

1 *Research Article*

2 **Population Dynamics of Spotted Wing *Drosophila*** 3 **(*Drosophila suzukii* Matsumura) in Maine Wild** 4 **Blueberry (*Vaccinium angustifolium* Aiton)**

5
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13 **Abstract:** A long-term investigation of *D. suzukii* dynamics in wild blueberry fields from 2012 - 2018
14 demonstrates relative abundance is still increasing seven years after initial invasion. Relative
15 abundance is determined by physiological date of first detection and air temperatures the previous
16 winter. Date of first detection of flies does not determine date of fruit infestation. The level of fruit
17 infestation is determined by year, fly pressure, and insecticide application frequency. Frequency of
18 insecticide application is determined by production system. Non-crop wild fruit and predation
19 influences fly pressure; increased wild fruit abundance results in increased fly pressure. Increased
20 predation rate reduces fly pressure, but only at high abundance of flies, or when high levels of wild
21 fruit are present along field edges. Male sex ratio might be declining over the seven years. Action
22 thresholds were developed from samples of 92 fields from 2012 - 2017 that related cumulative adult
23 male trap capture to the following week likelihood of fruit infestation. A two-parameter gamma
24 density function describing this probability was used to develop a risk-based gradient action
25 threshold system. The action thresholds were validated from 2016-2018 in 35 fields and were
26 shown to work well in two of three years (2016 and 2017).

27 **Keywords:** long-term, sex ratio, action threshold, pest management, insecticide use

28 **1. Introduction**

29 The Spotted Wing *Drosophila* (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is
30 an invasive vinegar fly unintentionally introduced from Asia. It was first documented in the
31 continental United States in 2008 [1]. After its introduction, SWD spread rapidly across much of the
32 country including Maine where it was first documented in 2012 [2]. Unlike native *Drosophila*, the
33 females have a serrated ovipositor that allows them to oviposit into intact ripe fruit. The larvae
34 develop inside the fruit. This causes softening and introduces bacteria that shorten shelf life and
35 renders fruit unmarketable [3]. The attack of undamaged ripe fruit along with its wide host range
36 has made it a serious insect pest in many fruit crops such as cherries, raspberries, blackberries,
37 strawberries, and blueberries [4,5].

38 Maine produces 10% of all blueberries in North America [6]. Maine's wild blueberry (lowbush)
39 crop (*Vaccinium angustifolium* Aiton) is produced on 42,500 acres of managed land. Production
40 contributes over \$250 million to the state's economy each year [6]. Wild blueberries are unique in
41 that they are grown on a two-year cycle; the first year is a prune year and is characterized by
42 vegetative growth and the development of flower buds. The second year is when bloom and berry
43 harvest occur [7]. Growers in Maine produce wild blueberries both organically and conventionally.
44 There are three levels of conventional production (low, medium, and high input [8,9]). These
45 production systems vary in the intensity of capital inputs used to produce the crop. Conventional
46 growers rely primarily on insecticide based pest management; there are fewer options for organic

47 control of *D. suzukii* [10]. *Drosophila suzukii* caused an estimated \$1.4 million crop loss in Maine wild
48 blueberry during 2012, the first growing season it was present in the state [11]. Falling blueberry
49 prices coupled with increased production costs for monitoring and managing *D. suzukii* have
50 presented challenges to Maine's blueberry growers [7].

51 Since its first detection in the continental United States in 2008, much research has been
52 conducted, though there are still gaps in knowledge that limit our understanding of this pest. To
53 date, most studies of *D. suzukii* population dynamics have only investigated population fluctuations
54 across one, and sometimes, two years [12-15]. One study, Briem et al. [16], analyzed adult trap catch
55 data in Germany from 2012-2018. Except for this study, data is lacking on long-term population
56 dynamics immediately following the invasion of this species. Invasive species population dynamics
57 can change after its initial establishment with short-term population dynamics quite different from
58 long-term population dynamics [17,18]. There have been several investigations that have evaluated
59 factors that appear to affect population growth and immature and adult survival. Predation and
60 parasitism of immature stages by natural enemies has been investigated both in Europe and North
61 America [19-21] and in its native range [22,23]. Winter temperatures appear to affect overwintering
62 survival of adults [24,28] and extreme high lethal temperatures have been shown to reduce longevity
63 of adults [29-31]. In addition, dispersal over long distances are likely maintain meta-populations of
64 *D. suzukii* [32]. Landscape level plant resources for reproduction and intrinsic rate of growth have
65 also been shown to affect population levels [5,33-38].

66 In order for growers to respond effectively to continued *D. suzukii* threats, they and pest
67 management specialists, need to understand factors that affect population increase in this invasive
68 pest. Our study examined population trends of *D. suzukii* in Maine wild blueberries over the course
69 of seven years. Studying *D. suzukii* over multiple years may help growers know what they can
70 expect from this invasive insect population so they can make appropriate decisions on monitoring
71 and management. We measured relative abundance of adults, the first date of *D. suzukii* adult
72 appearance and fruit infestation each year, sex ratio and the relationship between adult density and
73 fruit infestation levels across all seven years. We investigated potential relationships between
74 population dynamics and weather and what impact different management systems and production
75 stages (organic versus conventional, vegetative versus fruiting fields, and pesticide usage) have on
76 *D. suzukii* populations. Our hope is that this research provides growers with a better basis for
77 development of integrated pest management tools for monitoring, predicting, and controlling *D.*
78 *suzukii*, especially the practicality of action thresholds.

79 2. Materials and Methods

80 This study was conducted over a period of seven years (2012-2018) in Maine wild blueberry
81 fields to investigate the population dynamics of *D. suzukii*. Field studies were conducted in 12-20
82 managed wild blueberry fields per year in Hancock, Knox, Lincoln, Waldo, and Washington
83 counties, Maine, USA. Baited traps were used to estimate the relative abundance of adult fly
84 populations in wild blueberry fields. Fruit harvests followed by emersion of squashed fruits in a
85 saline solution were used to determine larval infestation levels.

86 2.1 Trapping

87 All adult sampling between 2012 - 2018 utilized spotted wing drosophila traps consisting of 473
88 ml red polystyrene Solo® (Dart Container Corp., Mason, MI) cups with 7-10, 0.48 cm diam. holes
89 punched evenly around the top rim and light-blocking lids [2,39]. Traps were baited with 118 ml
90 standard yeast/sugar bait (2014-2017) (5.07 g dry active yeast, 25.35 g sugar, 450 ml water) [2,39].
91 This bait is highly attractive to *D. suzukii* in the Maine wild blueberry landscape [39,40]. For all trials
92 in 2012, a yellow sticky card was also hung inside each trap to facilitate captured fly species
93 identification. In all sampling (2012-2018) traps were hung 0.30 to 0.61 m above the top of the
94 blueberry canopy using 0.91 m plant stands and were placed along wild blueberry field edges. Traps
95 were deployed at densities of 3-5 traps per field (more traps in larger size fields) and checked 1-2

96 times per week. At each visit, traps were replaced with new traps and fresh bait. Traps that were
 97 removed from the field were returned to the lab and drained through a fine mesh sieve to count the
 98 number of male and female *D. suzukii* [2]

99 2.2 Larval infestation

100 Wild blueberry infestation by larvae was determined by visiting fields weekly, once fruit was
 101 vulnerable to larval infestation, the first signs of individual ripe fruit [2]. Blueberries were sampled
 102 by collecting 1 L samples (ca. 500 berries / sample) at different locations (blueberry clones)
 103 representatively throughout the field. The berries were stored in a cooler with an ice pack for
 104 transfer to the laboratory. In the laboratory, fruit was added to a 4 L Zip Lock® (S. C. Johnson & Son,
 105 Inc., Racine, WI) polyethylene bag. A 1 L 10% saline solution was mixed (1 part salt:10 parts water,
 106 v:v) and ca. 236 ml was added to the bag containing the berries. The bag was sealed and the berries
 107 were gently pressed with a wooden block just enough to crack the skins. The split berries were
 108 allowed to remain in saline for 60 min. The berries and saline liquid were then strained through a
 109 coarse sieve into a dark colored tray. The fruit in the sieve was discarded and the strained liquid was
 110 inspected for larvae that sink to the bottom of the pan (modified from Drummond et al. [2]).

111 2.3 Statistical analysis and modeling

112 We used the statistical software JMP PRO version 14 [41] for all analysis. The specific models
 113 are mentioned under each objective below. Kaliedagraph® version 4.5.1 graphical software [42] was
 114 used for non-linear least squares, minimization of sums of squares was based upon partial deriva-
 115 tives derived by FAD.

116 2.4 Seasonal and annual *D. suzukii* relative abundance

117 In order to determine changes in seasonal occurrence in *D. suzukii* populations (2012 – 2018), we
 118 sampled adult population levels in wild blueberry fields using the trapping method previously
 119 described. The number of fields sampled ranged from 11 to 20 fields per year. Traps were deployed
 120 along the field / forest interface to determine adult relative abundance (see Trapping section above
 121 for details). Experimental details including number of sites, traps per field, and sampling intervals
 122 are in Table 1. In each field during each year, once fruit became susceptible to attack (ripe),
 123 blueberries were hand harvested and inspected for larval infestation (see Larval Infestation above
 124 for details, 2.2 Subsection).

125 **Table 1.** Sampling methods for *D. suzukii* seasonal and annual monitoring.

Year	Number of fields ^s	Traps per site	Starting date	Sampling interval (days)
2012	20 (19)	4 - 5	8 or 30 June	7
2013	17 (16)	2	3 - 5 July	7
2014	14 (11)	3*	8 - 9 July	7
2015	17 (13)	4	16 - 24 July	3 - 7
2016	14 (11)	3	15 - 21 July	5 - 7
2017	10 (7)	3	30 June	5 - 7

2018 20 (17) 3 2 - 6 July 3 - 7

126 § number of fields (total n=112), in parentheses are the number of fields that a biofix degree day value of 705
127 could be calculated.

128 * 7 traps deployed in Jonesboro, ME, field.

129

130 Across the seven years, using linear regression, we assessed *D. suzukii* seasonal rate of increase
131 (based upon trap capture of adults), annual trend in adult relative abundance (based upon
132 cumulative adult trap capture at degree day 705 (base 10°C). This degree day index corresponds to
133 average length of the wild blueberry growing season, 1 April to mid-August (harvest). We selected a
134 biofix or standardized degree day so that *D. suzukii* relative abundances could be compared between
135 years. Not all fields could be used in the standardized degree day comparison because some fields
136 were too distant from a local weather station and some fields were harvested long before the
137 accumulated degree days occurred. In addition, we investigated if the degree date of first adult
138 capture and the first fruit infestation varied over the seven-year period and if a linear trend of
139 increase or decrease existed.

140 We used a general linear model (Gaussian error term) to determine factors that predict
141 logarithm transformed cumulative fly captures per trap prior to harvest. Independent variables
142 investigated were mean spring and early summer air temperatures (June - July), mean winter air
143 temperatures prior to the growing season, degree day of first fly capture, and wild blueberry
144 production system. Logistic regression was used to determine factors that predict blueberry
145 infestation rate (proportion infested fruit) prior to harvest. The independent variables were
146 logarithm (base 10) transformed cumulative fly abundance / trap, year, and the number of late
147 summer insecticides (these are applied both to blueberry maggot fly, *Rhagoletis mendax* Curran, and
148 *D. suzukii*, sometimes simultaneously) applied to the field. The number of insecticides used in fields
149 that we sampled were recorded by us after interviewing each grower who agreed to provide this
150 information (n = 86 fields between 2012 and 2018).

151 2.5 Management system impact on *D. suzukii*

152 In order to determine the response of *D. suzukii* to management type (2012 - 2017), fifty-eight
153 fields were grouped by blueberry production system (organic or conventional: low, medium, or high
154 input). Typical levels of inputs used by growers in the four management systems for the production
155 of wild blueberries are given in Table 2. The cumulative number of adult *D. suzukii* per trap in each
156 field type was compared at degree day 705 and the degree day of first infestation using fixed model
157 analysis of variances (general linear model) with production system (categorical) and year
158 (continuous) as main effects along with their interaction. Poisson regression was used to determine if
159 insecticide application frequency was determined by production system. Multiple paired
160 independent Poisson contrasts (Bonferoni corrected) were used to determine which production
161 systems differed in insecticide application from each other.

162 2.6 Crop cycle

163 Ten fields were simultaneously sampled for adult *D. suzukii* relative abundance in vegetative vs
164 fruiting fields (five fruiting and five vegetative fields, a pair at each of five geographic locations) in
165 2012. Three traps were hung in each field located in Columbia, Columbia Falls, and Jonesboro,
166 Washington Co. ME on Aug 11 for one week. Analysis of variance (randomized complete block
167 design, paired field location as block) was used to compare trap capture of *D. suzukii* adults in crop
168 (fruit-bearing) versus prune (vegetative) fields. The dependent variables for three ANOVAs were
169 male, female, and total fly captures per trap.

170

171 **Table 2.** Typical* inputs associated with the four wild blueberry production management systems[§] [8,9].

Production factors	Organic	Low input	Medium input	High input
<i>Pruning</i>	Burned	Burned	Mowed	Mowed
<i>Land leveling</i>	Not land leveled [‡]	Not land leveled	Land leveled	Land leveled
<i>pH management</i>	pH managed	No pH management	pH managed	pH managed
<i>Fertility</i>	No fertilizer	No fertilizer	Reduced Fertilization (every other cycle)	Intensive Fertilization (fertilized every cycle)
<i>Pest, disease, and weed control</i>	Cutting woody weeds, no insecticides, no fungicides	Herbicide, blueberry maggot, mummy berry control with standard	Scouting, standard and reduced risk pesticides	Scouting, reduced risk pesticides
<i>Treatment of bare spots</i>	Mulch	No mulch	No mulch	Mulch
<i>Irrigation</i>	No irrigation	No irrigation	No irrigation	Irrigation
<i>Pollination (honey bees)</i>	0 - 2.5 hives / ha	0 - 5 hives / ha	5 - 10 hives / ha	15 - 25 hives / ha
<i>Harvest method</i>	Hand raked	Hand raked	Mechanical Harvest	Mechanical Harvest

172 * There is variation in grower production methods in each of the production systems.

173 [§] Production systems are not mutually exclusive. Some growers manage more than one production system.174 [‡] Land leveling involves rock removal and lifting the blueberry sod and grading the underlying soil.175 *2.7 Impact of natural enemy and wild fruits on D. suzukii abundance in wild blueberry fields*

176 We have previously shown that spotted wing drosophila utilize wild non-blueberry fruits along
 177 field edges to build up their populations prior to blueberry ripening [38]. We have also shown that
 178 predation of SWD pupae in wild blueberry fields can be quite high and appears to be mostly
 179 associated with insect predators, especially crickets [21]. This study, conducted in 2018, was
 180 designed to assess how wild fruit utilization and predation affect the population buildup of *D.*
 181 *suzukii* in wild blueberry fields. Twenty commercial wild blueberry fields were selected, 10 in each of

182 the two major growing regions (Midcoast and Downeast). Three traps (described above, *section 2.1*)
183 were placed in each field in early July, 2018. Traps were monitored at 5-7 day intervals until harvest.
184 Throughout the study, on each sample date, traps set the previous week were collected and returned
185 to the laboratory where male, female, and total abundance adults were determined and recorded.
186 New traps were deployed weekly. Using these data we calculated the total number of *D. suzukii*
187 adults and males per trap captured from each site for each date and the mean cumulative number of
188 adults and males over the entire sampling period.

189 An index of predation relied upon deploying sentinel *D. suzukii* pupae in the fields (see Ballman
190 et al. [21] for detailed methods). Pupae were removed from our laboratory colony, rinsed under
191 running water to remove media, and examined under magnification to verify the presence of a
192 developing fly. All pupae were frozen for 24 h prior to the start of the experiment. Killed pupae were
193 affixed to 9 cm white-painted Petri dishes by two, 7 cm rows of double-sided tape. Each piece of tape
194 had 10 pupae for a total of 20 pupae per plate. At each field site, three plates were placed along the
195 field edge 3 m apart and lightly covered with duff collected from the field. Plates were placed at a
196 total of 20 fields, 10 in the Mid-coast Region and 10 in the Downeast growing region. All plates were
197 left in the field for 24 hours and then retrieved. The numbers of intact pupae were counted in the lab
198 under magnification. Pupae that were obviously chewed, but not completely consumed, were
199 counted as predated.

200 Wild fruit host abundances were quantified along the edges of wild blueberry fields. Wild fruit
201 was surveyed 2-3 times in blueberry fields in late July, early August, and late August. Wild fruit
202 evaluations were not repeated once a field was harvested. To evaluate wild fruit, three, 30 m
203 transects were set up along each of four field edges. The presence or absence of wild fruit was
204 recorded at every other 0.3m along each transect for a total of 50 observations per transect. The
205 species of wild fruit along each field were also recorded (see Ballman et al. [21] for more details).

206 We constructed a general linear model to determine the field level factors that explained the
207 variation in the end of season total (male + female) *D. suzukii* captures per trap in each field. The
208 potential predictors that were considered for the model were: field size (ha), production system
209 (organic, and low, medium, and high conventional), the prevalence (% of transect landcover) of
210 non-crop wild fruit along the edges of the field, and the intensity (% of sentinel pupae predated) of
211 predation on pupae in the field. In addition, we included all of the two and three-way interactions in
212 the model.

213 2.8 Management - Early harvest tactic and action thresholds for *D. suzukii* in wild blueberry

214 The wild blueberry fields sampled in the seasonal occurrence study described above (*section 2.4*)
215 were used to determine if action thresholds for *D. suzukii* adults could be developed for growers.
216 Our approach was to base the action thresholds on adult male trap captures. Once pigmentation
217 develops in the wings of newly emerged males, they are easy to identify with little error by growers
218 in Maine. There are no other species of drosophila or other small sized Diptera in Maine that have
219 similar wing pigmentation and can be confused with male *D. suzukii*. A statistical model to
220 determine the relationship between male trap capture and percent fruit infestation was fit to all wild
221 blueberry fields sampled prior to harvest (n=92) between 2012 and 2017. Because the relationship
222 was non-linear in nature, we fit a linearized exponential model to the data to determine if a
223 significant relationship existed and then using the Levenberg-Marquardt's non-linear least squares
224 algorithm, we fit a three parameter exponential model to the data to construct a predictive equation
225 [43].

226 We first developed a tactic of early harvest. For this tactic we monitored fields for adult males
227 using baited traps and assessed larval infestation rate of fruit prior to harvest (see methods for
228 Seasonal and annual *D. suzukii* relative abundance section). In all years (2012 - 2018) fields that were
229 harvested prior to any *D. suzukii* males being captured were assessed as to their level of infested fruit
230 and the date of harvest. From this data, we determined if an early harvest tactic to avoiding damage
231 was practical. In 2016, the proportion of non-ripe fruit present was assessed at harvest in order to

232 show the cost / benefit of an early harvest tactic. A predictive linear model was developed to
233 determine the crop loss due to non-ripe fruit and the time of harvest.

234 Our second approach was to develop "risk based" action thresholds. This involved fitting a
235 probability density function to all fields (n=92). The density function was then used to determine the
236 probability or likelihood of infested fruit the week following a given mean cumulative male adult
237 trap capture. We tested several continuous variate density functions as models for our empirical
238 data. Depending upon the density function, a Kolmogorov-Smirnov cumulative frequency
239 distribution test or a Cramer-von Mises frequency distribution test was used to evaluate the
240 goodness of fit of the selected density function to the empirical data ($\alpha = 0.05$). This information was
241 then used to construct action thresholds for growers with varying risk aversion, based upon male *D.*
242 *suzukii* in trap captures. Validation of the early harvest tactic and action thresholds derived from the
243 probability model was performed on 14, 10, and 19 fields in 2016-2018. The 2016 validation was
244 upon a probability model derived from a subset of fields sampled between 2012 and 2016 (n=82);
245 whereas, the 2017 and 2018 validations were based upon the probability model derived from fields
246 sampled between 2012 and 2017 (n=92). Fields were pre-assigned with an action threshold. The
247 fields were monitored for the grower and with the assigned thresholds in mind, fields were either
248 harvested before or immediately after the threshold was reached, or the grower applied an
249 insecticide. The results were then compared to the expected probability derived from the probability
250 density function.

251 3. Results

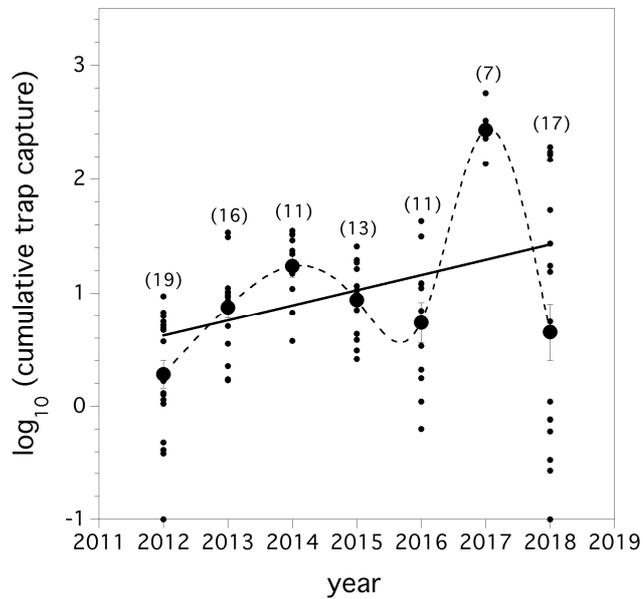
252 3.1 Seasonal occurrence of *D. suzukii*

253 The logarithm of the cumulative trap capture of *D. suzukii* flies at a biofix of degree-day 705
254 (harvest period) over the seven year period ($F_{(1,95)} = 4.602$, $P = 0.035$, $r^2 = 0.046$) is shown in Figure 1.
255 Because the Studentized residuals from the linear regression were not characteristic of a linear
256 increase in *D. suzukii* over time, we also evaluated a polynomial model with the hypothesis that
257 between 2012 and 2018, *D. suzukii* has been increasing, but in an oscillatory manner. This model, also
258 shown in Figure 1, explains more of the variance in logarithm cumulative trap captures ($F_{(4,92)} = 8.845$,
259 $P < 0.0001$, $r^2 = 0.271$), and the residuals suggest a better choice of hypothesis. Prediction of log
260 cumulative fly capture / trap at the biofix date was significantly dependent upon the degree day of
261 first fly trap capture ($F_{(1,86)} = 115.807$, $P < 0.0001$, $\beta = -0.003$) and the mean winter air temperature prior
262 to the growing season ($F_{(1,86)} = 6.097$, $P = 0.027$, $\beta = 0.012$). Year was not used in the model because we
263 wanted to test annual weather effects. The overall model explained 63.6% of the variance in log
264 cumulative fly capture per trap at the biofix of 705 degree days ($F_{(2,86)} = 58.656$, $P < 0.0001$, $r^2 = 0.636$).
265 We sought to determine if year to year variation in growing season accumulated degree-days for the
266 months June - August could explain cumulative *D. suzukii* trap capture at the biofix of degree-day
267 705, but there was no relationship ($P = 0.142$). Figure 2 illustrates the logarithmic increase in trap
268 captures over the growing season of *D. suzukii* in all the wild blueberry fields sampled and pooled
269 for each year between 2012 and 2018. The increasing logarithmic trends were significant ($F_{(3,1101)} =$
270 658.42 , $P < 0.0001$, $r^2 = 0.642$), but a year by date interaction exists ($P < 0.0001$), suggesting that the
271 rates of increase varied by year. When assessed by year, 2013 and 2014 rates of increase were less
272 than other years, while 2017 and 2018 were the highest rates of increase.

273 We calculated the rate of *D. suzukii* trap capture increase (proportion daily increase from first
274 detection to the degree day biofix) in each field. The average trap capture rate of increase for
275 2012-2018 was 2.15 ± 0.48 (s.e.). The highest rate of increase was in 2017 at 8.70 ± 2.52 . The factors that
276 were significant predictors of rate of trap catch were year ($P < 0.0001$) and spring degree day
277 accumulation (May and June, $P = 0.004$). The overall model explained 48.7% of the variance in rate of
278 increase in trap captures ($F_{(1,86)} = 19.786$, $P < 0.0001$). However, the slope of spring degree day
279 accumulation was negative ($\beta = -0.004 \pm 0.001$), which we can not explain. Therefore, spring degree
280 day accumulation might be negatively correlated with a predictor that we did not model.

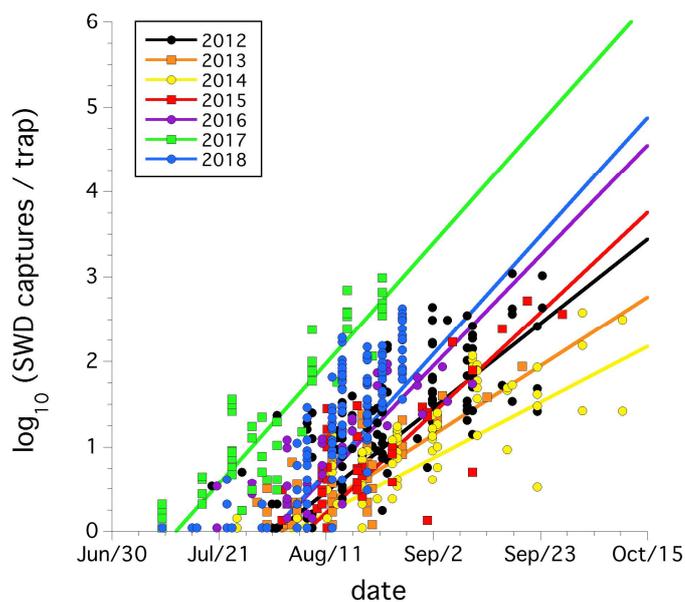
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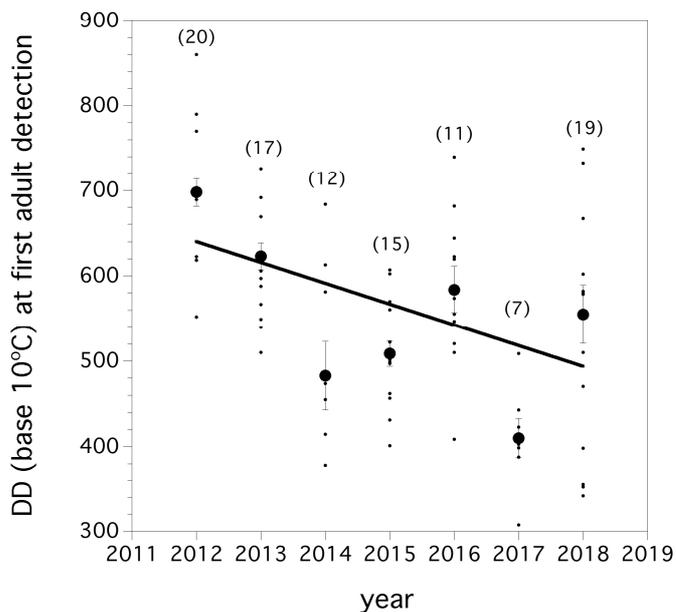
284 **Figure 1.** Cumulative trap captures (logarithm transformed) of *D. suzukii* at a biofix of 1270 DD (base 10°C).
 285 Small filled circles are individual field captures and large filled circles are annual means (error bars are
 286 standard errors). Parentheses above each year are the number of fields where degree-day information was
 287 available (total n = 94). Solid line is linear model and dashed line is fourth degree quadratic model.



288

289 **Figure 2.** Average trap catches of *D. suzukii* adults (logarithm transformed) over each growing season
 290 (2012-2018).

291 We found that over the seven year invasion period, first captures of flies occurred earlier,
 292 measured in degree days, each year ($F_{(1,99)} = 19.939$, $P < 0.0001$, $r^2 = 0.168$). This decline has a slope of
 293 -82.77 degree-days per year. The trend in the degree days at first detection of adults between 2012
 294 and 2018 is shown in Figure 3.
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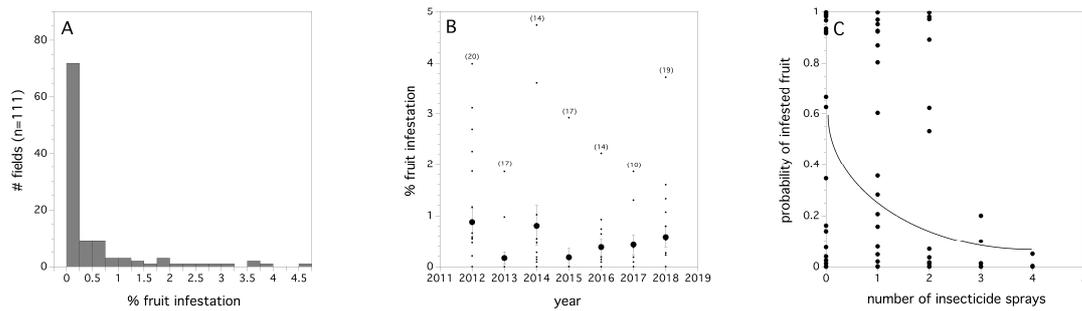


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297 **Figure 3.** Degree day (base 10°C) at first fly detection in a trap. Small filled circles are individual field captures
 298 and large filled circles are annual means (error bars are standard errors). Parentheses above each year are the
 299 number of fields where degree-day information was available ($n = 101$). Solid line is linear regression fit.

300 Many fields had a low percent of infested fruit prior to harvest. Figure 4A depicts the frequency
 301 distribution of fields with infested fruit over the seven-year study. The level of infestation by year
 302 can be seen in Figure 4B. There has been no trend in either increasing or decreasing infestation rates
 303 over time ($P = 0.864$, $\sin^{-1} \sqrt{\text{proportion}}$ transformed). Unlike first fly capture, we did not find any
 304 evidence to suggest that infestation of fruit occurred earlier, with respect to degree days, over time
 305 ($P = 0.667$). Logistic regression provided evidence that year ($\chi^2_{(6)} = 15.859$, $P = 0.017$, unit odds ratio
 306 among years: range of pairwise comparisons among years = 0.001 - 85.599), log (cumulative fly
 307 capture / trap) ($\chi^2_{(1)} = 60.559$, $P < 0.0001$, unit odds ratio = 1630.4), and the number of summer
 308 insecticide applications ($\chi^2_{(1)} = 4.553$, $P = 0.047$, unit odds ratio = 0.278), were all significant predictors
 309 of the proportion of blueberries in the field infested prior to harvest. Inspecting the odds ratios, it can
 310 be suggested that the most influential predictor is the relative fly abundance; year is sometimes
 311 highly influential and at other times not, and insecticide applications are influential but not as much
 312 as fly relative abundance / trap, or some year effects. Year in our model may represent weather
 313 effects. Figure 4C shows the relationship between the estimated probability of blueberry infestation
 314 and the number of insecticide applications.

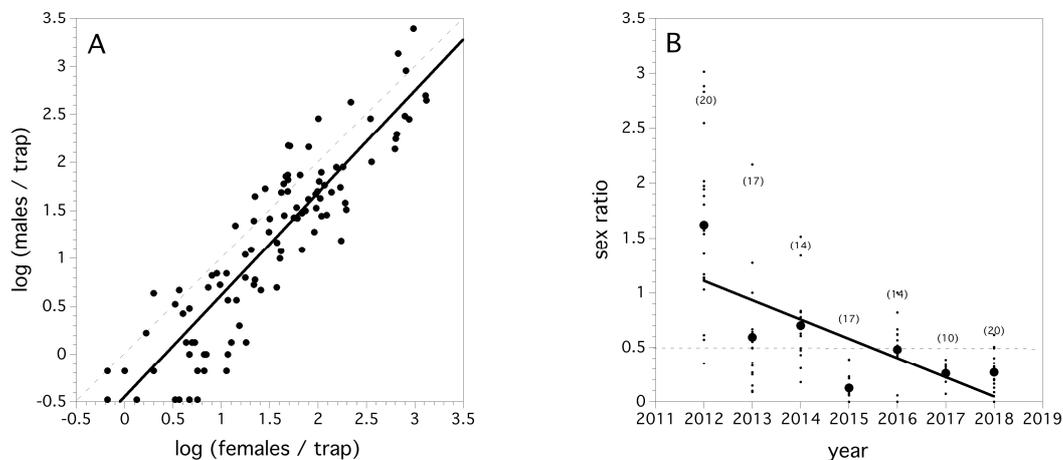
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316

317 **Figure 4.** Frequency of fruit infestation levels (%) per field (A); the percent fruit infestation in individual fields
 318 by year, small filled circles are individual field infestation rates and large filled circles are annual means (error
 319 bars are standard errors); and the relationship between model estimated probabilities of infested blueberry fruit
 320 in a field and the number of insecticide applications applied. Solid line is logistic regression fit (C).

321 We found that sex ratio of flies captured in traps was female biased; although, the slope of the
 322 regression relating $\log(\text{female flies / trap})$ to $\log(\text{male flies / trap})$ suggested that the sex ratio is
 323 constant across fly population density ($F_{(1,107)} = 440.271$, $P < 0.0001$, $r^2 = 0.815$, $\beta = +1.065$, Fig. 5A). Sex
 324 ratio (square root transformed) relative to the proportion of males to females declined in a linear
 325 fashion between 2012 and 2018 ($F_{(1,107)} = 51.617$, $P < 0.0001$, $r^2 = 0.325$, $\beta = -1.067$, Fig 5B).



326

327 **Figure 5.** Relationship between male and female trap capture, all fields and all years, solid line is least squares
 328 regression fit and dashed line is 1:1 relationship (A), and the sex ratio (male:female) of total flies captured in
 329 each field season by year, small filled circles are individual field sex ratios and large filled circles are mean sex
 330 ratios (error bars are standard errors) (B).

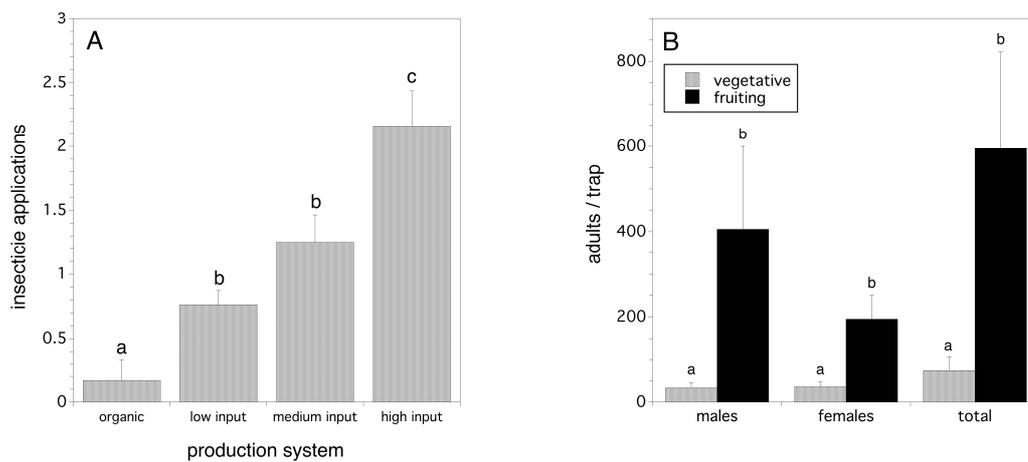
331 3.2 Management system impact on *D. suzukii*

332 We found that management system did not determine the logarithm of cumulative abundance
 333 of flies per trap ($P > 0.05$), nor the date of first fly detection and we have previously shown that
 334 management system does not directly affect fruit infestation rate. This was also the case when
 335 management systems were collapsed into conventional vs organic. However, Poisson regression
 336 suggested that wild blueberry management system does determine the frequency of pesticide
 337 applications in a model that includes both year and management system ($\chi^2_{(8)} = 45.551$, $P < 0.0001$)
 338 Organic systems used significantly fewer insecticide applications than the three conventional

339 systems (low, medium, and high). Figure 6A shows the results of individual Poisson contrasts
 340 separating the number of mean pesticides applied in each production system.

341 3.3 Crop cycle

342 We found that crop cycle determines adult relative abundance / trap. Significantly fewer males
 343 ($F_{(1,8)} = 6.346, P = 0.036$), females ($F_{(1,8)} = 7.803, P = 0.023$), and total flies per trap ($F_{(1,8)} = 6.905, P = 0.030$)
 344 were found in vegetative fields than in fruiting fields (Fig. 6B).



345

346 **Figure 6.** The effect of production system on the frequency (number) of insecticides used during the summer
 347 among the four production systems (A), and adult trap captures in vegetative compared to fruiting fields (B).
 348 Error bars are standard errors and the same letters associated with bars by treatment indicate that means are not
 349 significantly different from one another ($P > 0.05$).

350 3.4 Impact of natural enemy and wild fruits on *D. suzukii* abundance in wild blueberry fields

351 We found that field size and production system did not account for a significant proportion of
 352 the variation in adult trap capture. Predation and wild fruit hosts were significant predictors,
 353 resulting in 53.9% of the variation in the total *D. suzukii* adult trap capture relative abundance across
 354 all fields ($F_{(3,16)} = 6.258, P = 0.005, r^2 = 0.539$). Table 3 lists the model coefficients.

355 **Table 3.** Predictive model for determining abundance of SWD by the end of season.

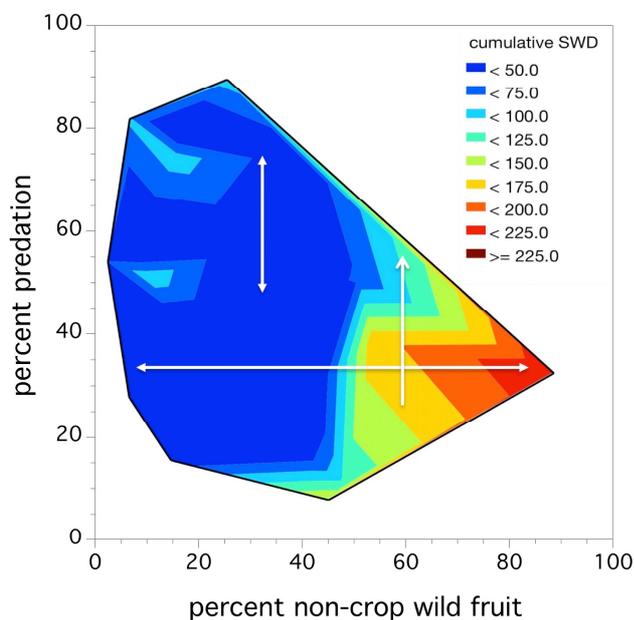
Terms in model	Estimate ¹	S.E. ²	T-ratio	Probability ³
Intercept	11.688	32.669	0.36	0.725
% Wild fruit	1.359	0.565	2.40	0.029
% Pupal predation	-0.024	0.430	-0.06	0.956
% Wild fruit X % pupal predation	-0.063	0.018	-2.22	0.042

356 ¹ estimate of model coefficient

357 ² standard error, precision of the coefficient

358 ³ probabilities in bold denote a coefficient significantly different than 0.

359 An inspection of Table 3 suggests that the two predictors do not operate independently, in fact,
 360 % pupal predation is ONLY of value in explaining relative abundance in the context of the wild fruit
 361 condition along the edge of a blueberry field. The abundance of wild fruit is a strong predictor on its
 362 own (explaining 39.5% of the variation in relative abundance. Figure 7 depicts this interaction
 363 between the two predictors as a thermal map. Inspection of this figure shows that the observed adult
 364 relative abundance increases from low (dark blue) to high (bright red) as the % wild fruit in a
 365 blueberry field increases (left to right of graph). However, an increase in % predation (bottom to top
 366 of graph) only results in a decrease in *D. suzukii* abundance when the percent wild fruit abundance is
 367 high or greater than 50%. This is the interaction: percent predation only appears to be influential and
 368 reduce *D. suzukii* relative abundance in fields with high levels of wild fruit. These fields also have
 369 higher adult relative abundances.



370

371 **Figure 7.** *Drosophila suzukii* captures in fruit-bearing fields as a function of non-crop wild fruit hosts and pupal
 372 predation. Darker blue regions are low and red regions are high adult relative abundances. White arrows show
 373 the change in adult relative abundance as non-crop wild fruit and predation changes.

374 3.5 Management - Early harvest tactic and action thresholds for *D. suzukii* in wild blueberry

375 The relationship between male *D. suzukii* cumulative trap capture (logarithm transform) just
 376 prior to harvest and percent fruit infestation was significant and exponential in form (linear model:
 377 $F_{(3,89)} = 88.044, P < 0.0001, r^2 = 0.494$). The coefficients of a non-linear fit exponential model of the
 378 form, % infestation = $a + b \times e^{(c \times \text{male trap capture})}$ were: $a = -0.675, b = 0.445,$ and $c = 1.129$. The model fit and
 379 observed data points are shown in Figure 8A.

380 The early harvest tactic has great promise for avoiding the consequences of fruit infestation due
 381 to *D. suzukii* attack. Table 4 shows that fields that are harvested prior to *D. suzukii* adults being
 382 captured in at least three traps per field are not likely to be characterized by infested blueberries.
 383 None of the fields that we monitored that were harvested early had any detectable fruit infestation
 384 (Table 4). However, we did find a cost to harvesting early.

385

386 **Table 4.** Results of early harvest as a tactic for averting risk of fruit infestation by *D. suzukii*.

Year(s)	Number of fields	Dates of harvest	Wild blueberry infestation rate
2012-2015	7	21 July - 28 July	0.0
2016	3	26 July - 4 Aug.	0.0
2017	1	18 July	0.0
2018	4	30 July - 8 Aug.	0.0

387

388 Figure 8B shows that non-ripe green fruit may more than offset the damage from *D. suzukii*. The
 389 relationship between date of harvest and the percentage of ripe (blue) berries harvested as a percent
 390 of the total berry yield is:

391 % ripe fruit = $100 / [1 + e^{(30.903 - 0.159 * \text{Julian Date})}]$, $P = 0.002$, $r^2 = 0.795$).

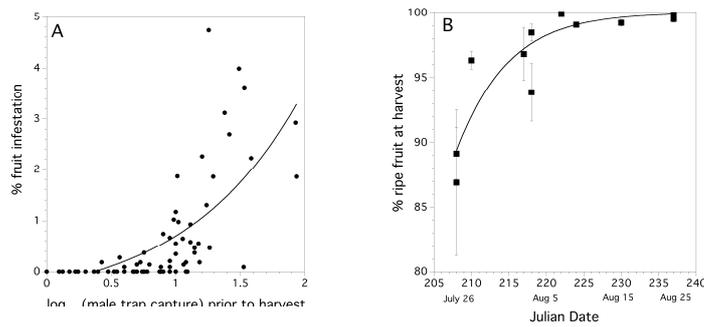
392 A two parameter Gamma probability density function best fit our empirical data comprising
 393 fields with mean numbers of cumulative male trap captures prior to harvest and the likelihood of
 394 not being infested the following week after the cumulative trap capture. Estimates for the two
 395 parameter Gamma distribution were: alpha (shape) = 1.6765 (1.0949-2.4512, 95% CI), and sigma
 396 (scale) = 9.43259 (6.1286 – 15.7678, 95% CI). The Cramer-von Mises W^2 test suggests that the
 397 empirical data is not significantly different from the theoretical frequency distribution ($W^2 = 0.153$, P
 398 = 0.25). Figure 8C shows the empirical frequency distribution and the theoretical model fit. Table 5
 399 shows the risk-based action thresholds derived from the Gamma probability density function. The
 400 series of action thresholds allow growers with different levels of aversion to risk to select the
 401 threshold that fits their philosophy or financial vulnerability.

402 **Table 5.** Action thresholds for cumulative male *D. suzukii* per trap and the expected probability of infestation
 403 the week following last trap capture (1- probability estimated from the 2 parameter Gamma density function).

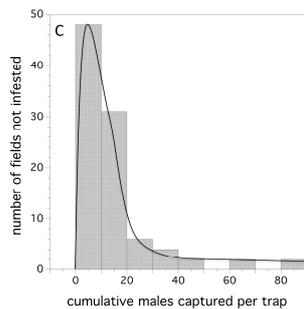
Action threshold: cumulative males captured / trap	Probability of infested fruit: the week following trap capture
0.25	0.001
0.50	0.005
1.0	0.01
2.0	0.05
3.5	0.1
7.0	0.25
16.0	0.5

404

405



406



407 **Figure 8.** Nonlinear exponential relationship between percent infested wild blueberry fruit and cumulative
 408 male *D. suzukii* trap capture (logarithmic transformed) prior to harvest (A), percentage ripe fruit as the harvest
 409 season progresses (2016), error bars are standard errors (B), gamma theoretical distribution and empirical
 410 frequency distribution (2012-2017, n=92 fields) comprising fields with mean numbers of cumulative male trap
 411 captures prior to harvest and the likelihood of not being infested the following week after the cumulative trap
 412 capture (C).

413 Validation of the thresholds from 2016-2018 is depicted in Table 6. It can be seen that in 2016
 414 and 2017 the observed infested fruit levels associated with the selected action thresholds were
 415 similar to the expected model predicted levels.

416 **Table 6.** Validation of action thresholds in commercial wild blueberry fields (2016-2018).

Year	Action threshold cumulative males per trap	Number of fields	Observed Proportion infestation rate ¹ (proportion fields ²)	Model predicted ³ proportion fields infested
2016	1	3	0, 0, 0 (0)	0.01
	3	3	0, 0, 0 (0)	0.08
	9	5	0, 0, 0, 0.003, 0.005 (0.40)	0.30
2017	1	3	0, 0, 0 (0)	0.02
	3	3	0, 0, 0 (0)	0.08
	6	4	0, 0, 0, 0.006 (0.25)	0.23
	1	3	0, 0, 0.01 (0.33)	0.01 ⁴
2018	3.5	6	0, 0, 0, 0.02, 0.03, 0.05 (0.50)	0.10
	7	2	0, 0.04 (0.50)	0.25

10

3

0.02, 0.01, 0.003 0.50
(1.00)417 ¹ Percent infested fruit sampled within a field.418 ² Sampled proportion of fields that were infested with *D. suzukii* larvae the week following the action threshold
419 was reached or the field was harvested.420 ³ Expected proportion of fields (predicted from probability model) associated with the selected action threshold
421 that will be infested with *D. suzukii* larvae.422 ⁴ Bold expectations differ in a large degree from the observed.423 **4. Discussion**

424 *Drosophila suzukii* is a major insect pest of commercial raspberry, blackberry, elderberry,
425 highbush blueberry and wild blueberry in Maine. However, the most widely grown fruit crop in
426 Maine is wild blueberry. This insect species was first observed as a damaging pest in 2012, despite
427 intensive sampling in 2010 and 2011. Briem et al. [16] in a long-term trapping study cautioned about
428 regarding trap capture as absolute population density. We agree that their warning is warranted. In
429 a previous study we showed that only 15-20% of flies in a caged population are captured by the
430 traps that we used in this study [44]. In addition, Briem et al. [16] show that trap captures for *D.*
431 *suzukii* can be affected by the environment surrounding the trap and weather conditions. This has
432 been shown for other insect species as well [45,46]. Therefore, in this study we refer to trap capture
433 as a relative abundance estimate of *D. suzukii* and that trap captures only provide an index of
434 population density. While we have observed its rate of trap capture to increase logarithmically,
435 within a growing season, this is not related to the production system or the rate of degree day
436 accumulation during the summer. Most crop production systems in other states also are
437 characterized by explosive logarithmic rates of trap captures during the stage that ripe fruit is
438 available for *D. suzukii* [47-49]. The rate of increase in trap capture during the growing season was
439 explained by a negative relationship with degree day accumulation in the spring. It is not clear why
440 cooler springs would result in a higher rate if trap capture increase in the late summer. We speculate
441 that either the rate of spring degree day accumulation is negatively correlated to a causal factor that
442 we did not measure or that it might be related to overwintered adult synchrony with spring
443 available food resources such as early ripening fruit (ex. *Cornus canadensis* L. (bunchberry), *Lonicera*
444 spp. (honeysuckles) and *Rubus* spp. (dewberry species) and/or early fungi that produce fruiting
445 bodies.

446 The relative abundance of *D. suzukii* (trap captures) is increasing annually in Maine. We found
447 that the date of first detection determined cumulative relative abundance of flies at a biofix of 705
448 DD. The earlier flies were captured in a field, the higher the relative abundance at this point in the
449 growing season. Pelton et al. [13] found that in higher altitude woodlands, flies were detected earlier
450 in the season than in low croplands, but unlike our study, they did not find any difference in fly
451 relative abundance due to earlier detection. Briem et al. [16] found that the rate of degree day
452 accumulation in the summer determined seasonal relative abundance of flies. We might be
453 witnessing the same phenomenon, but it might be confounded by the increasingly earlier detection
454 of flies in wild blueberry fields.

455 Our data also suggests that this increase is more likely to be oscillating and not a simple linear
456 trajectory. An annual increase over the seven-year period suggests that *D. suzukii* overwinters in
457 Maine; although, experimental studies of ours and others, suggest that the survival rate in the
458 northern U.S. is low [28]. The oscillating nature of increase might suggest that predation is
459 dampening *D. suzukii* population increase from one year to another. We have shown that predation
460 can reach 100% of deployed sentinel *D. suzukii* pupae in commercial wild blueberry fields [21]. Some
461 winters have been more severe for overwintering than others during the seven-year study. The
462 winter of 2017-2018 was one of the colder winters in recent years with extreme temperatures below
463 -26° C in southern Maine. This was 9-10° C colder than in 2016 or 2017. Therefore, the large drop in
464 relative abundance from 2017 to 2018 could be due to the extreme harsh winter. A negative
465 correlation between summer abundance of *D. suzukii* and the previous winter temperatures is
466 expected. There is evidence that while *D. suzukii* can over winter in north temperate US climate

467 zones, survival rates are very low, suggesting that northern states such as Maine might be close to
468 this pest's potential northern range [26-28]. Our analysis of relative abundance from one year to the
469 next showed that warmer winter temperatures resulted in higher relative abundance in wild
470 blueberry fields the subsequent year. A similar finding by Briem et al. [16] provided evidence in a
471 seven-year study in Germany that mild winters can influence relative abundance of flies.

472 Crop production system does not appear to affect relative abundance of *D. suzukii* adults in
473 wild blueberry fields, other than indirectly by determining the number of insecticides that are
474 applied during the period of *D. suzukii* buildup. One would think that because insecticide
475 applications reduce fruit infestation, that this would translate into lower *D. suzukii* relative
476 abundance. However, the explosive logarithmic increase in trap captures prior to harvest suggests
477 that insecticides are functioning more as crop protectants from fruit infestation than reducing
478 buildup of fly populations.

479 Crop stage does affect fly populations as measured by the relative abundance of flies. Fruit
480 bearing fields have significantly higher relative abundance than vegetative fields (no commercially
481 produced fruit present). In addition to showing the importance of wild fruits in the relative
482 abundance of *D. suzukii* in many crops, wild blueberry included [38], the presence of *D. suzukii* in
483 vegetative fields is another way that wild non-crop fruits serving as host reservoirs can maintain
484 populations in a field from one year to another, despite their being crop fruit present only every
485 other year. These vegetative field populations may be important if they migrate seasonally to crop
486 field habitats. Tait et al. [32] demonstrated that seasonal migration over large spatial scales (ca. 9 km)
487 in Italy might facilitate movement to exploit resources in patchy environments, such as crop fields in
488 the wild blueberry production regions in Maine. Briem et al. [16] also found that different habitats
489 affected relative abundance of *D. suzukii* in Germany, but in their case, forest edges and hedgerows
490 had higher population abundances than crop fields. This is opposite of what we found.

491 We found that the first detection of *D. suzukii* adults in traps was earlier with each year of the
492 infestation. We have not found this phenomenon reported by other researchers. This may reach a
493 constant date once the population becomes more adapted to Maine climates, as has been
494 documented with other invasive organisms [17]. This earlier detection of flies in traps was not
495 directly associated with earlier infestation rates in wild blueberry fields. However, we did find an
496 indirect relationship. Earlier detection results in higher relative abundance of flies, and higher
497 relative abundance of flies results in higher fruit infestation rates.

498 Fruit infestation rates are relatively low in Maine wild blueberry, ranging from 0-5%. Very little
499 has been reported in the literature about fruit infestation by *D. suzukii* in various crops Dal Fava et al.
500 [48] published an economic cost / benefit analysis for fruit production in Italy. Their conclusion was
501 that fruit infestation was not really the measure of crop loss. It was the frequency of insecticide
502 applications that made profit less likely, especially under high fly pressure. However, it is not the
503 absolute crop loss that many growers worry about, but more the detection of any larvae in fruit that
504 is bound for markets with low tolerance, such as foreign export markets [49]. As mentioned
505 previously, we did find that fruit infestation level is related to adult relative abundance, but
506 additional variation in fruit infestation is explained by year to year variation (we suspect this is due
507 to variation in weather conditions), and insecticide applications. Again, crop production system was
508 indirectly related to fruit infestation level because crop production system determines the number of
509 insecticide applications applied during the late summer. There has not been a lot of data
510 documenting commercial "field-level" effectiveness of multiple insecticide applications in growers
511 commercial fields in reducing fruit infestation. But more specifically, the relationship between
512 increased frequency of applications and the subsequent increased reduction of infestation. A few
513 studies in other states and crop systems have shown that insecticide applications in general reduce
514 fruit infestation [50-52]. However, in commercial wild blueberry fields, our data suggests that, on
515 average, more than two applications targeting *D. suzukii* during the growing season will have
516 limited added benefit in reducing fruit infestation.

517 A very interesting finding of ours is that sex ratio relative to males has been declining over the
518 seven year period (Fig. 5B). However, the regression on sex ratio appears to be highly leveraged by

519 the unusually high sex ratio in 2012. When male abundance is compared to female abundance over
520 the entire 7-year period, the proportions of male and female flies appear relatively constant as a
521 function of relative abundance (Fig. 5A). Therefore, we are not confident that the trend we have
522 observed in declining sex ratio relative to males is real. In addition, because we have been observing
523 an increase in relative abundance over the 7-year period, a drop in male sex ratio would not be
524 hypothesized to a cause such as *Wolbachia* spp. infection. *Wolbachia* bacteria has been observed in
525 high prevalence levels in Europe, but high infection rates and reproductive manipulation have not
526 been observed in North American *D. suzukii* populations [53]. We have not sampled and assayed
527 flies in Maine to verify this.

528 The invasion of *D. suzukii* has disrupted well-established effective integrated pest management
529 programs throughout the U.S. [50,51,54]. Some fruit production systems in North America increased
530 insecticide applications dramatically for the prevention of fruit infestation [51,55,56]. Biological
531 control and removal of non-crop fruit plants in the vicinity of crops have been presented as
532 alternatives or complimentary management tactics for this invasive pest [19,20,21,33,34,37]. We have
533 found from two studies in wild blueberry, this one and Ballman and Drummond [38], that increased
534 abundance of non-crop fruit along field edges results in increased fly pressure in the crop as
535 measured by trap capture. We have also found that predation of *D. suzukii* pupae is high in wild
536 blueberry fields [21], but before this study, we did not have any measure of the impact of natural
537 enemies on relative abundance of flies in fields. We showed that natural enemies do reduce fly
538 pressure, but only in fields that have high non-crop wild fruit abundance. We interpret this in the
539 following way. Fields that have high non-crop wild fruit abundance have higher fly pressure and
540 higher rates of reproduction in fields or in non-crop wild fruit refugia. Under high *D. suzukii* relative
541 abundance and reproduction, natural enemies numerically respond, and under this situation, reduce
542 pest populations. However, because the fly pressure is so high under this scenario, the amount of
543 reduction in the fly population does not appear to be capable, on its own, of reducing pest pressure
544 to a level that reduces risk for growers. This dynamic needs to be researched further and in other
545 crop systems.

546 We were able to develop action thresholds between 2012 and 2017. A risk aversion gradient
547 approach was taken [57]. A risk-based action threshold is appropriate given the different production
548 systems that exist in Maine for wild blueberry and given that markets range from local fresh market
549 roadside sales, to the export of frozen berries, to foreign markets. Therefore, growers can select the
550 threshold level that best suits their capital investment and perceived risk due to *D. suzukii* damage
551 potential. Other than, the first occurrence of flies in traps, we do not know of any other fruit crop
552 IPM programs that utilize action thresholds for management of *D. suzukii*. Wild blueberry growers
553 are currently using these thresholds [2]. The thresholds were well supported by the 2016 and 2017
554 validation studies. There was only a minimal departure between observed and expected proportion
555 of infested fields resulting from the selection of each specific threshold value. In 2018, while the early
556 harvest tactic was still highly effective, the thresholds did not prove to match up the expected with
557 the observed proportion of fields with infested fruit. So, in two of three years the thresholds worked
558 well. This threshold system does have a buffer for preventing unexpected losses. We recommend
559 that growers take a fruit sample to determine the percent fruit infestation before they reach
560 threshold levels. In this way growers can have confidence in this decision making tool and also catch
561 any damage before it gets too high if the thresholds break down. However, in all three years, the
562 infestation rate of 24 of 35 fields (68.6%) in our validation test were well predicted by our probability
563 model. Even in 2018, most of the infestation levels were between 1-3% and so infestation rates were
564 still relatively low in the absence of insecticides. Clearly, we need to conduct several more years of
565 validation in order to verify that these thresholds minimize the risk for growers, or at the very least,
566 determine how likely the thresholds are to provide accurate predictions. We can only speculate why
567 the thresholds did not perform well in 2018. That summer was one of the hottest summers on record
568 for Maine, with extreme high air temperatures of 35° C for both July and August. We hypothesize
569 that the fermentation bait we recommend either lost its attraction to flies, or that under extremely
570 hot conditions, flies are reluctant to enter the traps. In either case, the threshold based upon trap

571 capture would be underestimating cumulative male fly abundance. Briem et al. [16] showed
572 evidence of high temperatures diminishing trap efficacy for *D. suzukii*. This might be a phenomenon
573 that needs more attention in the future, especially as climate change is predicted to result in hotter
574 future summers in many north temperate fruit growing regions.

575 5. Conclusions

576 *D. suzukii* is a serious pest in Maine wild blueberry characterized by rapid increase during the
577 growing season. It is still increasing in abundance seven years after initial invasion in 2012 and
578 adults are detected in wild blueberry fields earlier in the growing season each year. The milder the
579 previous winter and the earlier the detection, the higher the fly pressure (cumulative trap capture at
580 biofix 705 DD) during the growing season. The level of fruit infestation is determined by year, fly
581 pressure, and insecticide application frequency. Insecticide frequency is determined by the
582 production system. Non-crop wild fruit and predation influence fly pressure in fields. Increase in
583 abundance of non-crop wild fruit along field edges results in increased fly abundance. Increased
584 predation in response to higher fly pressure reduces the rate of fly pressure increase. However,
585 predation only appears to operate significantly at high relative abundance of flies, or when high
586 levels of wild fruit are present along field edges and this may not be enough to avert risk of
587 infestation for growers. Early harvest is a highly effective means of avoiding fruit infestation, but
588 there is a cost due to harvest of immature fruit. This cost diminishes as the season progresses, but the
589 likelihood of infestation increases. Action thresholds that were developed and used by growers in 35
590 commercial fields, based upon cumulative male captures, worked remarkably well in two of three
591 years. Future research will be focused on continued evaluation and possible fine-tuning of the
592 risk-based thresholds.

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