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L. Moquet, H. Delatte. Host status of *Citrus hystrix*, *Citrus aurantifolia*, *Passiflora edulis* and *Litchi chinensis* for *Bactrocera dorsalis* (Tephritidae, Diptera) on Réunion Island. *Fruits*, 2021, 76 (6), pp.310 - 315. 10.17660/th2021/76.6.8 . hal-03606021

HAL Id: hal-03606021

<https://hal.univ-reunion.fr/hal-03606021>

Submitted on 11 Mar 2022

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Host status of *Citrus hystrix*, *Citrus aurantifolia*, *Passiflora edulis* and *Litchi chinensis* for *Bactrocera dorsalis* (Tephritidae, Diptera) on Réunion Island

L. Moquet^{1,a} and H. Delatte^{2,3}

¹ CIRAD, UMR PVBMT, F-97410 St. Pierre, Réunion, France

² CIRAD, UMR PVBMT, Ambatobe, 101 Antananarivo, Madagascar

³ FOFIFA CENRADERU-DRA, Ambatobe, 101 Antananarivo, Madagascar

Summary

Introduction – Determining the host status of fruit species for a given fruit fly species is essential to decide on its risk for the international trade of fresh fruits. This study aims to determine if *Bactrocera dorsalis*, the Oriental fruit fly, can infest fruits of four commercial species grown for exportation: *Citrus aurantifolia*, *Citrus hystrix*, *Passiflora edulis* and *Litchi chinensis* in La Réunion. **Materials and methods** – Fruits of *C. aurantifolia*, *C. hystrix*, *P. edulis* and *L. chinensis* were collected in the field to determine their natural infestation rates (natural hosts) and laboratory experiments were carried out to study insects' ability to oviposit and develop in the fruits (conditional hosts). **Results and discussion** – The two tested *Citrus* species were neither natural nor conditional hosts for *B. dorsalis*. *Passiflora edulis* was a conditional host for *B. dorsalis* on Réunion Island, as we observed infestations only in experimental conditions. Infestations in experimental and natural conditions by *B. dorsalis* were observed for *L. chinensis*. **Conclusion** – Our findings provided background information on the host status for *B. dorsalis* for four major fruits candidates for exportation from La Réunion. The risk for export is weak for tested *Citrus* species because they were neither natural nor conditional hosts for *B. dorsalis*. On the contrary, the risk for export is not null for *L. chinensis* and *P. edulis*, which present infestation by *B. dorsalis* either in field-collected fruit or in unperforated fruit in the laboratory. As infestations can be difficult to distinguish, post-harvest treatments should be considered to limit the risks.

Keywords

Bactrocera dorsalis, *Citrus*, Lychee, Passion fruit, Quarantine pest, La Réunion

Introduction

Frugivorous tephritid fruit flies represent one of the most significant threats to the cultivation of fruits and vegetables worldwide (White and Elson-Harris, 1992). Females lay their eggs under fruit skin, and the larvae develop in the pulp, causing damage by direct insect actions (feeding and oviposition) and subsequent decay by opportunistic pathogens.

^a Corresponding author: laura.moquet@cirad.fr.

Significance of this study

What is already known on this subject?

- *Bactrocera dorsalis* is one of the world's top invaders.
- Determining the host status of fruit species for export is crucial to evaluate the risks of spread through the international trade.

What are the new findings?

- *Citrus aurantifolia* and *Citrus hystrix* are not host of *B. dorsalis*.
- *Passiflora edulis* is a conditional host and *L. chinensis* is a natural host of *B. dorsalis*.

What is the expected impact on horticulture?

- The risk for export is not null for *L. chinensis* and *P. edulis*, so pre-harvest control for *B. dorsalis* must be done, and post-harvest treatments should also be considered.

Moreover, infested fruits can drop prematurely (Allwood and Leblanc, 1997; Kwasi, 2008). Fruit fly attack results in both a reduction in fruit production and problems with international trade. Their economic impact is so significant that countries impose quarantine restrictions to limit further spread of fruit fly pests or prohibit the import of fresh produce from countries where these pests are present (White and Elson-Harris, 1992).

One well-documented pest among Tephritidae is the Oriental fruit fly (*Bactrocera dorsalis* Hendel), regarded as one of the world's top invaders (Clarke *et al.*, 2005). This extremely polylectic species can infest more than 400 plant species of fruit and legume belonging to 73 families (Liquido *et al.*, 2015). Native to India, Southeast Asia and southern China, this species has spread rapidly throughout Africa after its first detection in 2003 in Kenya (De Villiers *et al.*, 2015; Lux *et al.*, 2003; Zeng *et al.*, 2019). *Bactrocera dorsalis* has a broad climatic tolerance range and currently occurs in over 65 countries in Asia, Oceania, America and Africa (Zeng *et al.*, 2019). Moreover, with climate change, the suitable area for *B. dorsalis* is expanding (Qin *et al.*, 2019). For example, this invader is moving into central areas of China, where the climate is similar to the temperate regions in Europe, and that were previously unsuitable to the survival of *B. dorsalis* (Han *et al.*, 2011; Stephens *et al.*, 2007). Moreover, incursions of *B. dorsalis* were recently detected in Europe (Egarter *et al.*, 2019; Nguen *et al.*, 2018).

La Réunion is a French overseas territory, but legally considered outside the European mainland area and subjected to the international trade regulations. In 2017, *B. dorsalis* was detected on the island and spread very quickly, becoming a major pest (Moquet *et al.*, 2021). If mango (*Mangifera indica* L.) constitutes one of the primary host plants of *B. dorsalis* in La Réunion (Moquet *et al.*, 2021), the host status of *Citrus aurantifolia* (Christm.) Swingle, *C. hystrix* DC., *Litchi chinensis* Sonn., and *Passiflora edulis* Sims is unclear. Previous studies in the laboratory showed that lemons (*Citrus limon* (L.) Osbeck) and lychee are relatively poor hosts for *B. dorsalis* (Armstrong and Follett, 2007; Manrakhan *et al.*, 2018). Ripe and unripe fruits of *Passiflora edulis* were reported as natural hosts in Comoros and Hawaii (Akamine *et al.*, 1974; Hassani, 2017).

Determining the host status of fruit species targeted for export to fruit flies is essential to evaluate the risks of spreading the pest through the international trade of fresh fruits (Follett *et al.*, 2021). Cowley *et al.* (1992) proposed to determine host status based on three experiments: 1) laboratory cage tests with punctured fruit, 2) laboratory cage tests with unpunctured fruit, and 3) field cage tests with unpunctured fruit attached to the tree. More recently, FAO (2016) described three categories for host plant status: natural host, conditional host and non-host. Natural hosts are infested by fruit fly larvae under natural conditions and able to sustain fly development to viable adults. A conditional host is not infested in natural conditions but can be infested in a semi-natural field. Trials may include the use of field cages, greenhouses (including glass, plastic and screen houses) and bagged fruit-bearing branches. A non-host is a plant species or cultivar that is not infested by the target fruit fly species under either natural or semi-natural conditions (FAO, 2016).

This study aims to determine if *B. dorsalis* can infest the following commercial fruit grown in La Réunion and targeted for potential export: *Citrus aurantifolia*, *Citrus hystrix*, *Passiflora edulis* and *Litchi chinensis*. If so, are infested fruits visually distinguishable from intact fruits? To answer these questions, we collected fruits in the field to determine the natural infestation rates and carried out infestations to study insects' ability to oviposit and develop in the fruits under laboratory conditions.

Materials and methods

Field sampling

In order to determine the natural infestation level by *B. dorsalis* in La Réunion, Kaffir lime (*C. hystrix*), lemon pebbles (*C. aurantifolia*), passion fruit (*P. edulis*) and lychee (*L. chinensis*) were collected from commercial farms and private gardens between 2018 and 2021. Whenever possible, at least 15 fruits were collected per plant species, site and date. We collected in total 188 fruits of *C. hystrix* (8 sites), 60 fruits of *C. aurantifolia* (5 sites), 105 fruits of *P. edulis* (6 sites), and 211 fruits of *L. chinensis* (13 sites) all over the island. We mainly collected the variety of lychee 'Kwai Mi' that is widely cultivated in La Réunion (171 fruits). In addition, one batch of varieties, 'Hoak-Ip' and 'Mauritius', was collected in the collection orchard of CIRAD, Saint Pierre La Réunion (20 fruits for each variety). The collected fruits were weighed and incubated individually in closed ventilated containers, lined with fine sand for pupation, in climatic chambers ($25 \pm 1^\circ\text{C}$, $80 \pm 10\%$ HR, 4,000 lux). Seven days after collection, and each week for three weeks, the sand was sieved in each individual container to check for the presence of pupae. Pupae were then isolated in a small plastic box ac-

cording to the fruit where they developed to sex and identify emerging adults. After identification, adults were stored in alcohol 90° at -20°C .

Bactrocera dorsalis laboratory colonies

Fruit flies used in simulations of infestations came from the Entomology Laboratory of the "Pole de Protection des Plantes" Saint-Pierre, La Réunion. Laboratory colonies were initiated from larvae of *B. dorsalis* present in various fruits collected in Réunion Island. The colonies are maintained under controlled conditions of ($25 \pm 1^\circ\text{C}$, $80 \pm 10\%$ RH) with natural light supplemented by artificial light (4,000 lux) to maintain a photoperiod of 12:12. Each week an artificial egg-laying device is proposed to mature mated adults of the rearing and those eggs are collected and transferred in an artificial carrot-based media (Duyck and Quilici, 2002). The larvae were then reared on this artificial diet. Adult flies were fed with sugar and enzymatic protein hydrolysate *ad libitum*. We used individuals of the F45 to F53 generation range, aged between 10 to 30 days, reared in cages of $30 \times 30 \times 30$ cm for our experiments.

Artificial infestation

To test the ability of *B. dorsalis* to oviposit and develop in the fruits of *C. hystrix*, *C. aurantifolia*, *L. chinensis* and *P. edulis*, we carried out experimental infestations under laboratory conditions with punctured and non-punctured fruits as the procedure described in Cowley *et al.* (1992).

Citrus and *Passiflora edulis*

Fruits used for these experiments were harvested at the usual stage targeted for export, *i.e.*, green for both species of *Citrus* fruits and, ripe and purple for *P. edulis*. Selected fruits were intact, healthy, untreated. Three treatments were realized:

- Control fruits, to observe the visual evolution of the fruits during ripening in the absence of infestation by *B. dorsalis* ($N=10$).
- Intact fruits were exposed to mature female *B. dorsalis*. Five fruits were placed in each of four laboratory colony cages with several hundred mature females for 150 minutes ($N=20$). In this treatment, we stimulated fruit fly oviposition as the density of females was very high and females had no alternative host plants. If *B. dorsalis* laid eggs and developed until the adult stage, we considered the plant species as a conditional host.
- The last treatment implicated fruits with ten perforations through the skin, made with a mounted needle, deep enough to reach fruit pulp. Five fruits were placed for 60 minutes in each of four cages with several hundred gravid female flies ($N=20$). This treatment simulated infestation on damaged fruits.

We chose infestation durations according to a preliminary experiment conducted on punctured artificial oviposition substrates (non-published data). We estimated that time needed to be higher in non-punctured fruits. After the exposure to flies, the incubation protocol for field-collected fruits described above was applied until possible adult emergence. Moreover, visual monitoring of the fruits was carried out by regularly photographing individual fruits (before infestation, just after the potential infestation, during the potential larval development).



FIGURE 1. Encaged fruits to test experimental infestation of lychee by *Bactrocera dorsalis*. a) A lychee tree with experimental set up, and b) Lychees in 30×30×30 cm cages before the addition of 100 gravid females.

Litchi chinensis

Lychee fruits deteriorate quickly after harvest, so the experiments were carried out in field cages directly on the tree. Four branches of about 30 lychees were put in a 30×30×30 cm cage with two sleeve openings (4M3030D, BugDorm, MegaView Science, Taiwan) at least one week before the experiment to avoid infestation by wild fruit flies. One of the cage sleeves was attached to the branch so that the fruits hung in the cage without touching the walls (Figure 1). Each fruit was individually marked with a label on the peduncle. When the fruits were ripe, one hundred gravid *B. dorsalis* females from the laboratory colonies were placed in the cages for 24 h. Three cages were set up for the infestations, and an additional cage served as a control. After the flies were removed, the fruits were left in the cages for a week before being collected and brought back to the laboratory for incubation. Because infestations were already recorded in field-collected samples, no conditions with manually perforated fruit skins were conducted.

Results

Field sampling

We collected in total 188 fruits of *C. hystrix* (8 sites), 60 fruits of *C. aurantifolia* (5 sites), 105 fruits of *P. edulis*

(6 sites), and 211 fruits of *L. chinensis* (13 sites) all over the island. No flies emerged from the field-collected fruits of *C. hystrix*, *C. aurantifolia* and *P. edulis*. A weak infestation was observed on *L. chinensis* with four of the 211 infested fruits collected (Table 1). We observed one to four adults of *B. dorsalis* emerging per infested fruit.

Citrus

The flies were observed in oviposition position (probing or undifferentiated egg laying) for all conditions tested (with or without perforations). However, we did not observe any fruit fly development in intact fruits of either species. On the contrary, when the skin of the fruits was perforated, 80% of the fruits of *C. aurantifolia* were infested by *B. dorsalis* with on average 857 ± 660 pupae per kg of fruit and 40% of the fruits of *C. hystrix* with on average 238 ± 281 pupae per kg (Table 1). On average, $62.6 \pm 34.4\%$ of the pupae survived until the adult stage after their development on *C. hystrix* and $64.0 \pm 29.9\%$ on *C. aurantifolia*.

Passiflora edulis

When fruits were intact, only 10% of the fruits (two out of 20 fruits) were infested by *B. dorsalis*. For these two fruits, we found the equivalent of 2,072 pupae per kg of fruit. When fruits were perforated, 100% of the fruits were infested by

TABLE 1. Emergences of *Bactrocera dorsalis* from fruits of *Citrus hystrix*, *C. aurantifolia*, *Passiflora edulis* and *Litchi chinensis* collected in the field or exposed to flies in laboratory.

		<i>Citrus hystrix</i>	<i>Citrus aurantifolia</i>	<i>Passiflora edulis</i>	<i>Litchi chinensis</i>
Field	Nr. infested fruits	0 / 188	0 / 60	0 / 105	4 / 211
	Nr. pupae kg ⁻¹	–	–	–	87 ± 58
Experimental unperforated fruits	Nr. infested fruits	0 / 20	0 / 20	2 / 20	11/90
	Nr. pupae kg ⁻¹	–	–	2,072	135.6 ± 86.9
Experimental perforated fruits	Nr. infested fruits	8 / 20	16 / 20	20 / 20	–
	Nr. pupae kg ⁻¹	238 ± 281	857 ± 660	4,569 ± 1,302	–

Nr. Pupae kg⁻¹: Number of pupae per kilogram of infested fruits, +/- standard error.

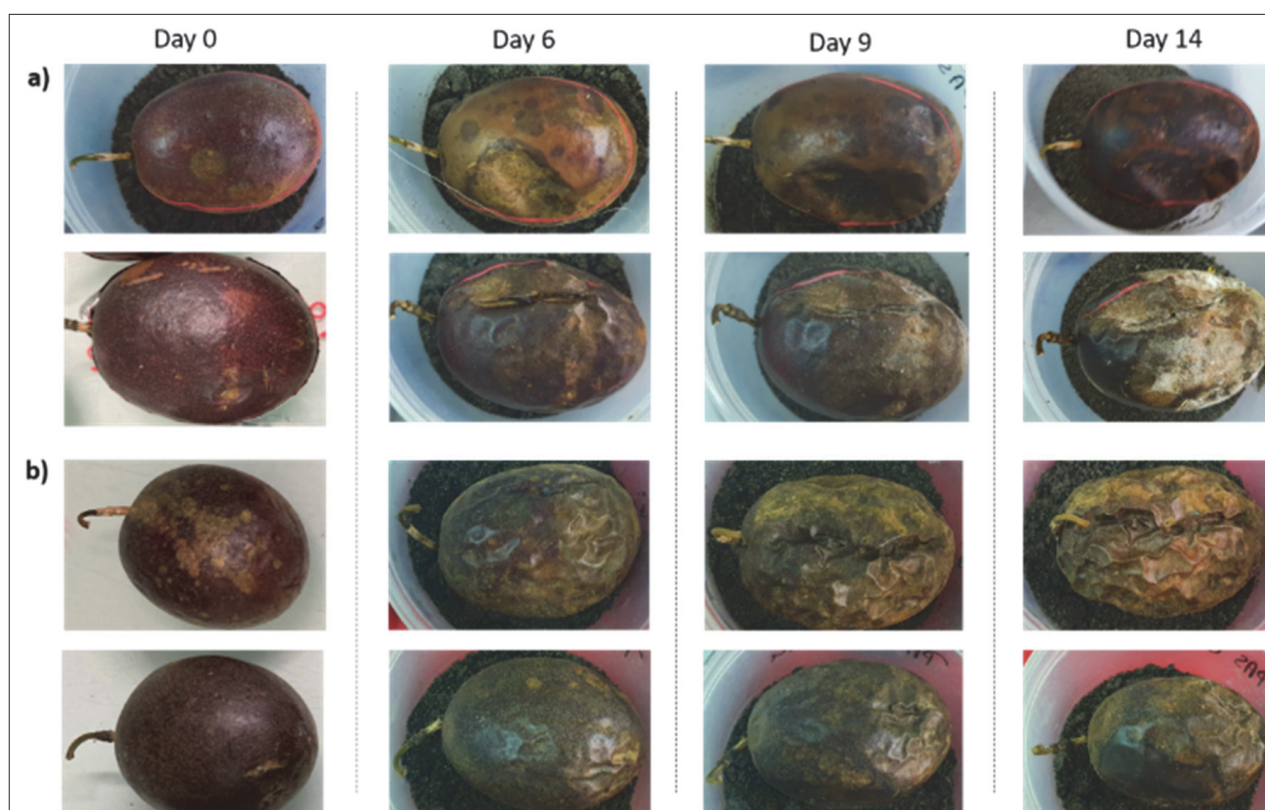


FIGURE 2. Example of fruit ripening of *Passiflora edulis*. a) Infested by *Bactrocera dorsalis* (emergences recorded), and b) Not infested (control) according to the day after exposure to *B. dorsalis* females.

B. dorsalis with an average of $4,569.0 \pm 1,302.4$ pupae per kg of fruit, i.e., 388.9 ± 128.3 pupae per fruit (Table 1). Visually, infested fruits were not distinguishable (Figure 2).

Litchi chinensis

Pupae of *B. dorsalis* developed from 12% of the unperforated lychee tested in the laboratory, yielding 135.6 ± 86.9 pupae kg^{-1} (Table 1). After seven days, fruits with oviposition punctures were more degraded than the healthy fruits, with brown spots and mould in the oviposition area (Figure 3). Observations have shown that *B. dorsalis* can lay eggs between the fruit scales (Figure 4). Just after the infestations, the oviposition mark was discreet, and only a slight oozing was visible. Some fruits showed all the signs of oviposition by *B. dorsalis* (spots and moulds) but without emergence.

Discussion

Field sampling and laboratory experiments allow us to determine the host status of *C. hystrix*, *C. aurantifolia*, *P. edulis* and *L. chinensis* for *B. dorsalis*. We did not observe natural infestation for two species of *Citrus*. Based on our sampling, undamaged fruits of *Citrus hystrix* and *C. aurantifolia* are regarded as non-hosts under natural conditions under La Réunion environmental conditions. Until now, *C. hystrix* was cited only once as a host of *B. dorsalis* in the field (Allwood *et al.*, 1999). On the contrary, according to the study of Ndiaye (2009), some varieties of *C. aurantifolia* could be infested by *B. dorsalis* in the field. In the laboratory tests, our results show that *B. dorsalis* can develop in fruits of both species when punctures facilitate access to the fruit pulp. However, it appears that flies were unable to pass the skin barrier in unperforated fruit. These results were consistent with the absence of flies emerging from fruits collected in the field.

Studies have shown that *Citrus* fruits, such as lemon or grapefruit, can exhibit chemical resistance mechanisms in the skin via the secretion of essential gum and oil limiting infestations by fruit flies (Greany *et al.*, 1983; Ruiz *et al.*, 2014). Thus, the risk for export is weak if the exported fruits are subjected to rigorous post-harvest grading to ensure the export of fruits without defects.

None of the 90 field-collected fruits of *P. edulis* were infested by fruit flies. In other studies, passion fruits were observed as infested in natural conditions by *B. dorsalis* (Allwood *et al.*, 1999; Hassani, 2017; Leblanc *et al.*, 2012; Ndiaye, 2009; Vargas *et al.*, 2007). The absence of infestation in the present study could result from the stage of maturity of collected fruits. We mainly collected mature fruits, while according to Akamine *et al.* (1974), the most infested stage is the immature stage. Nevertheless, we observed infestations and adult emergences from intact fruits in artificial conditions (10% of fruits were infested). These results showed that *P. edulis* is a conditional host plant species in La Réunion. Moreover, the very high number of pupae formed per kg of fruit ($4,569.0 \pm 1,302.4$ pupae per kg of punctuated fruits) suggests that *P. edulis* is a host of high nutritional quality. Thus, the experimental infestations showed that the risk of transporting *B. dorsalis* larvae during export exists.

On the 211 fruits of *L. chinensis* collected, 1.9% were infested by fruit flies. This species can be considered as a natural host plant species of *B. dorsalis* in La Réunion. *Litchi chinensis* was recorded as a natural host to *B. dorsalis* in Asia by Allwood *et al.* (1999). Lychee infested with *B. dorsalis* were intercepted by the plant quarantine at Narita Airport in Japan (Iwaizumi, 2004). In Hawaii, McQuate and Follett (2006) found that the proportion of fruit infested by *B. dorsalis* varied from 0 to 8.65%, according to orchard and treatments. In

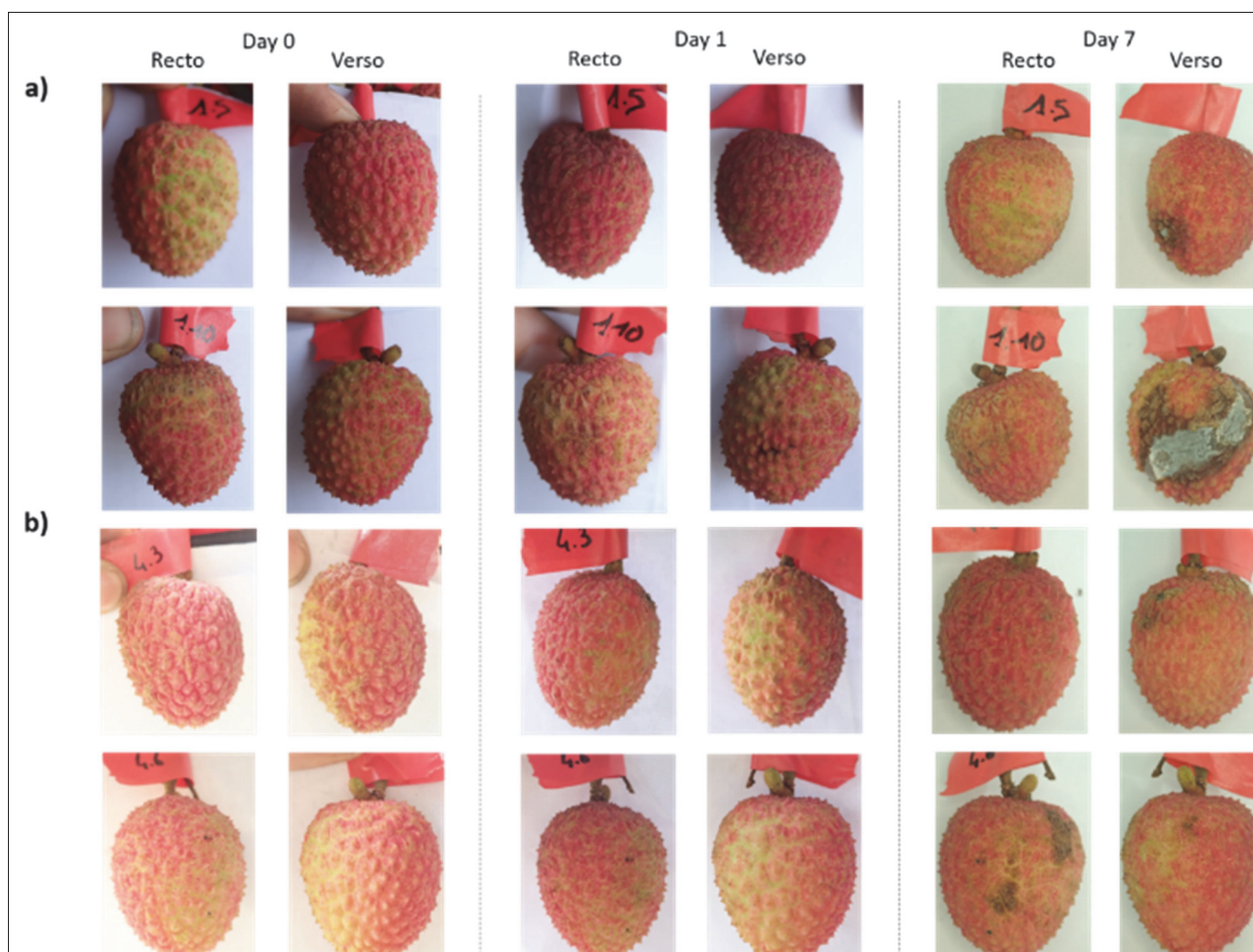


FIGURE 3. Example of fruit ripening of *Litchi chinensis*. a) Infested by *Bactrocera dorsalis* (emergences recorded), and b) Not infested (control) according to the day after exposure to *B. dorsalis* females.

our experimental conditions, pupae of *B. dorsalis* developed from 12% of the tested lyches. These results showed the capacity of *B. dorsalis* to lay and develop in unpunctuated fruits under field conditions. Contrary to potential secondary infestation that could have been made by other pests, this fruit fly species is able to pierce the skin of the fruit between the scales (Figure 4). However, judging from the low infestation rate, *L. chinensis* seems to be a low preference but natural host for *B. dorsalis*, and the risk for export is not null.

In conclusion, the two tested *Citrus* species were neither

natural nor conditional hosts for *B. dorsalis*. *Passiflora edulis* was a conditional host for *B. dorsalis*. Based on our sample size, the failure to detect *B. dorsalis* in Passion fruits collected in the field does not imply that the species is always absent in these host fruits. Moreover, in experimental conditions, interactions between insects and fruits were amplified, and the absence of alternative host plants for females proved to be suitable hosts on damaged fruits in laboratory conditions. Thus, because of the large number of flies that can develop per fruit, it is essential to take precautions by applying pre-harvest fruit fly control. Finally, *L. chinensis* is a natural host of *B. dorsalis* in La Réunion. As infestations can be difficult to detect for these two host plant species, post-harvest treatments should be considered to limit the risks for export.

Acknowledgments

I would like to thank Marie-Ludders Moutoussamy and Emma Dieudonné for fruits sampling, and Timothé Lin, Sarah Turpin, Léa Mussard and Dorian Govindinchetty for their help with laboratory experiments. Thanks to ARIFEL (Mathieu Picard), who provided fruits for the experiments. The authors also acknowledge the Plant Protection Platform (3P, IBISA) for hosting all lab experiments and fruit fly rearing. This research was conducted within the framework of the UMT BAT: "Biocontrôle en Agriculture Tropicale" and was funded by CIRAD, the French Ministry of Agriculture (MAAF), Région Réunion, and the European Union: European Agricultural Funds for Rural Development (EAFRD) and European Funds for Rural Development (EFRD).

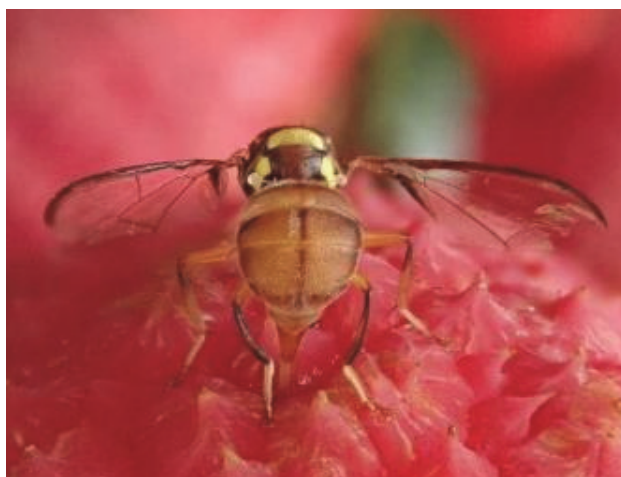


FIGURE 4. *Bactrocera dorsalis* laying in fruit of *Litchi chinensis*.

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Received: Jun. 27, 2021

Accepted: Sep. 1, 2021