid	0.64	-25.50	0.65	-25.51	0.65	-25.53
		Winter	Min Humid	0.07	-20.35	0.05	-20.19	0.03	-20.08
			Average Humid	0.35	-22.82	0.33	-22.65	0.31	-22.46
			Max Humid	0.63	-26.80	0.65	-27.21	0.66	-27.48
2015	M	Summer	Min Humid	-0.01	-40.00	0.05	-40.53	0.11	-41.07
			Average Humid	0.09	-40.87	0.15	-41.52	0.20	-42.02
			Max Humid	0.15	-41.49	0.18	-41.81	0.20	-42.06
		Winter	Min Humid	-0.04	-46.87	0.02	-47.39	0.08	-48.00
			Average Humid	0.07	-47.85	0.15	-48.66	0.21	-49.37
			Max Humid	0.17	-48.97	0.21	-49.40	0.24	-49.77
	F	Summer	Min Humid	-0.22	-52.64	-0.27	-52.33	-0.30	-52.11
			Average Humid	-0.29	-52.18	-0.26	-52.37	-0.23	-52.63
			Max Humid	-0.03	-54.18	-0.03	-54.22	-0.02	-54.28
		Winter	Min Humid	-0.25	-56.62	-0.29	-56.32	-0.32	-56.12
			Average Humid	-0.26	-56.57	-0.23	-56.76	-0.20	-57.00
			Max Humid	0.03	-58.85	0.03	-58.84	0.03	-58.86

Comparisons of linear models based on monthly data.

[†]Temperature and humidity factors are used at their minimum (Min Temp, Min Humid), average (Mean Temp, Mean Humid), and maximum (Max Temp, Max Humid).

Results for the best predictor variable within each sex/morph are in bold.

[‡]Males are denoted as 'M' and females are denoted as 'F'.

Table 3. Best predictor variables based on lowest AICs for proportional abundance of *D. suzukii* males and females of each seasonal morph for 2014 and 2015

Year	Sex	Morph	Predictor variables		
			Temperature	Humidity	Combined 'Temperature + Humidity'
2014	Males	Summer	-	Max Humidity	-
		Winter	-	-	'Max Temperature + Max Humidity'
	Females	Summer	-	Max Humidity	-
		Winter	-	-	'Max Temperature + Max Humidity'
2015	Males	Summer	-	-	'Max Temperature + Max Humidity'
		Winter	-	-	'Max Temperature + Max Humidity'
	Females	Summer	-	Max Humidity	-
		Winter	-	Max Humidity	-

that humidity might potentially affect the successful establishment of *Drosophila* populations in temperate regions (Aggarwal et al. 2013). Acclimation to low-humidity conditions seemed to facilitate cold tolerance in *D. melanogaster* (Aggarwal et al. 2013) and further work should address the impact of relative humidity on cold tolerance and how this may explain the establishment of *D. suzukii* in colder regions.

Recent studies have suggested that the combined effect of abiotic factors could help better understand population dynamics (Wiman et al. 2016, Tochen et al. 2016, Eben et al. 2018). In this study, the combined effect of maximum temperature and maximum humidity turned out to be the best predictor for *D. suzukii*

proportional abundance, consistently across both years. Under controlled conditions, extreme high temperature and low humidity were shown to decrease *D. suzukii* adult survival in the laboratory (Eben et al. 2018). A combined effect of temperature and humidity on organism survival and fecundity has been shown for other insect species, such as the Banks grass mite *Oligonychus pratensis* (Banks) (Perring et al. 1984) and the mosquito *Aedes aegypti* L. (Costa et al. 2010). To the best of our knowledge, this is the first study addressing the combined effect of these two abiotic factors on *D. suzukii* seasonal population abundance in the field, and our results provide a better understanding of the seasonal dynamics of *D. suzukii* in the Midwest.

Table 4. Reproductive output of *D. suzukii* females collected at three time points in 2015: week 1 (first trap catches), week 13 (population peak), and week 20 (last trap catches)

Weeks/morphs	Fat content (mean index)	Number of mature eggs (mean ± SE)	Proportion of females with immature eggs	Proportion of mated females
Week 1 (first trap catches)	1.9 ± 0.07	1.9 ± 0.4	0.8	1
Week 13 (Population peak)	1.8 ± 0.09	1 ± 0.6	0.7	0.9
Week 20 (last trap catches)	2.4 ± 0.07	0.4 ± 0.27	0.2	0.6
Data comparisons	1.8 ± 0.09	0.7 ± 0.37	0.6	0.9
Weeks	$F(2,35) = 0.33, P = 0.72$	$F(2,35) = 2.87, P = 0.07$	$X^2(2, N = 39) = 3.661, P = 0.16$	$X^2(2, N = 39) = 0.19, P = 0.18$
Morphs	$F(1,35) = 2.89, P = 0.10$	$F(1,35) = 1, P = 0.32$	$X^2(1, N = 39) = 3.104, P = 0.08$	$X^2(1, N = 39) = 1.96, P = 0.16$

It has been suggested that the reproductive status of *D. suzukii* females changes during the season (Mitsui et al. 2010, Zerulla et al. 2015, Arno et al. 2016, Wallingford et al. 2016, Grassi et al. 2018). The number of mature eggs in *D. suzukii* females trapped in the field has been shown to decrease over time, suggesting that *D. suzukii* undergoes a reproductive diapause (Arno et al. 2016, Wallingford et al. 2016, Grassi et al. 2018). In Wisconsin, we did not observe seasonal changes in the number of mature eggs or the presence of immature eggs in trapped *D. suzukii* females, regardless of morphotype. In recent studies, populations of *D. suzukii* were found with mature eggs in late fall and winter (Zerulla et al. 2015, Kaçar et al. 2015, Grassi et al. 2018), supporting our results and suggesting that overwintering winter morph females, at least in Wisconsin, tend to be reproductively mature before going into diapause. This finding should be further investigated with other populations in the Midwest to confirm the reproductive status of winter morph females.

In this study, the majority of the females collected were mated, regardless of the time of the season and morphotype, supporting recent findings suggesting that *D. suzukii* females are able to store viable sperm through the winter (Ryan et al. 2016, Rossi-Stacconi et al. 2016, Grassi et al. 2018). The mating status before winter exposure affects spring fecundity, as females mated before winter were able to lay viable eggs after overwintering (Arno et al. 2016, Ryan et al. 2016). The reproductive status of *D. suzukii* females was recently shown to affect the females' response to baits and lures and to vary with the time in the season and the developmental stage of fruit (Jaffe et al. 2018, Wong et al. 2018). Although females with mature eggs seem to be more attracted to fermenting baits (such as the bait used in our study) than females without mature eggs, the mating status, reproductive status, and seasonal morph status did not affect their attraction to fermenting baits (Wong et al. 2018). Such findings have important implications for management decisions the following spring and should focus on control strategies that target mated overwintering females seeking feeding and oviposition sites.

Here, *D. suzukii* females had similar fat content throughout the season, suggesting that females are able to feed all the way into December. In a recent study, *D. suzukii* were found to emerge and reproduce on dropped fruit in the Fall in Michigan (Bal et al. 2017), providing reproduction hosts for winter morph populations later in the season. In addition, dropped fruit wastes could be utilized by *D. suzukii* adults as a source of food. In fact, *D. suzukii* females were shown to feed on decaying or damaged fruit and females feeding on these were in turn able to produce oocytes and increase their longevity (Plantamp et al. 2017).

This study focused on describing the seasonal abundance of *D. suzukii* in Wisconsin, and identified the seasonal phenology of summer and winter morphs, the female reproductive output, and the effect of temperature and humidity on seasonal abundance. Seasonal phenology and abundance varied between years, emphasizing the importance for commercial growers to monitor populations early in susceptible crops and for research efforts to address the correlation between degree days and first occurrence in the Midwest to help better predict population dynamics. This is the first report of *D. suzukii* wintermorph in the Midwest and this finding combined with their reproductive status will help better understand their overwintering ability in this region. Overall, the variation observed in *D. suzukii* abundance was best explained by the combined effect of maximum temperature and maximum humidity and maximum humidity alone, providing a better understanding of the abiotic factors affecting populations in Wisconsin, and emphasizing the importance of management strategies that reduce humidity levels in susceptible crops.

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