

Click here to view linked References

1 Laboratory experiment of *Stigmatomyces majewskii* (Laboulbeniales:
2 Laboulbeniaceae) infection on *Drosophila suzukii* (Diptera: Drosophilidae)

3
4 Izumi Yamazaki¹, Moe Onuma¹, Tomohiko Ri^{1,2}, Izumi Okane³, Natsumi Kanzaki⁴,
5 Yousuke Degawa^{2,3}, Kyoichi Sawamura^{3,*}

6 ¹ Degree Programs in Life and Earth Sciences, Graduate School of Science and
7 Technology, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

8 ² Sugadaira Research Station, Mountain Science Center, University of Tsukuba, 1278-
9 294 Sugadaira Kogen, Ueda, Nagano 386-2204

10 ³ Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai,
11 Tsukuba, Ibaraki 305-8572, Japan

12 ⁴ Kansai Research Center, Forestry and Forest Products Research Institute, 68
13 Nagaikyutaroh, Momoyama, Fushimi, Kyoto, Kyoto, 612-0855 Japan

14
15 ORCID ID

16 I Yamazaki: <https://orcid.org/0000-0003-4697-2633>

17 M Onuma: <https://orcid.org/0000-0002-2971-7289>

18 T Ri: <https://orcid.org/0000-0002-8308-956X>

19 I Okane: <https://orcid.org/0000-0002-2365-9989>

1
2
3 20 N Kanzaki: <https://orcid.org/0000-0001-8752-1674>
4
5

6 21 Y Degawa: <https://orcid.org/0000-0002-5955-1187>
7
8

9 22 K Sawamura: <https://orcid.org/0000-0002-4229-3150>
10
11

12 23
13

14 24 *Corresponding Author: Kyoichi Sawamura, Faculty of Life and Environmental Sciences,
15
16

17 25 University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan
18
19

20 26
21
22

23 27 **Abstract**
24

25
26 28 Originally endemic to Asia, *Drosophila suzukii* (Matsumura, 1931) is a serious
27

28
29 29 agricultural pest that is rapidly spreading globally. To suppress the rising *D. suzukii*
30

31 30 populations, several pest management programs have been implemented. Researchers
32
33

34 31 have searched for natural enemies, such as predators, parasites, parasitoids, and
35
36

37 32 pathogens. We discovered an obligate ectobiont fungus, *Stigmatomyces majewskii* H. L.
38
39

40 33 Dainat, Manier & Balazuc, 1974, that is associated with *D. suzukii*. We conducted
41
42

43 34 laboratory experiment of *S. majewskii* infection and revealed successful transmission via
44
45

46 35 self-grooming and sexual contact during the courtship behavior of *D. suzukii*, although
47
48

49 36 non-sexual contact through other behaviors may also be involved. We sought to measure
50
51

52 37 the fitness of the fungus infected flies. The longevity of infected flies was comparable to
53
54

55 38 non-infected flies, but further research is required to test other fitness traits.
56
57

39

40 **Keywords**

41 Ectobiont fungus, Invasive species, Natural enemy, Pest control, Spotted wing

42 drosophila

43

44 **Introduction**

45 The spotted wing drosophila, *Drosophila suzukii* (Matsumura, 1931), is one of the worst
46 agricultural pests in the world today; the females of this species have large, serrated
47 ovipositors that cause serious damage to ripening fruits and berries (Walsh et al. 2011;
48 Atallah et al. 2014; Asplen et al. 2015). Originally endemic to East and Southeast Asia,
49 the distribution of *D. suzukii* has since spread worldwide after its invasion of Europe and
50 the Americas in the late 2000s (Hauser 2011; Lee et al. 2011; Walsh et al. 2011; Calabria
51 et al. 2012; Cini et al. 2012; Rota-Stabelli et al. 2013; Deprá et al. 2014; Asplen et al.
52 2015; Haye et al. 2016; dos Santos et al. 2017). To suppress *D. suzukii* populations,
53 control measures, including insecticides, attract-to-kill traps, altered cultivation
54 environment, sterilized male release, and engineered gene drive systems have been
55 explored (Hamby et al. 2012; Mori et al. 2017; Buchman et al. 2018; Schetelig et al. 2018;
56 Tait et al. 2021), but have not succeeded. Introducing natural enemies (predators,
57 parasites, parasitoids, and pathogens) is an additional, clean strategy to combat invasive

species, which can cause pandemics as they may be free from the natural enemies present in their original habitat (Roy et al. 2011; Schulz et al. 2019). Natural enemies applicable to *D. suzukii* have been sought in their original distribution range as well as among commercial products of biopesticides (Kasuya et al. 2013; Renkema et al. 2015; Cuthbertson and Audsley 2016; Becher et al. 2018; Garriga et al. 2018; Girod et al. 2018; Foye and Steffan 2020; Bing et al. 2021; Sario et al. 2021). In this paper, we introduce an ectobiont fungus, *Stigmatomyces majewskii* H. L. Dainat, Manier & Balazuc, 1974, which was recently discovered on *D. suzukii* in Japan, the original distribution range of the fly (Yamazaki et al. 2023).

The genus *Stigmatomyces* s. str. (Laboulbeniales: Laboulbeniaceae) comprises 171 species, the majority of which are obligate ectobionts found on Dipteran cuticles (Benjamin 1973; Tavares 1985; Haelewaters et al. 2020a, 2021). Among them, ten are known to be associated with drosophilid flies (Index Fungorum 2022; Yamazaki et al. 2023). *S. majewskii* was originally described by Dainat et al. (1974), and six drosophilid host species have been reported in Europe, Africa, and Asia (Dainat et al. 1974; Christian 2001; Rossi et al. 2010, 2013; Haelewaters et al. 2012). We recently added two host species from Japan, *D. suzukii* and *D. rufa* Kikkawa & Peng, 1938 (Yamazaki et al. 2023). The infection frequency is high in wild *D. suzukii* populations in Japan: 16.5% in males

and 3.3% in females (Yamazaki et al. 2023). Thus, we hypothesize that *S. majewskii* may affect native *D. suzukii* populations.

The first step in understanding the host-ectobiont relationship is to conduct the laboratory experiment of infection. Laboulbeniales infection has been analyzed in several host-ectobiont systems. Classical observations indicate that transmission between host individuals primarily occurs via direct contact (Richards and Smith 1955; Whisler 1968). However, in the case of infection on carabid ground beetle, indirect transmission via the substrate (soil) has also been suggested (De Kesel 1995). Several insects have been analyzed to study the parasitic effects on the host, such as fitness outcomes of the infections and parasitic manipulations of behaviors (Strandberg and Tucker 1974; Csata et al. 2014; Báthori et al. 2015, 2017; Konrad et al. 2015; Haelewaters et al. 2020b; Szentiványi et al. 2020; Tartally et al. 2021; Awad et al. 2023).

An extremely advanced study of Laboulbeniales infection was carried out on the ladybird *Harmonia axyridis* (Pallas, 1773), examining the system of a parasitic *Hesperomyces* species (reviewed by Riddick et al. 2009; Ceryngier and Twardowska 2013; Haelewaters et al. 2017, 2022). This Asian ladybird was introduced to the USA and Europe as a biocontrol agent against aphids and scale insects. However, these introduced populations have now expanded worldwide causing several negative impacts, threatening native prey species as well as agricultural production and human health; the introduced

control agents have themselves become invasive pests (Kock and Galvan 2008; Roy et al. 2016; Brown and Roy 2017). Therefore, in search for biological controls, parasites and pathogens of *H. axyridis* were studied. Previous analyses have indicated that a number of behavioral and life history traits of the ladybird promote the spread of the parasite within the population and that infection mostly occurs during sexual contact in the mating/feeding season (Riddick and Schaefer 2005; Riddick 2006; Harwood et al. 2006; Nalepa and Weir 2007; Riddick and Cottrell 2010; Cottrell and Riddick 2012). The parasite was observed to have negative effects on host activity and survival (Nalepa and Weir 2007; Riddick 2010), especially when an entomopathogenic fungus is co-infected in the American native ladybird species tested (Haelewaters et al. 2020b).

In the present study, we conducted laboratory experiments on *S. majewskii* infection in live *D. suzukii* flies and measured the fitness of *S. majewskii*-infected individuals. This may provide fundamental knowledge in managing invasive populations of *D. suzukii* in the future.

Materials and Methods

Flies

We used a standard strain of *D. suzukii* (SGD001) which was derived from a female collected at Sugadaira highland, Ueda, Nagano Pref. in 2017. Wild flies were constantly

collected from three localities – i.e., Kyoto (34.94°N, 135.77°E), Sugadaira (36.52°N, 138.35°E), and Tsukuba (36.12°N, 140.10°E) (Yamazaki *et al.*, 2023) and *S. majewskii*-infected *D. suzukii* were used for the infection experiments.

Infection Experiments

In the first experiment, an infected fly (a wild-caught male; donor) and non-infected flies (SGD001; recipient) were confined in a vial (diameter, 3 cm; height, 10 cm) containing the fly medium (cornmeal/glucose/yeast extract/agar); number of recipient flies depends on trials as mentioned below. The flies were aspirated weekly from the vial into a Petri dish (diameter, 3.6 cm; height, 1.0 cm), examined under a binocular stereo microscope (SMZ-U, Nikon, Japan), and transferred to a fresh vial. Dead flies were collected from the vial, preserved in 70% ethanol, and later examined for infected body parts. We conducted the experiments under different conditions (temperature, lightning, and number and sex of recipient flies), which are summarized in Table 1. After the pilot tests, further experiments were conducted at 18 °C under constant light, and the infected flies ($n = 16$) were photographed weekly using a camera (EOS Kiss X10, Canon, Japan) connected to the binocular microscope. For the second experiment, we used a laboratory-infected male donor (SGD001) and an uninfected, virgin female recipient (SGD001).

1
2
3 132 They were confined in a vial for 4 days and reared separately thereafter. This experiment
4
5
6 133 was replicated five times.
7
8
9 134

10 11 135 **Longevity Experiments** 12 13

14 136 An infected fly (wild-caught or SGD001; donor) and five non-infected flies (SGD001;
15
16
17 137 recipient) were confined in a vial; the latter were 6–8 days old, with unmated males or
18
19
20 138 females (Table S1). One week later, the donor flies were sacrificed and preserved in 70%
21
22
23 139 ethanol, and the recipient flies were individually reared transferring into a new vial at 1-
24
25
26 140 week intervals. The recipient flies were examined every 1–3 days to check if they were
27
28
29 141 alive, and preserved in 70% ethanol after death. The presence/absence of the *S. majewskii*
30
31
32 142 infection was also checked. The experiment was conducted at 18 °C under constant light.
33
34
35 143

36 37 144 **Results** 38 39

40 41 145 **Infection succeeded in laboratory** 42 43

44 146 In a pilot test, we successfully transmitted *S. majewskii* to three male and two female *D.*
45
46
47 147 *suzukii* flies (Table 1). The infection frequency in males was 4.3% (2/47) at 18 °C under
48
49
50 148 constant light, and 2.1% (1/47) at 25 °C under a light/dark cycle (L:D=14h:10h). The
51
52
53 149 infection frequency in females was 8.3% (2/24) and 0% (0/27) in respective conditions.
54
55
56 150 The positions of *S. majewskii* in the donors and recipients are summarized in Figure 1. As
57
58
59
60
61
62
63
64
65

the frequency was higher in the former condition, we used that condition (at 18 °C under constant light) in the following experiments. We successfully identified three infection patterns here: (1) male → male, (2) male → female, and (3) female → male. Furthermore, by using newly infected *D. suzukii* as the secondary donor, we were able to achieve up to three successive passages of *S. majewskii* transmission on *D. suzukii*: female → male → female. Infection was detected (thalli were just observed irrespective of maturity) as early as 2 weeks after confinement in a female host.

Monitoring of *S. majewskii* on *D. suzukii*

From our observations of 16 infected-*D. suzukii* flies, we collected sufficient data on visual changes in *S. majewskii* in 12 cases (Table 2). The following events were observed: (1) maturation (before completion of the appendage), (2) elongation of matured thalli, (3) propagation (offspring production), (4) no growth, (5) decrease of immature thalli, and (6) decrease of matured thalli. The developmental staging of *S. majewskii* has been described in Yamazaki *et al.* (2023).

When we started observation (day 1), host F (male) exhibited multiple mature thalli on the right foreleg femur. While resting, the tip of the thalli contacted the posterior region of the right compound eye (Figure S1a). He groomed his head with his forelegs, and the neck of the thalli, which is where the ascospores were released, was rubbed around

the right eye. No new *S. majewskii* were detected by day 7 (Figure S1b), but immature thalli were observed on the margin of his right eye on day 13 (Figure S1c), which matured by day 22 (Figure S1d). In host B (male), multiple mature thalli were observed on the dorsal abdomen on day 1 (Figure S2a). The thalli elongated (Figure S2b), and the size almost doubled by day 25 (Figure S2c). However, the number of thalli decreased by day 32 (Figure S2d).

In contrast, we also observed cases in which the infected thalli stopped growing. Host A (female) exhibited an immature thallus in front of the head on day 1 (Figure S3a, b). The thallus did not develop by day 15 (Figure S3c) and was lost by day 20 (Figure S3d). Host G (male) exhibited an immature thallus on the tarsus of the left foreleg (Figure S4a). The thallus did not develop by day 36 (Figure S4b) and was lost by day 39 (Figure S4c).

We observed a case of male-to-female transmission in one of the five pairs. In the donor male, multiple thalli were restricted to the position between the margin of the left compound eye and maxillary pulp (Figure 2a). After 18 days of isolation, we observed an immature thallus on the dorsal abdomen (right side between segments T4 and T5) of the recipient female (Figure 2b). This process is schematically shown in Fig. 2c.

Effect of *S. majewskii* on *D. sukuzii* longevity

We measured the longevity of 80 female and 63 male *D. suzukii* flies (Table S1). Among the recipient flies five males (7.9%) and no females were infected with *S. majewskii*. As shown in Figure 3, uninfected females ($n = 80$) survived longer than uninfected males ($n = 58$); however, the difference was not statistically significant (log-rank test: $p = 0.09$). Similarly, infected males ($n = 5$) survived longer than uninfected males ($n = 58$); however, the difference was not statistically significant (log-rank test: $p = 0.5$). Each experimental batch included five recipient flies, and longevity was compared between them (Figure S5). Three of the five infected males were ranked in the top two in terms of longevity, suggesting that *S. majewskii* infection did not reduce male longevity under the given conditions.

Discussion

To understand biology of Laboulbeniales, infected sites on the hosts collected from the wild have been investigated (e.g., Hedström 1994; Nalepa and Weir 2007; Sundberg et al. 2018; Doorenweerd et al. 2020; Szentiványi et al. 2020), and laboratory experiments of infection have been conducted (e.g., Richards and Smith 1955; Whisler 1968; De Kesel 1995; Haelewaters et al. 2020b). In the present study, we examined the transmission of *S. majewskii* among *D. suzukii* individuals via laboratory experiments (Table 1; Figure 1).

Based on data obtained from continuous monitoring (Table 2), we hypothesized the infection mechanisms.

Stigmatomyces majewskii propagated within *D. suzukii* (host F; Figure S1). This is presumably an auto-infection caused by the self-grooming behavior of *D. suzukii* (Yamazaki et al. 2023), and a similar infection has been suggested in other host-ectobiont systems: *Herpomyces* species on cockroaches (Richards and Smith 1955), *Stigmatomyces* species on lesser house fly (Whisler 1968), *Hesperomyces* species on ladybird (Nalepa and Weir 2007), and *Rickia* species on *Myrmica* ants (Haelewaters et al. 2015). Mature *S. majewskii* thalli exhibited elongation, but most of them disappeared later (host B; Figure S2). Grooming might also cause thalli to be removed from the exoskeleton, although this can be an active process of *S. majewskii*. The development of *S. majewskii*, in some cases, ceased (hosts A and G; Figure S3, S4). This might depend on the host condition, paucity of nutrients for the parasite, or difference in resistance against the parasite.

In the case of a male-female pair of *D. suzukii*, *S. majewskii* seemed to be transmitted from the front of the male's head to the dorsal abdomen of the female (Figure 2). These two parts were in contact owing to the courtship behavior of *D. suzukii* (see Figure 1 of Revadi et al. 2015). This strongly suggests that *S. majewskii* can be transmitted from *D. suzukii* males to females via sexual contact. Transmission during sexual contact

has been suggested in several host-ectobiont systems: *Laboulbenia* species on *Benbidion* ground beetle (Benjamin and Shanor 1952), *Stigmatomyces* species on lesser house fly (Whisler 1968), *Filariomyces* species on predatory earwig (Strandberg and Tucker 1974), *Stigmatomyces* species on guava fruit flies (Hedström 1994), *Laboulbenia* species on *Pogonus* ground beetle (De Kesel 1995), and *Coreomyces* species on aquatic Hemiptera (Sundberg et al. 2018). Another possible mechanism of *S. majewskii* transmission on *D. suzukii* is through aggressive behavior, where legs are used for fencing and males perform flying attachments in some social situations (Belenioti and Chaniotakis 2020). As males are more aggressive than females, such behavior may lead to male-to-male transmission. We do not have evidence of such a mechanism; however, it is worth examining this possibility in the future.

In the aforementioned male-female pair, we detected immature thalli in the recipient female 18 days after isolation. As it takes at least 9 days for the *S. majewskii* maturation (as seen in the case of host F), the life cycle of *S. majewskii* is hypothesized to be approximately 27 days, which is similar to previous reports (2–3 weeks) for other Laboulbeniales fungi (Richards and Smith 1955; Whisler 1968; Haelewaters et al. 2021). This is much shorter than the longevity of *D. suzukii* (Figure 3; see also Cloutier et al. 2021). Although *S. majewskii* can breed on a single host individual, *S. majewskii* must be transmitted to a new host for the continuation of generations. The rate of successful

transmission is still low in the laboratory (maximum 8%), and it must be improved in the future by searching for suitable conditions for transmission. It should also be noted that the infected flies used as donors do not always carry *S. majewskii* at the reproductive stage. The term of donor/recipient confinement (7 days for the longevity experiment) might be too short for *S. majewskii* maturation on the donor.

The sample size of the longevity experiment was apparently too small; $n = 5$ for the infected flies. But the present experiment is important because most of the previous reports concerning longevity used wild-collected, already-infected individuals (Riddick 2010; Csata et al. 2014; Konrad et al. 2015; Szentiványi et al. 2020; Tartally et al. 2021). This could have led to sampling bias, as researchers may have selectively collected infected survivors. The exceptions were *Stigmatomyces* species on lesser house fly (Whisler 1968) and *Hesperomyces* species on ladybirds (Haelewaters et al. 2020b), where laboratory-infected materials were used. Our preliminary data (Figure 3, S5) do not suggest that *S. majewskii* infection reduced the longevity of *D. sukii*. Although *S. majewskii*-infected *D. sukii* produced offspring both in males and females (data not shown), it is essential to measure other fitness traits, such as reproductive activity and the degree of fertility. This is important because some behaviors are suppressed in heavily infected individuals (Strandberg and Tucker 1974; Nalepa and Weir 2007; Riddick 2010). It is also possible that *S. majewskii* infection has an effect on the immune system of *D.*

suzukii, which might enhance or suppress resistance to other parasites and pathogens. Changes in mortality in doubly infected hosts have been observed in some host–parasite systems (Konrad et al. 2015; Haelewaters et al. 2020b; Tartally et al. 2021). Introducing a second parasite or pathogen into *S. majewskii*-*D. suzukii* system is needed in future studies.

Acknowledgements

MO was supported by the Support for Pioneering Research Initiated by the Next Generation from the Japan Science and Technology Agency (JPMJSP2124). The authors thank two anonymous reviewers for their helpful comments to the manuscript.

References

- Asplen MK, Anfora G, Biondi A, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Kárpáti Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Véték G, Vogt H, Walton VM, Yu Y, Zappalà L, Desneux N (2015) Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci 88:469–494. <https://doi.org/10.1007/s10340-015-0681-z>

- 282 Atallah J, Teixeira L, Salazar R, Zaragoza G, Kopp A (2014) The making of a pest: the
283 evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species.
284 Proc R Soc B 281:20132840. <https://doi.org/10.1098/rspb.2013.2840>
- 285 Awad M, Piálkova R, Haelewaters D, Nedvěd O (2023) Infection patterns of ladybird
286 *Harmonia axyridis* (Coleoptera: Coccinellidae) by ectoparasitic microfungi and
287 endosymbiotic bacteria. J Invertebr Pathol 197: 107887.
288 <https://doi.org/10.1016/j.jip.2023.107887>
- 289 Báthori F, Csata E, Tartally A (2015) *Rickia wasmannii* increases the need for water in
290 *Myrmica scabrinoides* (Ascomycota: Laboulbeniales; Hymenoptera: Formicidae). J
291 Invertebr Pathol 126: 78–82. <https://jhr.pensoft.net/articles.php?id=13253>
- 292 Báthori F, Radai Z, Tartally A (2017) The effect of *Rickia wasmannii* (Ascomycota,
293 Laboulbeniales) on the aggression and boldness of *Myrmica scabrinoides*
294 (Hymenoptera, Formicidae). J Hymenopt Res 58: 41–52.
295 <https://jhr.pensoft.net/articles.php?id=13253>
- 296 Becher PG, Jensen RE, Natsopoulou ME, Verschut V, De Fine Licht HH (2018) Infection
297 of *Drosophila suzukii* with the obligate insect-pathogenic fungus *Entomophthora*
298 *muscae*. J Pest Sci 91:781–787. <https://doi.org/10.1007/s10340-017-0915-3>

- 299 Belenioti M, Chaniotakis N (2020) Aggressive behaviour of *Drosophila suzukii* in
300 relation to environmental and social factors. Sci Rep 10:7898.
301 <https://doi.org/10.1038/s41598-020-64941-1>
- 302 Benjamin RK (1973) Laboulbeniomyces. In: Ainsworth GC, Sparrow FK, Sussman AS,
303 (eds) The Fungi – An Advanced Treatise, vol 4a. New York, Academic Press, pp
304 223–246
- 305 Benjamin RK, Shanor L (1952) Sex of host specificity and position specificity of certain
306 species of *Laboulbenia* on *Bembidion picipes*. Am J Bot 39:125–131.
307 <https://doi.org/10.1002/j.1537-2197.1952.tb14255.x>
- 308 Bing XL, Winkler J, Gerlach J, Loeb G, Buchon N (2021) Identification of natural
309 pathogens from wild *Drosophila suzukii*. Pest Manage Sci 77:1594–1606.
310 <https://doi.org/10.1002/ps.6235>
- 311 Brown PMJ, Roy HE (2017) Native ladybird decline caused by the invasive harlequin
312 ladybird *Harmonia axyridis*. Insect Conserv Divers 11: 230–239.
313 <https://doi.org/10.1111/icad.12266>
- 314 Buchman A, Marshall JM, Ostrovski D, Yang T, Akbari OS (2018) Synthetically
315 engineered *Medea* gene drive system in the worldwide crop pest *Drosophila suzukii*.
316 Proc Natl Acad Sci USA 115:4725–4730. <https://doi.org/10.1073/pnas.1713139115>

- 1
2
3 317 Calabria G, Maca J, Bächli G, Serra L, Pascual M (2012) First records of the potential
4
5
6 318 pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J Appl Entomol
7
8
9 319 136:139-147. <https://doi.org/10.1111/j.1439-0418.2010.01583.x>
10
11
12 320 Ceryngier P, Twardowska K (2013) *Harmonia axyridis* (Coleoptera: Coccinellidae) as a
13
14
15 321 host of the parasitic fungus *Hesperomyces virescens* (Ascomycota: Laboulbeniales,
16
17
18 322 Laboulbeniaceae): A case report and short review. Eur J Entomol 110:549–557.
19
20
21 323 <https://doi.org/10.14411/eje.2013.075>
22
23
24 324 Christian E (2001) The coccinellid parasite *Hesperomyces virescens* and further species
25
26
27 325 of the order Laboulbeniales (Ascomycotina) new to Austria. Ann Nathist Mus Wien
28
29
30 326 B 103:599–603
31
32
33 327 Cini A, Ioriatti C, Anfora G (2012) A review of the invasion of *Drosophila suzukii* in
34
35
36 328 Europe and a draft research agenda for integrated pest management. Bull Insectology
37
38
39 329 65:149–160
40
41
42 330 Cloutier C, Guay JF, Champagne-Cauchon W, Fournier V (2021) Overwintering survival
43
44
45 331 of *Drosophila suzukii* (Diptera: Drosophilidae) in temperature regimes emulating
46
47
48 332 partly protected winter conditions in a cold-temperate climate of Québec, Canada.
49
50
51 333 Can Entomol 153:259–278. <https://doi.org/10.4039/tce.2021.6>
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 334 Cottrell TE, Riddick EW (2012) Limited transmission of the ectoparasitic fungus
335 *Hesperomyces virescens* between lady beetles. J Entomol 814378.
336 <https://doi.org/10.1155/2012/814378>
- 337 Csata E, Erős K, Markó B (2014) Effects of the ectoparasitic fungus *Rickia wasmannii*
338 on its ant host *Myrmica scabrinodis*: changes in host mortality and behavior. Insectes
339 Soc 61:247–252. <https://doi.org/10.1007/s00040-014-0349-3>
- 340 Cuthbertson AGS, Audsley N (2016) Further screening of entomopathogenic fungi and
341 nematodes as control agents for *Drosophila suzukii*. Insects 7:24.
342 <https://doi.org/10.3390/insects7020024>
- 343 Daane KM, Wang XG, Biondi A, Miller B, Miller JC, Riedl H, Shearer PW, Guerrieri E,
344 Giorgini M, Buffington M, Van Achterberg K, Song Y, Kang T, Yi H, Jung C, Lee
345 DW, Chung BK, Hoelmer KA, Walton VM (2016) First exploration of parasitoids
346 of *Drosophila suzukii* in South Korea as potential classical biological agents. J Pest
347 Sci 89:823–835. <https://doi.org/10.1007/s10340-016-0740-0>
- 348 Dainat H, Manier JF, Balazuc J (1974) *Stigmatomyces majewskii* n.sp., *Stigmatomyces*
349 *papuanus* Thaxter 1901, parasitic Laboulbeniales of acalypterous Diptera. Bull
350 Trimmest Soc Mycol Fr 90:171–178.
- 351 De Kesel A (1995) Relative importance of direct and indirect infection in the transmission
352 of *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales). Belg J Bot 128:124–130.

- 353 Deprá M, Poppe JL, Schmitz HJ, Toni DCD, Valente VLS (2014) The first records of the
354 invasive pest *Drosophila suzukii* in the South American continent. J Pest Sci 87:379–
355 383. <https://doi.org/10.1007/s10340-014-0591-5>
- 356 Doorenweerd C, Sievert S, Rossi W, Rubino D (2020) The paradoxical rarity of a fruit
357 fly fungus attacking a broad range of hosts. Ecol Evol 10:8871–8879.
358 <https://doi.org/10.1002/ece3.6585>
- 359 dos Santos LA, Mendes MF, Krüger AP, Blauth ML, Gottschalk MS, Garcia FRM (2017)
360 Global potential distribution of *Drosophila suzukii* (Diptera, Drosophilidae). PLoS
361 ONE 12:e0174318. <https://doi.org/10.1371/journal.pone.0174318>
- 362 Foye S, Steffan SA (2020) A rare, recently discovered nematode, *Oscheius onirici*
363 (Rhabditida: Rhabditidae), kills *Drosophila suzukii* (Diptera: Drosophilidae) within
364 fruit. J Econ Entomol 113:1047–1051. <https://doi.org/10.1093/jee/toz365>
- 365 Garriga A, Morton A, Garcia-del-Pino F (2018) Is *Drosophila suzukii* as susceptible to
366 entomopathogenic nematodes as *Drosophila melanogaster*? J Pest Sci 91:789–798.
367 <https://doi.org/10.1007/s10340-017-0920-6>
- 368 Girod P, Borowiec N, Buffington M, Chen G, Fang Y, Kimura MT, Peris-Felipo FJ, Ris
369 N, Wu H, Xiao C, Zhang J, Aebi A, Haye T, Kenis M (2018) The parasitoid complex
370 of *D. suzukii* and other fruit feeding *Drosophila* species in Asia. Sci Rep 8:11839.
371 <https://doi.org/10.1038/s41598-018-29555-8>

- 372 Haelewaters D, van Wielink PS, van Zuijlen JW, Verbeken M, De Kesel A (2012) New
373 records of Laboulbeniales (Fungi, Ascomycota) for the Netherlands. *Entomolog Ber*
374 72:175–183. <https://natuurtijdschriften.nl/pub/1011617>
- 375 Haelewaters D, Boer P, Gort G, Noordijk J (2015) Studies of Laboulbeniales (Fungi,
376 Ascomycota) on *Myrmica* ants (II): variation of infection by *Rickia wasmannii* over
377 habitats and time. *Anim Biol* 65:219–231. [https://doi.org/10.1163/15707563-](https://doi.org/10.1163/15707563-00002472)
378 00002472
- 379 Haelewaters D, Verhaeghen S, González TAR, Bernal J, Saucedo RV (2017) New and
380 interesting Laboulbeniales from Panama and neighboring areas. *Nova Hedwigia*
381 105:267–299. https://doi.org/10.1127/nova_hedwigia/2017/0410
- 382 Haelewaters D, Dima B, Abdel-Hafiz AII, Abdel-Wahab MA, Abul-Ezz SR, Acar I,
383 Aguirre-Acosta E, Aime MC, Aldemir S, Ali M et al (2020a) Fungal systematics
384 and evolution: FUSE 6. *Sydowia* 72: 231–356. [https://www.verlag-](https://www.verlag-berger.at/detailview?no=2870)
385 [berger.at/detailview?no=2870](https://www.verlag-berger.at/detailview?no=2870)
- 386 Haelewaters D, Hiller T, Kemp EA, van Wielink PS, Shapiro-Ilan DI, Aime MC, Nedvěd
387 O, Pfister DH, Cottrell TE (2020b) Mortality of native and invasive ladybirds co-
388 infected by ectoparasitic and entomopathogenic fungi. *PeerJ* 8.
389 <http://doi.org/10.7717/peerj.10110>

- 1
2
3 390 Haelewaters D, Blackwell M, Pfister DH (2021) Laboulbeniomyces: intimate fungal
4
5
6 391 associates of arthropods. Annu Rev Entomol 66:257–276.
7
8
9 392 <https://doi.org/10.1146/annurev-ento-013020-013553>
10
11
12 393 Haelewaters D, Van Caenegem W, De Kedel A (2022) *Hesperomyces harmoniae*, a new
13
14 394 name for a common ectoparasitic fungus on the invasive alien ladybird *Harmonia*
15
16
17 395 *axyridis*. Sydowia 75: 53–74. <https://doi.org/10.12905/0380.sydowia75-2022-0053>
18
19
20 396 Hamby KA, Hernández A, Boundy-Mills K, Zalom, FG (2012) Associations of yeasts
21
22
23 397 with spotted-wing *Drosophila* (*Drosophila suzukii*; Diptera: Drosophilidae) in
24
25
26 398 cherries and raspberries. Appl Environ Microbiol 78:4869–4873.
27
28
29 399 <https://doi.org/10.1128/AEM.00841-12>
30
31
32 400 Harwood JD, Ricci C, Romani R, Pitz KM, Weir A, Obrycki JJ (2006) Prevalence and
33
34
35 401 association of the Laboulbenian fungus *Hesperomyces virescens*
36
37
38 402 (Laboulbeniales: Laboulbeniaceae) on Coccinellid hosts (Coleoptera:
39
40
41 403 Coccinellidae) in Kentucky, USA. Eur J Entomol 103:799-804.
42
43
44 404 <https://doi.org/10.14411/eje.2006.109>
45
46
47 405 Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura)
48
49
50 406 (Diptera: Drosophilidae) in the continental United States, with remarks on their
51
52
53 407 identification. Pest Manage Sci 67:1352–1357. <https://doi.org/10.1002/ps.2265>
54
55
56
57
58
59
60
61
62
63
64
65

- 408 Haye T, Girod P, Cuthbertson AGS, Wang XG, Daane KM, Hoelmer KA, Baroffio C,
409 Zhang JP, Desneux N (2016) Current SWD IPM tactics and their practical
410 implementation in fruit crops across different regions around the world. J Pest Sci
411 89:643–651. <https://doi.org/10.1007/s10340-016-0737-8>
- 412 Hedström I (1994) *Stigmatomyces* species on guava fruit flies in seasonal and nonseasonal
413 neotropical forest environments. Mycol Res 98:403–407.
414 [https://doi.org/10.1016/S0953-7562\(09\)81196-9](https://doi.org/10.1016/S0953-7562(09)81196-9)
- 415 Index Fungorum (2022) Index Fungorum Partnership. <http://indexfungorum.org/>.
416 Accessed June 6, 2022
- 417 Kasuya N, Mitsui H, Ideo S, Watada M, Kimura MT (2013) Ecological, morphological
418 and molecular studies on *Ganaspis* individuals (Hymenoptera: Figitidae) attacking
419 *Drosophila suzukii* (Diptera: Drosophilidae). Appl Entomol Zool 48:87–92.
420 <https://doi.org/10.1007/s13355-012-0156-0>
- 421 Koch RL, Galvan TL (2008) Bad side of a good beetle: The North American experience
422 with *Harmonia axyridis*. BioControl 53:23–35. [https://doi.org/10.1007/s10526-007-](https://doi.org/10.1007/s10526-007-9121-1)
423 9121-1
- 424 Konrad M, Grasse AV, Tragust S, Cremer S (2015) Anti-pathogen protection versus
425 survival costs mediated by an ectosymbiont in an ant host. Proc R Soc B
426 282:20141976. <https://doi.org/10.1098/rspb.2014.1976>

- 427 Lee JC, Bruck DJ, Curry H, Edwards D, Haviland DR, Van Steenwyk RA, Yorgey BM
 428 (2011) The susceptibility of small fruits and cherries to the spotted-wing drosophila,
 429 *Drosophila suzukii*. Pest Manage Sci 67:1358–1367.
 430 <https://doi.org/10.1002/ps.2225>
- 431 Mori BA, Whitener AB., Leinweber Y, Revadi S, Beers EH, Witzgall P, Becher PG
 432 (2017) Enhanced yeast feeding following mating facilitates control of the invasive
 433 fruit pest *Drosophila suzukii*. J Appl Ecol 54:170–177.
 434 <https://doi.org/10.1111/1365-2664.12688>
- 435 Nalepa CA, Weir A (2007) Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae)
 436 by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): Role of mating status
 437 and aggregation behavior. J Invertebr Pathol 94:196–203.
 438 <https://doi.org/10.1016/j.jip.2006.11.002>
- 439 Renkema JM, Telfer Z, Garipey T, Hallett RH (2015) *Dalotia coriaria* as a predator of
 440 *Drosophila suzukii*: Functional responses, reduced fruit infestation and molecular
 441 diagnostics. Biol Control 89:1–10.
 442 <http://dx.doi.org/10.1016/j.biocontrol.2015.04.024>
- 443 Revadi S, Lebreton S, Witzgall P, Anfora G, Dekker T, Becher PG (2015) Sexual
 444 behavior of *Drosophila suzukii*. Insects 6:183–196.
 445 <https://doi.org/10.3390/insects6010183>

- 1
- 2
- 3 446 Richards GA, Smith MN (1955) Infection of cockroaches with *Herpomyces*
- 4
- 5
- 6 447 (Laboulbeniales). I. Life history studies. Biol Bull 108:206–218
- 7
- 8
- 9 448 Riddick EW (2006) Influence of host gender on infection rate, density and distribution of
- 10
- 11
- 12 449 the parasitic fungus, *Hesperomyces virescens*, on the multicolored Asian lady beetle,
- 13
- 14
- 15 450 *Harmonia axyridis*. J Insect Sci 6. <https://doi.org/10.1673/031.006.4201>
- 16
- 17
- 18 451 Riddick EW (2010) Ectoparasitic mite and fungus on an invasive lady beetle: parasite
- 19
- 20
- 21 452 coexistence and influence on host survival. Bull Insectology 63:13–20
- 22
- 23
- 24 453 Riddick EW, Cottrell TE (2010) Is the prevalence and intensity of the ectoparasitic fungus
- 25
- 26
- 27 454 *Hesperomyces virescens* related to the abundance of entomophagous coccinellids?
- 28
- 29
- 30 455 Bull Insectology 63:71–78
- 31
- 32
- 33 456 Riddick EW, Schaefer PW (2005) Occurrence, density, and distribution of parasitic
- 34
- 35
- 36 457 fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on multicolored
- 37
- 38
- 39 458 Asian lady beetle (Coleoptera: Coccinellidae). Ann Entomol Soc Am 98:615–624.
- 40
- 41 459 [https://doi.org/10.1603/0013-8746\(2005\)098\[0615:ODADOP\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2005)098[0615:ODADOP]2.0.CO;2)
- 42
- 43
- 44 460 Riddick EW, Cottrell TE, Kidd KA (2009) Natural enemies of the Coccinellidae:
- 45
- 46
- 47 461 Parasites, pathogens, and parasitoids. Biol Control 51:306–312.
- 48
- 49
- 50 462 <https://doi.org/10.1016/j.biocontrol.2009.05.008>
- 51
- 52
- 53 463 Rossi W, Máca J, Vavra J (2010) New records of Laboulbenials (Ascomycota) from the
- 54
- 55
- 56 464 Czech Republic and Slovakia. Polish Bot Stud 55:343–351
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

- 1
2
3 465 Rossi W, Santamaria S, Andrade R (2013) Notes on the Laboulbeniales (Ascomycota)
4
5
6 466 parasitic on Diptera from Portugal and other countries. Plant Biosyst 147:730–742.
7
8
9 467 <https://doi.org/10.1080/11263504.2012.753132>
10
11
12 468 Rota-Stabelli O, Blaxter M, Anfora G (2013) *Drosophila suzukii*. Curr Biol 23:R8–9.
13
14
15 469 <https://doi.org/10.1016/j.cub.2012.11.021>
16
17
18 470 Roy HE, Lawson Handley LJ, Schönrogge K, Poland RL, Purse BV (2011) Can the
19
20
21 471 enemy release hypothesis explain the success of invasive alien predators and
22
23
24 472 parasitoids? BioControl 56:451–468. <https://doi.org/10.1007/s10526-011-9349-7>
25
26
27 473 Roy HE, Brown PMJ, Adriaens T, Berkvens N, Borges I, Clusella-Trullas S, Comont RF,
28
29
30 474 De Clercq P, Eschen R, Estoup A et al. (2016) The harlequin ladybird, *Harmonia*
31
32
33 475 *axyridis*: global perspectives on invasion history and ecology. Biol Invasions 18:
34
35
36 476 997–1044. <https://doi.org/10.1007/s10530-016-1077-6>
37
38
39 477 Sario S, Santos C, Gonçalves F, Torres L (2021) DNA screening of *Drosophila suzukii*
40
41
42 478 predators in berry field orchards shows new predatory taxonomical groups. PLoS
43
44
45 479 ONE 16:e0249673. <https://doi.org/10.1371/journal.pone.0249673>
46
47
48 480 Schetelig MF, Lee KZ, Otto S, Talmann L, Stökl J, Degenkolb T, Vilcinskis A,
49
50
51 481 Halitschke R (2018) Environmentally sustainable pest control options for
52
53
54 482 *Drosophila suzukii*. J Appl Entomol 142:3–17. <https://doi.org/10.1111/jen.12469>
55
56
57
58
59
60
61
62
63
64
65

- Schulz AN, Lucardi RD, Marsico TD (2019) Successful invasions and failed biocontrol: The role of antagonistic species interactions. *BioScience* 69: 711–724. <https://doi.org/10.1093/biosci/biz075>
- Strandberg JO, Tucker LC (1974) *Filariomyces forficulae*: Occurrence and effects on the predatory earwig, *Labidura riparia*. *J Invertebr Pathol* 24:357–364. [https://doi.org/10.1016/0022-2011\(74\)90144-X](https://doi.org/10.1016/0022-2011(74)90144-X)
- Sundberg H, Kruys Å, Bergsten J, Ekman S (2018) Position specificity in the genus *Coreomyces* (*Laboulbeniomyces*, *Ascomycota*). *FUSE* 1:217–228. <https://doi.org/10.3114/fuse.2018.01.09>
- Szentiványi T, Estók P, Pigeault R, Christe P, Glaizot O (2020) Effects of fungal infection on the survival of parasitic bat flies. *Parasites Vectors* 13:1–9. <https://doi.org/10.1186/s13071-020-3895-8>
- Tait G, Mermer S, Stockton D, Lee J, Avosani S, Abrieux A, Anfora G, Beers E, Biondi A, Burrack H et al (2021) *Drosophila suzukii* (Diptera: Drosophilidae): a decade of research towards a sustainable integrated pest management program. *J Econ Entomol* 114:1950–1974. <https://doi.org/10.1093/jee/toab158>
- Tartally A, Szabó N, Somogyi AÁ, Báthori F, Haelewaters D, Mucsi A, Fürjes-Mikó Á, Nash DR (2021) Ectoparasitic fungi of *Myrmica* ants alter the success of parasitic butterflies. *Sci Rep* 11:24031. <https://doi.org/10.1038/s41598-021-02800-3>

502 Tavares II (1985) Laboulbeniales (Fungi, Ascomycetes). Mycol Mem 9:1–627
 503 Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O’Neal
 504 SD, Zalom FG (2011) *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of
 505 ripening soft fruit expanding its geographic range and damage potential. J Integr Pest
 506 Manage 2:G1–7. <https://doi.org/10.1603/IPM10010>
 507 Whisler HC (1968) Experimental studies with a new species of *Stigmatomyces*
 508 (Laboulbeniales). Mycologia 60:65–75.
 509 <https://doi.org/10.1080/00275514.1968.12018548>
 510 Yamazaki I, Onuma M, Omiya H, Ri T, Kanzaki N, Degawa Y, Sawamura K (2023) First
 511 record of *Stigmatomyces* (Ascomycota: Laboulbeniales) on Drosophilidae from
 512 Japan. Fly submitted
 513
 514 **Figure legends**
 515 **Fig. 1**
 516 Sites of *Stigmatomyces majewskii* infection on donor and recipient of *Drosophila suzukii*
 517 (a) at 25 °C under light/dark cycle (L:D=14h:10h). (b)–(e) at 18 °C under constant light.
 518 (a)–(c) male → male; (d) and (e) male → female. Left, donor. Right, recipient. Arrow in
 519 (c) indicates scattered, black dots (truncated *S. majewskii*); see also Figure S7b’ of
 520 Yamazaki *et al.* (2023). The infection on the thorax of the recipient in (c) is ventral.

Fig. 2

A case of *S. majewskii* transmission in a pair of *D. sukuzii* (male → female). (a) the male on day 1. (b) the female at day 19; arrow indicates the position of *S. majewskii*. (c) schematic drawing.

Fig. 3

Survival curves of *D. sukuzii*. Red, uninfected females ($n = 80$). Blue, uninfected males ($n = 58$). Black, males infected with *S. majewskii* ($n = 5$).

Fig. S1

S. majewskii infection on host F (male) of *D. sukuzii*. (a) day 1. (b) day 7. (c) day 13. (d) day 22.

Fig. S2

S. majewskii infection on host B (male) of *D. sukuzii*. (a) day 1. (b) day 8. (c) day 25. (d) day 32.

Fig. S3

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

540 *S. majewskii* infection on host A (female) of *D. suzukii*. (a), (b) day 1. (c) day 15. (d) day

541 20. (b) frontal view; (a), (c), (d) top view. Arrow indicates the position of *S. majewskii*.

542

543 **Fig. S4**

544 *S. majewskii* infection on host G (male) of *D. suzukii*. (a) day 1. (b) day 36. (c) day 39.

545

546 **Fig. S5**

547 Longevity of five *D. suzukii* males from the same batch. Blue, uninfected. Orange,

548 infected with *S. majewskii*. Data from three batches are shown. 5-1, 5-2 etc. are individual

549 flies.

550

551 **Table 1**

552 Tests of *S. majewskii* infection on *D. suzukii*

553

554 **Table 2**

555 Events observed in *S. majewskii* on individual *D. suzukii*

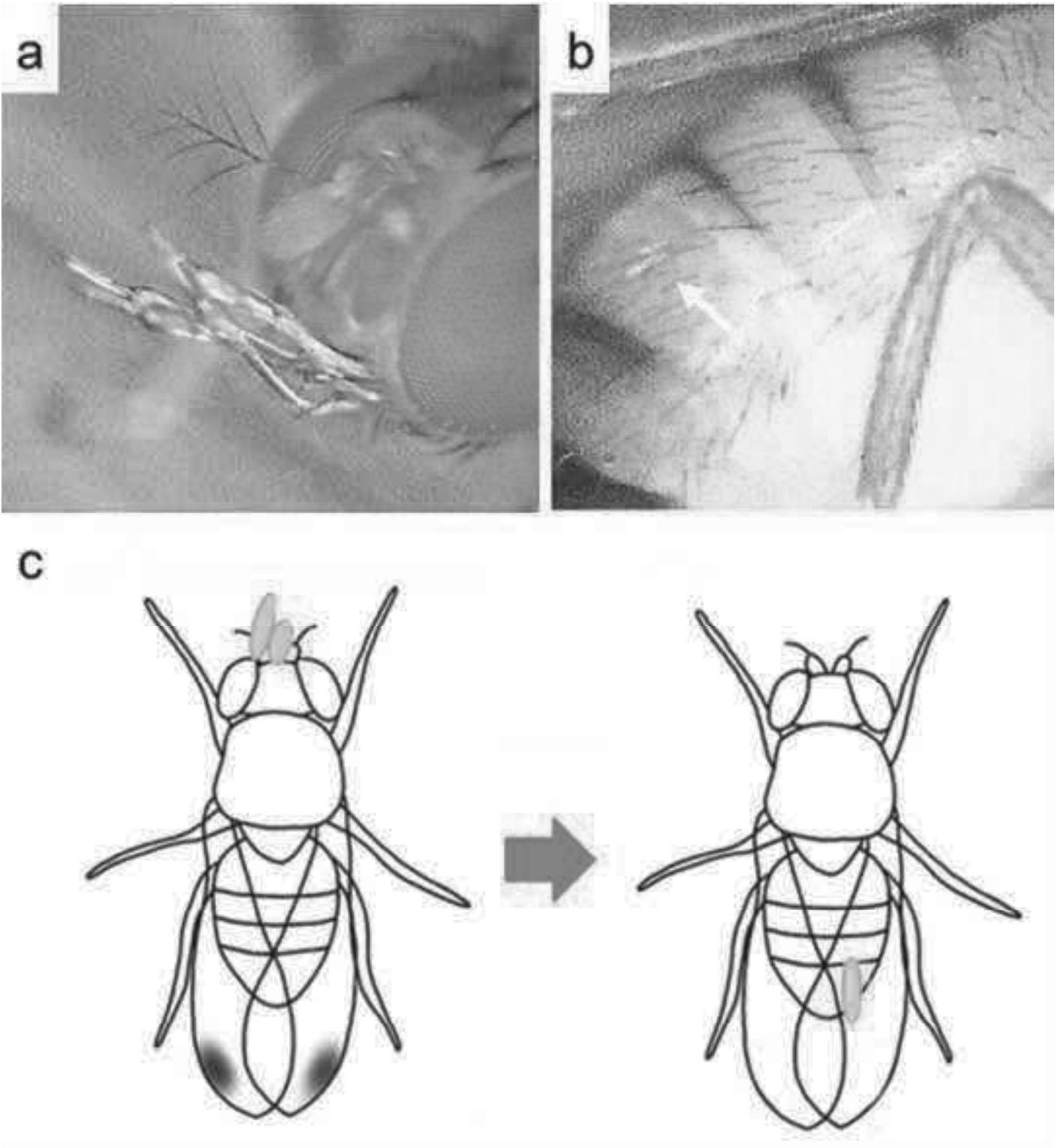
556

557 **Table S1**

558 Combinations of donors and recipients in the longevity experiment

Figure 2

[Click here to access/download;Figure;Yamazaki et al2_Fig2.png](#)



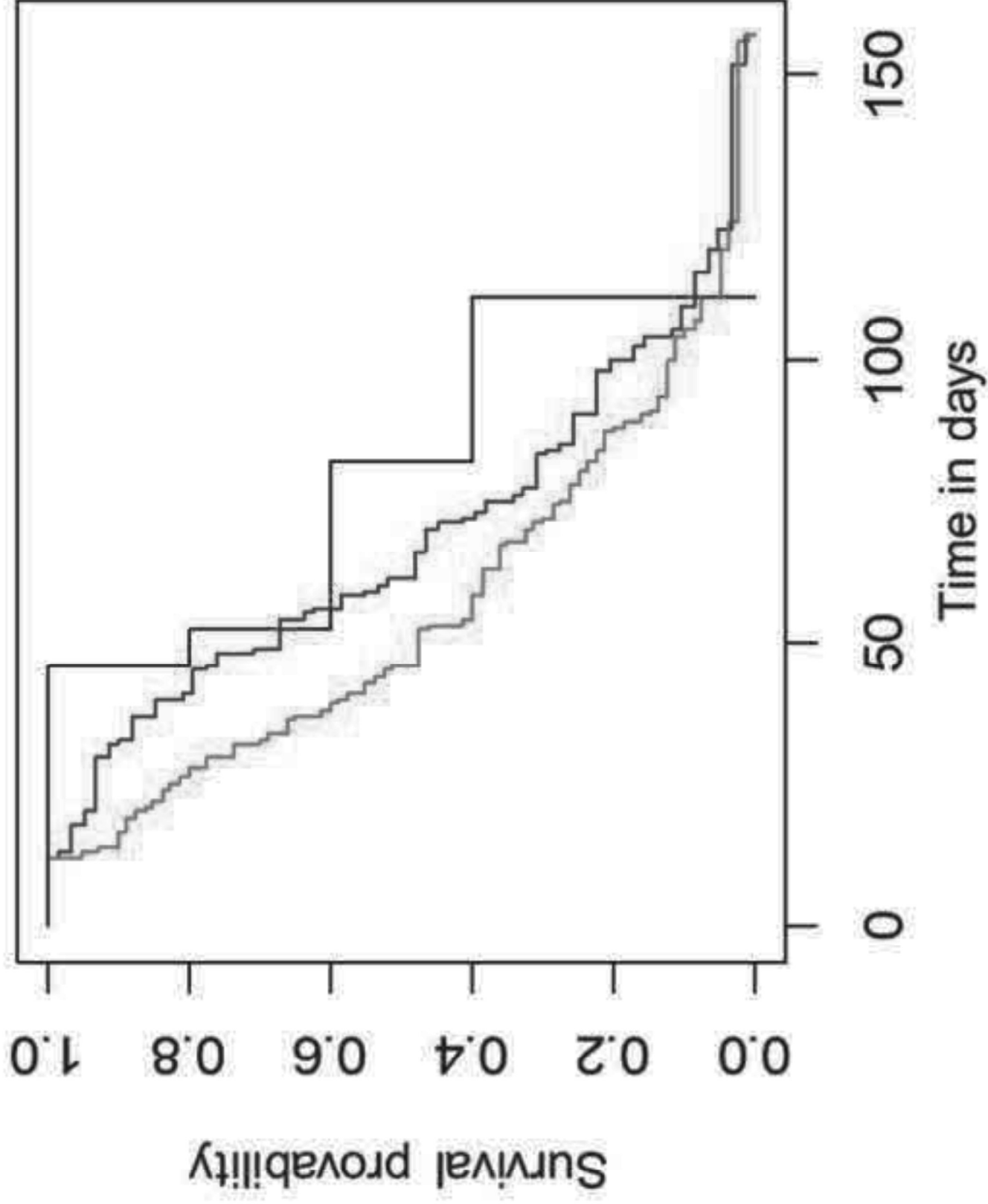


Figure 3

Table 1 Tests of *S. majewskii* infection on *D. suzukii*

Temperature	Light (L : D)	No. recipient flies per test	No. replicate	No. males tested	No. males infected	No. females tested
25°C	14h : 10h	3-5 males	10	39	1	-
		3-5 females	5	-	-	20
		2 males + 2 females	4	8	0	7
18°C	24h : 0h	3-5 males	10	39	2	-
		3-5 females	4	-	-	16
		2 males + 2 females	4	8	0	8

No. females infected
-
0
0
-
1
1

Table 2 Events observed in *S. majewskii* on individual *D. sukuzii*

Host	Sex	Maturation	Elongation	Propagation	No growth	Decese of immatured	Decrease of matured
A	Female				✓	✓	
B	Male		✓				✓
D	Male	✓		✓			
E	Male			✓		✓	
F	Male	✓		✓			
G	Male				✓	✓	
I	Female				✓	✓	
J	Female				✓	✓	
K	Female			✓			
L	Male		✓	✓			
M	Male		✓	✓			✓
O	Male				✓		