

## ***Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry***

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## ORIGINAL CONTRIBUTION

**Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry**S. Tochen<sup>1</sup>, J. M. Woltz<sup>1</sup>, D. T. Dalton<sup>1</sup>, J. C. Lee<sup>2</sup>, N. G. Wiman<sup>1</sup> & V. M. Walton<sup>1</sup><sup>1</sup> Department of Horticulture, Oregon State University, Corvallis, OR, USA<sup>2</sup> USDA-ARS Horticultural Crops Research Unit, Corvallis, OR, USA**Keywords**

fecundity, longevity, ovarian maturation, relative humidity, spotted wing drosophila

**Correspondence**

Daniel T. Dalton (corresponding author), Department of Horticulture, Oregon State University, 4017 ALS, Corvallis, OR 97331, USA. E-mail: daniel.dalton@oregonstate.edu

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**Abstract**

Temperature and humidity affect insect physiology, survival, fecundity, reproductive status and behaviour. Complementing previous work investigating the effects of temperature on adult survival and fecundity of the invasive frugivorous pest, *Drosophila suzukii* (Matsumura), this study was conducted to determine the effect of humidity on *D. suzukii* larval development, adult survival, fecundity and reproductive status using blueberry as a host substrate. The five constant humidity levels in laboratory bioassays were 20, 33, 71, 82 and 94% RH at  $20.6 \pm 0.2^\circ\text{C}$ . As RH increased, fecundity and longevity increased. At the higher humidity levels, RH had limited impact on mean generation times ( $T$ ), larval development and eclosion times. The highest net reproductive rate ( $R_o = 68$ ) and highest intrinsic rate of population increase ( $r_m = 0.17$ ) were both recorded at 94% RH. The reproductive status of females, as indicated by the number of mature oocytes per female, was significantly greater at 82 and 94% RH, compared to 71% RH. In addition to the laboratory procedures, we correlated field trap captures over an 81-day summer period to relative humidity (RH) levels in close proximity to those traps. In the field, low ambient humidity levels resulted in decreased trap captures. A humidity-dependent population model predicted lower densities of *D. suzukii* relative to populations at higher humidity. This study supports the hypothesis that cultural practices that minimize lower humidity levels in crops can contribute to the management of *D. suzukii*. Such methods may include open pruning, drip irrigation and field floor management.

**Introduction**

*Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is an invasive pest of soft-skinned fruit in North America, South America and Europe (Lee et al. 2011b; Calabria et al. 2012; Deprá et al. 2014). *D. suzukii* lays eggs in intact, ripe fruit (Walsh et al. 2011), making the fruit unmarketable and necessitating frequent insecticide sprays and other cultural control measures to protect crops (Van Timmeren and Isaacs 2013). Additionally, *D. suzukii* has a wide host range, including raspberries, blackberries, strawberries, blueberries and many other commercially grown fruits (Lee et al. 2011a), endangering

commercial fruit production in affected regions around the world.

Insects can colonize and persist under a wide range of environmental conditions including extremes in temperature and humidity. Patterns of insect abundance and distribution can be limited by plant diversity, water availability and temperature, as insects are prone to heat stress and water loss due to small body size and inherent cost of gas exchange (Heinrich 1993; Addo-Bediako et al. 2001; Chown et al. 2011). To overcome thermal extremes and water loss, insects utilize physiological mechanisms such as heat-shock proteins in drosophilid flies (Goto and Kimura 1998; Hoffmann et al. 2003; Bublly et al. 2013) and

adaptations such as waterproofed cuticles (Bradley 2009). Insects also exhibit behavioural mechanisms such as foraging behaviour to increase consumption of water relative to nutrients (Contreras et al. 2013) and relocating to preferred temperature and humidity conditions (Perttunen and Salmi 1956; Heinrich 1993; Tichy 2003). In laboratory studies, *Drosophila* flies were found to avoid highly saturated environments where relative humidity (RH) exceeded 87% (Perttunen and Salmi 1956), and in a choice test, *Drosophila melanogaster* Meigen adults clearly favoured air conditions of 3% RH over air saturated at 99% RH (Sayeed and Benzer 1996). Because regulation of temperature and water balance is so important for homeostasis, such mechanisms may even divert resources away from other aspects of individual fitness (Chown et al. 2011).

Relative humidity affects diverse physiological parameters in insects. For example, low RH can lead to water loss, with resultant costs to fecundity (Yadav and Sharma 2014). In contrast, excessive RH can result in proliferation of fungal pathogens (Roca and Lazzari 1994). Longevity and fecundity of olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae), increased as RH increased up to 75%, while female *B. oleae* failed to develop mature oocytes at low-humidity levels (Broufas et al. 2009). Similarly, juvenile and adult survival and fecundity of *Apolygus lucorum* (Meyer-Dür) (Heteroptera: Miridae) increased with increasing humidity levels, resulting in increased population growth and outbreaks of this pest after periods of heavy rainfall (Lu and Wu 2011).

An understanding of insect thermal and humidity tolerances will provide direction to refine predictive population models that typically account for temperature effects alone. Temperature and humidity have been shown to strongly influence behaviour and physiology of *D. melanogaster* (Sayeed and Benzer 1996; Aggarwal et al. 2013). Understanding the effects of such factors on development of *D. suzukii* may result in an improved understanding of habitats where this species will most likely be found. This information would be applicable to integrated pest management in situations where managers can alter habitats to reduce pest pressure. In situations where habitat modification is unfeasible, knowledge of specific environmental conditions can be used as a guide to enact timely pest management activities.

Temperature-related life table parameters for *D. suzukii* have recently been determined using blueberries and cherries as hosts (Tochen et al. 2014). However, data have not been published on the effect of humidity on developmental parameters and overall

population growth for this species. The aim of this study was to determine the effect of humidity on larval development, adult longevity, fecundity and status of ovarian maturity for *D. suzukii*. We also correlated microclimatic RH effects with *D. suzukii* trap captures and integrated the life table parameters into a humidity-dependent population model.

## Materials and Methods

### Insect colonies and humidity manipulation

Stock colonies of *D. suzukii* were established and supplemented from wild-caught adults and pupae on Himalaya blackberries, *Rubus armeniacus* Focke, near Corvallis, Oregon. These cultures were maintained in the laboratory at  $22 \pm 2^\circ\text{C}$ , 65% RH and a photoperiod of 16:8 (L:D). Flies in the colony were provided with a water wick and artificial diet (Dalton et al. 2011) that served as both a food source and an oviposition medium.

BugDorm rearing units measuring  $30 \times 30 \times 30$  cm (MegaView Science, Taichung, Taiwan) were modified to create sealed containers (henceforth called RH chambers) in which constant RH levels were maintained. The manufactured screened portions of the rearing units were covered with plastic sheets, and the mesh sleeves were replaced with plastic sleeves and closed with a clamp. Saturated salt solutions were used to maintain different constant RH levels in each RH chamber, using different salt compounds to create specific RH levels (Winston and Bates 1960). Five saturated salt solutions were prepared by boiling 100 ml water and adding 50 grams of salt. Upon cooling, more salt was added to each solution as necessary to saturate the solution. The solutions were subsequently placed in four large petri dishes at the bottom of each RH chamber to allow for maximal surface exposure of the solution. A  $30 \times 30$  cm section of egg crate lighting panel (Home Depot, Atlanta, GA) was placed on top of the four petri dishes to protect flies from encountering the salt solution and to provide a platform for *D. suzukii* rearing containers within each chamber. All replicates for each experimental comparison were housed within the appropriate RH chamber for each treatment.

Temperature and RH were recorded using data loggers (HOBO U-12-012, Onset Computer Corporation, Bourne, MA) affixed to the midpoint of the wall of each chamber. These logger data indicated that the salt solutions resulted in distinct, non-overlapping RH levels (Table 1). The mean temperature

**Table 1** Mean developmental period, longevity, and oocyte production of *Drosophila suzukii* at five constant humidity levels on commercially sourced blueberry

Salt solution	RH Treatment	Measured RH $\pm$ SEM	Days to eclosion $\pm$ SEM	% Females with mature oocytes <sup>1</sup>	Mature oocytes per female $\pm$ SEM <sup>1</sup>	Female longevity (days) $\pm$ SEM	Lifetime egg production $\pm$ SEM	Male longevity (days) $\pm$ SEM
LiCl*H <sub>2</sub> O	20	19.8% $\pm$ 0.3	16.2 $\pm$ 0.3 a (26)	0.0% c (19)	0.0 $\pm$ 2.6 b (19)	1.5 $\pm$ 0.3 b (19)	0.0 $\pm$ 0 c (19)	1.4 $\pm$ 0.6 bc (7)
MgCl <sub>2</sub> *6H <sub>2</sub> O	33	32.5% $\pm$ 0.2	16.7 $\pm$ 0.2 a (45)	4.2% c (24)	0.3 $\pm$ 1.7 b (24)	2.5 $\pm$ 0.7 b (24)	0.5 $\pm$ 0.5 c (24)	0.6 $\pm$ 0.2 c (21)
Mg(NO <sub>3</sub> ) <sub>2</sub> *6H <sub>2</sub> O	71	70.7% $\pm$ 0.2	16.3 $\pm$ 0.3 a (42)	42.9% b (14)	5.1 $\pm$ 2.8 b (14)	20.8 $\pm$ 4.2 a (19)	19.9 $\pm$ 3.5 b (19)	19.0 $\pm$ 4.0 a (23)
NaCl	82	82.2% $\pm$ 0.3	15.2 $\pm$ 0.2 b (32)	95.5% a (22)	13.9 $\pm$ 2.5 a (22)	20.9 $\pm$ 3.3 a (20)	24.8 $\pm$ 3.9 b (20)	15.5 $\pm$ 5.0 ab (14)
KNO <sub>3</sub>	94	93.7% $\pm$ 0.1	16.0 $\pm$ 0.2 a (38)	92.9% a (28)	17.4 $\pm$ 2.3 a (28)	27.5 $\pm$ 3.4 a (21)	84.8 $\pm$ 11.2 a (21)	20.3 $\pm$ 3.8 a (17)

Saturated salt solutions and corresponding relative humidity (RH) of each solution are given in the left-most two columns. Emerging individuals to adulthood for each sex are indicated in the according column. Means within a column followed by different lowercase letters are significantly different. Values in parentheses indicate numbers of insects in statistical analysis.

<sup>1</sup>For the 20 and 33% RH levels, females from the longevity experiment were frozen directly after death and subsequently dissected at 1.5  $\pm$  3.8 and 2.5  $\pm$  2.6 days, respectively, as opposed to 7 days at the 71, 82, and 94% RH levels.

was 20.6  $\pm$  0.2°C in all chambers during the duration of the experiments. Additionally, measurements were taken with a calibrated hygrometer (VWR International, Radnor, PA) every time that a chamber was opened to ensure that the RH remained at the specified level for each treatment. These measurements never showed large deviations from the target RH values, indicating that the salt solutions did not need to be replenished or adjusted during the experiments.

#### Demographic parameters and ovarian maturation

Commercially sourced organic blueberries were exposed to the *D. suzukii* stock colony for 30-minute periods to obtain a total of 500 eggs for experimental purposes. The number of eggs laid in each blueberry was determined by counting egg respiratory filaments under magnification. Single berries infested with 1 to 5 eggs each were individually placed into an egg-rearing container consisting of a 30-ml plastic cup (Solo<sup>®</sup>, Urbana, IL) with a mesh fabric lid. Multiple egg-rearing containers with eggs were then placed into each RH chamber to achieve a total of 100 *D. suzukii* eggs per RH chamber. The egg-rearing containers were monitored every 48 h for adult *D. suzukii* eclosion. Dates were recorded in order to calculate the number of days from oviposition to adult eclosion.

Adult *D. suzukii* that eclosed within egg-rearing containers were transferred daily into separate containers that were maintained within the same RH chambers. The adults were allowed 3 days to reach sexual maturity. Mature individuals were then placed into adult-rearing containers within the RH chambers. The adult-rearing container consisted of a 163-mL plastic cup (Dart Container Corporation, Mason, MI) with two 6.35  $\times$  2.5 cm windows with fabric mesh screens affixed by hot glue (Arrow Fastener Co., LLC, Saddle Brook, NJ) and contained a 1-ml microcentrifuge tube that was filled with water and fitted with a cotton wick. Two different groups of adult-rearing containers were used: one to measure adult longevity and fecundity and one to measure egg maturation within ovaries. Approximately half of all adults emerging within the 71, 82 and 94% RH chambers were assigned to each of the two groups. Due to limited adult emergence and short longevity within the 20 and 33% RH chambers, only one set of adult-rearing containers was established for the low-humidity treatments. For these two treatments, all of the emerging adults were used to measure longevity and fecundity and no adults were reserved for measuring egg maturation.

Longevity and fecundity of adult *D. suzukii* were determined by placing one male and one female into adult-rearing containers together with a single blueberry fruit. Blueberry fruit was replaced every 48 h until females died. Dead males were replaced for the duration of the experiment to allow continuous mating. The number of eggs laid in each blueberry fruit over the 48-h period was determined under magnification. Developmental, reproductive and survival parameters were calculated using the measured mean survival and fecundity values. The mean generation times ( $T$ ) of *D. suzukii* on blueberries were estimated using the equation,  $T = \sum l_x m_x / R_o$  (Price 1997), where  $x$  = age in days,  $l_x$  = the proportion of females surviving on day  $x$  and  $m_x$  = the mean number of eggs produced on day  $x$ . Net reproductive rate was obtained using the equation,  $R_o = \sum l_x m_x$ . The intrinsic rate of population increase ( $r_m$ ) was estimated using the equation,  $r_m = \log_e R_o / T$  (Price 1997). Calculations were produced using Statistica (StatSoft, Inc., Tulsa, OK).

The ovarian maturation of *D. suzukii* females was determined at the highest three RH levels. Egg maturation at 71, 82 and 94% RH was determined by placing approximately half of the eclosed males and females from egg-rearing containers into adult-rearing containers within RH chambers. Artificial diet media and a 1-mL microcentrifuge tube filled with water and a cotton wick were provided to allow for feeding and hydration. Each female *D. suzukii* was dissected 7 days after placement into adult-rearing containers to determine the presence and number of mature eggs. An egg was considered mature if it contained a fully formed chorion on the outer surface (Figure S1) (Klowden 2002). Because of rapid mortality at the low-humidity levels, flies from 20 to 33% RH treatments were frozen after death at  $1.5 \pm 3.8$  and  $2.5 \pm 2.6$  days, respectively, after placement into longevity adult-rearing containers. Thus, flies from 20 to 33% RH were dissected at a younger age than 7 days.

#### Relating humidity to field trap captures

*Drosophila suzukii* populations were monitored along with environmental data in seven locations within a commercial blueberry field near Jefferson, Oregon (89 m elevation) from 1 June to 21 August 2013. Hourly ambient temperature and RH were recorded during the monitoring period using Hygrochron model DS1923-F5 dataloggers (Maxim Integrated, San Jose, CA) that were secured in a shaded interior portion of a blueberry bush canopy ca. 1 m above

ground level. *D. suzukii* populations were monitored using four traps placed near each datalogger. In each row containing a datalogger, one trap was placed ca. 1 m away on each side of the datalogger. An additional trap was placed ca. 2 m away in both rows directly adjacent to the datalogger. Traps were constructed using clear 946-ml plastic cups and lids (Dart Container Corporation, Mason, MI). Fifteen 0.5 cm diameter holes were punched around the perimeter into the upper 5 cm of each cup to allow entry of *D. suzukii* (Lee et al. 2012). Traps were baited with ca. 150 ml of natural apple cider vinegar (H.J. Heinz Company, L.P., Pittsburgh, PA) and 0.25-ml unscented liquid dish soap to break surface tension. For each week of the study, the contents of traps were collected and the apple cider vinegar solution was replaced. The number of male and female *D. suzukii* from each weekly sample was counted under magnification in the laboratory.

#### Population modelling

Population estimates related to humidity were conducted for *D. suzukii* by adapting the population model from Wiman et al. (2014), using R 3.1.2 (R Development Core Team 2014). Survival and fecundity at each tested humidity level were calculated separately for four age groups of flies aged 0 to 50 days and used in the time-varying matrix model. These humidity-related survival and fecundity data were then fitted to linear, quadratic and nonlinear regression models, and the model with the highest coefficient of determination was selected for each age group (Table 3). Regression equations for humidity-dependent survival and fecundity for each age class were incorporated into a  $50 \times 50$  Leslie matrix that was multiplied by a population vector for each iteration of the model. Simulations were used to estimate relative population growth of *D. suzukii* population data from the seven monitored locations for 82 days (Wiman et al. 2014). The population vectors were initiated by arbitrarily placing 100 flies into each adult age class (17–50 days old).

#### Statistical analysis

Laboratory data were statistically analyzed using the R 3.1.2 'Stats' package (R Development Core Team 2014) unless otherwise stated. Days to eclosion, longevity of adult males and females, and the numbers of oocytes per female during the first week of adult life were compared among RH treatments using analysis of variance (ANOVA). Male and female longevity, as



well as mature oocytes per female, were square root transformed to achieve homoscedasticity of error variance. The total mean number of eggs laid per female was compared among RH treatments using ANOVA, with female longevity included as a covariate to account for the effect of lifespan on egg-laying potential. Total eggs laid per female were log-transformed to achieve homoscedasticity of error variance. Significant differences between means were separated using Tukey's HSD test at the 95% confidence level ( $P < 0.05$ ). The percentage of females with mature oocytes was compared among RH treatments using a chi-square test, with a Bonferroni adjustment for the comparisons between individual treatments. Non-parametric Kruskal–Wallis ANOVA rank-sum tests were used to compare adult female survival for each humidity level using Statistica. Nonlinear regression was performed for each RH to estimate the lower and upper levels of the intrinsic rate of population increase ( $r_m$ ) (Briere et al. 1999).

Three correlation analyses were conducted, the first to determine the relationship between RH and seasonal *D. suzukii* trap counts, the second to determine the relationship between temperature and seasonal *D. suzukii* trap counts, and the third to determine the relationship between humidity-related *D. suzukii* population estimates from the population model projections and seasonal *D. suzukii* trap counts. The mean humidity, temperature and seasonal *D. suzukii* trap counts for each of the seven locations were calculated over an 82-day period from 1 June 2013 to 21 August 2013, as this was the period when crop was present within the blueberry field. Total fly captures were combined for the four traps that surrounded each datalogger. The first and second analyses to determine the relationship between season-long *D. suzukii* field captures and ambient RH and temperature were evaluated using a polynomial regression in Statistica. The relationship between season-long *D. suzukii* field captures and population increase, estimated by simulations, was evaluated with multiple regression in Statistica. Two illustrative simulations, that is, one at the highest (77.0%) RH and one at the lowest (68.3%) RH, were conducted.

## Results

### Demographic parameters and ovarian maturation

The 82% RH regime resulted in the earliest eclosion of adults from egg-rearing containers relative to

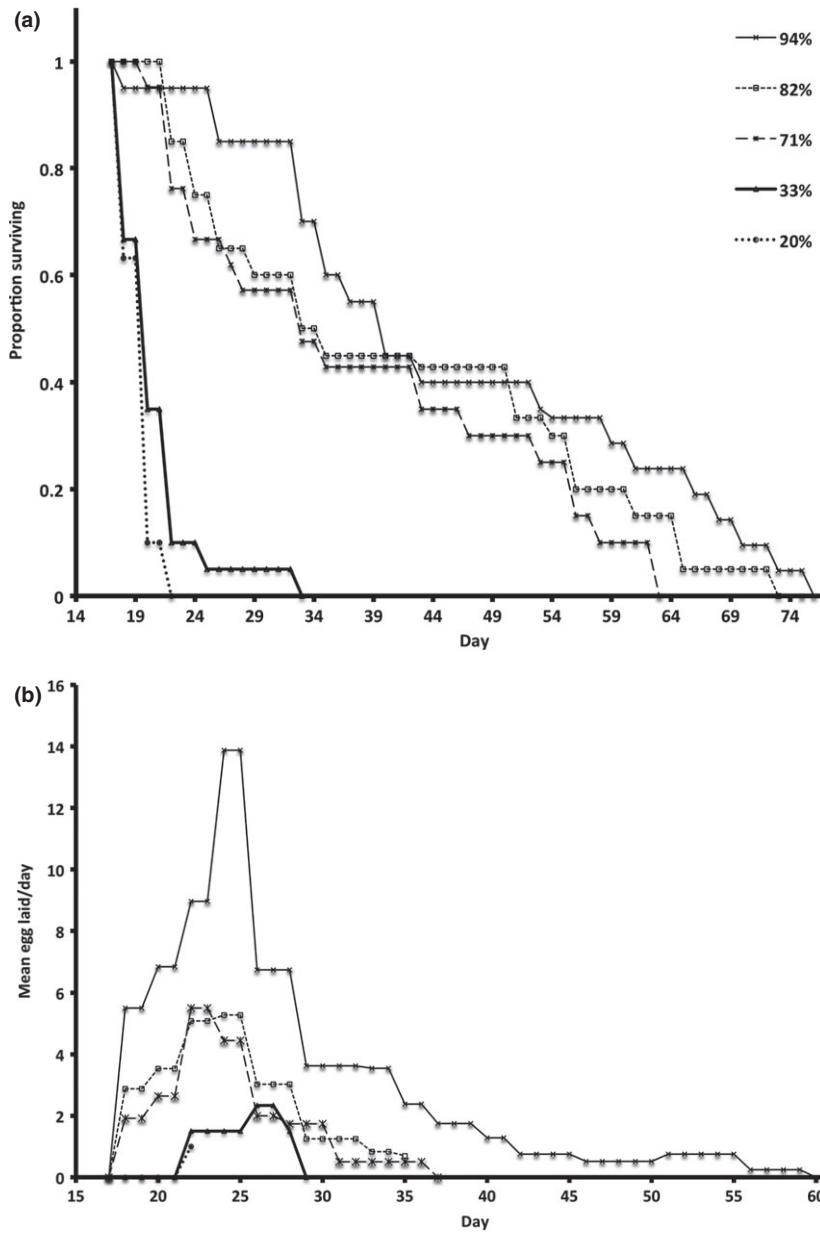
all other RH levels assayed. No other significant differences of eclosion were observed among treatments ( $F = 7.08$ , d.f. = 4,180;  $P < 0.001$ ; Table 1).

Differing relative humidity regimes resulted in significant differences in mean survival of adult females ( $F = 33.61$ , d.f. = 4,98,  $P < 0.001$ ; Table 1) and males ( $F = 9.524$ , d.f. = 4,76,  $P < 0.001$ ; Table 1). Mean survivorship of females at 20 and 33% RH levels was less than 3 days, whereas at 71, 82 and 94% RH females survived an average of 20 or more days (Table 1). Log-rank tests for adult female survival curves displayed significant differences for blueberry ( $H_{4,186} = 12.72$ ,  $P = 0.013$ , fig. 1a). Relative humidity similarly affected male mean survival; adult males survived for longer periods at higher RH levels. Egg-laying rates increased with increasing RH ( $F = 92.27$ , d.f. = 5,97;  $P < 0.001$ ), even when accounting for longer adult female survival at higher RH. Oviposition was lowest at 20 and 33% RH, intermediate at 71 and 82% RH and highest at 94% RH (Table 1, fig. 1b). Generation time ( $T$ ) ranged from 24 to 25 days over the range of trialled humidity levels (Table 2). Both  $R_0$  and  $r_m$  increased from 20 to 94% RH levels.

The percentage of females with mature oocytes ( $\chi^2 = 78.20$ , d.f. = 4,  $P < 0.001$ ) and the number of mature oocytes in ovaries increased with RH ( $F = 32.96$ , d.f. = 4,102;  $P < 0.001$ ; Table 1). At 20 and 33%, RH egg maturation periods were shorter due to short longevity of adult female flies at these humidity levels. Numerically lower mature oocytes were counted at 20 and 33% RH compared to 71% RH, although it must be noted that flies at the two lowest RH levels did not survive the entire 7-day adult period before dissection. At 71% RH, oocyte counts were significantly lower compared to 82% and 94% RH. At these two highest RH levels, nearly all females had mature oocytes present. The functions used to describe both survival and fecun-

**Table 2** Developmental and reproductive parameters related to relative humidity (RH) for *Drosophila suzukii* on blueberry.  $R_0$  = the net reproductive rate;  $T$  = mean generation time in days;  $r_m$  = intrinsic rate of population increase

RH%	$R_0$	$T$	$r_m$
20	-	-	-
33	10	25	0.09
71	25.4	24	0.14
82	35.1	24	0.15
94	68	25	0.17



**Fig. 1** Proportion of adult female *Drosophila suzukii* survival (a) and mean daily oviposition (b) at five constant humidity levels on blueberry.

dity provided good fit to the analyzed data (Table 3).

#### Relating humidity to field trap captures

Ambient RH significantly influenced seasonal adult *D. suzukii* captures. The function,  $y = 0.0189x^2 - 87$  ( $R^2 = 0.79$ ;  $F = 2.99$ ; d.f. = 1,5;  $P = 0.03$ ), describes the role of RH on seasonal *D. suzukii* trap captures, with a clear increase in seasonal trap captures associated with increasing humidity (fig. 2a). Ambient temperature showed no correlation to seasonal adult *D. suzukii* captures ( $R^2 = 0.09$ ;  $F = 0.62$ ; d.f. = 1,5;

$P = 0.46$ ). The trap data were also significantly correlated ( $R^2 = 0.63$ ;  $F = 8.4$ ; d.f. = 1,5;  $P = 0.033$ ) with log-transformed *D. suzukii* population estimates from the humidity population model (fig. 2b). The function describing the relationship of model-predicted *D. suzukii* population and actual field captures is  $y = 0.0347x + 2.98$  (fig. 2b). For illustrative purposes, the model outputs are presented together representing two mean daily humidity levels of  $68.3 \pm 5\%$  RH and  $77.0 \pm 5\%$  RH. Estimated populations at the lesser field humidity level remained relatively low compared to the higher field humidity level (fig. 3a, b).

## Discussion

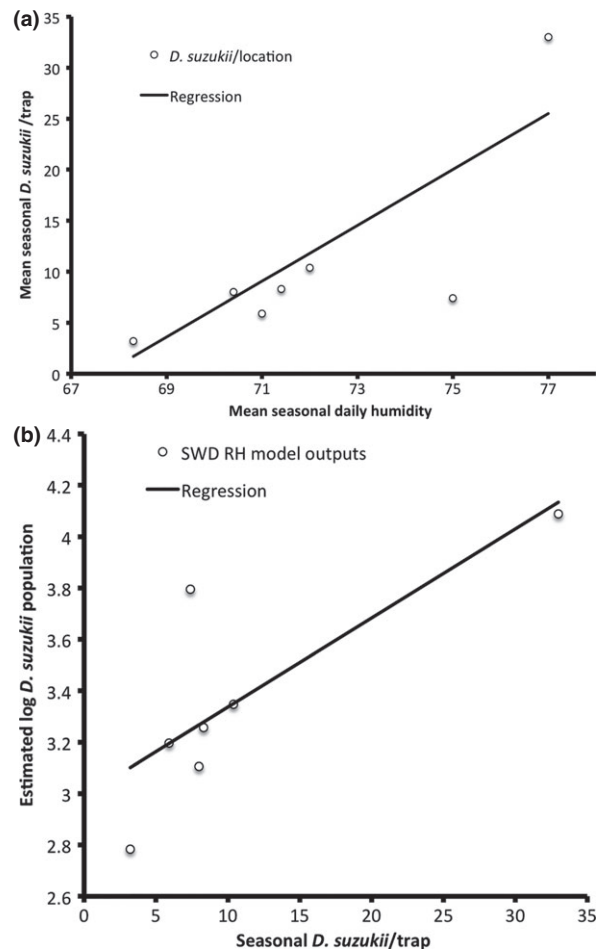
A previous study examined the relationship between temperature, fruit host, survival and fecundity of *D. suzukii* (Tochen et al. 2014), and life table data at

**Table 3** Functions describing survival and fecundity of *Drosophila suzukii* for each of four age periods (days) on blueberry

Day	Function	R <sup>2</sup>	F Value	P Value	d.f.
Survival					
0–16	Immature probability of survival = 0.86				
17–25	$y = 0.0085x + 0.198$	0.96	82.3	0.002	1, 3
26–35	$y = 0.0106x - 0.234$	0.97	109.2	0.002	1, 3
36–50	$y = 0.0069x - 0.1553$	0.94	56	0.004	1, 3
Fecundity					
0–16	0				
17–25	$y = 0.0015x^2 - 0.0643x + 0.8425$	0.98	131.07	0.0075	1, 3
26–35	$y = 0.001171x^2 - 0.02802x$	0.99	210.17	0.0006	1, 3
36–50	$y = 0.013x - 0.35$	0.97	85.39	0.0027	1, 3

22°C were comparatively similar to our findings reported in the current study. The data in the current study provide survival, developmental and reproductive parameters for *D. suzukii* mating pairs kept at different RH levels on commercially sourced blueberry fruit. Age-related female survival and oviposition at each RH level indicate that higher humidity is more suitable for adult *D. suzukii* longevity and reproduction. Temperature and humidity likely affect *D. suzukii* in an interactive fashion. High humidity can increase survival of many arthropods at high temperatures (Parsons 1979; Perring et al. 1984). For the mosquito *Aedes aegypti* (Diptera: Culicidae), increasing temperatures negatively affect oviposition, but the magnitude of this effect is lessened by concomitant increases in RH (Costa et al. 2010). Given the likelihood of interactive effects between temperature and humidity, additional research is recommended to examine the combined effects of these two environmental parameters on the reproductive capacity and field behaviour of *D. suzukii*.

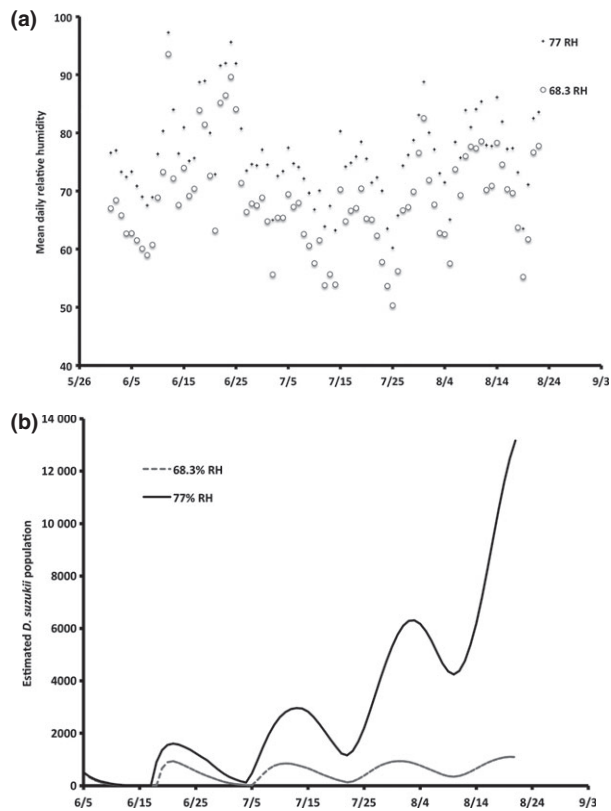
The intrinsic rate of population increase ( $r_m$ ) values indicates that RH levels above 20% resulted in increased reproductive potential, with greater RH leading to higher  $r_m$  values. In the current study, short survival times and lack of reproduction recorded at 20% RH indicate that this humidity is below the conditions under which *D. suzukii* can reproduce. The developmental and reproductive parameters related to humidity are realistic in comparison with tephritids (Quesada-Moraga et al. 2012), initial data on



**Fig. 2** Correlation between mean seasonal humidity and mean seasonal *Drosophila suzukii*/trap (a). Correlation between seasonal *D. suzukii*/trap and estimated logarithmic *D. suzukii* population level (b).

*D. suzukii* (Kanzawa 1939; Kimura 2004), and more recent data for *D. suzukii* developing on blueberry (Tochen et al. 2014). Other unrelated insect species show similar trends of higher survival and fecundity at higher RH. For example, adult longevity and fecundity of the mirid bug *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae) are higher at 70 and 80% RH than at 40 and 50% RH. For *A. lucorum*, the intrinsic rate of population increase decreased as RH decreased below the optimum of 70% (Lu and Wu 2011). Adult female longevity and fecundity of the olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) similarly increased with increasing RH up to 75% (Broufas et al. 2009). The initial condition or age of the study insects may also affect behavioural or physiological responses to humidity (Perttunen and Salmi 1956). For some other species, humidity may have little or no effect on behaviour. In the leaf beetle, *Longitarsus bethae* Savini and Escalona (Chrysomelidae:





**Fig. 3** Daily mean relative humidity levels in two locations (68.3 and 77.0% RH) (a) and *Drosophila suzukii* estimated population levels at 68.3 and 77.0% RH at 20.6°C over an 82-day period (b).

Alticinae), oviposition rates were similar when comparing low and high RH (Simelane 2007).

We show that humidity-related *D. suzukii* trap counts and modelled population estimates provide some insights into the impact of field humidity when comparing less and more optimal conditions. Initial CLIMEX and MAXENT models were generated to predict areas of the U.S. where *D. suzukii* would be likely to reproduce (Damus 2009). These models predicted highly suitable conditions across the eastern U.S., as well as along the Pacific Coast from Southern California to British Columbia, where temperatures and humidity levels match conditions favoring reproduction. However, the CLIMEX and MAXENT models indicated poor suitability of arid regions of the Western U.S. that are known today to have significant *D. suzukii* pest pressure (Beers et al. 2011; Dalton et al. 2011; Wiman et al. 2014). While summer temperatures and humidity create extremely hot and dry environments in these inland Western areas, conditions in agricultural fields may not match those of the prevailing surrounding environment. Canopy cover in arid climates has a moderating effect on soil surface

temperatures resulting in a decrease of soil water evaporation rates (Breshears et al. 1998). In the agroecosystem, artificially large shaded canopies and irrigated soils may provide refuge for *D. suzukii* to persist in otherwise unsuitable habitats. *Drosophila* migration was documented towards suitable habitats from fly release points within 100 m of a desert oasis (Coyné et al. 1987). Additionally, protein-marked *D. suzukii* were recaptured within 90 m of suitable host plants along agricultural field margins (Klick et al. 2015). Thus, the behavioural adaptation of short-distance migration towards favourable microclimates likely enhances the ability for *D. suzukii* to survive and reproduce in otherwise marginal environments.

Whereas humidity increased *D. suzukii* population estimates in the current study, no such correlations were found between temperature and *D. suzukii* trap counts. We believe that higher *D. suzukii* trap counts and population estimations translate to increased pest pressure owing to increased reproduction and adult longevity. Manipulation of field characteristics to decrease humidity within the field may result in lower *D. suzukii* pressure by physiological or behavioural mechanisms. However, behavioural mechanisms (avoidance) may prove temporary as *D. suzukii* could re-orient towards crops during favourable conditions (Klick et al. 2015).

While laboratory results from this study show a strong relationship between RH and potential *D. suzukii* population growth, the field results are less clear but nonetheless supportive. Our field data show a positive relationship between trap captures and RH. This is partially due to a mismatch in the temporal scales of the RH and *D. suzukii* field data. *D. suzukii* were collected over weekly intervals, making trapping data too coarse to capture differences in *D. suzukii* activity on an hourly basis, yet fluctuations in ambient RH were averaged to produce mean daily values. Nonetheless, the field data do indicate that, averaged over several weeks, *D. suzukii* are more active at high ranges of RH. Other factors likely affect *D. suzukii* field activity, including temperature, host volatiles, suitable oviposition and feeding sites, suitable microhabitat availability and proximity to alternate hosts and other moisture resources (Cha et al. 2012; Tochen et al. 2014; Lee et al. 2015). Furthermore, although published data are not yet available, there is a general sense from growers and researchers alike that *D. suzukii* population pressure is worse in fields with high humidity, or in close proximity to water bodies (H. Heiser, T.J. Hafner pers. comm). Therefore, a better assessment of the relationship between RH and *D. suzukii* activity in the field would be to compare trap

catches over time in fields that have consistently different RH due to irrigation practices, pruning practices, or surrounding sources of water or non-crop vegetation.

In addition to direct effects evaluated in this study, the relationship between RH and population parameters may be mediated through the effects of RH on nutrient availability. For example, RH can alter nutrition sources for flies, including bacterial communities on leaf surfaces (O'Brien and Lindow 1989) or sucrose concentrations of nectar (Corbet and Delfosse 1984). Feeding history may also affect an insect's physiological response to RH (Andersen et al. 2010). This study describes the role of relative humidity and its impact on survival and population growth of *D. suzukii*. In certain cropping systems and production regions, it may be feasible to manipulate humidity in crops with pruning, irrigation practices and ground cover manipulations. These practices may be valuable to limit *D. suzukii* population increase. Placing traps within high-humidity regions of a field may improve their predictive capacity, and incorporating humidity-dependent survival and fecundity data could further improve the predictive capability of *D. suzukii* matrix population model (Wiman et al. 2014) and lead to more refined management decisions.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Mature (left) and non-mature (right) *Drosophila suzukii* oocytes.