



## Where and what to feed? Differential effects on fecundity and longevity in the invasive *Drosophila suzukii*

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### Abstract

Successful establishment of invasive species requires that the species meet environmental conditions favouring their longevity and fecundity. When juveniles and adults consume different resources, gravid females may have to choose whether to feed or to reproduce. We used a successful invasive species to test whether female life history traits are affected by the potential nutrients found in reproductive sites. The pest species, *Drosophila suzukii*, lays eggs in fruits that could provide nutrients since fruit exudates are generated during oviposition. We demonstrated that *D. suzukii* adults cannot survive when they have only access to reproductive sites (*i.e.* undamaged fruits). But they can find the nutrients necessary for longevity on these reproductive sites if there are larger holes in the exocarp. Egg maturation is low when *D. suzukii* feeds on nutrients from fruits, even when damaged, suggesting that females cannot acquire sufficient nutrients for egg maturation where they lay their eggs. Finally, a field experiment supported our laboratory results: wild females have a low degree of egg maturation, even when captured near reproductive sites. Our study hence shows constraints on egg production, and therefore on fruit infestation, determined by the nutritional ecology of pest females.

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### Introduction

Nutrition constrains most biological processes by affecting growth, survival, movement and reproduction, each a key component of fitness (Barrett, Hunt, Moore, & Moore 2009; Joern & Behmer 1997; Slansky 1982). Nutritional

ecology – the study of nutritional interactions between organisms and their environment – is gaining increased interest from researchers (Lihoreau et al. 2015; Llandres et al. 2015; Raubenheimer, Simpson, & Mayntz 2009). Initially focusing on the energy intake for an individual (optimal foraging theory, MacArthur & Pianka 1966; Ydenberg, Welham, Schmid-Hempel, Schmid-Hempel, & Beauchamp 1994; Raubenheimer et al. 2009), studies on nutrition have shifted toward a more realistic approach, taking into account the nutrient composition of food and that animals must optimize their food intake of both energetic macronutrients

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(carbohydrates, proteins, lipids) and/or non-energetic nutrients (water, mineral salts, vitamins, antioxidants). Dealing with the multi-dimensionality of food sources, some species are able to regulate their intake of food to reach an optimal ratio of nutrient intake (Fanson, Yap, & Taylor 2012; Kyriazakis, Emmans, & Whittemore 1991; Lee et al. 2008; Raubenheimer & Simpson 1997; see Behmer (2009) for review of insect herbivores).

In insects, the decision to select a specific resource depends on factors such as nutritional status, previous feeding experience, mating status, and egg load in the ovary (Behmer 2009; Jervis, Eilers, & Harvey 2008; Minkenberg, Tatar, & Rosenheim 1992). The heterogeneous spatial repartition of resources in the environment is thus an important constraint on the foraging strategy of insects. As food resources are usually unbalanced and fluctuating, suitable nutrients may be locally unavailable or in limited quantity, causing physiological trade-offs (Roff 1992; van Noordwijk & de Jong 1986). Furthermore, if the resources associated with the two main fitness components, survival and reproduction, are spatially separated, another trade-off is expected between the amounts of time allocated to searching each of these specific resources. Females will thus need to decide between searching for food (which leads to a delayed fitness gain), mating or searching for suitable sites for their progeny (direct fitness gain) (Desouhant, Driessen, Amat, & Bernstein 2005; Refsnider & Janzen 2010). Such a trade-off would be even more important if adults and juveniles do not feed on the same resources (de Roos & Persson 2013) and if the energy-expensive activity of flying was necessary to achieve these functions (Amat et al. 2012; Harrison & Roberts 2000).

In the context of invasion, foraging strategies contribute to the successful establishment and spread of alien species (Evans, Inta, & Lai 2011; Sol & Lefebvre 2000). Davis, Grime, and Thompson (2000) predict that a successful invasion will be favoured by a high density of resources due to either a large amount of unused resources or a low utilization by resident species. This prediction has been validated in plants but in few animal species (Catford, Jansson, & Nilsson 2009; Elton 1958; Stachowicz & Tilman 2005). Our main objective is to understand the nutritional ecology of an invasive pest species, the spotted wing drosophila *Drosophila suzukii*.

This Asian species is a successful invader: it was observed for the first time in Europe and North America in 2008 (Asplen et al. 2015; Cini, Ioriatti, & Anfora 2012), and since then, it has spread further, even reaching Mexico in 2011, Brazil in 2013, and La Reunion in 2014. The nutritional constraints of *Drosophila* are well known in *Drosophila melanogaster*. This species is particularly sensitive to desiccation and starvation (Aguila, Suszko, Gibbs, & Hoshizaki 2007; David, Allemand, Van Herreweghe, & Cohet 1983; Hoffmann & Harshman 1999). Moreover, *Drosophila* eggs are yolk-rich, primarily composed of proteins and secondarily of lipids (Beenackers, Van der Horst, & Van Marrewijk 1985) and thus costly to produce. Most frugiv-

orous *Drosophila* species aggregate on decaying fruits for feeding, mating and egg laying. On decaying fruits, they find carbohydrates (in the juice) to survive and proteins (in yeasts) to mature eggs (Begon 1982; Markow & O'Grady 2008). However, unlike these other *Drosophila* species, *D. suzukii* can also insert eggs in ripening and healthy fruits (Bellamy, Sisterson, & Walse 2013; Kim et al. 2015; Lee et al. 2011, 2015; Poyet et al. 2015; Steffan et al. 2013; Stewart, Wang, Molinar, & Daane 2014), thanks to its serrated ovipositor (Atallah, Teixeira, Salazar, Zaragoza, & Kopp 2014), and can be found on fungi (Kimura 1976; Kimura, Toda, Beppu, & Watabe 1977) and occasionally on flowers (*Styrax japonicus*, Mitsui, Beppu, & Kimura, 2010).

Regarding the successful invasion of *D. suzukii*, we expected that healthy fruits provide sufficient nutrients to fuel the adult's reproductive and maintenance functions. To investigate this question, we tested the effects of different artificial and natural food resources on longevity and fecundity usually involved in a trade-off. We first determined nutritional resources necessary for these two traits and whether healthy fruits, used as reproductive sites, are also feeding sites. In a second set of experiments, we test if the oviposition behaviour could improve the access to nutrients from the reproductive sites. In *D. suzukii*, sawing of the fruit exocarp during oviposition may permit access to the fruit juice of healthy fruit. Finally, we investigated the nutritional status of females caught in the field to test whether they find nutrients, particularly in the absence or presence of reproductive sites.

## Materials and methods

### Biological model

Adult *Drosophila* females need specific nutrients, such as water, carbohydrates and yeast, to survive and mature their eggs (Anagnostou, Dorsch, & Rohlf 2010; Becher et al. 2012; Drummond-Barbosa & Spradling 2001; Rohlf & Kürschner 2010), but *Drosophila*'s feeding behaviour and needs outside of the lab are poorly documented. After piercing the exocarp of the fruit to lay one egg in the hole, *D. suzukii* females often drink a drop of the fruit juice that leaks from the wound (pers. obs. See Appendix A- Supplementary material: Video).

We used a *D. suzukii* strain founded by approximately 20 females trapped in Sainte-Foy-les-Lyon (France; North: 45°44'23.98", East: 4°47'26.79") in May 2012. This strain was maintained on *D. melanogaster* medium (=DM medium; David & Clavel 1965) in a climatic chamber SANYO (MLR-351H) at 20 °C (min = 19.5 °C, max = 20.5 °C), a relative humidity of 60 % (min = 57%, max = 65%) and a 12 h:12 h light/dark cycle. The experiments were carried out in the spring of 2014.

**Table 1.** Experimental feeding treatments (labelled from “A” to “H”) that differ in the content and quality of nutritional sources. Two types of fruit maturation (fresh and decaying) were provided to *Drosophila suzukii* females (treatments D–H). Fruit access represents the surface of fruit that was accessible to females for feeding. Access to the fruit was manipulated (from a hole of 1 mm in diameter to total access in a cut fruit). N represents number of flies used for longevity measurement in Experiment 1.

Feeding treatment	Type	Composition	Fruit access	N
A	Control	Distilled water	–	28
B	Control of longevity	Distilled water + Sucrose 2 %	–	33
C	Control of egg maturation	Distilled water + Sucrose 2 % + Yeast 5 %	–	33
D	Undamaged fruit	Fresh blueberry	–	28
E	Damaged fruit	Fresh blueberry	With a hole of 1 mm	20
F	Damaged fruit	Fresh blueberry	With a hole of 3 mm	12
G	Damaged fruit	Fresh blueberry	Two halves of a cut fruit	31
H	Damaged decaying fruit	Decaying blueberry	Two halves of a cut fruit left outside the fridge for 3–5 days	32

### Experiment 1 – effects of reproductive and feeding sites on longevity and fecundity

This experiment investigated the influence of various nutritive resources on female longevity and fecundity and whether the presence of physical damages on the reproductive resources (healthy fruits) or the presence of decay could modulate the resource effects. The entire experiment was conducted at once, but for clarity reasons, we present results in two sections. Controls are thus the same in each section.

We used blueberries of the *Blue Aroma* and *Royal Blue* cultivars, produced in Morocco in March 2014. Blueberries are good oviposition sites for *D. suzukii* (Bellamy et al., 2013). We established 8 feeding treatments labelled from “A” to “H” (Table 1). Comparing specific treatments allowed for testing precise hypotheses. Yeasts were provided by Lynside® Nutri (Lesaffre Brand), the composition of which is in Appendix A- Supplementary material: Table 1. Decaying fruits were fruits left outside of the refrigerator individually put in plastic boxes for 3–5 days. Resources were renewed every 2 days to maintain their state constant throughout the experiment. “Water + Sucrose” (B) and “Water + Sucrose + Yeast” (C) were controls for low quantity and maximum quantity, respectively, for oocyte maturation (Drummond-Barbosa & Spradling 2001).

The tested individuals were randomly chosen from among freshly emerged females from the mass rearing population. Females had no access to food before experiments. We used virgin flies to avoid the potential effect of oviposition on longevity and fecundity. *D. suzukii* adults were sexed after brief ether anaesthesia within 3 h after emergence. Individuals were randomly assigned to the feeding treatments by “box group”, i.e., by groups of 4 females in plastic boxes (diameter 83 mm × height 53 mm). Due to limited emergence number of flies, females were not assigned equally among all feeding treatments. The females were introduced into the plastic boxes containing one of the resource treatments. Each nutritive resource was put in a Petri dish (diameter ca. 55 mm)

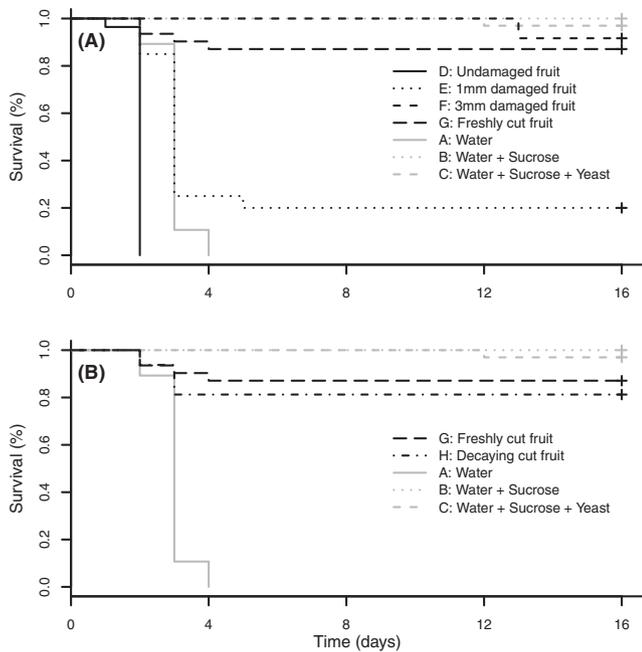
and was placed in a climatic chamber SANYO (MLR-351H) at a temperature of 21 °C (min = 20.5 °C, max = 21.5 °C), a relative humidity of 75% (min = 69 %, max = 79%) and a 12 h:12 h light/dark cycle.

Longevity was estimated daily by counting the number of live individuals in each experimental box for 16 days. Potential fecundity was estimated, after female dissection, as the number of “mature oocytes” (stage 14, last stage of maturation (King, 1970)), present in one randomly chosen ovary. Stage 14 was chosen because, at this stage, oocytes are not subjected to resorption in *D. melanogaster* (Drummond-Barbosa & Spradling, 2001) and are ready to be fertilized and laid. Oocyte counts were carried out 8 days and 16 days after female emergence on surviving females from 7 to 8 randomly chosen boxes per feeding treatment and per date (in total, 61 females for Treatment B, 63 females for Treatment C, 51 females for Treatment G and 53 females for Treatment H. See Table 1 for names). Since we had to sacrifice females to count oocytes, we dissected for each treatment an extra group of 8-day-old females whose longevity was not recorded. Oocytes were counted after dissection under a stereo microscope.

To control for the effect of body size on longevity and fecundity, the length of the right wing (i.e., distance between vein r-m and the end of vein R<sub>4+5</sub> at the wing tip) of a sample of 16-day-old females was measured. This distance is known to correlate with thorax length, longevity and fecundity in *D. melanogaster* and *D. simulans* (Tantawy & Rakha, 1964). Feeding treatments did not differ significantly in terms of wing size (ANOVA; F<sub>3,133</sub> = 2.08; P = 0.106). In total, the longevity of 248 individuals was measured, and 228 individuals were dissected for estimation of potential fecundity.

### Experiment 2 – effects of reproduction behaviour on the longevity of females

The second experiment tested whether egg laying sites can be used as feeding sites after oviposition. Thus, we tested

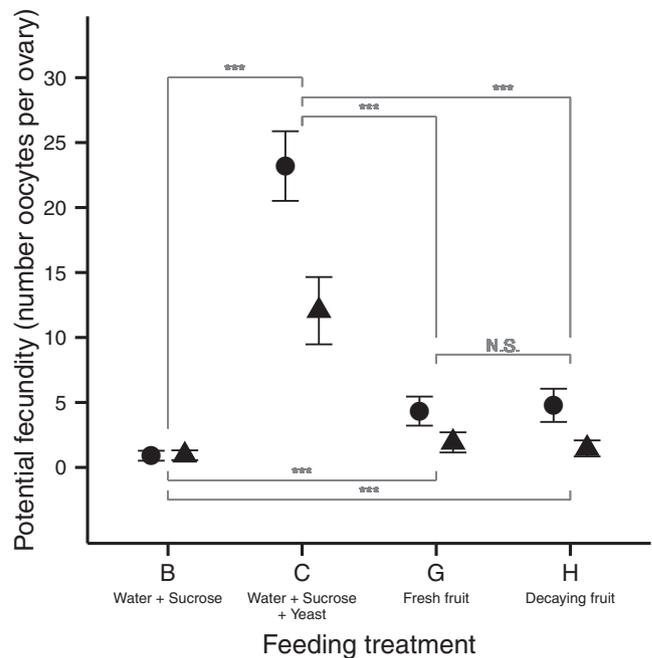


**Fig. 1.** Survival curves of virgin females of *Drosophila suzukii* feeding on different feeding treatments (Experiment 1): (A) effect of the access size (B) effect of fruit decay. The observation lasted 16 days. Survival curves are estimated using parametric models of survival with a Weibull distribution. For more information about feeding treatments and sample sizes, see Table 1.

whether perforation of the fruit exocarp by mated females permits feeding and thus influences longevity. Conditions in this experiment were similar to those in Experiment 1. Newly emerged females were sexed and assigned to either “Mated” or “Unmated” treatments. In the “Mated” treatment, groups of 4 females were placed with 2 males, and in the “Unmated” treatment, groups of 4 females had no access to males. Matings were allowed for three days, during which the flies were fed sucrose *ad libitum* (agarose + 1% of sucrose + nipagine medium). The 4 females in each vial, but not the males, were then exposed to “Undamaged fruit” (D) and the controls “Water” (A), “Water + Sucrose” (B) and “Water + Sucrose + Yeast” (C) (14 groups of 4 females per treatment using the same boxes as in Experiment 1 with fresh cherry instead of blueberry). Cherries were of the *Regina* cultivar and had been produced in Bessenay (France) in July 2014. Resources were renewed every two days to maintain their state constant throughout the experiment. Longevity was measured over 7 days (441 individuals in total).

### Experiment 3 – nutritional status of wild females

We characterized the nutritional status of wild caught *Drosophila* females by observing their ovaries. Ovary state in *D. melanogaster* is known to mirror nutritional intake in the previous 24 h (Drummond-Barbosa & Spradling 2001). Maturation of oocytes occurs irrespective of the mating status (Bouletreau-Merle 1973), and the degree of maturation is

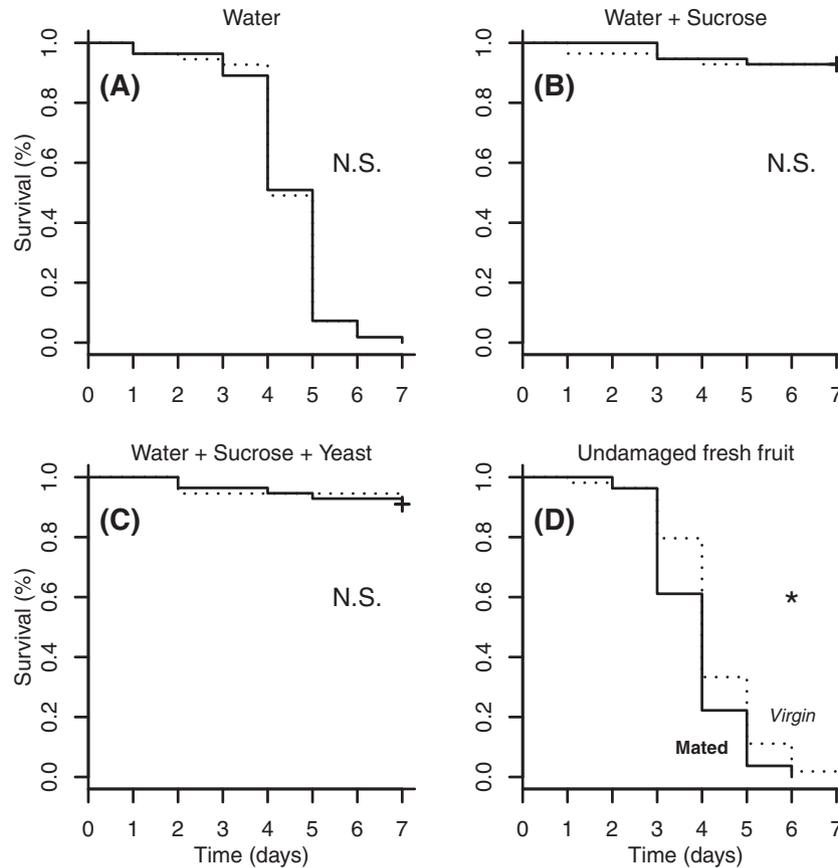


**Fig. 2.** Potential fecundity of virgin females fed on different feeding treatments and at 8 days (circles) and 16 days (triangles; mean  $\pm$  95% CI). Potential fecundity is measured as the number of mature oocytes found per ovary. Feeding treatments correspond to “Water + Sucrose” (B,  $n = 61$ ), “Water + Sucrose + Yeast” (C,  $n = 63$ ), “Freshly cut fruit” (G,  $n = 51$ ) and “Decaying cut fruit” (H,  $n = 53$ ). Statistical tests were performed by the contrast method in the Generalized Estimating Equations model, where age classes were pooled in each treatment. \*\*\*:  $P < 0.001$ ; N.S.: not significant.

limited mostly by protein intake (Mahowald & Kambyzellis 1980). Thus, the degree of oocyte maturation in the ovaries of females caught in natural conditions was compared with that of females reared under controlled conditions (*i.e.*, laboratory experiments).

We first characterized the maturation of *D. suzukii* ovaries of virgin females fed in the laboratory (under the same conditions as Experiment 1) on four different diets varying in nutrient composition. The females were fed either on poor resource “S” (a filter paper with distilled water + 2% sucrose), intermediate resource “FR” or “DM” (FR: red fruit *Drosophila* medium; DM: *D. melanogaster* medium; see Appendix A- Supplementary material: Table 2 for composition) or rich resource “Y” (a filter paper with distilled water + 2% of sucrose + 5% of yeast). One ovary of each female was randomly chosen, and 4 measurements were taken with an optical microscope (ZEISS, Axio Images Z1, Axiovision Software v4.8.1) as follows: length, width and area of the ovary and number of mature oocytes in the ovary.

In a second step, the ovary structures of 86 wild females were analysed to assess nutritional status. The same measurements as described for laboratory individuals were made. We observed in Experiment 2 that ovary structure, and therefore oocyte maturation, were similar in virgin and mated females (see Appendix A- Supplementary material: Text-Figure-



**Fig. 3.** Survival curves of females of different mating status (dotted lines for virgin, solid lines for mated flies) on different feeding treatments: (A) on “Water” (A,  $n = 110$ ), (B) on “Water + Sucrose” (B,  $n = 112$ ), (C) on “Water + Sucrose + Yeast” (C,  $n = 111$ ), and (D) on “undamaged fresh fruit” (D,  $n = 108$ ). Survival curves are estimated using parametric models of survival with a Weibull distribution. Statistical tests in each panel were Log Rank tests. \*:  $P = 0.034$ ; N.S. is not significant for mating status effect.

Table 3). Comparing virgin lab individuals and likely-mated, field individuals is hence relevant. Wild females were trapped in October 2014 (during the period of highest density in this area) in three landscape types, including the reproductive sites of the insects: a landscape of apple and cherry orchards without flowers and fruits (26 females in Saint-Marcel-lès-Valence, France; North:  $44^{\circ}58'19.3''$ , East:  $4^{\circ}55'39.2''$ ), a landscape of forest (40 females in Saint-Marcel-lès-Valence, France; North:  $44^{\circ}58'30.2''$ , East:  $4^{\circ}55'41.9''$ ) and a landscape of kiwi orchards with fruits (20 females in Etoile sur Rhône, France; North:  $44^{\circ}49'03.48''$ , East:  $4^{\circ}52'53.25''$ ). Females were trapped alive using a white plastic trap full of cotton soaked in a mixture of red wine and apple cider vinegar (Landolt, Adams, & Rogg 2012). The traps remained in the field for 24 h, and the insects were dissected less than 12 h post-collection.

### Statistical analysis

Longevity was analysed using parametric models of survival (Weibull distribution) with “feeding treatment” as a fixed factor and “box group” as a random factor in Experiment 1 and with “feeding treatment” and “mating status”

(and their interaction) as fixed factors and “box group” as random factors in Experiment 2. The random factor “box group” was integrated using a shared frailty model (Therneau & Grambsch 2000) to tackle the correlation between observations of the same box group. The shared frailty model considers the shared risk for distinct categories of dying due to unmeasured variables. The survival models allowed consideration of the censored data, *i.e.*, individuals not dead before the end of the experimental observation (Kleinbaum & Klein 2012). Potential fecundity in Experiment 1 was analysed using Generalized Estimating Equations (GEE, Liang & Zeger (1986)), a model (Poisson distribution with a log link) for integrating the box group as clusters (random factor). The GEE is useful when clusters are small. The explanatory variables were “feeding treatment” and “age” as fixed factors. Potential fecundity in Experiment 3 was analysed using a Generalized Linear Model (Poisson distribution with a log link) with “landscape type” as a factor.

To analyse the effect of landscape on wild females, we performed a linear discriminant analysis (LDA). This allowed for determining how ovary maturation (based on morphological measurements: length, width and area) contributes to the correct classification of individuals into the diet. This classi-

fication, which is based on insects in controlled conditions, helped predict the nutritional status of wild females. We built predictive models using two-thirds of the laboratory insects. The remaining third of the laboratory insects was used to estimate the proportion of flies correctly assigned to the correct diets. This process was repeated 100 times (using different subsets of flies), and on average, 68% of the laboratory flies were assigned to their original diets. We retained a model with an assignment rate of 68%, which was used to assign wild flies to categories of food quality.

R software (version 3.1.3, R Core Team 2015) was used to perform the statistical analyses. Packages survival 2.38–1, geepack 1.2–0 and MASS 7.3–39 were used for the survival analysis, the fecundity analysis and LDA, respectively.

## Results

### Experiment 1 – effects of reproductive and feeding sites on longevity and fecundity

#### Effect of fruit damage on longevity

The longevity of virgin females was significantly affected by feeding treatment ( $\chi^2 = 336$ ;  $df = 8.6$ ;  $P < 0.001$ ) (Fig. 1). Two distinct groups of feeding treatments emerged (Fig. 1A): a group with a high mortality (A, D, and E, see Table 1) and a group with a low mortality (B, C, F, G). “Water + Sucrose” (B) belonged to this second group, with medians greater than 16 days, showing that sucrose is an important nutrient for longevity.

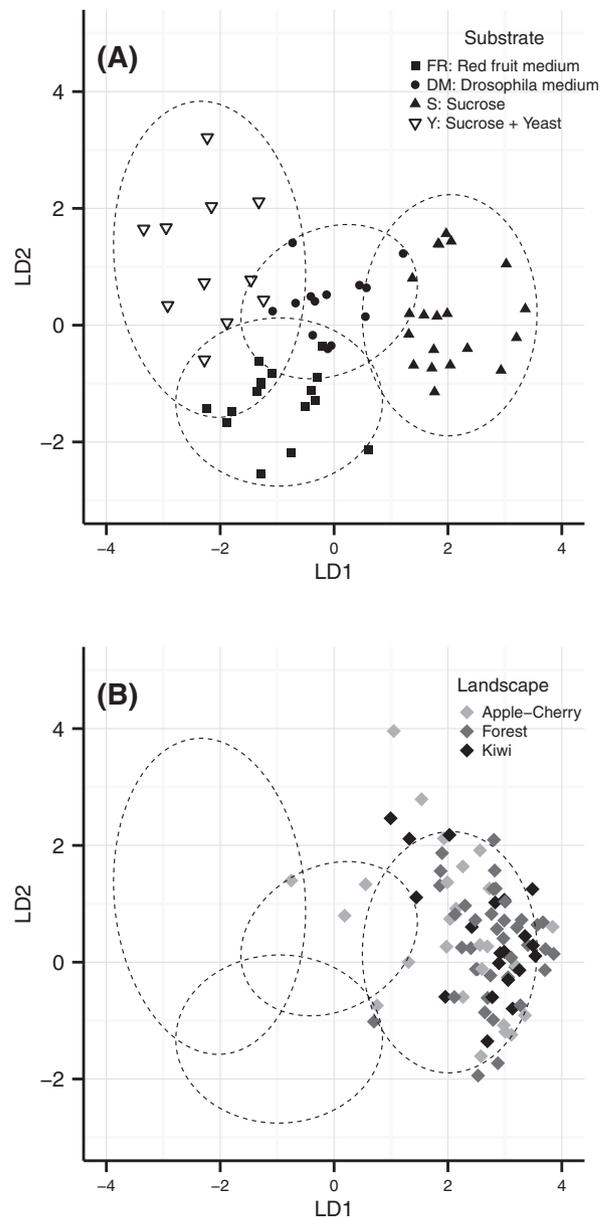
Access to water also had a significant, beneficial effect on survival, as revealed by the comparison between the treatments “Water” (A) and “Undamaged fruit” (D) ( $z = -3.5$ ,  $P < 0.001$ ). Females in Treatment A lived longer (median = 3 days, Fig. 1A) than the females in Treatment D (median = 2 days). This suggests that virgin females that did not lay eggs did not use water inside the fruit.

When comparing fruits without wounds (D), with a 1 mm hole (E) and with a 3 mm hole (F), significant differences were observed (D vs E ( $z = 8.8$ ,  $P < 0.001$ ); E vs F ( $z = 3.9$ ,  $P < 0.001$ )). Larger holes helped flies live longer. However, there was no significant longevity difference between the flies exposed to fruits with 3 mm holes (F) and the flies exposed to cut fruits (G) ( $z = -0.47$ ,  $P = 0.64$ ).

#### Effect of fruit decay on longevity and fecundity

Longevity on “Freshly cut fruit” (G) was similar to that on “Decaying cut fruit” (H) ( $z = -0.75$ ,  $P = 0.45$ , Fig. 1B) and on “Water + Sucrose” (B) ( $z = 1.1$ ,  $P = 0.29$ ). Thus, in damaged fruits, females found nutrients that allowed for longevity similar to sugar alone, and this was independent of the state of decay.

Both feeding treatment and female age had significant effects on potential fecundity (Fig. 2). In general, there were significantly fewer oocytes in the 16-day-old females



**Fig. 4.** Linear Discriminant Analysis on morphometrics of ovaries of females (A) reared on four different diets in the laboratory (FR = “Red fruit medium”, DM = “DM medium”, S = “Sucrose”, Y = “Yeast”). Two-thirds of these individuals were used to build the LDA model. LD1 and LD2 are the first (LD1) and second (LD2) axes of the LDA analyses (explaining 81% and 15% of the variance, respectively). Panel (B) contains individuals caught in the field in three different landscapes (apple–cherry, forest, kiwi). In both panels, each ellipse corresponds to diet membership estimated from the laboratory flies (centred on the mean and size proportional to standard deviation).

than in the 8-day-old females (mean number of mature oocytes per ovary  $\pm 95\%$  CI:  $4.4 \pm 1.1$  vs  $8.7 \pm 1.9$ ; Wald  $\chi^2 = 76$ ;  $df = 1$ ;  $P < 0.001$ ; Fig. 2). However, this effect differed according to the feeding treatment (interaction: Wald  $\chi^2 = 16$ ;  $df = 3$ ;  $P = 0.001$ ). No significant differences were

found between the two ages in the flies of the poor quality treatment “Water + sucrose” (B; number of mature oocytes per ovary:  $0.9 \pm 0.3$ ). A significant decrease between 8-day-old and 16-day-old females was observed when females received a rich “Water + Sucrose + Yeast” treatment (C: from  $23.2 \pm 2.7$  to  $12.1 \pm 2.6$  mature oocytes per ovary), a “Freshly cut fruit” treatment (G: from  $4.3 \pm 1.1$  to  $1.9 \pm 0.8$  mature oocytes per ovary) or a “Decaying cut fruit” treatment (H: from  $4.8 \pm 1.3$  to  $1.5 \pm 0.6$  mature oocytes per ovary). Surprisingly, no significant difference was observed between “freshly cut” and “decaying cut” fruits (treatments G and H, Wald  $z = 0.24$ ,  $P = 0.62$ ). However, these treatments (*i.e.*, real fruit) led to significantly greater numbers of oocytes than Treatment B with “Water + Sucrose” (Wald  $z = 68.7$ ,  $P < 0.001$ ) and significantly fewer oocytes than treatment with “Water + Sucrose + Yeast” (Treatment C: Wald  $z = 317.2$ ,  $P < 0.001$ ). Females on fruit treatments G and H matured 5-fold fewer oocytes than females on rich food (Treatment C).

### Experiment 2 – effects of reproduction behaviour on the longevity of females

Longevity was significantly influenced by the feeding treatment ( $\chi^2 = 641$ ;  $df = 32.8$ ;  $P < 0.001$ ) but not by the mating status of the flies ( $\chi^2 = 0.9$ ;  $df = 0.23$ ;  $P = 0.077$ ) or by their interaction ( $\chi^2 = 0.7$ ;  $df = 1.43$ ;  $P = 0.56$ ). As in the previous experiment, females survived longer when feeding on “Water + Sucrose” (B) and “Water + Sucrose + Yeast” (C) (median  $> 7$  days, Fig. 3B and C) than on “Water” (A) and “Undamaged fruit” (D) (median, equal to 4.5 days and 4 days, respectively; Fig. 3A and D).

Despite the lack of significance of the interaction between mating status and feeding treatment, we investigated a specific case for which we had a clear prediction. When females were exposed to “Undamaged fruit” (Treatment D), we expected greater survival of mated females than virgin females because the former may feed on fruit juice after oviposition (see biological model). There was indeed a significant difference in this treatment (Log Rank test:  $\chi^2 = 5$ ;  $df = 1$ ;  $P = 0.033$ ), but the pattern was opposite of our prediction because mated females had lower longevity than virgins (Fig. 3D).

### Experiment 3 – nutritional status of wild females

Females from the field harboured a small number of mature oocytes in their ovaries (mean  $\pm 95\%$  CI:  $0.34 \pm 0.2$  per ovary). Landscape origin significantly influenced mature oocyte numbers ( $\chi^2 = 16$ ;  $df = 2$ ;  $P < 0.001$ ). Females from “forests” had the fewest oocytes ( $0.15 \pm 0.2$ ;  $max = 4$ ), tightly followed by “kiwi orchard” females ( $0.2 \pm 0.2$ ;  $max = 1$ ), and females captured in the “apple–cherry orchard” had significantly more oocytes ( $0.73 \pm 0.6$ ;  $max = 6$ ;  $z = 3.6$ ,  $P < 0.001$ ).

A Linear Discriminant Analysis (LDA) model was parametrized with data from insects fed on four diets in the laboratory (Table 2 and Fig. 4A). Pictures of typical ovaries obtained on the four diets are available in the photos (see Appendix A– Supplementary material: Pictures). Wild females were assigned to the different diets based on ovary morphometrics and the LDA model (Table 2 and Fig. 4B). Independently of their landscape origin, most individuals appeared closest to lab individuals fed on the poor resource. More precisely, 80.8% of females from “apple–cherry orchard”, 85.0% of females from “kiwi orchard” and 97.5% of females from “forest” had ovaries similar to those of flies from “S” treatment (*i.e.*, water and sucrose). None of the females were assigned to either rich resource “Y” or intermediate resource “FR” containing red fruits.

## Discussion

We investigated nutritional ecology in the invasive *D. suzukii* and tested whether reproductive sites could provide enough nutrients for reproduction and survival. We found carbohydrates to be mandatory for adult survival and proteins for egg production. Moreover, it appeared that healthy fruits, where females oviposit, cannot serve as feeding sites. When fruits were decaying or largely damaged, females could feed and produce oocytes.

Our first experiment revealed the nutritional requirements of *D. suzukii* females for longevity and reproduction. Feeding on sugar and fruit pulp significantly increased the longevity of *D. suzukii* compared to water only. This fly species requires proteins and micronutrients to mature eggs, which were provided by yeast in our experiments, as in *D. melanogaster* (Bownes, Scott, & Shirras, 1988). To our surprise, decaying fruits, which should contain more yeast than fresh fruits, did not sustain elevated oocyte maturation (Fig. 2), but a potential negative effect of other microorganisms (*i.e.* moulds) or a positive effect by fermentation of fresh fruit cannot be excluded. This suggests that wild *D. suzukii* adults find carbohydrates and proteins on different substrates. For instance, *D. suzukii* could feed on plant leaves that harbour free carbohydrates, proteins, bacteria and yeast (Beattie & Lindow 1999; Lindow & Brandl, 2003). *Tephritidae* flies, which have similar ecology and invasive characteristics as *D. suzukii*, are known to graze plant leaves (Hendrichs, Lauzon, Cooley, & Prokopy 1993; Yee 2008). *D. suzukii* could also feed on floral (Tochen, Walton, & Lee 2016) and extra-floral nectars, which are common among its larval breeding plants (Koptur, 1992); this has been observed for two other *Drosophila* species (Hespenheide, 1985; Naganuma & Hespenheide, 1988). This hypothesis is supported by a recent paper suggesting that *D. suzukii* adults are more attracted to leaf than fruit volatiles of strawberry (Keeseey, Knaden, & Hansson 2015). As recently shown in *D. melanogaster* (Anagnostou et al., 2010; Stamps, Yang, Morales, & Boundy-Mills, 2012; Yamada, Deshpande,

**Table 2.** Assignment number of lab and wild females of *Drosophila suzukii* to four diets (FR=“Red fruit”, DM=“DM medium”, S=“Sucrose”, Y=“Yeast”). The LDA model was estimated using a subsample of two-thirds of the laboratory insects. The assignments of the remaining third of laboratory individuals by the model in the diets are in grey boxes on the left panel. On average, 68% of the remaining laboratory individuals were assigned to the right diet. This assignment rate was close to the average assignment rate estimated from hundreds of models. The assignments of wild females are on the right panel.

LDA groups	Laboratory diets				Landscape type		
	FR	DM	S	Y	Apple–cherry	Forest	Kiwi
FR	3	0		2		1	
DM	1	5	1		5		3
S	1	1	6		21	39	17
Y	2	1		5			
True assignment rate	0.43	0.71	0.86	0.71			

Bruce, Mak, & Ja, 2015), the nature of the microorganisms responsible for fruit decay can largely influence nutrient availability possibly explaining the low degree of oocyte maturation in the laboratory. Microorganisms can be specific and deposited by *Drosophila* species (e.g., faecal deposits and surfaces of oviposited eggs; Bakula, 1969; Gilbert, 1980; Hamby, Hernández, Boundy-Mills, & Zalom, 2012; Rohlf & Kürschner, 2010). Finally, rotting or decaying fruits may also serve as both feeding and oviposition sites despite the casual observation that *D. suzukii* females that oviposit in damaged fruit tend to do so in the undamaged parts of the fruit. Even if our conclusions are drawn from a single population, we believe that our interpretation is valid for the species. Indeed, the effects of nutrients on fecundity and longevity should be specific and thus similar among populations.

Our second main result is that females exposed to undamaged fruits died the fastest (Fig. 1A treatment D). Although we observed that mated females feed on exudate from the hole they shape for egg laying (see Appendix A- Supplementary material: Video), we were surprised to find that mated females died faster than virgins (which do not lay eggs). This result suggests that the quality or amount of food in this juice droplet does not counterbalance the physiological cost of oocyte production or oviposition behaviour. When we artificially increased the size of the exocarp hole, mimicking a hole made during oviposition (mean diameter observed on cherry  $\pm 95\%$  CI:  $197 \mu\text{m} \pm 11.4$ ,  $n=44$ ), longevity increased. The effect was particularly clear when the hole diameter reached 3 mm, which is larger than the approximate width of females and could therefore allow them to enter the fruit and feed further down. In nature, we can expect that only fruits that have already been damaged by, for example, insects or birds will constitute suitable feeding sites for *D. suzukii*.

Our results suggest that healthy fruits cannot be used as feeding sites by *D. suzukii*, which may cause a trade-off in the amount of time allocated to searching each type of site (e.g., Desouhant et al. 2005). The availability of feed-

ing sites depends on the timing of the fruit infestation: it is high if the fruits are already exploited by conspecific larvae or damaged by other phytophagous animals, such as hornets and birds. Season and fruit density should also affect this availability. In temperate latitudes, host density is low in winter and early spring (Poyet et al. 2015), and potential feeding sites, such as plant leaves, floral and extra-floral nectars and/or damaged fruits, are scarce. As the season progresses and leaves and fruits appear, feeding sites become more frequent, decreasing the amount of time required for searching. We found that wild females caught in October exhibited low reproductive potential, with few oocytes and ovary structures similar to those of lab females fed on sucrose only. This suggests that wild *D. suzukii* females had little access to proteins despite high fruit densities when they were caught (Poyet et al. 2015). It is unlikely that these females had already laid all their eggs right before capture because most of the ovaries contained no oocytes under active development. This nutritive status matches a field study from Japan (Mitsui et al. 2010). In both cases, it is possible that the females entered reproductive diapause before overwintering (Zhai et al. 2016), leading to altered feeding strategies to improve longevity.

To conclude, despite access to fruit juices (through artificial wounds in fruit exocarp or natural openings due to oviposition), *D. suzukii* females could not sustain egg production on reproductive sites in the absence of an additional protein source and they have thus to find appropriate resources elsewhere to be able to mature eggs. Further studies investigating *D. suzukii*'s nutritional ecology should thus span several seasons and involve direct measurements of nutrient content in different food sources as well as the physiological state of flies captured nearby.

## Author contributions

PG, ED and CP conceived the study. CP and VE performed the experiments. CP led the literature review, data analysis

and writing. Important contributions to the data analysis were made by ED, and to the writing of the manuscript, by ED, PG and SF.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2016.10.005>.

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