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RESEARCH PAPER

First record of *Zaprionus tuberculatus* (Diptera: Drosophilidae) in mainland France

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Abstract

As most drosophilid species are not considered as primary pest, studies of their communities in natura, with standardized surveys, are relatively sparse. However, Drosophila suzukii and its rapid expansion through the world led to the implementation of many monitoring programs. As part of a research project on D. suzukii, we set up in 2022 an annual fly monitoring in 16 fruits plantations in France to understand population's dynamics of D. suzukii and to broadly survey drosophilid communities. The captures led to the first observation of Zaprionus tuberculatus in mainland France. Over the whole monitoring, we trapped a total of 111 specimens in a fig orchard located in southern France (Salses-le-Château), both in fig trees and nearby hedgerow. The first detection of Zaprionus tuberculatus occurred in July 2022 in the hedgerow, and captures continued until January 2023 with an interruption in November and December. In addition, in this orchard we collected overripe figs in September 2023 from which over 20 Z. tuberculatus have emerged in the following 2 weeks, confirming the establishment of a permanent population. The pest status of Z. tuberculatus and its potential risk for agriculture is not yet clear, but the pest behavior of the close-relative species Zaprionus indianus, especially on figs, should be a warning point for the entry of Z. tuberculatus into the EU and France, as they may have similar polyphagous habit. The pest status and range expansion of Z. tuberculatus should be assessed to estimate risk to fruit's production.

Key words: France, fruit fly, invasion, pest risk, Zaprionus tuberculatus

Introduction

Drosophilidae Rondani is a very diverse family with almost 4,700 described species distributed in 77 genera (Bächli 2023). The largest genus of Drosophilidae is *Drosophila* Fallén which comprises 1,676 described species. There is a strong heterogeneity in the existing knowledge on Drosophilidae, with a huge literature on genetics as well as cellular and developmental biology of *Drosophila* species, mainly *Drosophila melanogaster*, but little knowledge on other genera. Studies of the whole drosophilid natural communities, including other genera than *Drosophila*, with standardized surveys are relatively sparse (de Toni et al. 2007; Delbac et al. 2020; Mateus et al. 2006). A possible reason for this lack

of interest is likely the ecology and lifecycle of Drosophilidae. For most drosophilid species, larvae grow on decaying plant material and forage on associated microbial communities (Carson 1971; Markow & O'Grady 2008) and are therefore not considered as primary pest. In addition, these flies are small and cause no trouble to humans or animals, so they received relatively less attention by scientific community. However, the spotted-wing drosophila *Drosophila suzukii* (Matsumura, 1931) and its rapid expansion through the world changed the situation. The damage caused to cultivated fruits by this invasive species and the huge economic loss have led to a large number of studies in the last decade about its ecology and control measures (Tait et al. 2021). Many fly monitoring

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programs have been set up in various countries to better understand and predict populations dynamics of D. suzukii (Briem et al. 2018; Hamby et al. 2014; Harris et al. 2014; Kremmer et al. 2017). As part of a research project on D. suzukii (DroFramb action within the framework of ANR Drothermal project, https://www.drothermal.cnrs.fr/), in 2022, we set up an annual fly monitoring in various regions of France. The main objective was to conduct a vast monitoring program of D. suzukii during a whole year, including during winter months, to get data on D. suzukii occurrence and abundance in various areas and climates. This monitoring effort also provided the opportunity to survey Drosophilidae communities in fruit plantations. Regular field monitoring is crucial to detect novel invaders and potential novel pests. Indeed, early detection is key to successful preventative strategies. Here, we will not present the whole capture data of D. suzukii across all the various sites of the national monitoring program. Instead, we present the data of a single site in the south of France where we document the first occurrence, and likely establishment, of a new invasive and potential pest fly, Zaprionus tuberculatus Malloch, 1932 (Diptera: Drosophilidae) in mainland France.

Materials and Methods

From March 2022 to February 2023, we conducted a national participative monitoring program of *D. suzukii* (Matsumura, 1931) within 14 raspberry plantations. Because there were

no raspberry growers willing to participate in the south of France, where raspberry cultivation is much less developed, we decided to expand our sampling effort to cherry and fig orchards. As a result, we monitored a total of 16 fruit plantations along a latitudinal gradient in France (Fig. 1). In each orchard, three traps were installed at least 10 m apart within the plantation at fruiting height and three others in a hedgerow or a grove nearby at approximately 1 m height which corresponds to the height at which most potential wild fruits are available for D. suzukii (Rubus spp., Sambuccus spp., Eleagnus spp.). Traps were opened for 10 days at the end of each month resulting in twelve 10-days trapping sessions across seasons. Flies were captured using home-made upside down red-colored water bottle traps (320 mm height-ø 79.5 mm, Badoit®, France) pierced with six holes (Ø 5 mm each) in the larger part of the bottle (see Figure S1). The trap contained a liquid bait (80 mL) in an open but inaccessible plastic container positioned inside the bottle. Additionally, a drowning solution (30 mL) was present in a collection tube screwed to the neck of the bottle (see Figure -S1). For the bait, we used a mixture of 80% apple cider vinegar (5% acidity, Bouton d'Or®, France) and 20% of cane sugar syrup (Canadou[®], France). Flies caught in the bottles fell in the drowning solution that was made of salt water (40 g.L⁻¹) with a drop of odorless soap (L'Arbre Vert[®]-Peaux Sensibles sans parfum, France). Bait and drowning solution were renewed at each trapping session. Local temperatures at each site were monitored during all the experiment in plantation and hedgerow using two TMS-4 Standard

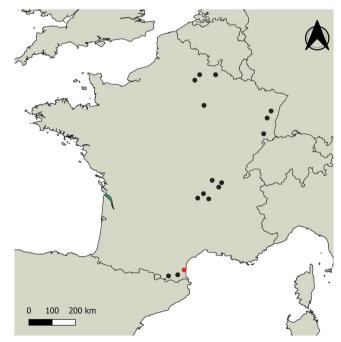


FIGURE 1 French locality (red dot) where specimens of Zaprionus tuberculatus were collected. Black dots represent the other monitored localities where no Z. tuberculatus specimens were collected during the same period (© EuroGeographics for the administrative boundaries).

dataloggers (TOMST[®], Czech Republic) which collected data every 15 min in three different levels: 6 cm into the soil, at the surface and 15 cm above the soil surface. These data allowed gathering microclimate thermal measurements at a biologically relevant scale and allowed exploring whether the level of *D. suzukii* captures responded to soil or surface temperature as well as air temperature, especially during winter months.

Results and Discussion

While identifying *D. suzukii* specimens collected in different regions of France, we came across flies with white longitudinal stripes at a specific location within a fig orchard in southern France (Salses-le-Château; 42°50/0.169"N 2°55/5.447"E; see Figs. 1 and 2). Flies with these morphological features typically belong to the *Zaprionus* Coquillet, 1902 genus. According to the Köppen-Geiger classification (Geiger 1954; Kottek et al. 2006), the climate of this region is type Csa, corresponding to hot-summer Mediterranean climate with mild winters averaging above 0°C and hot summers averaging above 25°C. In this location, over the whole monitoring period in 2022, we trapped 111 specimens of *Zaprionus* sp. out of a total of 3766 drosophilids (Table 1).

Species identification was first done by R. Georges and then sent to A. Yassin for confirmation based on external morphological criteria. The genus *Zaprionus* Coquillett,

1902 exhibits characteristic white longitudinal stripes on the frons and the mesonotum (Fig. 2A,B) and we could also easily distinguish a protruding tubercle bearing a bristle on the forefemur of all our specimens (Fig. 2C). This feature is characteristic of a clade of seven Afrotropical species, known as the Zaprionus tuberculatus subgroup (Yassin & David 2010), in reference to the first species that has been discovered in the clade, that is, Zaprionus tuberculatus Malloch, 1932. Only one of the seven species, the species Zaprionus tuberculatus, has invasive capacities and has expanded its geographical range to the palearctic region during the last four decades (Yassin 2008). The seven species can be distinguished on the basis of morphological characters of male and female genitalia and internal reproductive organs as well as immature stages (Tsacas et al. 1977; Yassin & David 2010). Of these characters, male and female genitalia provide the best diagnoses on non-living specimens, with DNA barcoding being of limited utility due to shared mitochondrial sequences among closely-related species (Yassin 2008). Dissection of a sample of captured specimens from Salses-le-Château supported the morphological identification of the introduced flies as Zaprionus tuberculatus. Female spermathecae (Fig. 3A) have predominantly smooth surface except on the tip wherein the surface becomes slightly rigged, whereas the phallotrema of the male aedeagi (Fig. 3B) has a conspicuous ventral process and a densely teethed flap surrounding its border. Individuals are now kept in 70% alcohol and are available upon request from R.G. and H.C. (see authors affiliation for address).

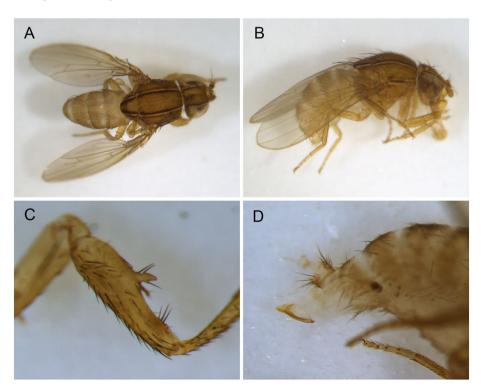


FIGURE 2 Zaprionus tuberculatus: dorsal view (A), lateral view (B), detail of the protruding tubercle on the forefemur of a male (C) and detail of the ovipositor of a female (D).

Table 1 Occurrence of Zaprionus tuberculatus collected in France. Number of male and female Z. tuberculatus and all other Drosophilidae trapped in
a fig orchard and a hedgerow nearby during 1-year monitoring in 2022-23. Temperatures indicated are monthly air average, minimum (min) and
maximum (max) of average daily temperature recorded every 15 min by TMS-4 dataloggers (TOMST, Czech Republic) at 15 cm above the ground
surface

Date collected	Fig orchard				Hedgerow			
	Zaprionus tuberculatus		Other Drosophilidae	Daily temperature (°C) average; min—max	Zaprionus tuberculatus		Other Drosophilidae	Daily temperature (°C) average; min—max
	Males	Females			Males	Females		
March 2022	0	0	36	11.8; 8.9–17.6	0	0	67	11.9; 8.9–17.6
April 2022	0	0	58	15.1; 6.5–22.2	0	0	33	15.1; 5.9–22.3
May 2022	0	0	1	20.9; 17.1–26.2	0	0	32	20.2; 17.3–25.6
June 2022	0	0	131	25.5; 21.3–30.3	0	0	172	24.4; 20.2–29.3
July 2022	0	0	650	28.7; 24.7–32.7	11	19	1,356	27.5; 23.2–32.1
August 2022	12	13	390	28.3; 24.0–33.4	13	15	479	27.7; 23.7–32.6
September 2022	0	0	5	22.4; 16.2–27.7	4	4	168	22.4; 16.3; 27.7
October 2022	4	2	227	19.5; 17.2–21.8	6	6	330	19.6; 17.3–21.9
November 2022	0	0	2	13.0; 7.6–19.0	0	0	81	13.6; 8.6–19.9
December 2022	0	0	0	9.2; 2.7–14.1	0	0	2	10.0; 4–15.3
January 2023	0	0	27	7.6; 3.0–12.8	2	1	24	8.1; 3–13.8
February 2023	0	0	71	8.9; 3.6–14.4	0	0	50	9.5; 3.5–15.2



FIGURE 3 Detail of female spermatheca (A) and male aedeagus (B) of trapped individuals of *Zaprionus tuberculatus* (afb: aedeagal flap border; avp: aedeagal ventral process).

Zaprionus tuberculatus is widespread in Africa and the islands of the Indian Ocean (Madagascar, Comoro, Seychelles, Réunion and Mauritius) and Atlantic Ocean (St Helena and Cap Verde) (Chassagnard 1988; Chassagnard & Kraaijeveld 1991; Tsacas 1980; Tsacas et al. 1977; Yassin & David 2010). At the end of 20th century, *Z. tuberculatus* was recorded in Palearctic region from Canary Islands, Egypt, Cyprus, Malta and Israel (Chassagnard & Kraaijeveld 1991; Ebejer 2001). More recently, *Z. tuberculatus* occurred in Turkey (Patlar et al. 2012), Italy (Raspi et al. 2014), Romania (Chireceanu et al. 2015), Tunisia (Ben Halima Kamel et al. 2020) and Brazil (Cavalcanti et al. 2022).

The first detection of *Z. tuberculatus* occurred in July 2022 in the hedgerow and captures continued until January 2023

with an interruption in November and December (Table 1). In the fig orchard, we trapped *Z. tuberculatus* in August 2022 and then in October 2022, corresponding respectively to the beginning and the end of figs harvest. These results suggest that *Z. tuberculatus* could remain in natural woody habitats and colonize orchards when fruits come to maturity. To verify whether this observation was the result of a temporary accidental introduction or an established population, we revisited the plantation the following year, in September 2023, to harvest 100 ripe and 100 overripe figs directly from the trees at different locations within the fig orchard. The collected fruits were placed on vermiculite layer in emergence cages (four cages with ripe figs and four cages with overripe figs). Cages were then placed in a quarantine

chamber regulated at 20°C and 70% RH to assess emergence for 1 month. Subsequently, we identified and counted the *Z*. *tuberculatus* individuals that emerged. Among the different fly species emerging from ripe and overripe figs (mainly *D*. *suzukii* and *Ceratitis capitata*, not counted), we found 13 females and 10 males of *Z*. *tuberculatus*, but only from the overripe figs. The emergence of *Z*. *tuberculatus* occurred during the 2 weeks following the harvest. The presence of *Z*. *tuberculatus* for two consecutive years led us to posit that this invasive fly may have permanently established itself, at least at this specific location.

Compared to other first reports, our trapping results show a longer period of occurrence of Z. tuberculatus in newly colonized countries. In Turkey, the first records of Z. tuberculatus were reported to be in August (Patlar et al. 2012; Zengin 2020), whereas Raspi et al. (2014) in Italy and Constantina et al. (2015) in Romania reported that Z. tuberculatus flies were found in the traps only during autumn from September to October. To our knowledge, until now the latest captures were reported in mid-December in Turkey and Italy (Amiresmaeili et al. 2019; Zengin 2020), so this is the first time that adults of Z. tuberculatus are trapped in winter in Europe. During January, mean daily temperature recorded in the surveyed hedgerow was 8.1°C with a minimum of 3°C (Table 1). All daily temperature data for the air, ground surface or ground is available in Table S1. Several studies showed evidence of climatic niche shift in exotic species during their expansion (Hill et al. 2017; Medley 2010; Petersen 2013; Wei et al. 2017). Such niche shift was also demonstrated in other invasive Drosophilidae, D. suzukii (Ørsted & Ørsted 2019) and Zaprionus indianus (da Mata et al. 2010). Our results suggest that Z. tuberculatus may experience a climatic niche shift to accommodate to climate colder than in its tropical origin, as also suggested by Cavalcanti et al. (2022) Yet, additional monitoring of Z. tuberculatus in others areas and for several consecutive seasons are highly needed to substantiate this "niche shift" assumption. With climate projection indicating that global warming will result in a progression of Mediterranean climate to the north of France during the next decades (Dubreuil 2022), climatic conditions might become more suitable to a possible northward expansion of Z. tuberculatus.

The pest status of *Z. tuberculatus* and its potential risk for agriculture is not yet clear. In its native range, *Z. tuberculatus* develops on decaying fruits, particularly on fallen rotting figs (Lachaise et al. 1982) and was thus not considered as a pest. However, the spread of this species and the fact that it has successfully been reared on 49 different species of tropical fruits (Lachaise & Tsacas 1984) should be a point of vigilance. This is particularly important in view of the first emergences we have observed from overripe figs. Moreover, Balmès and Mouttet (2019) reported emergences of *Z. tuberculatus* from imported fruits of *Citrus sinensis* from South Africa and *Litchi sinensis* from Reunion.

Zaprionus indianus is an invasive species that is closely related to Z. tuberculatus (Yassin & David 2010). The pest behavior of Z. indianus should also be a warning point for the entry of Z. tuberculatus into the EU and France, as they may have similar nutritional ecology and invasion routes. In Brazil, Z. indianus was reported attacking figs and was responsible for 50% of fig losses of fruits that were still on trees (Dettler et al. 2021; Vilela et al. 1999). The entry in Central and South America and the damages to fig orchards (van der Linde et al. 2006; Vilela 1999) led to its inscription on the alert list of the European and Mediterranean Plant Protection Organization (EPPO) in 2016. Under laboratory conditions, Bernardi et al. (2017) observed that Z. indianus could oviposit in ripe strawberries and the larvae were then able to develop in the berries. Yet, the ability of Z. indianus to oviposit and generate offspring in healthy strawberry fruit was clearly facilitated by injuries caused by D. suzukii or by mechanical injuries (Bernardi et al. 2017). Although no specific report of damage by Z. indianus in the EU has been reported so far, considering the damage caused to figs in South America, Z. indianus establishment and spread was considered as a threat with possible large economic consequences in Europe (EFSA P on PH (PLH) et al. 2022). Consequently, in 2022, Z. indianus was considered by European Food Safety Authority as a potential Union quarantine pest (EFSA P on PH (PLH) et al. 2022). Our results suggest that a particular attention should also be paid on Z. tuberculatus. Indeed, we detected males and females Z. tuberculatus in a fig orchard during the harvest period where fruits are systematically removed before rotting as a prophylactic measure against D. suzukii (personal communication). This may suggest a potential capacity to develop in healthy fruits (to be confirmed). Indeed, Zaprionus flies can infest figs on trees via oviposition on ostiole base (EFSA P on PH (PLH) et al. 2022). Moreover, climatic conditions in many EU member states and host plant availability in those areas are conducive for establishment of both Z. indianus and Z. tuberculatus.

Our monitoring of D. suzukii enabled the first detection of Z. tuberculatus in southern France in summer 2022, at a singular site (a fig orchard and its adjacent hedgerow). This finding was reaffirmed the subsequent year, based on fruit samples collected in late summer 2023 in the same fig orchard. We will conduct additional sampling effort, over multiple years and at various locations to better characterize the phenology and formally confirm the establishment of this species in France. Our study also highlights a potential risk associated with the discovery of Z. tuberculatus, a new invasive drosophilid that may have potential economic consequences on fruit production. We are currently raising individuals collected on the invasive front in quarantine to carry out research into their ecology and biology in order to clarify their pest potential, particularly on figs, citrus fruit and strawberries.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Homemade trap used for the *Drosophila suzukii* monitoring (DroFramb action within the framework of ANR Drothermal project, https://www.drothermal.cnrs.fr/).

Table S1. Temperature data collected by TMS-4 Standard dataloggers (TOMST[®], Czech Republic) in the fig orchard and the hedgerow nearby. Indicated values are average \pm standard deviation; minimum - maximum of daily temperature recorded every 15 minutes at 6 cm into the ground (T_ground), at the surface (T_surface) and 15 cm above the ground surface (T_air).