Egg-stage desiccation reduces developmental recovery and reveals strain-dependent *Wolbachia*-associated costs in the Mediterranean fruit fly, *Ceratitis capitata*

Kamilari M[1†*], Giannatos G[1,2†], Tsiamis, G[2], Augustinos AA[1*]

[1] Department of Plant Protection Patras, Institute of Industrial and Forage Crops, Ellinikos Georgikos Organismos DIMITRA, 26442 Patras, Greece

[2] Laboratory of Systems Microbiology and Applied Genomics, Department of Sustainable Agriculture, School of Agricultural Sciences, University of Patras, 2 George Seferi St., 30131 Agrinio, Greece

[†] equal contribution

[*] Corresponding authors

Abstract

The Mediterranean fruit fly (medfly) (*Ceratitis capitata* (Wiedemann, 1824) is a major agricultural pest, and egg desiccation is a critical constraint during handling and mass-rearing, as even short periods without moisture may compromise developmental success and downstream adult performance. The *Wolbachia*–medfly symbiosis is a relatively recently established artificial association, generated less than three decades ago using *Rhagoletis cerasi* as the *Wolbachia* donor. In this study, we evaluated the effects of egg-stage desiccation on developmental success and subsequent adult performance in three medfly lines differing in *Wolbachia* status: the uninfected Benakeion line, the *w*Cer2-infected 88.6 line, and the *w*Cer4-infected S10.3 line. Eggs were exposed to desiccation for 0–24 h at 4-h intervals before transfer to larval diet, and hatching, pupation, and adult emergence were recorded. We additionally assessed adult survival under stress for flies

emerging from the 0, 8, and 10 h egg-desiccation treatments. Under control conditions, Benakeion showed the highest hatching and developmental recovery, S10.3 the lowest, and 88.6 intermediate performance. Across all strains, short desiccation exposures were comparatively well tolerated, whereas prolonged exposure sharply reduced hatching, pupation, and adult emergence, with the clearest decline at 20–24 h. Strain-dependent differences were expressed mainly at the hatching stage, while later developmental transitions were more similar among strains once larvae had hatched. In the adult follow-up, strain, rather than moderate egg-stage desiccation, was the main determinant of short-term survival and survival under extreme stress, with S10.3 again showing the weakest performance. These results indicate that *Wolbachia*-associated fitness costs in medfly are strain dependent and that egg-stage desiccation primarily acts at the embryonic bottleneck. Beyond providing insight into the *Wolbachia*–medfly artificial symbiosis, our findings are directly relevant to egg-handling and strain-evaluation protocols in medfly mass-rearing systems.

Keywords

Ceratitis capitata; desiccation stress; Wolbachia; egg viability; mass rearing; quality control; Tephritidae

1. Introduction

Water balance is a central determinant of insect performance, affecting survival, development, behaviour, and ecological persistence. In terrestrial insects, desiccation stress arises when evaporative water loss exceeds the capacity for water acquisition and physiological compensation, and coping with this imbalance depends on integrated responses involving the cuticle, excretory system, gut, metabolism, and behaviour. Because all terrestrial insects experience fluctuating temperature and humidity, they may encounter desiccation stress continuously or intermittently, with consequences ranging from reduced activity and altered behaviour to developmental

disruption and mortality (Crowe, 2015; McCluney, 2017; McCluney et al., 2012; Moss et al., 2024; Weldon et al., 2018, 2016). At the physiological level, water loss can lead to a hyperosmotic state that impairs protein folding, enzyme activity, and cellular homeostasis, making osmoregulation and dehydration tolerance fundamental components of insect resilience (Addo-Bediako et al., 2001; Chown et al., 2011; Halberg and Denholm, 2024; Thorat and Nath, 2018; Weldon et al., 2019, 2018). Although the general principles of insect osmoregulation are well established, responses to desiccation remain insufficiently characterized for many economically important non-model species.

Insects exhibit diverse physiological and behavioural strategies for coping with water scarcity. At the physiological level, variation in cuticular permeability, renal and digestive function, and metabolic responses can all contribute to the maintenance of water and ion balance under dehydration stress (Chown et al., 2011; Halberg and Denholm, 2024; Lee, 2002; Thorat and Nath, 2018). At the behavioural level, insects may modify activity patterns, seek out more humid microhabitats, or alter reproductive investment in response to desiccating conditions(Bartlett et al., 2019; Bujan et al., 2016; Johnson et al., 2023). Such strategies are ecologically significant because temperature and humidity often fluctuate together in nature, and periods of high temperature coupled with low relative humidity can reduce survival and population persistence if water loss cannot be compensated (Fischer and Kirste, 2018; Holmes and Benoit, 2019; Schmidt et al., 2018; Simmons et al., 2023; Wang et al., 2021). Understanding how insects respond to desiccation stress is therefore important not only from a physiological perspective, but also for interpreting species distributions, host use, and performance in changing environments.

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is among the most economically important tephritid pests worldwide and a major target of area-wide control programmes. Native to temperate regions but now widely established in subtropical and tropical

areas, medfly continues to expand its geographic range, with its seasonal biology and invasion success shaped by temperature, humidity, and host availability (Szyniszewska *et al*, 2024; Papadogiorgou *et al*, 2024; Szyniszewska & Tatem, 2014). Because the species exploits fleshy fruit and other humid microhabitats for oviposition and larval development, the egg and larval stages are especially sensitive to water balance, whereas adults may experience water stress outside the host or under suboptimal environmental conditions (Eskafi and Fernandez, 1990; Giunti et al., 2023; Sinclair et al., 2024; Weldon et al., 2016). Earlier work showed that medfly eggs are highly sensitive to low-humidity exposure, with hatchability declining sharply under severe drying conditions, indicating that embryonic development constitutes a major bottleneck in the response to water loss (Shoukry and Hafez, 1979). From an applied perspective, egg survival and the quality of resulting adults are directly relevant to the efficiency of mass-rearing systems and to standard quality-control frameworks used in tephritid production programmes (FAO/IAEA/USDA., 2019).

Medfly is also a tractable and highly informative model within Tephritidae. Its economic importance and cosmopolitan distribution have made it a key system in genetics, molecular biology, biotechnology, behaviour, and environmentally friendly insect control approaches (Bravo and Zucoloto, 1998; Dyck et al., 2021; Franz et al., 2021; Giunti et al., 2023). Medfly was the first fruit fly in which polytene chromosome maps were developed, providing evidence for the conservation of Muller elements in Diptera and facilitating later cytogenetic and genomic advances (Zacharopoulou et al., 2017). It was also the first non-drosophilid insect to be genetically transformed (Loukeris et al., 1995; Zwiebel et al., 1995). In addition, medfly has served as a model species for the development of the sterile insect technique (SIT), including extensive work on artificial rearing and genetic sexing strains for male-only releases (Franz et al., 2021). These characteristics make medfly particularly suitable for examining how physiological stress at the egg stage may affect developmental success and the biological quality of resulting adults.

94 The medfly also provides a useful system for studying the fitness consequences of artificial
95 *Wolbachia* associations. *Wolbachia* is a widespread intracellular α-proteobacterium associated
96 with diverse reproductive phenotypes in arthropods, including parthenogenesis, feminization, male
97 killing, and cytoplasmic incompatibility (CI) (Hilgenboecker et al., 2008; O'Neill and Karr, 1990;
98 Saridaki and Bourtzis, 2010; Yen and Barr, 1971; Zug and Hammerstein, 2012). Stable
99 transinfections with the *Wolbachia* strains *w*Cer2 and *w*Cer4 were established previously in *C.*
100 *capitata*, using *Rhagoletis cerasi* as donor, in the context of exploring the possible exploitation of
101 Wolbachia-induced CI in agricultural pest control. Both strains induce strong CI in the novel host,
102 including unidirectional incompatibility against uninfected females and bidirectional incompatibility
103 between the two infected lines (Zabalou et al., 2009, 2004). More broadly, medfly has become one
104 of the best tephritid systems for evaluating both the biological and applied consequences of
105 *Wolbachia* infection in fruit flies (Mateos et al., 2020).

106 However, the medfly–Wolbachia symbiosis does not come without fitness costs. Initial and follow-
107 up studies have shown that the effects of *Wolbachia* on medfly biology are strain dependent, with
108 infected lines showing reduced hatching, altered immature performance, increased sensitivity to
109 elevated temperatures, and reduced male sexual signalling, among other effects (Dionysopoulou et
110 al., 2020; Kyritsis et al., 2022, 2019; Sarakatsanou et al., 2011; Zabalou et al., 2009, 2004). In
111 particular, the *w*Cer4-infected line S.10.3 has repeatedly shown stronger costs than the *w*Cer2-
112 infected line 88.6, indicating that host genotype and *Wolbachia* strain interact to shape life-history
113 traits and tolerance to challenging developmental conditions (Dionysopoulou et al., 2020; Kyritsis et
114 al., 2022, 2019). Because transient drying of eggs may occur during handling and transfer in rearing
115 facilities, any Wolbachia-associated reduction in desiccation tolerance could influence both
116 developmental recovery and the biological quality of resulting adults. Despite this, the interaction
117 between egg-stage desiccation and Wolbachia infection in medfly has not been examined directly.

118 In the present study, we investigated whether desiccation duration affects hatching, pupation, and
119 adult emergence in three medfly lines differing in Wolbachia status, namely the uninfected
120 Benakeion line and the *w*Cer2- and *w*Cer4-infected lines 88.6 and S.10.3. We also examined whether
121 moderate egg-stage desiccation has downstream effects on adult survival under stress. We
122 predicted that prolonged egg desiccation would reduce developmental recovery, that the strongest
123 strain-dependent divergence would be expressed at hatching, and that the *w*Cer4-infected line
124 would show the greatest performance cost. By addressing these questions, the study links stress
125 physiology with an applied problem in medfly strain evaluation and egg-handling protocols.

126 **2. Materials and Methods**

127 ***2.1 Insect strains and rearing conditions***

128 All experiments were conducted at the Department of Plant Protection of Patras (ELGO-DIMITRA,
129 Greece) using *C. capitata* strains maintained routinely in the insectary. Adults were kept in plastic-
130 framed cages (30 × 30 × 10 cm) with mesh-covered sides for oviposition under controlled conditions
131 of 25 ± 1°C, 55% relative humidity (RH), and a 14:10 h light:dark photoperiod.

132 Three medfly lines differing in Wolbachia status were used: (i) Benakeion, a long-established
133 laboratory line free of Wolbachia; (ii) 88.6, a Benakeion-derived transinfected line carrying the *w*Cer2
134 Wolbachia strain; and (iii) S.10.3, a Benakeion-derived transinfected line carrying the *w*Cer4
135 Wolbachia strain. The transinfected lines were originally generated by artificial transfer of Wolbachia
136 from *Rhagoletis* cerasi into the medfly background, as described previously (Zabalou et al., 2004).
137 The infected lines have since been maintained through inbreeding under laboratory conditions.

138 ***2.2 Verification of Wolbachia infection status***

139 Prior to the experiments, the Wolbachia status of all three strains was verified by diagnostic PCR.
140 Genomic DNA was extracted from six adult individuals per strain using a CTAB-based protocol

141 (Kamilari et al., 2025). DNA quality was first checked using universal 12S rRNA primers (Hanner and
142 Fugate, 1997). Wolbachia infection status was then determined using strain-specific primer pairs
143 targeting the *wsp* variants diagnostic for *w*Cer2 and *w*Cer4 (Arthofer *et al*, 2009). PCR products were
144 separated on 2% agarose gels and visualized under UV illumination. All individuals yielded the
145 expected amplification pattern, confirming the infection status of the three experimental lines.

146 ***2.3 Egg collection and desiccation treatments***

147 Eggs were collected during the early morning (08:00–11:00 h) from cages at peak oviposition activity
148 (days 4–7). Freshly collected eggs were transferred onto dry black filter paper and arranged in
149 replicate batches of approximately 30 eggs each. For each strain, replicate egg batches were
150 assigned to one of seven desiccation treatments: 0, 4, 8, 12, 16, 20, or 24 h of exposure. Finally,
151 replicate batches from all runs were pooled for analysis. For the control treatment (0 h), three
152 replicate egg batches per strain were transferred immediately to Petri dishes containing standard
153 larval diet. For the desiccation treatments, eggs remained on dry filter paper without an external
154 moisture source for the assigned duration and were then transferred to larval diet. Thus, for each
155 strain, developmental performance was assessed after a graded series of egg-stage desiccation
156 exposures from 0 to 24 h at 4-h intervals (**Fig.1).**

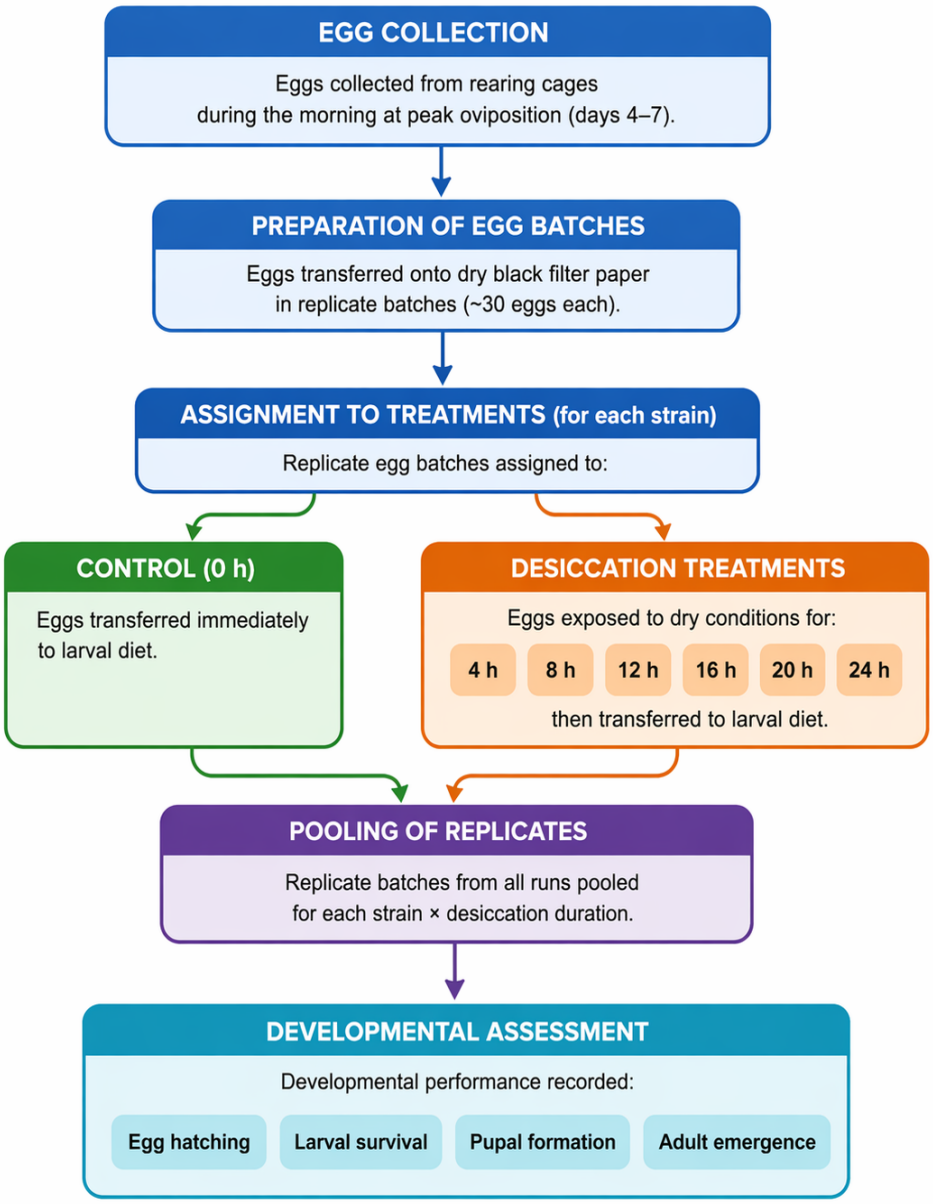

Figure 1. Experimental workflow for egg-stage desiccation treatments and developmental assessment in *Ceratitis capitata*.

2.4 Assessment of hatching, pupation, and adult emergence

Hatching was scored four days after oviposition. At that point, each Petri dish was transferred to an individual plastic box containing sand as pupation substrate. Seven days later, pupae were recovered by sieving, counted, and transferred to new Petri dishes for adult emergence. Adult

emergence was considered complete after 11 days, and the total number of emerged adults was recorded.

For each replicate, developmental performance was quantified using both egg-based and conditional-stage metrics. The following response variables were calculated: i) Hatching (%) = number of hatched eggs / initial number of eggs × 100; ii) Pupation on eggs (%) = number of pupae / initial number of eggs × 100; iii) Emergence on eggs (%) = number of emerged adults / initial number of eggs × 100; iv) Pupation on hatched eggs (%) = number of pupae / number of hatched eggs × 100; v) Emergence on pupae (%) = number of emerged adults / number of pupae × 100. This two-level approach allowed us to distinguish overall developmental recovery from stage-specific survival after successful completion of the preceding stage.

2.5 Effects of egg-stage desiccation on adult survival under stress

To evaluate whether moderate egg-stage desiccation had downstream effects on adult quality, a targeted adult follow-up assay was conducted using flies emerging from three egg treatments: control (0 h), 8 h desiccation, and 10 h desiccation. The 8 h and 10 h treatments were selected intentionally based on the main developmental assay, which indicated that the transition from limited to more pronounced detrimental effects occurred within the 8–12 h interval. Thus, 8 h was included as the upper bound of relatively tolerated exposure, whereas 10 h was selected as an intermediate exposure within this biologically relevant transition zone. Longer desiccation exposures were not included in the adult assay because they yielded too few surviving individuals for a meaningful assessment of post-emergence performance.

Following emergence, 20–40 adult flies per strain and treatment group were randomly selected and individually housed in vials without access to food or water. Each fly's survival under extreme stress, defined as the number of hours from adult emergence to death, was recorded. In addition, survival

at 48 h post-emergence was scored as a binary variable (1 = alive at 48 h; 0 = dead before 48 h), as a practical proxy of adult biological quality in line with quality-control approaches used in tephritid mass-rearing programmes. Mortality was checked twice daily at 12 h intervals (FAO/IAEA/USDA., 2019).

2.6 Statistical analysis

As a preliminary step, we analyzed the three non-desiccated control strains to establish baseline developmental performance under unstressed conditions. For each strain, hatching, pupation, and adult emergence were calculated both relative to the initial number of eggs and relative to the preceding developmental stage.

To assess the effects of egg-stage desiccation on development, we analyzed hatching, pupation, and adult emergence across the full desiccation series (0–24 h) in the three medfly strains. The primary analysis focused on egg-based endpoints, namely hatching (%), pupation on eggs (%), and emergence on eggs (%), because these variables capture overall developmental recovery from the initial egg cohort. Conditional-stage endpoints, namely pupation on hatched eggs (%) and emergence on pupae (%), were analyzed separately to determine whether strain effects persisted after successful completion of earlier developmental transitions.

Normality of residuals was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene's test. When assumptions of normality and homoscedasticity were met, parametric analyses were used. Specifically, baseline control comparisons among strains were evaluated by one-way ANOVA, followed by Tukey's honestly significant difference (HSD) test for pairwise contrasts. When assumptions were not met, non-parametric tests were applied. In those cases, differences among strains or among desiccation durations were tested using the Kruskal–Wallis test, followed by Dunn's post hoc comparisons with Bonferroni correction. For the desiccation-

210 duration analyses, hatching, pupation on eggs, and emergence on eggs were compared across the
211 seven time points (0, 4, 8, 12, 16, 20, and 24 h). For strain-level comparisons across treatments,
212 hatching, pupation on hatched eggs, and emergence on pupae were compared among Benakeion,
213 88.6, and S.10.3.

214 For the adult follow-up assay, two response variables were analyzed: survival at 48 h and total
215 survival under extreme stress under stress. This targeted assay included only the control, 8 h, and
216 10 h egg-desiccation treatments, because these exposure levels were chosen to represent the
217 biologically relevant transition zone identified by the main developmental dataset while still
218 providing enough surviving adults for downstream analysis. Survival at 48 h was analyzed using
219 binomial logistic regression, with strain, egg-desiccation treatment, and their interaction included
220 as fixed effects. Adult survival under extreme stress was analyzed using a two-way ANOVA with strain
221 and egg-desiccation treatment as factors after checking model assumptions with the Shapiro–Wilk
222 and Levene's tests. When ANOVA assumptions were not met, non-parametric alternatives (Kruskal–
223 Wallis test followed by Dunn's post hoc test with Bonferroni correction) were applied.

224 All statistical analyses were performed in SPSS v16. Dunn's post hoc tests were carried out using
225 StatsCalculators (2025) ("StatsCalculators.com - Free Online Statistics Calculators," n.d.). The
226 significance threshold was set at $\alpha = 0.05$, with Bonferroni adjustment applied where appropriate for
227 multiple pairwise comparisons.

228 **3. Results**

229 ***3.1. Wolbachia-status verification***

230 All individuals screened from the three medfly strains yielded the expected amplicon pattern in the
231 diagnostic PCR assays, confirming the Wolbachia status of the experimental lines: Benakeion as
232 uninfected, 88.6 as infected with *w*Cer2, and S.10.3 as infected with *w*Cer4.

3.2. Response to egg-stage desiccation stress

3.2.1. Baseline developmental performance of the three strains under control conditions

Under non-desiccating conditions, the three *Ceratitis capitata* strains differed markedly in overall developmental performance (**Table 1; Figure 2**). Benakeion showed the highest hatching, pupation, and adult emergence when these endpoints were calculated relative to the initial number of eggs, whereas S.10.3 consistently showed the lowest values and 88.6 was intermediate. Mean hatching reached 94.47 ± 3.87% in Benakeion, compared with 73.37 ± 1.82% in 88.6 and 58.33 ± 3.82% in S.10.3. A similar ranking was observed for pupation on eggs and emergence on eggs.

When developmental success was expressed relative to the preceding developmental stage, differences among strains were greatly reduced. Pupation among hatched larvae remained high in all strains, ranging from 95.77 ± 4.35% in S.10.3 to 97.73 ± 1.96% in Benakeion, while emergence among pupae ranged from 93.87 ± 6.90% in S.10.3 to 100.00 ± 0.00% in Benakeion (**Table 1**).

The inferential analyses supported the same pattern (**Table 2**). Significant strain differences were detected for hatching, pupation on eggs, and emergence on eggs, whereas no significant strain effect was detected for pupation on hatched eggs or emergence on pupae. Thus, under control conditions, the major among-strain divergence was concentrated at the egg-to-larva transition rather than in later developmental stages. Full descriptive statistics are provided in **Table S1**, and the corresponding conditional-stage control profiles are shown in **Figure S1**.

Table 1. Baseline control performance summary (mean ± SD) for the three Ceratitis **capitata *strains.***

*Mean ± SD values for hatching, pupation, and adult emergence in the Benakeion, 88.6 (w*Cer*2), and S.10.3 (w*Cer*4) strains under non-desiccating conditions. Developmental success is expressed both relative to the*

initial number of eggs and relative to the preceding developmental stage, allowing comparison of overall developmental recovery and conditional-stage survival.

Strain	Hatching (%)	Pupation on eggs (%)	Emergence on eggs (%)	Pupation on hatched eggs (%)	Emergence on pupae (%)
Benakeion	94.47 ± 3.87	92.20 ± 1.91	92.20 ± 1.91	97.73 ± 1.96	100.00 ± 0.00
88.6	73.37 ± 1.82	70.63 ± 2.37	69.43 ± 4.45	96.23 ± 1.31	98.23 ± 3.06
S.10.3	58.33 ± 3.82	55.83 ± 3.82	52.50 ± 6.61	95.77 ± 4.35	93.87 ± 6.90

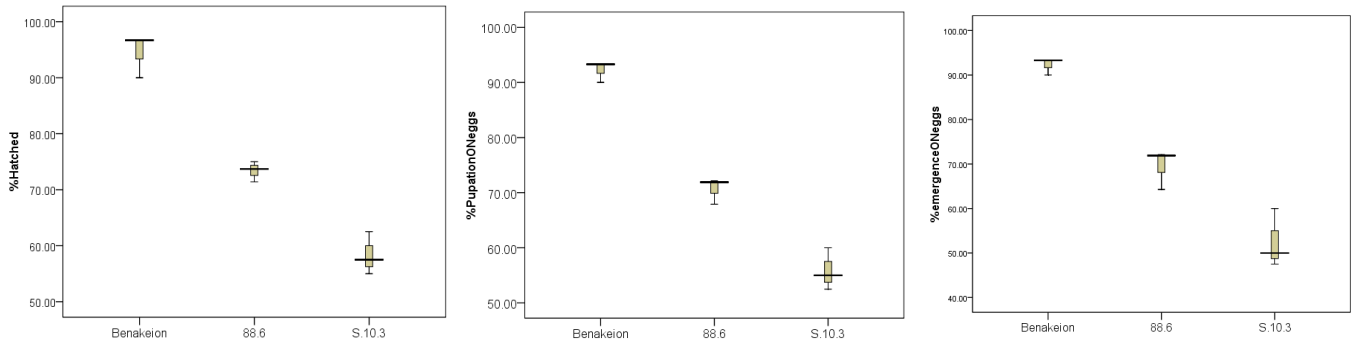

Figure 2. *Baseline developmental performance under control conditions. Panels show mean percentage hatching, pupation, and adult emergence in the three strains under non-desiccating conditions; the clearest between-strain separation is observed for egg-based endpoints.*

Table 2*. *Summary of baseline control-condition comparisons among the three *Ceratitis* **capitata *strains.***

Statistical comparison of developmental endpoints under control conditions. The table summarizes the test

used, test statistic, p-value, and overall outcome for hatching, pupation, and adult emergence expressed either relative to the initial number of eggs or to the preceding developmental stage.

Endpoint	Test	Statistic	p-value	Interpretation
Hatching (%)	One-way ANOVA	F = 90.211	<0.001	All three strains differ; Benakeion > 88.6 > S.10.3.
Pupation on hatched eggs (%)	One-way ANOVA	F = 0.388	0.695	No significant strain effect.
Pupation on eggs (%)	One-way ANOVA	F = 126.331	<0.001	All three strains differ.
Emergence on pupae (%)	One-way ANOVA	F = 1.576	0.282	No significant strain effect.
Emergence on eggs (%)	One-way ANOVA	F = 53.188	<0.001	All three strains differ.

3.2.2. Effects of desiccation duration on developmental success calculated against the initial egg number

Desiccation duration had a strong negative effect on developmental success across all three strains. When hatching, pupation, and adult emergence were assessed relative to the initial number of eggs, Kruskal-Wallis tests showed highly significant effects of desiccation duration for all three endpoints (**Table 3; Figure 3**).

Across strains, developmental performance remained comparatively stable during short desiccation exposures and then declined progressively with increasing duration. No significant differences were detected among the 0, 4, and 8 h treatments for hatching, pupation on eggs, or emergence on eggs. By contrast, prolonged exposure resulted in a marked loss of developmental recovery, with the clearest and most consistently supported reductions observed at 20 and 24 h. Thus, medfly eggs tolerated short desiccation exposures relatively well under the conditions tested, whereas extended water deprivation sharply reduced developmental success. Full Dunn pairwise comparisons for the desiccation-duration analysis are provided in **Table S2**.

Table 3. Summary of desiccation-duration effects on egg-based developmental endpoints in *Ceratitis* **capitata.** *Kruskal–Wallis results for hatching, pupation on eggs, and adult emergence on eggs across the seven egg-desiccation treatments (0, 4, 8, 12, 16, 20, and 24 h). The table reports the test statistics, degrees of freedom, p-value, and the principal post hoc conclusion for each endpoint.*

Endpoint	Kruskal–Wallis χ^2	df	p-value	Key post hoc conclusion
Hatching (%)	47.098	6	<0.001	No difference among 0, 4, and 8 h; strong decline at 20 and 24 h relative to early time points.
Pupation on eggs (%)	53.506	6	<0.001	Short exposures are tolerated; prolonged desiccation sharply reduces success.
Emergence on eggs (%)	53.074	6	<0.001	Long exposures, especially 20–24 h, cause strong loss of developmental recovery.

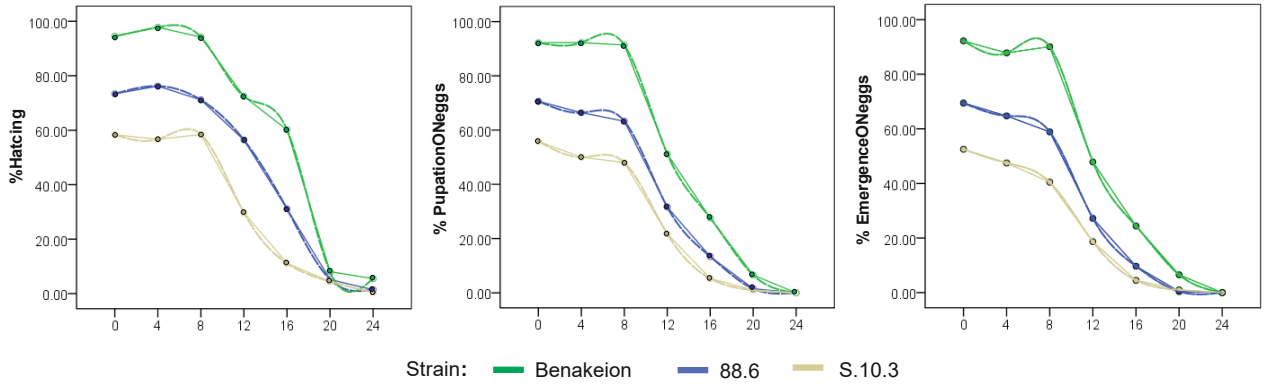

Figure 3. *Estimated marginal means or corresponding summary profiles for hatching, pupation on eggs, and adult emergence on eggs across the seven egg-desiccation treatments (0, 4, 8, 12, 16, 20, and 24 h). The figure depicts the overall temporal pattern of developmental recovery across the desiccation gradient, highlighting the relatively limited effects of short exposures and the marked decline associated with prolonged desiccation.*

3.2.3. Strain-specific differences under desiccation and stage-specific conditional survival

Across all desiccation treatments combined, strain significantly affected hatching success, whereas effects on downstream developmental transitions were weaker. For hatching percentage, the Kruskal–Wallis test was significant and Dunn's post hoc comparisons showed that Benakeion differed significantly from S.10.3, while 88.6 did not differ significantly from either strain. No significant strain differences were detected for pupation among hatched larvae or emergence among pupae.

Benakeion consistently showed the highest developmental recovery across desiccation treatments, S.10.3 the lowest, and 88.6 intermediate values. However, once larvae successfully hatched, subsequent completion of pupation and adult emergence was broadly similar among strains. These findings indicate that the strain-dependent component of the desiccation response was expressed primarily at the level of egg viability and hatching. The full sequential-stage statistical results are provided in **Table S3**, and the corresponding conditional-stage profiles across desiccation treatments are shown in **Figure S3**.

3.2.4. Adult survival under extreme stress (no food and water provision) following egg-stage desiccation

Because the main developmental assay indicated that the biologically relevant transition from limited to more pronounced detrimental effects occurred between 8 and 12 h of egg desiccation, the adult follow-up focused on control, 8 h, and an intermediate 10 h exposure. This design allowed evaluation of post-emergence performance within the most informative applied range while avoiding longer egg-desiccation treatments that yielded too few surviving adults for meaningful downstream analysis.

To assess whether egg-stage desiccation had downstream effects on adult quality, we evaluated survival at 48 h after emergence and total adult survival under extreme stress under starvation and dehydration. Survival at 48 h remained high in Benakeion and 88.6 across treatments, whereas S.10.3 showed lower survival, particularly after egg desiccation **(Table 4**). Logistic regression identified a significant overall effect of strain on 48 h survival. Relative to S.10.3, the odds of survival were significantly higher in 88.6 and even higher in Benakeion (Table 5). Full logistic regression coefficients and confidence intervals are provided in **Table S4.**

Adult survival under extreme stress showed a similar pattern (**Figure 4**). Mean survival under extreme stress remained relatively stable across treatments within Benakeion and 88.6, whereas S.10.3 consistently showed lower values. The statistical analyses supported a significant main effect of strain on adult survival under extreme stress, whereas the main effect of egg-desiccation treatment was not significant (**Table 5**). Likewise, the currently reported model output for 48 h survival did not support a significant treatment effect or a significant strain × treatment interaction.

Taken together, the adult follow-up assays indicate that post-emergence performance under stress was determined mainly by strain identity rather than by moderate egg-stage desiccation. Among the three lines, S.10.3 consistently showed the weakest adult performance, whereas Benakeion and 88.6 were comparatively robust.

Table 4. Adult descriptive outcomes after moderate egg-stage desiccation in three *Ceratitis* ***capitata strains.*** *Percentage of adults alive at 48 h, mean adult survival under extreme stress, and median adult survival under extreme stress for the Benakeion, 88.6, and S.10.3 strains after egg treatments of 0, 8, and 10 h*

desiccation. Adults were maintained without food or water after emergence to assess post-emergence performance under stress.

Strain	Treatment	Alive at 48 h (%)	Mean survival under extreme stress (h)	Median survival under extreme stress (h)
Benakeion	Control	95	70.86	72
Benakeion	8 h desiccation	93	70.57	72
Benakeion	10 h desiccation	95	73.43	72
88.6	Control	95	66.00	66
88.6	8 h desiccation	100	73.80	72
88.6	10 h desiccation	90	72.00	72
S.10.3	Control	91	66.00	66
S.10.3	8 h desiccation	73	63.82	60
S.10.3	10 h desiccation	82	61.64	60

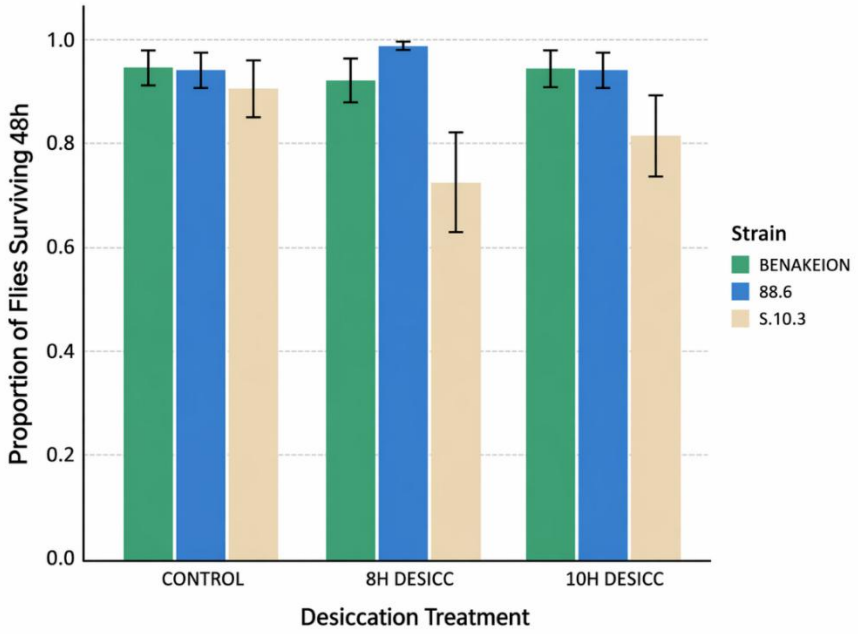

Figure 4. *Mean adult survival under extreme stress by strain and egg-desiccation treatment. Benakeion and 88.6 maintain comparatively robust adult performance across treatments, whereas S.10.3 shows consistently lower survival under extreme stress.*

Table 5: Statistical summary of the adult follow-up assays for 48 h survival and survival under extreme stress after egg-stage desiccation. *Summary of the statistical models used to assess post-emergence performance in adults originating from eggs exposed to 0, 8, or 10 h desiccation. The table reports the model used, the principal test results, and the overall outcome for survival at 48 h and total adult survival under extreme stress under stress.*

Outcome	Model	Key result	Interpretation
48 h survival	Logistic regression	Omnibus $\chi^2 = 12.52$, $p = 0.002$; OR 88.6 vs S.10.3 = 3.59 ($p = 0.015$); OR Benakeion vs S.10.3 = 9.00 ($p = 0.005$).	Short-term adult survival differs mainly by strain, with S.10.3 performing worst.
48 h survival	GLM terms	Treatment $p = 0.625$; strain × treatment $p = 0.944$.	No significant treatment effect or strain × treatment interaction was detected in the current model output.
survival under extreme stress	Two-way ANOVA	Strain: $F(2,244) = 13.82$, $p < 0.001$; treatment: $F(2,244) = 2.04$, $p = 0.132$.	Strong strain effect; no clear downstream treatment effect.

4. Discussion

Egg-stage desiccation had a strong negative effect on developmental recovery in *Ceratitis capitata*, but this effect was not uniform across strains. Across the three lines examined here, developmental performance remained comparatively stable during short desiccation exposures and then declined sharply after prolonged drying, with the clearest reductions observed at the longest exposure times. At the same time, the three strains differed substantially in both baseline and stress-associated performance: Benakeion consistently showed the highest values, S.10.3 the lowest, and 88.6 an

intermediate phenotype. Importantly, the main among-strain divergence was concentrated at the hatching stage. Once larvae had hatched successfully, subsequent pupation and adult emergence were much more similar among strains. This indicates that egg viability and successful completion of embryogenesis represent the major bottleneck in the response to desiccation in this system (Halberg and Denholm, 2024; Shoukry and Hafez, 1979).

This interpretation is consistent with general principles of insect water balance. Desiccation stress arises when water loss exceeds the insect's capacity for physiological compensation, and the ability to withstand such stress depends on the coordinated function of the cuticle, excretory system, gut, and metabolic homeostasis. In egg stages, where developmental progression depends on maintaining sufficient hydration for embryogenesis, water loss is expected to impose particularly strong constraints. Our data fit this framework well, because the clearest effect of desiccation was expressed before or at hatching rather than during later immature development. The results therefore support the view that, under the conditions tested here, egg-stage dehydration primarily compromises embryonic viability rather than causing a generalized reduction in survival across all later developmental stages (Halberg and Denholm, 2024).

The temporal pattern of the response is also biologically informative. Short desiccation exposures caused little measurable reduction in hatching, pupation, or adult emergence, whereas prolonged exposure led to a marked decline in developmental recovery. This pattern suggests a threshold-like deterioration in performance rather than a strictly linear response across time. Earlier work on medfly biology reported that egg desiccation under severe low-humidity conditions causes a strong loss of hatchability, with little or no recovery after prolonged exposure (Chapman et al., 1998; McDermott and Mullens, 2014). Although the exposure conditions in the present study were less extreme than those historical assays, the qualitative pattern is consistent: medfly eggs tolerate only a limited period of drying before developmental recovery collapses. The present results therefore

386 extend earlier observations by showing that this sensitivity is also modulated by *Wolbachia* strain
387 background in transinfected medfly lines.

388 The baseline control data reinforce this interpretation. Even in the absence of desiccation,
389 Benakeion outperformed both *Wolbachia*-infected lines when developmental success was
390 calculated from the initial number of eggs, whereas differences among strains largely disappeared
391 when pupation and emergence were expressed relative to the preceding developmental stage. This
392 pattern suggests that the main cost associated with the infected lines, particularly S.10.3, is not a
393 generalized reduction in performance across all immature stages but rather a stronger effect on the
394 earliest transition from egg to larva. Among the two infected lines, S.10.3, which carries *w*Cer4,
395 consistently showed the weakest performance, with the lowest baseline hatching, the lowest
396 developmental recovery under desiccation, and the poorest adult survival and survival under
397 extreme stress under stress. In contrast, the 88.6 line carrying *w*Cer2 was much closer to the
398 uninfected Benakeion line, especially in the adult assays. The most parsimonious interpretation is
399 therefore that the cost of the artificial medfly–Wolbachia association is strain dependent, with
400 *w*Cer4 imposing a stronger burden on the host than *w*Cer2 under the conditions tested here. The
401 present data do not resolve mechanism, but they are compatible with the hypothesis that Wolbachia
402 strain identity affects embryonic robustness and thereby alters the capacity of eggs to withstand
403 temporary water loss. This interpretation is consistent with the original medfly transinfection work,
404 which demonstrated stable establishment and strong cytoplasmic incompatibility in both lines, and
405 with later studies showing that the phenotypic consequences of infection differ substantially
406 between *w*Cer2 and *w*Cer4 backgrounds (Kyritsis et al., 2022, 2019; Zabalou et al., 2004).

407 From an applied perspective, this result is relevant beyond the specific question of Wolbachia
408 biology. In medfly rearing systems, the egg stage is operationally important because egg collection,
409 temporary holding, transfer, and handling conditions can influence downstream productivity. The

410 present findings indicate that short periods of drying may be tolerated under the laboratory
411 conditions used here, whereas prolonged desiccation rapidly reduces developmental yield. They
412 also show that host genotype and symbiotic background can influence this response. This is
413 important because mass-rearing systems, and especially programmes that evaluate novel lines for
414 operational use, benefit from knowing whether hidden fitness costs are expressed at the egg stage.
415 The study therefore contributes directly to the biological basis for egg-handling and strain-evaluation
416 protocols in medfly rearing facilities (Bourtzis and Vreysen, 2021; Elaini et al., 2020;
417 FAO/IAEA/USDA., 2019).

418 The adult stress assay was designed as a targeted follow-up focused on the most informative applied
419 exposure range, adding an important second layer to the interpretation. Specifically, adults were
420 assessed after control, 8 h, and 10 h egg desiccation because the main developmental dataset
421 indicated that the 8–12 h interval represented the transition from relatively tolerated exposure to
422 increasingly detrimental effects on developmental recovery. This is also the exposure range of
423 greatest practical interest for facilities, because it captures the boundary at which temporary egg
424 handling may begin to compromise developmental yield while still permitting enough recovery to
425 evaluate the biological quality of resulting adults. Selecting an intermediate 10 h treatment therefore
426 allowed assessment of downstream adult performance within this biologically relevant window,
427 while avoiding longer desiccation durations that yielded too few survivors for informative adult-
428 quality analysis. Within this range, adults emerging from desiccated eggs did not show a clear
429 reduction in 48 h survival or total survival under extreme stress attributable to treatment itself.
430 Instead, post-emergence performance was explained mainly by strain identity, with S.10.3 again
431 showing the lowest robustness and Benakeion and 88.6 performing better. This suggests that, within
432 the range of egg desiccation examined in the adult follow-up, survivors did not carry a strong
433 detectable downstream penalty in this particular assay. That point is important because survival

434 under stress is commonly used as a practical biological-quality indicator in tephritid rearing,
435 including in the evaluation of sterile males destined for SIT release purposes. Thus, the present
436 results suggest that moderate egg-stage desiccation reduced developmental yield, but did not
437 produce a strong additional deterioration in this adult-quality proxy among those individuals that
438 completed development (FAO/IAEA/USDA., 2019; Shelly, 2018).

439 With respect to IIT-based or broader Wolbachia-based control strategies, the present study is best
440 viewed as a contribution to understanding the biology and hidden costs of an artificially established
441 medfly–Wolbachia association and to the evaluation of rearing-relevant traits before operational
442 deployment. The data indicate that not all Wolbachia infections are equivalent from a host-fitness
443 perspective and that *w*Cer2 may be less costly than *w*Cer4 under desiccation-related stress.
444 However, whether such differences translate into operational advantages in control programmes
445 will depend on programme-specific needs, the applicable regulatory framework, and biological
446 performance under production and field conditions. For that reason, the emphasis of the present
447 study should remain on artificial symbiosis and strain evaluation rather than on strong claims about
448 immediate operational application (Bourtzis and Vreysen, 2021; Mateos et al., 2020; Saridaki and
449 Bourtzis, 2010; Zabalou et al., 2009).

450 In summary, this study shows that egg-stage desiccation sharply reduces developmental recovery
451 in *C. capitata* after prolonged exposure, that strain-specific differences are expressed mainly at the
452 hatching stage, and that the *w*Cer4-infected line S.10.3 bears the strongest cost under both control
453 and stress conditions. By contrast, the 88.6 line remains much closer to the uninfected background,
454 particularly in adult performance. These findings refine our understanding of the fitness
455 consequences of artificial Wolbachia associations in medfly and provide directly relevant
456 information for the design of egg collection, temporary holding, and strain-evaluation protocols in
457 mass-rearing systems (Cáceres et al., 2007; FAO/IAEA/USDA., 2019).

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