Insect population dynamics under *Wolbachia*-induced cytoplasmic incompatibility: puzzle more than buzz in *Drosophila suzukii*

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Abstract

Wolbachia pipientis is an intracellular bacterium that infects many insects and other arthropods. Cytoplasmic incompatibility (CI) is one of several effects that *Wolbachia* has on its hosts. In its simplest form, CI causes host reproductive failure when an uninfected female mates with an infected male. Theoretically, this has two consequences. First, when an incompatible *Wolbachia* strain is introduced into a recipient host population, uninfected females are selected against, triggering *Wolbachia* invasion. Second, reproductive failures during *Wolbachia* invasion lead to a transient decrease in population growth rate. Modelling studies combining *Wolbachia* invasion and host population dynamics show that these two processes could interact to cause population decline and even extinction. However, these effects could be sensitive to density dependence, with competition reducing the demographic impact of CI and Allee effect increasing the probability of extinction. We tested these predictions with laboratory experiments in the fruit fly *Drosophila suzukii* and the exogenous *Wolbachia* strain *w*Tei. Surprisingly, the introduction of *w*Tei into *D. suzukii* populations at carrying capacity did not result in the expected invasion of *w*Tei and transient population decline. In parallel, we found strong negative density dependence, but no Allee effect. From these results, we propose that negative density dependence is an important but underappreciated driver of the eco-evolutionary feedback underlying *Wolbachia* invasion and insect population dynamics. If future models and data support this hypothesis, pest management strategies using *Wolbachia*-induced CI should target early-season populations with negligible competition but a possible Allee effect.

Keywords: Density dependence, Allee effect, intraspecific competition, *Drosophila suzukii*, *Wolbachia*, cytoplasmic incompatibility, eco-evolutionary dynamics.

Introduction

Wolbachia pipientis is an endosymbiotic bacterium found in a large number of nematode and arthropod species, including insects [1,2]. The overall record of interactions between *Wolbachia* and

their hosts is highly variable, ranging from parasitism to mutualism. The ability of *Wolbachia* to manipulate the reproduction of their hosts at the advantage of its own transmission is the most widespread facet of these interactions [3]. Cytoplasmic incompatibility (CI) is one such manipulation. CI is the consequence of a sperm modification that occurs during spermatogenesis in the infected male. When an infected male mates with an infected female, the modification is restored and the cross produces offspring. If the female is uninfected, the modification is not restored which, in diploid species, causes a malfunction during the first mitotic divisions, resulting in embryo death [4]. Hence, infected females have more offspring on average than uninfected females because they can produce offspring with all types of males, be they infected or not. Since *Wolbachia* is transmitted maternally and vertically, these females are more likely to transmit the incompatible strain, thus promoting its spread [5].

The high invasion success of *Wolbachia* combined with its ability to reduce the transmission of viruses or other pathogens [6,7], as piqued the interest of epidemiologists. Disease-vector control strategies have been developed based on the introduction of individuals artificially infected with a virus-blocking exogenous incompatible strain of *Wolbachia* into a target population. The reproductive advantage of infected females favors the invasion of the virus-blocking strain, which increases in frequency until it becomes the majority or fixed strain. In the event of a resident strain not inducing CI is present in the population, it would be replaced by the introduced incompatible strain, which results in a host population infected with a *Wolbachia* strain with traits of interest for pest control. For instance, such a replacement strategy has been shown to be effective in suppressing dengue transmission by the mosquito *Aedes aegypti* [8].

In order to optimize the success of such *Wolbachia* replacement programs, it is necessary to identify the factors that influence *Wolbachia* invasion dynamics. Historically, most theoretical models of CI dynamics are analogous to population genetic models and have focused on the evolution of the frequencies of an incompatible strain within the host population, neglecting the specificities of host population dynamics [9–13]. Only a limited number of host demographic parameters directly affecting changes in frequencies were considered, and assumed to be constant [8,13,14]. These models predict the existence of a critical infection frequency, referred to as the invasion threshold, above which an

incompatible strain will deterministically invade the host population. The threshold depends on three parameters: the intensity of infection-induced CI, the success of maternal transmission and the costs resulting from infection, such as reduced fertility. More recently, Hancock *et al.* [15–17] added density dependence on host demographic parameters in order to study *Wolbachia* invasion dynamics with more realistic assumptions. They concluded from different models that the intensity of competition, by varying the reproductive success of individuals, significantly reduces the ability of *Wolbachia* to invade a population [16,17].

The ability of *Wolbachia* to invade may serve another pest management strategy, alternative to the drive of interesting characters. In a landmark publication, Dobson *et al.* [18] have proposed a novel CI-based insect pest suppression strategy based on *Wolbachia* invasion. They extended classical CI models by assuming realistic host population dynamics with density dependent survival of immature stages and parameter values taken from the insect literature. They simulated the introduction of an incompatible *Wolbachia* strain into a host population that had reached carrying capacity. While the incompatible strain spreads through the population, the frequency of incompatible crosses increased, resulting in a transient reduction in host population size. Population size declined to a minimum when the proportions of infected and uninfected individuals were similar, and then increased again when the incompatible strain became the majority. One of their predictions is that the minimum population size reached during invasion is influenced not only by *Wolbachia*'s own invasion parameters, but also by demographic parameters intrinsic to host populations. For instance, under conditions of high host reproductive rate and intraspecific competition, the demographic consequences of CI may go unnoticed.

The transient population decline caused by *Wolbachia*-induced cytoplasmic incompatibility, as predicted by Dobson et al. [18], is a form of demographic destabilization that, in conjunction with other processes, could cascade towards population extinction. As other process, the Allee effect is an excellent candidate. It is characterized by population growth rate decreasing with the decrease of the population size, which triggers an extinction vortex if the growth rate becomes negative below a critical number of individuals (the Allee threshold) or if the growth rate remains positive but combines with stochasticity. The Allee effect has thus been envisioned as a catalyst for extinction upon which

novel tactics could be developed to eradicate pest populations [19–23]. Building on these ideas, Blackwood *et al.* [24] assumed an Allee effect to investigate the conditions under which CI expression would serve pest eradication. To this end, they simulated *Wolbachia* introductions into populations affected by a more or less intense Allee effect. When the Allee effect was strong and the population reproduction rate low, the model predicted that the minimum population size reached during *Wolbachia* invasion would fall below the Allee threshold, leading to population extinction. On the other hand, at high reproduction rates, the transient reduction induced by CI was not large enough to bring the population size below the Allee threshold.

The theoretical models of Dobson *et al.* [18] and Blackwood *et al.* [24] are of major interest for insect pest management but, to our knowledge, they have not been tested yet. We are unaware of any empirical evidence of either transient population declines caused by the invasion of an incompatible *Wolbachia* strain and subsequent increase in CI or population extinctions if CI combines with an Allee effect. We therefore initiated a research program using these models as a general theoretical framework and *Drosophila suzukii* as a model organism. The present article reports our first findings.

There are several reasons for choosing *Drosophila suzukii* as a model organism. First, *D. suzukii* is a major invasive pest of berry and stone fruit crops, including cherries, blueberries, raspberries, blackberries, peaches, nectarines, apricots, grapes, etc… [25]. Native from Southeast Asia, *D. suzukii* was first reported in the USA and Europe in the late 2000s and continues to spread worldwide (e.g. Africa; [26–28]). Unlike other *Drosophila* species, the serrated ovipositor of *D. suzukii* allows females to lay eggs under the skin of ripening or mature fruits, causing significant damage to cultivated fruits and consequently, huge economic losses [25]. This calls for research to develop environmentally friendly methods to control this major agricultural pest. Second, *D. suzukii* is naturally infected with *Wolbachia*. Contrary to the common observation of "most-or-few" infection frequencies, the prevalence of the resident *w*Suz strain in European populations is highly variable, averaging 46% [29]. This infection polymorphism could be a consequence to the fact that *w*Suz induced no or weak CI [29,30] as well as the variable benefits and costs of *w*Suz protection from viruses [31]. Third, we capitalized on a strong body of experimental work based on the transinfection of *D. suzukii* with various *Wolbachia* strains, including *wHa* and *w*Tei, which produce cytoplasmic incompatibility [32–34]. Finally,

the expression of negative density dependence on individual performance has already been observed in *D. suzukii* [35]. Under experimental conditions with high larval densities, some individuals were observed to leave the breeding site, which was interpreted as a response to intraspecific competition for resources [36]. While the Allee effect has not yet been demonstrated in *D. suzukii*, it has been documented in other closely related *Drosophila* species [37].

The aim of this study is to estimate the strength of positive and negative density dependence in *D. suzukii* and to test the effect of *Wolbachia*-induced CI on the dynamics of experimental populations. In this article, we report the absence of an Allee effect under our laboratory conditions. In contrast, we found strong negative density dependence resulting in logistic population growth. Finally, we found no effect of introducing an incompatible *Wolbachia* strain on *D. suzukii* population dynamics. We discuss these results in the light of the models developed by Dobson *et al.* [18] and Blackwood *et al.* [24] and hypothesize that negative density dependence may have much stronger influences than expected on *Wolbachia* invasion and the demographic consequences of cytoplasmic incompatibilities.

Materials and methods

Insect rearing

Two lines of *D. suzukii* have been maintained in the laboratory since 2018, *i.e.*, for around 24 generations before experiments. These lines were infected with either *w*Suz, the strain naturally present in *D. suzukii* [29,30,38] or the exogenous *Wolbachia* strain *w*Tei, the natural symbiont of *D. teissieri*. The latter was selected because of the incompatibility induced by transinfected *D. suzukii* males when crossed with *w*Suz-infected females[32,34]. The three lines were reared on cornmeal-based food medium containing, per 1 liter of water: 10 g agar, 80 g corn flour, 80 g brewer's yeast and 60 ml moldex (100 g methyl hydroxybenzoate in 1L 70% ethanol). Rearing, as well as all

experiments, occurred in a climatic chamber at 23-24°C, 50-60% humidity and a photoperiod of 12h-12h.

Experiment 1. Estimation of population size

In order to estimate accurately the number of flies in population cages, we developed a rapid method based on insect counts on photographed cages. Prior to experiments, we assessed the relevance of this method. For this, between 4 and 396 individuals were introduced into 59 cubic experimental cages (21 cm x 21 cm x 21 cm) made of fine-mesh fabric and a transparent plastic wall. The cages were then photographed using a Nikon N750D camera and a Nikon lens (AF-S Micro Nikkor 60mm f/2.8G ED). Visible Individuals were counted on a computer using the software ImageJ 1.52 [39] on three predefined areas (S1 Supporting information) : (i) individuals appearing only on the back wall (area n°1), (ii) individuals appearing on the whole surface except the left side and the cage floor (area n°2), or (iii) only individuals appearing on the front wall (area n°3) . Areas n°1 and n°2 were respectively used for the experiments 2 and 3. Area n°3 was not used in the present study, but it will be used in follow-up experiments. For each area, linear regressions between the numbers of individuals on pictures and the total numbers in the cage were fitted, and the coefficients of determination R^2 estimated. Kolmogorov-Smirnoff tests, from the DHARMa package in R, were used to test normality of the residual distribution. These and the following statistical analyses were carried out using R 4.2.2 software (core team 2022).

Experiment 2. Early dynamics of small populations

Founding populations

This experiment aimed at quantifying a demographic Allee effect, i.e., positive density dependence at low population size. To this end, we founded *D. suzukii* populations with a range of initial number of individuals and estimated subsequent growth rates. Fifty-two populations were initiated with pairs of <24 hour-old virgin individuals. We manipulated the number of pairs: 1 (N=13, N being the number

of replicated populations), 3 (N=13), 10 (N=10), 30 (N=8) and 100 (N=8). For each population, the pairs were introduced into a cage as described above. Replicates were distributed across four blocks spaced one week apart. Weekly, flies were provided with a new oviposition patch consisting of 100 ml (75 mm diameter) rearing medium supplemented with a fresh raspberry. As the generation time was around two weeks in our rearing conditions, each patch was exposed three weeks to ascertain that eggs laid in the patch yielded new adults emerging into the cages. Immature drosophila developed by feeding on the rearing medium whereas adult flies were supplied with water and honey three times a week. To estimate population size, each cage was photographed once a week. Populations were followed nine weeks post foundation; generations were completely overlapping, but if they were discrete, nine weeks would represent 4 to 5 generations of *D. suzukii*.

Fitting an exponential growth model

The early dynamics of populations founded with a small number of individuals is expected to depend on initial population size and the strength of an Allee effect. In the absence of an Allee effect, exponential population growth is expected. With a weak Allee effect, exponential growth is also expected, but the growth rate should be lower for initially small populations (e.g., 1 pair, 3 pairs, etc.) than for larger ones (e.g., 100 pairs). With a strong Allee effect, populations of initial size smaller than the Allee threshold should go extinct whereas populations initially above the Allee threshold should increase exponentially, with a growth rate again depending on population size. We tested these predictions by counting populations that went extinct and estimating the growth rate of those that persisted. The growth rate (r) was estimated by fitting a simple exponential growth model such as $N_t = N_0 \exp(r\ t)$ to each population time series, excluding the last week at which the largest populations had stopped increasing. Also, due to missing values, 5 populations out of the 52 founded were discarded. In practice, we fitted weekly counts with a generalized linear model based on a Poisson distribution and a log link function, i.e., $\log(N_t) = \log(N_0) + r\ t$. The initial number of individuals observed just after the populations had been founded was defined as an offset ($\log N_0$), that is, a parameter included in the model but not estimated. Growth curves and estimated growth

rates r were obtained for each population. A linear regression was then used to analyze the effect of the number of founders on growth rate.

Testing density dependence

A complementary method to test for positive density dependence is to assess the relation between the proportion p of populations growing between t and t+1 and the population size at time t. Analyzing p rather than individual counts circumvents the high sampling variance at low numbers and the consequent spurious estimations of growth rates [40]. With an Allee effect, we expect p to increase with population size at low population size, with p=0.5 being the Allee threshold. Independently of the experimental manipulation of initial population size, we associated all possible pairs of N_t and N_{t+1} for each of the 48 populations at each weekly interval. A population was considered stable or growing for $N_{t+1} \geq N_t$ (further referred to as a growing population given the low number of pairs, 14 of 410 pairs, with $N_{t+1} = N_t$) or decreasing otherwise. We then calculated the proportion p of growing populations within 18 classes of population sizes (1-49, 50-99, …, 850-899). This proportion was fitted with a logistic regression to test three alternative hypotheses: a null model suggesting no relation between population size and growth ($p = \beta_0$); a standard model suggesting a linear relation ($p = \beta_0 + \beta_1 N_t$); a polynomial model allowing a non-linear relationship ($p = \beta_0 + \beta_1 N_t + \beta_2 N_t^2$). The third model is well suited to describe bell-shaped curves that characterize populations with both an Allee effect and negative density dependence. The AIC was used to select the best model.

Experiment 3. *Wolbachia* invasion and consequent population dynamics

Experimental populations

The aim of this experiment was to assess changes in *D. suzukii* population dynamics caused by the introduction of individuals infected with an incompatible *Wolbachia* strain. To this end, we founded

218 eight populations with 100 *w*Suz-infected individuals (sex ratio 1:1) aged 3 to 5 days. Each population
219 was studied continuously across three distinct periods, for a total of 23 weeks: a first period of
220 population growth (5 to 8 week); a seven-week period of dynamic equilibrium around the carrying
221 capacity, starting upon observation of the first decrease in number; a post-introduction period (8 to
222 11 weeks) starting upon the introduction of infected flies. Populations were maintained as in the
223 previous experiment, with a new reproduction patch provided weekly and exposed during three
224 weeks, and water and honey to feed adults three times a week. Cages were again photographed every
225 week for estimation of population sizes. The experiment could have last longer but was terminated
226 due to the covid crisis.

227 Introducing *Wolbachia* and characterizing infection status

228 Individuals introduced after the seven-week period of dynamic equilibrium were infected by either
229 *w*Suz for the four "Control" populations or *w*Tei for the four "IC" populations. The number introduced
230 was equal to 10% of the mean number of individuals across the period of equilibrium. Before
231 introduction, we verified the fixation of *w*Suz for all populations by genotyping 20 individuals per
232 population (methods below). After introduction, we monitored the evolution of *w*Tei frequencies over
233 time in the four CI populations. For this, we randomly sampled 20 individuals per week and stored
234 them in 99% ethanol at -20°C until analysis. Individual infection status was assessed via HRM (High
235 Resolution Melting; [41], a genotyping method that allows the detection of changes in the melting
236 profiles of double-stranded DNA. For each individual, DNA was extracted using Macherey Nagel's
237 NucleoSpin kit. *Wolbachia*-specific primers 81f (5'- TGGTCCAATAAGTGATGAAGAAAC-3'; Braig *et al*,
238 1998) and 2R (5'- CAGCAATTTCAGGATTAG -3'; [41] were used to amplify the *wsp* ("*Wolbachia*
239 Surface Protein") gene. PCRs were performed in a 10 µL volume containing 500nM primers, 1X
240 Precision Melt Supermix (Biorad), containing dNTPs, iTaqTM DNA polymerase, MgCl2, EvaGreen
241 buffer, stabilizers, and 2 µL of 10-fold diluted DNA. Cycle conditions were 95°C for 2 min, then 30 sec
242 at 95°C, 30 sec at 54°C and 30 sec at 72°C for 40 cycles, followed by 30 sec at 95°C and 1 min at 60°C
243 (Biorad CFX96).

Estimating carrying capacity

The eight populations were studied over enough time to estimate the carrying capacity *K* in our environmental conditions. For this, we fitted non-linear regressions to the number of adults observed each week, from foundation to the introduction of infected flies (first and second time periods). Three candidate population growth models were fitted: Gompertz, logistic, and Weibull. SSlogis, SSgompertz and SSweibull functions in R were used to obtain the initial parameter values for each candidate model (so-called "selfStart" models). All these models share one parameter, the asymptotic population size defined as the carrying capacity. The AIC and root-mean-square error (RMSE) were then used to select the best of the three models fitted to each population.

Testing the consequences of CI on population dynamics

Evolution of the frequency of *w*Tei-infected individuals was analyzed using a generalized linear mixed model based on a negative binomial distribution and a logit link function, with time as a fixed effect and population as a random effect. We excluded the first period of population growth to focus on the influence of introducing incompatible *Wolbachia* on the dynamics of populations that had reached carrying capacity. The main prediction tested here is that the introduction of individuals infected by the incompatible *w*Tei *Wolbachia* strain should lead to a decline in population size, co-occurring with the invasion of *w*Tei and subsequent increase in the proportion of incompatible crosses. This decline should not be observed in control populations with introductions of individuals infected by *w*Suz. We thus fitted the following model:

$$N_{ijt} = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot intro + \beta_3 \cdot t \cdot intro + \beta_4 \cdot t \cdot intro \cdot strain + \varepsilon_{ijt}$$

where N_{ijt} is the number of individuals in populations of introduction status *i* (before vs after introduction; variable *intro* in the model) having undergone treatment *j* (introduction of *w*Tei or *w*Suz; variable *strain* in the model) at time *t* relative to the introduction (rescaled so that *t*=0 upon introduction). This model allowed to test four predictions (Fig 1).

- H1: Control and CI populations were at equilibrium before introduction so that there should be no effect of time during the seven weeks prior to the introduction ($\beta_1 = 0$).
- H2: Introductions should result in an immediate but small increase in population size ($\beta_2 \neq 0$).
- H3: Introductions may destabilize populations from equilibrium and hence, induce a change in the effect of time on population size ($\beta_3 \neq 0$).
- H4: The latter change is expected to be affected by the strain, with *w*Tei, contrary to *w*Suz, inducing a decrease in population size ($\beta_4 \neq 0$).

These four hypotheses were tested with likelihood ratio tests between models with and without the variable or interaction concerned.

Results

Estimation of population size (experiment 1)

In order to estimate population size, we counted adult flies on pictures of three different areas of the experimental cages. Whatever the area considered, we found a clear relationship between the number counted on pictures and the total number of flies in the cages (Fig 2). Linear regressions were characterized by high coefficients of determination: R^2=0.99 for the first two areas and R^2=0.86 for the last area. Kolmogorov-Smirnov tests confirmed the normal distribution of errors, and hence, an absence of sampling bias (Fig 2; KS test: p=0.83, 0.85, and 0.34 for areas 1, 2 and 3 respectively).

Early dynamics of small populations (experiment 2)

None of the newly founded populations went extinct, even introducing a single pair. Population growth during the first eight weeks was well described by an exponential model, regardless of the number of founding pairs (Fig 3). The estimated growth rate *r* was influenced by initial population size

(Anova, $F_{4,42}$ = 14.6, p =1.47 · 10^{-7}). Contrary to expectation with an Allee effect, the growth rate r was highest at smallest population sizes, from 1.73 ± 0.03 for single pairs to 1.4 ± 0.04 for 100 pairs.

The detailed analysis of week-to-week variations in population sizes confirmed the absence of Allee effect. Among the three alternative models, the second order polynomial logistic regression best fitted the proportion of increasing populations (AIC=61.3, versus 82.9 for the null model and 63.5 for linear regression). The resulting model (logit p = 1.814 + 2.014 · 10^{-03} N_t – 1.056 · 10^{-05} N_t^2) suggests that the proportion of populations growing between consecutive weeks (p) peaks at a population size of about Nt=95 individuals. This is the population size at which the derivative of the above equation equals zero. However, the predicted value of p for this "optimal" population size (p=0.87) is not different from that predicted for Nt=0, i.e. the intercept: p=0.86 [0.77; 0.92]. Thus, despite a second-order logistic regression best fitting the data, there is no evidence of an Allee effect. This also appears clearly in Fig 4.

Altogether, these results suggest an absence of Allee effect in our conditions. Rather, the decrease in population growth with increasing population size reflects negative density dependence.

Reaching carrying capacity (Experiment 3)

The eight experimental populations studied over a longer time period were well described by Gompertz, Logistic or Weibull population growth models (Table 1). Notwithstanding slightly different shapes, these three models are characterized by a plateau, the carrying capacity, K (Fig 5). Although the estimated values of K varied between populations, they were of similar order of magnitude, between 455 and 593 (Table 1). This carrying capacity was reached after five to eight weeks.

Consequences of cytoplasmic incompatibilities at carrying capacity (Experiment 3)

To test the effect of introducing CI into a recipient population of *D. suzukii*, we fitted a generalized linear model to population size, with a negative binomial distribution of errors and a log link function (Fig 6). The variance in numbers within replicate was low (5.3×10^{-12}) so we added no random effect. Hypotheses H1 to H4 were tested *via* likelihood ratio tests for comparisons of models with and without parameters under scrutiny.

- H1: as expected from the design of the experiment, prior to the introductions of infected flies, the numbers of individuals varied at random around the carrying capacity (β_0=6.21; test for parameter β_1=-0.02; $LR_{1,135}$=0.98, p=0.32).
- H2: as expected, introducing infected flies had an immediate, positive influence on population size (test for parameter β_2=0.24; $LR_{1,135}$=6.13, p=0.01).
- H3: following the immediate increase in numbers, the introduction of infected individuals had no general influence on the variation over time (test for parameter β_3=-0.009; $LR_{1,135}$=0.18, p=0.67).
- H4: most importantly, this absence of effect of introduction on population dynamics was consistent for the two strains of *Wolbachia*, i.e., the interaction time × strain after introduction was not significant (test for parameter β_4=-0.02; $LR_{1,135}$=3.11, p=0.08). A trend in the expected direction can be observed and could be responsible for this marginally significant test, but this trend seems to be partly driven by only few data points (Populations 6 and 8; Fig 6).

Given the importance of this latter test and the low p-value, we complemented the parametric analysis with a chi-square test to compare the proportion of weekly observations where population sizes were below and above the carrying capacity. Before introduction, 53% (15/28) and 64% (18/28) observations were below K for control and IC populations respectively, and these proportions did not differ (χ^2= 0.30, p=0.59). After introduction 38% (14/37) observations were below K for IC populations, a proportion that, contrary to theoretical predictions, was not higher than that observed for control populations (47%, 18/38; χ^2=0.36, p=0.55).

Altogether, these results suggest that introducing the incompatible *Wolbachia* strain *w*Tei into a population of *D. suzukii* at carrying capacity produced an immediate and limited burst in population size but no consistent decrease in population size over time.

Invasion dynamics of the incompatible strain (Experiment 3)

After introducing flies infected by *w*Tei at an initial frequency of 10% of the estimated population size, the prevalence of *w*Tei did not change consistently over time (χ^2=3.5473, df=1, p=0.059). The prevalence of *w*Tei reached up to 25% in two populations, which probably produced the marginally significant test, but it was below the initial frequency introduced in the other two (Fig 7). For populations with weak evidence for invasion of *w*Tei, weekly variations in the frequency of infected individuals were high, which could reflect random effects such as sampling variance, drift, or the combination of both processes.

Discussion

The endosymbiotic bacterium *Wolbachia pipientis,* widespread in insects, often causes cytoplasmic incompatibility, a reproductive failure that occurs when males and females do not have the same infection status. This has led to novel and interesting ideas for insect pest management based on the introduction of an incompatible *Wolbachia* strain into a target population, with the assumption that the resulting incompatibilities will affect insect population dynamics. The reasoning is that if the incompatible strain is introduced at an initial frequency above its invasion threshold, it will invade the population, which should then lead to an increase in the proportion of incompatible crosses and, in turn, a decrease in the population growth rate. This basic idea has been formalized in the theoretical models of Dobson *et al.* [18] and Blackwood *et al.* [24] which predict that the introduction of one or more incompatible *Wolbachia* strains into a target population could lead to a transient decrease in

359 population size [18]. Furthermore, assuming that a pre-existing Allee effect is enhanced by
360 complementary tactics such as mating disruption, the unwanted population could be driven to
361 extinction [24]. Although these models are of great interest for their efforts to link *Wolbachia* invasion
362 to insect population dynamics, their hypotheses and predictions have not been tested with
363 appropriate experiments. Using *Drosophila suzukii* as a model organism, we present here a first step
364 in this direction.

365 Our main result is that introducing the incompatible *Wolbachia* strain *w*Tei into a recipient *Drosophila*
366 *suzukii* population at carrying capacity does not result in an observable decrease in population size.
367 This finding, with this specific *Wolbachia* strain and insect host, contradicts theoretical predictions
368 [18,24], and moderates the perspective on using cytoplasmic incompatibility as a lever for insect pest
369 management. In the remainder of the discussion, we explore the various hypotheses that could explain
370 this result.

371 The most trivial hypothesis to explain the lack of influence of *Wolbachia*-induced IC on population
372 dynamics is that contrary to expectations, the introduced incompatible *Wolbachia* did not invade the
373 recipient populations and therefore, did not produce enough incompatible crosses to produce an
374 effect. This is a hypothesis that we cannot firmly reject. Although the prevalence of individuals infected
375 with *w*Tei reached up to 25% in some populations, weekly estimates revealed important variations,
376 with frequencies that were sometimes lower than the 10% fixed at introduction. This result contrasts
377 with the published data, which show that incompatible *Wolbachia* strains introduced above their
378 invasion threshold easily invade recipient populations. In *Aedes aegypti*, the introduction of 20
379 *Wolbachia* (*w*AlbB)-infected females into an experimental population of 100 uninfected individuals
380 resulted in the fixation of the introduced strain in seven generations [42]. In another trial on the same
381 species, 65% of *w*Mel-infected mosquitoes were introduced in two populations of 1680 uninfected
382 individuals, and again, the introduced strain became fixed 30 and 80 days after introduction [43]. In
383 *Drosophila simulans*, cage populations were established with a mixture of uninfected individuals and
384 either 35 % of *w*Au-infected individuals or 4 % of *w*Ri-infected individuals (for a total of 650 and 1440
385 adult females, respectively). A significant increase in *w*Au and *w*RI frequencies was observed for all
386 populations from the 10th generation onwards, except in two cages where *w*Ri was either lost or at low

frequency [44]. With a similar invasion dynamic, our seven-to-ten-week experiment (i.e., 4-5 generations) should have revealed the invasion of *w*Tei more clearly than it did. In this respect, our results are surprising.

Invasion may have failed if the number of infected drosophila introduced was too low to trigger invasion. Theoretically, *Wolbachia* invasion depends on the intensity of CI, the rate of transmission, and the cost of infection [9–13]. Assuming perfect transmission, the incompatible strain should invade if it is introduced above a threshold frequency equal to the ratio of infection cost (*sf* in the above-mentioned models) to incompatibility (*sh*). In our system of *D. suzukii* and *w*Tei, incompatibility is only incomplete, with an egg hatch rate of about 33% after incompatible crosses (i.e., *sh* = 0.67; [34]. In parallel, the analyses of traits such as fecundity, longevity, egg hatch rate and developmental time all suggest an absence of cost of *w*Tei infection on *D. suzukii* ([32,34]). This apparent absence of infection costs (*sf* = 0), even with incomplete incompatibility, suggests a theoretical invasion threshold of 0. This means that in practice the introduction of very few infected individuals infected with *w*Tei should result in the invasion of this strain. Under these conditions, the number of flies we introduced experimentally - between 76 and 100 individuals in populations that were an order of magnitude larger - should have been high enough to induce invasion.

The observed frequencies of *w*Tei in the experimental populations with CI can alternatively be interpreted as a successful but slow invasion. In this context, the generational overlap inherent in our biological model could have allowed older males, inducing lower CI, to participate in reproduction. Indeed, CI intensity decreases rapidly with male age in several *Drosophila* species [45–47]. In *D. suzukii*, CI intensity is reduced to 27% in mass crosses between *w*Suz-infected females and *w*Tei-infected males aged 9-10 days, compared to 53% when males are 2-3 days old [32,34]. If older males are as competitive for access to females as younger ones, the negative relationship between CI intensity and male age could reduce the demographic consequences of CI, particularly in species such as *D. suzukii*, where individuals can survive for over 58 days at 23-24°C [34].

A final hypothesis, not exclusive to the previous arguments, is that the influence of negative density dependence on host population dynamics overwhelms that of *Wolbachia*-induced CI. In our

414 experiment, after an initial phase of exponential growth, the populations were well described by
415 logistic-type models characterized by a carrying capacity estimated between 450 and 600 adults. This
416 upper limit to population growth is probably a consequence of competition among larvae for limited
417 food resources. Indeed, intraspecific competition certainly occurs in natural populations of *D. suzukii*.
418 Our data suggest that the number of larvae developing in small fruits can be high (for instance, we
419 could find up to 21 individuals at emergence from raspberry or blackberry exposed 48 h in the field;
420 S2 Supporting information). This suggests that larval intraspecific competition may be severe *in natura*,
421 in particular when fruit abundance is weak, *i.e.*, at the beginning or at the end of the season. Laboratory
422 experiments are consistent. According to Bezerra Da Silva *et al*. [36], the recurrent observation of *D.*
423 *suzukii* pupae outside oviposition patches is a sign of high sensitivity to larval competition. The number
424 of eggs and larvae developing in damaged fruits affects development time and further reduces adult
425 size and fecundity [48,49]. In adults, increasing density results in females laying in fewer eggs laid per
426 fruit, a behavior interpreted as an adaptation to alleviate competition among immature stages [50].

427 If larval survival depends on the intensity of competition within the fruit, a reduction in egg hatch rate
428 resulting from *Wolbachia*-induced CI should, at high densities, alleviate competition and therefore,
429 cause an immediate increase in larval survival. Reduced competition would offset incompatibility, and
430 if this were true, CI would not cause the predicted decline in population size. Such a verbal argument
431 echoes a specific section of the theoretical analysis of Dobson *et al.* ([18], Fig. 2b), where the effect of
432 density on immature survival is scaled by the parameter γ [51]. Increasing the influence of density by
433 increasing γ made the effects of introducing incompatible strains disappear [18]. Other modelling
434 studies have highlighted similar influence of competition among hosts on *Wolbachia* invasion [15–17].
435 More generally, the strength of competition may alter the benefits of gene drive [52] and of the sterile
436 insect technique [53] with undesirable consequences such as increasing (rather than decreasing) the
437 size of pest populations.

438 The extent to which negative density dependent survival may jeopardize attempts to reduce pest
439 reproduction appears to be a key issue. However, empirical evidence for the critical role of density
440 dependence in *Wolbachia* invasion and consequences for host population dynamics is completely
441 lacking. In *D suzukii*, we suggest that negative density dependence may explain the discrepancy

between prior expectations and observed population dynamics. However, further species-specific models and experiments are needed to better understand the interplay between density dependence, the invasion of incompatible *Wolbachia* strains and the resulting population dynamics.

Positive density dependence, *i.e.*, the Allee effect, is also being proposed as an important process in pest management. The basic reasoning is that eradication can be achieved via a combination of treatments that reduce population size (pesticides, predators, etc.) and tactics that enhance a pre-existing Allee effect (for instance, mating disruption). Theoretical models, verbal arguments and reviews are numerous [19,20,54,55] and, for insect pests, well supported by widespread evidence of Allee effects [21,56]. A keystone example is that of the gypsy moth *Lymantria dispar*, whose spread is slowed by a combination of insecticides, sterile insect technique and mating disruption [57]. From this perspective, an important finding of our study is the absence of a demographic Allee effect in *D. suzukii*. Despite founding populations with the smallest possible number of individuals (one male and one female) we observed no positive relationship between population size and growth rate, and no extinction. This finding contrasts with observations of Allee effects in closely related species such as *D. melanogaster* [37,58]. In our experimental conditions with population cages, relatively high densities may have prevented an Allee effect. Alternatively, a component Allee effect could have occurred at the individual level, but was offset by negative density dependence and thus did not translate to the population level.

In conclusion, many of our results are puzzling with respect to prior expectations. The absence of a transient decrease in *D. suzukii* population size after the introduction of the incompatible *Wolbachia* strain *w*Tei is most easily explained by the slow invasion of this introduced strain, which, in itself, lacks clear explanations. Nevertheless, there were some incompatible crosses in populations where the proportion of *w*Tei was sometimes higher than 20%, but these did not influence the host population dynamics. We argue that the strong negative density dependence could be a key driver of insect population dynamics, counterbalancing the planned effect of cytoplasmic incompatibility, and possibly hampering pest management programs based on reproductive failure.

Acknowledgments

We thank P. Decoeur, E. Deleury, E. Desouhant, P. Gibert, C. Lopes, L. van Oudenhove for useful discussion on data.

References

1. Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*? – a statistical analysis of current data: *Wolbachia* infection rates. FEMS Microbiology Letters. 2008;281(2):215-20.

2. Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc R Soc B. 2015;282(1807):20150249.

3. Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol. 2008;6(10):741-51.

4. Werren JH. Biology of *Wolbachia*. Annu Rev Entomol. 1997;42(1):587-609.

5. Turelli M, Hoffmann AA. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature. 1991;353(6343):440-2.

6. Flórez LV, Biedermann PHW, Engl T, Kaltenpoth M. Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. Nat Prod Rep. 2015;32(7):904-36.

7. Ross PA, Turelli M, Hoffmann AA. Evolutionary ecology of *Wolbachia* releases for disease control. Annu Rev Genet. 2019;53(1):93-116.

8. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature. 2011;476(7361):454-7.

490 9. Caspari E, Watson GS. On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution.
491 1959;13(4):568-70.

492 10. Fine PEM. On the dynamics of symbiote-dependent cytoplasmic incompatibility in culicine mosquitoes.
493 Journal of Invertebrate Pathology. 1978;31(1):10-8.

494 11. Hoffmann AA, Turelli M, Harshman LG. Factors affecting the distribution of cytoplasmic incompatibility in
495 *Drosophila simulans*. Genetics. 1990;126(4):933-48.

496 12. Hurst L D. The incidences and evolution of cytoplasmic male killers. Proc R Soc Lond B. 1991;244(1310):91-9.

497 13. Turelli M. Evolution of incompatibility-inducing microbes and their hosts. Evolution. 1994;48(5):1500-13.

498 14. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, et al. The *w*Mel
499 *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature.
500 2011;476(7361):450-3.

501 15. Hancock PA, Sinkins SP, Godfray HCJ. Population dynamic models of the spread of *Wolbachia*. The American
502 Naturalist. 2011;177(3):323-33.

503 16. Hancock PA, White VL, Callahan AG, Godfray CHJ, Hoffmann AA, Ritchie SA. Density-dependent population
504 dynamics in *Aedes aegypti* slow the spread of *w*Mel *Wolbachia*. J Appl Ecol. 2016;53(3):785-93.

505 17. Hancock PA, White VL, Ritchie SA, Hoffmann AA, Godfray HCJ. Predicting *Wolbachia* invasion dynamics in
506 *Aedes aegypti* populations using models of density-dependent demographic traits. BMC Biol.
507 2016;14(1):96.

508 18. Dobson SL, Fox CW, Jiggins FM. The effect of *Wolbachia*-induced cytoplasmic incompatibility on host
509 population size in natural and manipulated systems. Proc R Soc Lond B. 2002;269(1490):437-45.

510 19. Liebhold AM, Tobin PC. Population ecology of insect invasions and their management. Annu Rev Entomol.
511 2008;53(1):387-408.

512 20. Tobin PC, Berec L, Liebhold AM. Exploiting Allee effects for managing biological invasions. Ecology Letters.
513 2011;14(6):615-24.

514 21. Fauvergue X. A review of mate-finding Allee effects in insects: from individual behavior to population
515 management. Entomol Exp Appl. 2013;146(1):79-92.

516 22. Liebhold AM, Berec L, Brockerhoff EG, Epanchin-Niell RS, Hastings A, Herms DA, et al. Eradication of
517 invading insect populations: from concepts to applications. Annu Rev Entomol. 2016;61(1):335-52.

518 23. Lampert A, Liebhold AM. Combining multiple tactics over time for cost-effective eradication of invading
519 insect populations. Ecology Letters. 2021;24(2):279-87.

520 24. Blackwood JC, Vargas R, Fauvergue X. A cascade of destabilizations: combining *Wolbachia* and Allee effects
521 to eradicate insect pests. J Anim Ecol. 2018;87(1):59-72.

522 25. Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, et al. Invasion biology of spotted wing Drosophila
523 (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci. 2015;88(3):469-94.

524 26. Hassani IM, Behrman EL, Prigent SR, Gidaszewski N, Ravaomanarivo LHR, Suwalski A, et al. First occurrence
525 of the pest *Drosophila suzukii* (Diptera: Drosophilidae) in the Comoros Archipelago (Western Indian Ocean).
526 African Entomology. 2020;28(1):78.

527 27. Boughdad A, Haddi K, El Bouazzati A, Nassiri A, Tahiri A, El Anbri C, et al. First record of the invasive spotted
528 wing Drosophila infesting berry crops in Africa. J Pest Sci. 2021;94(2):261-71.

529 28. Kwadha CA, Okwaro LA, Kleman I, Rehermann G, Revadi S, Ndlela S, et al. Detection of the spotted wing
530 drosophila, *Drosophila suzukii*, in continental sub-Saharan Africa. J Pest Sci. 2021;94(2):251-9.

531 29. Cattel J, Kaur R, Gibert P, Martinez J, Fraimout A, Jiggins F, et al. *Wolbachia* in European populations of the
532 invasive pest *Drosophila suzukii*: regional variation in infection frequencies. PLoS ONE.
533 2016;11(1):e0147766.

534 30. Hamm CA, Begun DJ, Vo A, Smith CCR, Saelao P, Shaver AO, et al. *Wolbachia* do not live by reproductive
535 manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Mol Ecol.
536 2014;23(19):4871-85.

537 31. Cattel J, Martinez J, Jiggins F, Mouton L, Gibert P. *Wolbachia*-mediated protection against viruses in the
538 invasive pest *Drosophila suzukii*. Insect Mol Biol. 2016;25(5):595-603.

539 32. Cattel J, Nikolouli K, Andrieux T, Martinez J, Jiggins F, Charlat S, et al. Back and forth *Wolbachia* transfers
540 reveal efficient strains to control spotted wing Drosophila populations. J Appl Ecol. 2018;55(5):2408-18.

541 33. Nikolouli K, Colinet H, Renault D, Enriquez T, Mouton L, Gibert P, et al. Sterile insect technique and
542 *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. J Pest Sci.
543 2018;91(2):489-503.

544 34. Nikolouli K, Sassù F, Mouton L, Stauffer C, Bourtzis K. Combining sterile and incompatible insect techniques
545 for the population suppression of *Drosophila suzukii*. J Pest Sci. 2020;93(2):647-61.

546 35. Hardin JA, Kraus DA, Burrack HJ. Diet quality mitigates intraspecific larval competition in *Drosophila suzukii*.
547 Entomol Exp Appl. 2015;156(1):59-65.

548 36. Bezerra Da Silva CS, Park KR, Blood RA, Walton VM. Intraspecific competition affects the pupation behavior
549 of spotted-wing Drosophila (*Drosophila suzukii*). Sci Rep. 2019;9(1):7775.

550 37. Wertheim B, Marchais J, Vet LEM, Dicke M. Allee effect in larval resource exploitation in *Drosophila* : an
551 interaction among density of adults, larvae, and micro-organisms. Ecological Entomology.
552 2002;27(5):608-17.

553 38. Siozios S, Cestaro A, Kaur R, Pertot I, Rota-Stabelli O, Anfora G. Draft genome sequence of the *Wolbachia*
554 endosymbiont of *Drosophila suzukii*. Genome Announc. 2013;1(1):e00032-13.

555 39. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods.
556 2012;9(7):671-5.

557 40. Tobin PC, Whitmire SL, Johnson DM, Bjørnstad ON, Liebhold AM. Invasion speed is affected by geographical
558 variation in the strength of Allee effects. Ecology Letters. 2007;10(1):36-43.

559 41. Henri H, Mouton L. High-Resolution Melting technology: a new tool for studying the *Wolbachia*
560 endosymbiont diversity in the field. Molecular Ecology Resources. 2012;12(1):75-81.

561 42. Xi Z, Khoo CCH, Dobson SL. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory
562 population. Science. 2005;310(5746):326-8.

563 43. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, et al. The *w*Mel strain
564 blocks dengue and invades caged populations. NATURE. 2011;476(7361):450-U101.

565 44. Kriesner P, Hoffmann AA. Rapid spread of a *Wolbachia* infection that does not affect host reproduction in
566 *Drosophila simulans* cage populations. Evolution. 2018;72(7):1475-87.

567 45. Turelli M, Hoffmann AA. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter
568 estimates from natural populations. Genetics. 1995;140(4):1319-38.

569 46. Reynolds KT, Hoffmann AA. Male age, host effects and the weak expression or non-expression of
570 cytoplasmic incompatibility in *Drosophila* strains infected by maternally transmitted *Wolbachia*. Genet Res.
571 2002;80(2):79-87.

572 47. Shropshire JD, Hamant E, Cooper BS. Male age and *Wolbachia* dynamics: investigating how fast and why
573 bacterial densities and cytoplasmic incompatibility strengths vary. mBio. 2021;12(6):e02998-21.

574 48. Kienzle R, Groß LB, Caughman S, Rohlfs M. Resource use by individual *Drosophila suzukii* reveals a flexible
575 preference for oviposition into healthy fruits. Sci Rep. 2020;10(1):3132.

576 49. Reyes-Ramírez A, Belgaidi Z, Gibert P, Pommier T, Siberchicot A, Mouton L, et al. Larval density in the
577 invasive *Drosophila suzukii* : immediate and delayed effects on life-history traits [Internet]. 2022 oct [cited
578 2023 july 30]. Available from: http://biorxiv.org/lookup/doi/10.1101/2022.10.06.511120

50. Elsensohn JE, Schal C, Burrack HJ. Plasticity in oviposition site selection behavior in *Drosophila suzukii* (Diptera: Drosophilidae) in relation to adult density and host distribution and quality. Journal of Economic Entomology. 2021;114(4):1517-22.

51. Slatkin M, Smith JM. Models of coevolution. The Quarterly Review of Biology. 1979;54(3):233-63.

52. Dhole S, Lloyd AL, Gould F. Gene drive dynamics in natural populations: the importance of density dependence, space, and sex. Annu Rev Ecol Evol Syst. 2020;51(1):505-31.

53. Yakob L, Alphey L, Bonsall MB. *Aedes aegypti* control: the concomitant role of competition, space and transgenic technologies. Journal of Applied Ecology. 2008;45(4):1258-65.

54. Blackwood JC, Berec L, Yamanaka T, Epanchin-Niell RS, Hastings A, Liebhold AM. Bioeconomic synergy between tactics for insect eradication in the presence of Allee effects. Proc R Soc B. 2012;279(1739):2807-15.

55. Suckling DM, Tobin PC, McCullough DG, Herms DA. Combining tactics to exploit Allee effects for eradication of alien insect populations. Jnl econ entom. 2012;105(1):1-13.

56. Kramer AM, Dennis B, Liebhold AM, Drake JM. The evidence for Allee effects. 2009;51(3):341-54.

57. Lampert A, Liebhold AM. Combining multiple tactics over time for cost-effective eradication of invading insect populations. Ecology Letters. 2021;24(2):279-87.

58. Elliott KH, Betini GS, Norris DR. Fear creates an Allee effect: experimental evidence from seasonal populations. Proc R Soc B. 2017;284(1857):20170878.

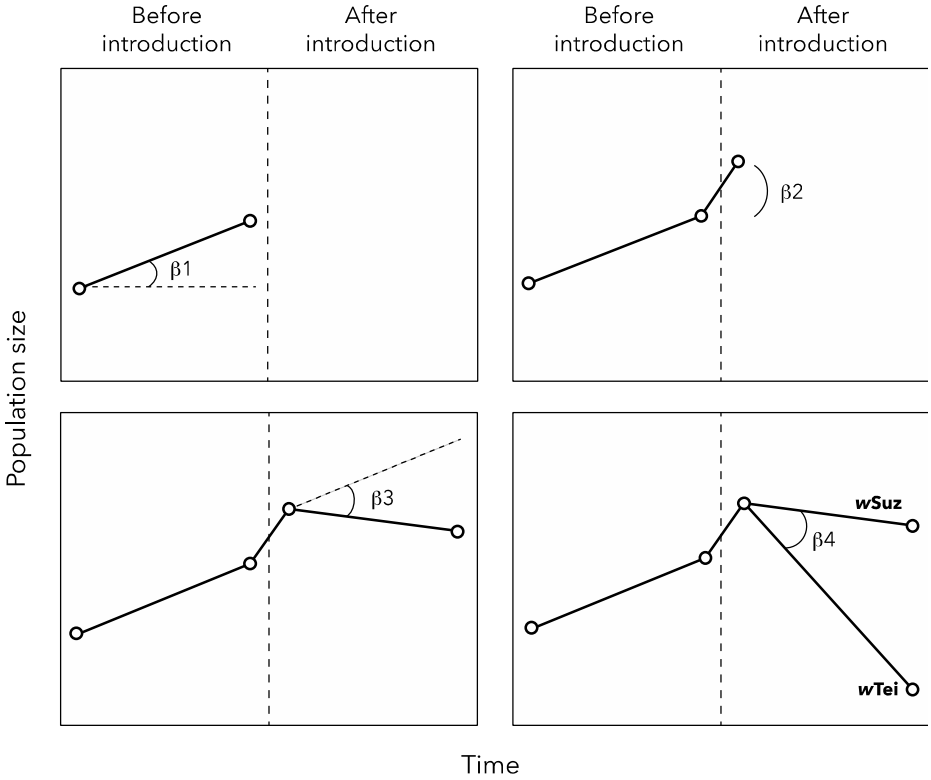

Figure 1. Schematic representation of the statistical model fitted to time variations in number of *D. suzukii* in order to test the effect of introducing insects infected with either incompatible (*w*Tei) or compatible (*w*Suz) *Wolbachia* into a recipient population. Four hypotheses were tested, based on estimation of four model parameters. H1: parameter β1 population growth before introduction; populations were at carrying capacity and we therefore expected β1=0; H2: parameter β2 represents the immediate effect of introduction on population size; the number of flies introduced was set at 10% of the carrying capacity so we expected β2>0. H3: parameter β3 represents the effect of introduction on time variation of population size; if β1=0 and β2>0, we expected β3<0 reflecting a return to carrying capacity. H4: parameter β4 represents the effect of the *Wolbachia* strain introduced on the variation of population size after introduction; theoretical models predict that the introduction of an incompatible strain (*w*Tei) should result in a transient decrease in the population size, which should not be observed in control populations (introduction of *w*Suz), so that we expect β4<0.

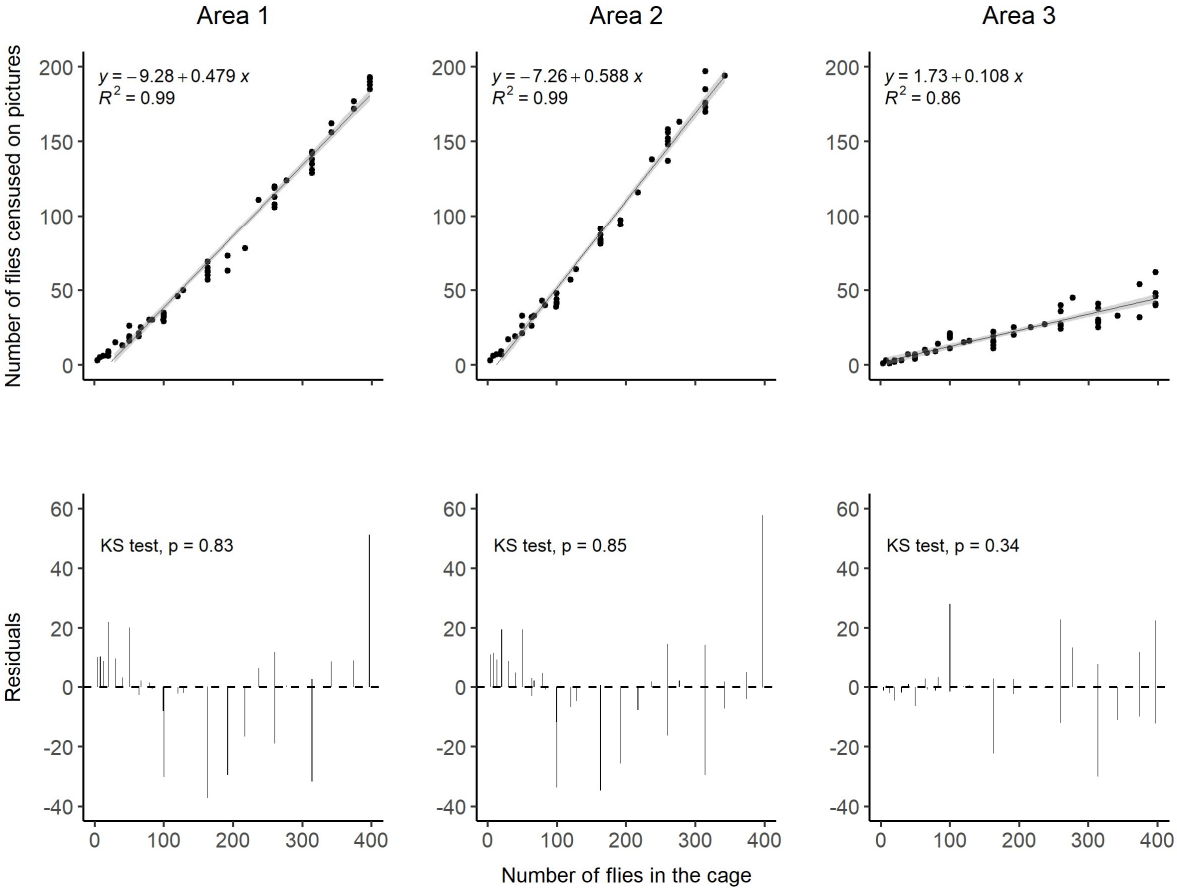

Figure 2. Estimation of population size. Linear regressions between the number of adult *D. suzukii* censused on pictures of three different areas of the experimental cages and the total number of individuals in the cage.

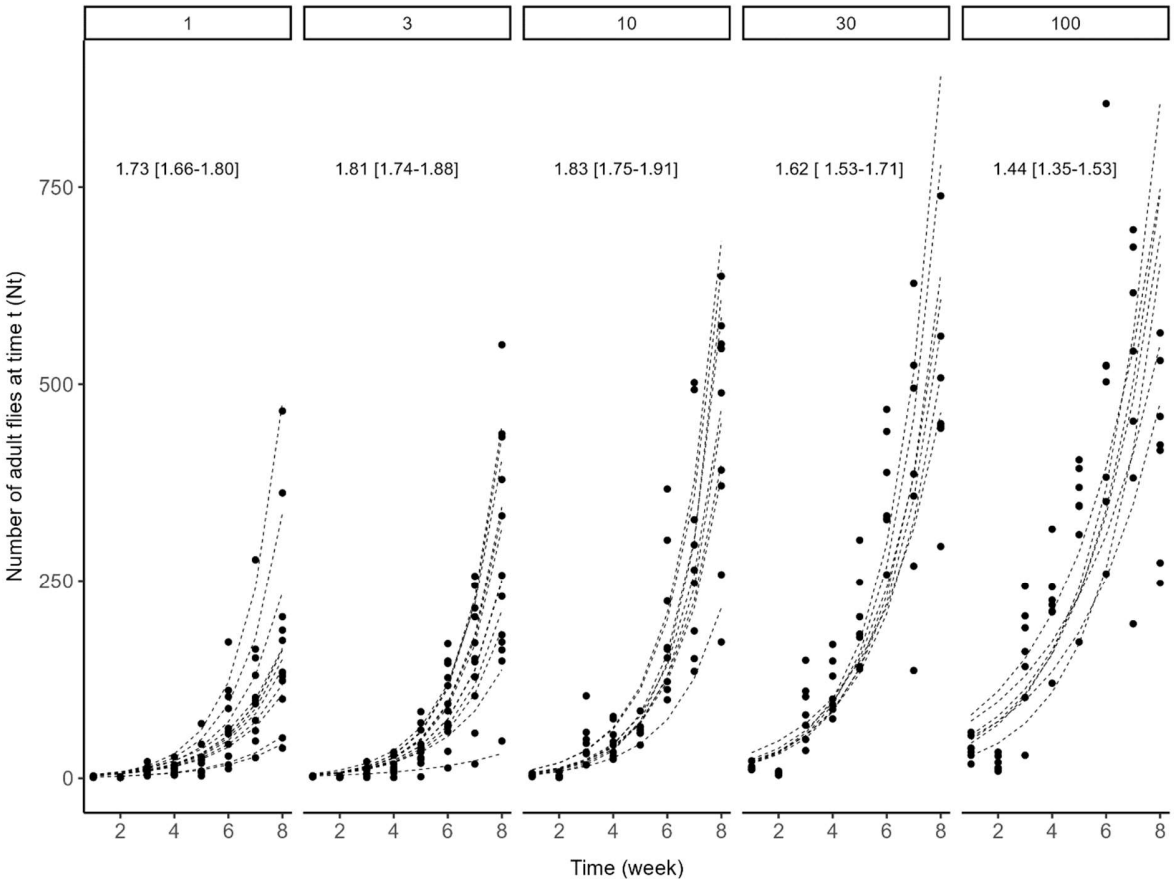

Figure 3. Early dynamics of small *D. suzukii* populations initiated with 1, 3, 10, 30 or 100 male-female pairs. For each replicated population, the eight-week time series of observed number of individuals (circles) was fitted with an exponential growth model $N_t = N_0 \exp(r\,t)$ (lines). Estimated marginal means of population growth rate r and their 95% confidence intervals are shown.

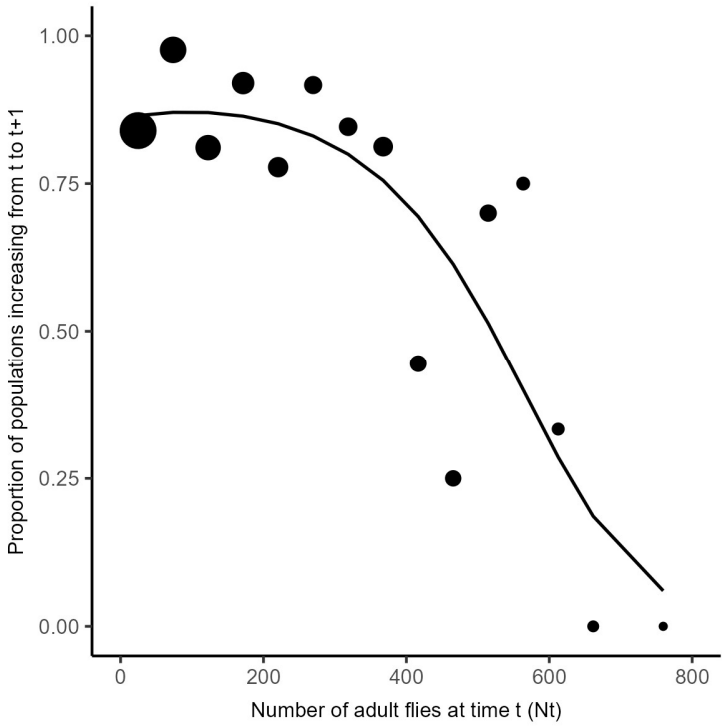

Figure 4. Early dynamics of small *D. suzukii* populations. For each range of population size at time *t* (N_t: 0-49, 50-99, etc.) circles represent the proportion *p* of pairs for which the number of individuals increased ($N_{t+1} \geq N_t$). Circle size represent the number of pairs available to estimate each proportion (largest circle: x pairs; smallest circle: y pairs). The curve represents the second-order polynomial regression that best fitted the data (logit $p = 1.814 + 2.014 \cdot 10^{-03} N_t - 1.056 \cdot 10^{-05} N_t^2$).

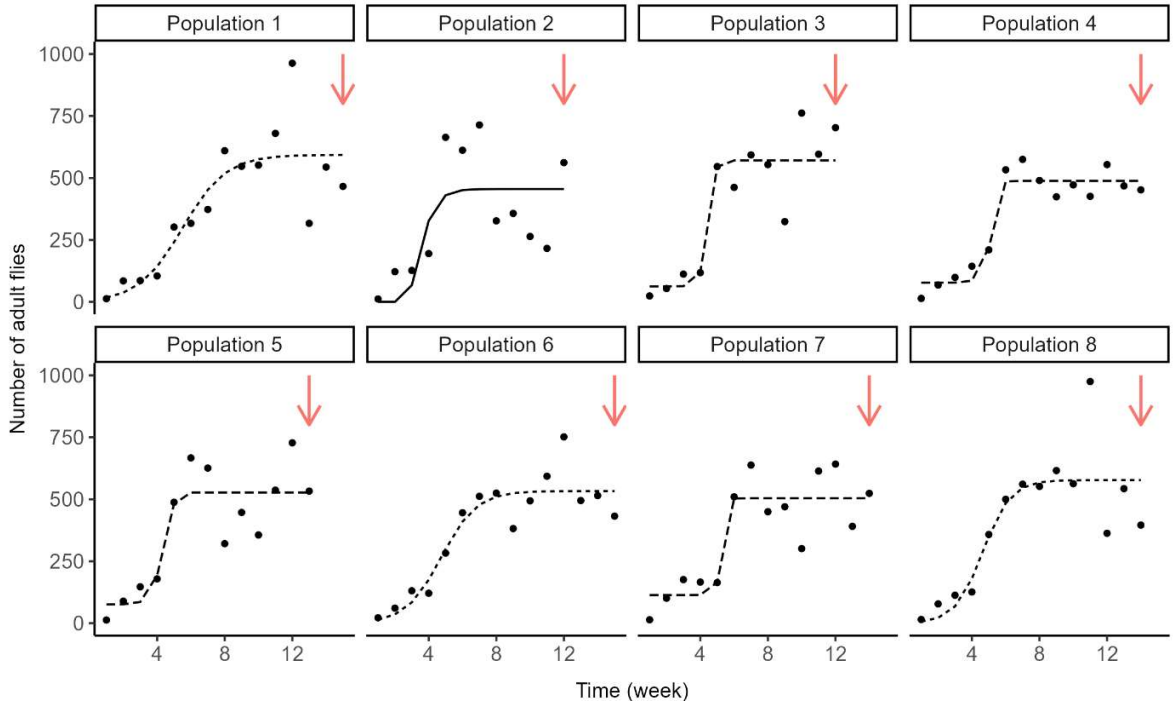

Figure 5. Reaching the carrying capacity. Observed and predicted time variations of *D. suzukii* population size for eight replicated populations before the introduction of Wolbachia-infected individuals. Line types represent the logistic population growth model that best fitted the data (dashed = Weibull, solid= Gompertz, dotted= logistic). Red arrows indicate the time at which infected flies were introduced, i.e., seven weeks after the first observed decrease in population size.

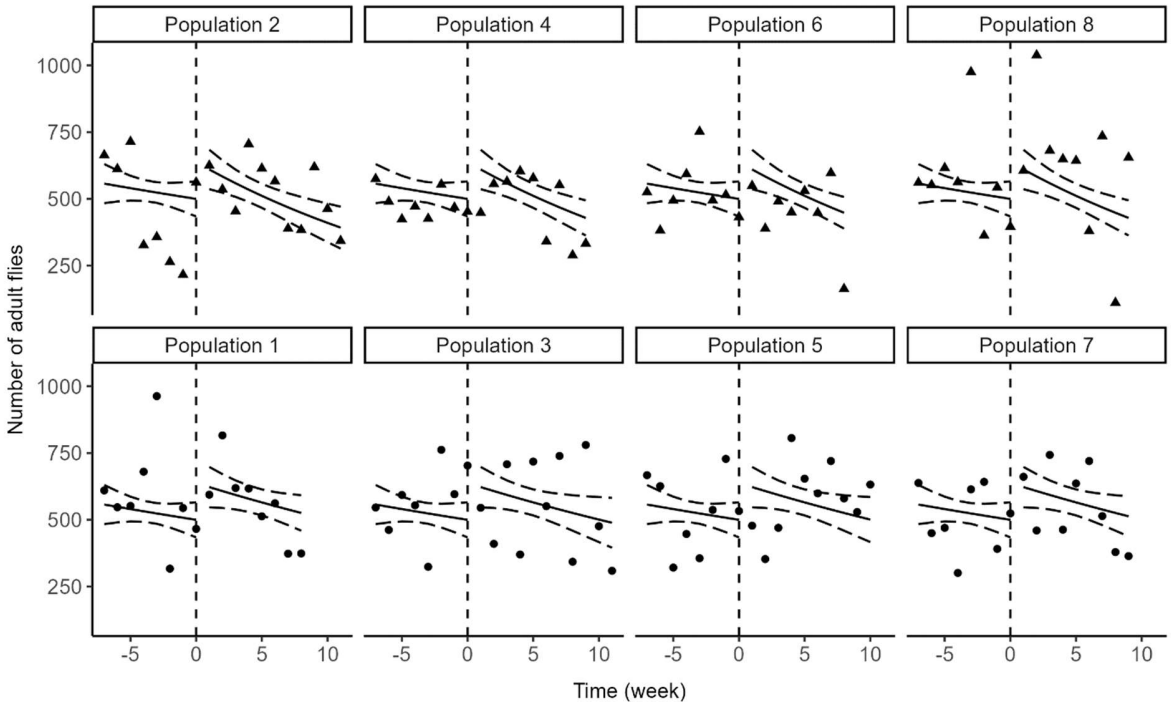

Figure 6. Impacts of introduced CI-inducing flies. Observed and predicted number of adult *D. suzukii* in populations with CI (triangles; introduction of individuals infected by *w*Tei) and without CI (circle; introduction of individuals infected by *w*Suz). Time is scaled so that introductions occur at t=0.

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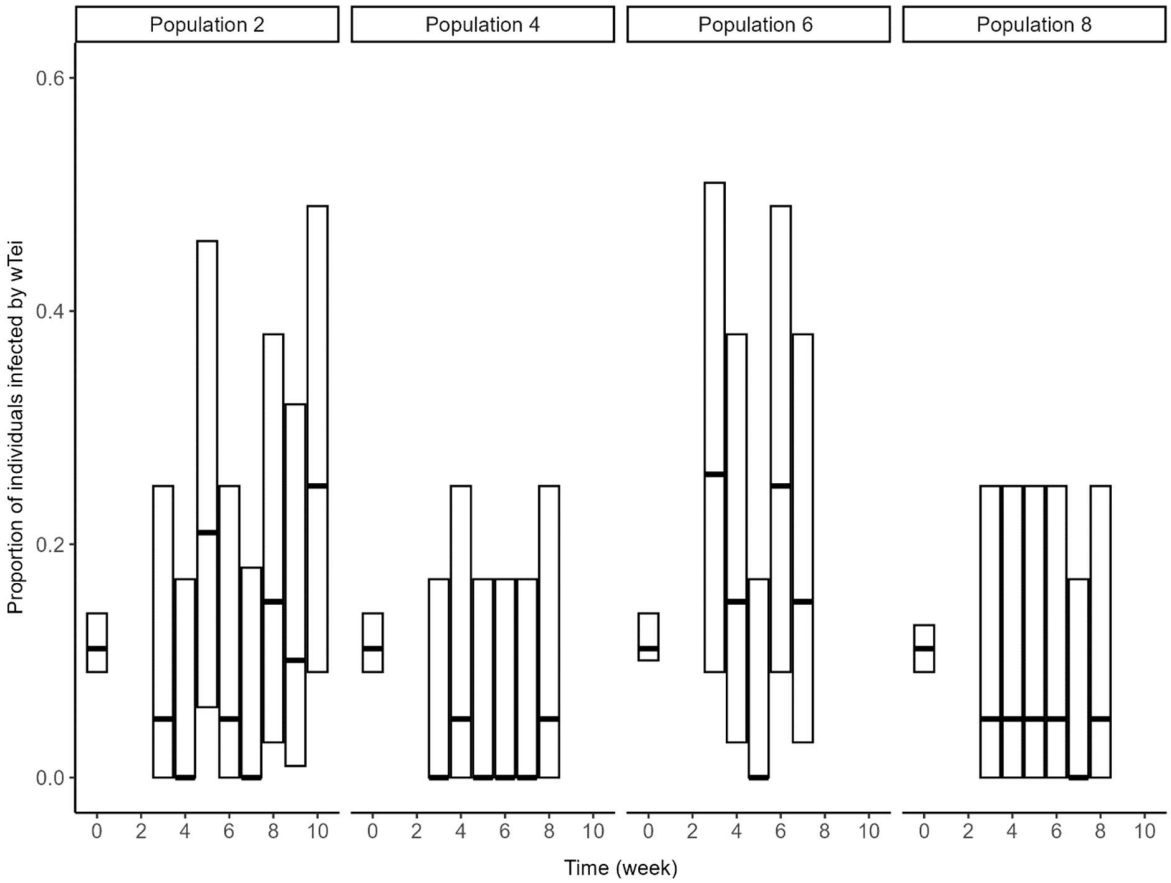

Figure 7. Dynamics of Wolbachia infection. Evolution of the proportion of *D. suzukii* infected by the introduced *Wolbachia* strain *w*Tei. Each proportion is presented with its theoretical 95% confidence interval, based on the binomial distribution. The first bar at t=0 corresponds to the proportion of *w*Tei-infected flies actually introduced (10 %). Subsequent prevalence was estimated from the third week post-introduction, after emergence of offspring from the introduced individuals.

Table 1- Gompertz, logistic and Weibull population growth models fitted to each of the eight experimental population. K (referred as Asym in the models) is the parameter common to all three models and that corresponds to the asymptotic value of the population size when time tends to ∞, or the carrying capacity. Values of K and 95% confidence intervals were estimated. Models in bold are those that best fit the observed data (lowest AIC and RMSE).

Population	Model	AIC	RMSE	K estimate
1	Gompertz	197.9	135.7	597.2 (452.6,741.8)
	Logistic	**197.3**	**133.3**	**593.5(466.3,720.6)**
	Weibull	_	_	_
2	**Gompertz**	**164.2**	**162.1**	**455.7(317.1,594.3)**
	Logistic	_	_	_
	Weibull	_	_	_
3	Gompertz	_	_	_
	Logistic	154.7	109.4	572.7(478.5,666.9)
	Weibull	**155.7**	**104.9**	**570.7(475.5,665.9)**
4	Gompertz	165.3	66.6	489.0(433.7,544.3)
	Logistic	_	_	_
	Weibull	**158.1**	**47.9**	**488.2(449.6,526.8)**
5	Gompertz	_	_	_
	Logistic	_	_	_
	Weibull	**169.0**	**109.6**	**527.4(436.2,618.7)**
6	Gompertz	182.6	81.5	543.3(464.9,621.7)
	Logistic	**181.7**	**79.2**	**533.4(467.4,599.4)**
	Weibull	183.4	78..2	523.8(462.0,585.5)
7	Gompertz	179.5	110.6	520.0(400.2,639.9)
	Logistic	178.6	106.9	510.4(415.5,605.3)
	Weibull	**177.0**	**94.2**	**504.5(427.3,581.7)**
8	Gompertz	185.2	135.7	579.3(459.6,699.0)
	Logistic	**184.7**	**133.4**	**577.6(464.9,690.3)**
	Weibull	_	_	_